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Emerging and Reemerging Virus Diseases of Vertebrates[☆]

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Glossary

Amplicon The product nucleic acid obtained from a polymerase chain reaction.

Bocavirus A genus of the family *Parvoviridae* containing bovine, canine, and human species.

Kaposi's sarcoma A skin tumor which frequently develops in young males infected with HIV.

Kawasaki disease A mucocutaneous lymph node syndrome that has features of a virus infection but so far no causative agent has been discovered. There are about 120 cases per

100 000 population in Japan, a sixfold higher incidence than in the USA.

Picobirnavirus A virus containing a genome consisting of two segments of double-stranded RNA, 2.6 and 1.5 kb in length.

Sigmodontinae A subfamily of rodents in the family Muridae that contains over 500 species, confined to the American continent.

Vero cells A heteroploid cell line derived from the kidney of a normal African green monkey (*Cercopithecus aethiops*).

Zoonosis A disease spread from animals to humans

Introduction

It became apparent during the last two decades of the twentieth century that new infectious diseases were increasingly being recognized in the human and animal populations. This led to the establishment of a formal committee of the Institute of Medicine of the National Academy of Sciences, USA, who reported on their deliberations in 1992, in a report edited by Joshua Lederberg and Richard Shope. This was followed 10 years later by a second report, edited by Mark Smolinski, Margaret Hamburg, and Joshua Lederberg, which appeared in 2003. Among the factors they cited as contributing to emergence were microbial adaptation and change, human susceptibility to infection, climate and weather, changing ecosystems, economic development and land use, human demographics and behavior, technology and industry, international travel and commerce, breakdown of public health measures, poverty and social inequality, war and famine, lack of political will, and finally intent to harm.

Recognition of Emerging and Reemerging Virus Diseases

The advent of highly specific molecular techniques such as the polymerase chain reaction (PCR) in the early 1980s permitted the detection and grouping of viruses on the basis of genome nucleotide sequence analysis, and in several respects these techniques have replaced serological analyses for the characterization of viruses. Although it is still important to isolate viruses in cell culture for their complete characterization, it is now possible directly to detect viruses in diseased tissues by PCR, then, by sequencing the amplicon, to determine whether a new virus has emerged to cause the disease. In fact, many viruses which do not readily grow in cell culture can only be differentiated by sequence analysis. The papillomaviruses are an example. Their study was very difficult until the advent of sequence analysis, which now has revealed more than 100 types in humans, and many more in animals and birds. For differentiation, three virus genes (E6, E7, and L1) are sequenced, and if the combined sequence of these three genes differs by more than 10% from known papillomaviruses, the virus is considered to be a new type. Other viruses which have not been grown in cell culture include many caliciviruses, and the ubiquitous anelloviruses, such as torque-teno (TT) virus, which can be detected and sequenced in the blood of most humans and many other vertebrate species.

Hepatitis C virus was originally described as non-A, non-B hepatitis virus because of the severe disease it caused but the virus would not grow in cell culture, and eventually was detected in blood known to be infected with the virus by reverse transcription of the RNA present using random primers then expressing the resultant DNA in the bacteriophage lambda gt 11. Thousands of clones were screened using patient blood as a source of antibody before positive clones were detected, which then allowed the

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development of enzyme immunoassays that could detect the virus in blood and so were used to screen blood destined for transfusion, saving millions of lives worldwide. Once the complete genome of hepatitis C virus was sequenced, it became apparent that there are many different genotypes circulating in the world, with different pathogenic properties.

Nucleotide sequence analysis has also been extremely useful in tracing the origins of viruses. For example, when hantavirus pulmonary syndrome, caused by a bunyavirus of rodents, Sin Nombre virus, was initially detected in 1993 in the Four Corners region of Western USA, it was found that rodents inside a house where people had been infected carried a virus identical in sequence to virus isolated from human cases. However, rodents caught at various distances from the house had increasingly variable genome RNA sequences, providing strong evidence that these rodents, deer mice (*Peromyscus maniculatus*), were the source of the infection. Subsequently more than 30 other hantaviruses, some of which also cause hantavirus pulmonary syndrome in humans, were isolated from rodents throughout North and South America. Each new virus seems to be associated with a different genetic variant of rodent host, and all rodents that carry the virus belong to the subfamily Sigmodontinae, unique to the American continent.

A particularly powerful tool for the initial recognition of an emerging virus is the application of immunohistochemistry to diseased tissues. Provided a comprehensive collection of antibodies is available, the particular virus or related group of viruses can often be detected. For example, when Hendra virus first appeared in 1995 in Australia, causing the death of a horse trainer and 14 of his horses, antibody against the virus was sent to the Centers for Disease Control and Prevention (CDC). Then, in 1999, CDC was asked to investigate a newly emerged epidemic that had appeared in Malaysia, killing more than 100 people and causing disease in many pigs. Initially, it was suspected to be caused by a virus related to Japanese encephalitis virus, but a virus was isolated from the pigs that replicated in Vero cells, and reacted in an immunofluorescence test against the Hendra virus antiserum. This could subsequently be used on patient tissues to study the pathogenesis of the disease, and after comparison of the genome sequences of Hendra virus and Nipah virus they were found to be closely related and are now classified in the genus *Henipavirus* of the *Paramyxoviridae*.

Finally, molecular methods can be used to detect new, emerging viruses in the absence of disease in the host. In 2001, Allander and colleagues searched for RNA viruses in human respiratory secretions using random primer PCR, and discovered a hitherto unknown parvovirus with a sequence related to the bovine and canine parvoviruses, which are grouped together in the genus *Bocavirus*. The new virus was called human bocavirus, and many research groups worldwide have now confirmed the presence of the virus, particularly in pediatric samples, although it is still not certain how important this virus is in causing morbidity and mortality. Their method also amplified a human coronavirus from the respiratory samples, and when sequenced this turned out to be HKU1, a recently emerged coronavirus detected by scientists at Hong Kong University. It is possible that a systematic search of human samples using such molecular techniques might reveal more hitherto unknown human viruses.

Human Demographics and Behavior

In some cases, the emerging viruses themselves have contributed to other viruses emerging and reemerging in the population. This is especially true of human immunodeficiency virus (HIV), the cause of acquired immune deficiency syndrome (AIDS), which rapidly spread following its emergence in the early 1980s to infect more than 40 million people worldwide by the end of the twentieth century. Because of its severe effects on the immune system, the virus leads to numerous other infections in the HIV-infected population. For example, picobirnaviruses, that had been detected in fecal samples from chickens and rabbits, were difficult to detect in human fecal samples until a cohort of men with AIDS was examined, and in these humans picobirnavirus was detected for the first time. Some rare diseases have become common in persons with AIDS. For example, the human polyomavirus known as JC virus can cause the rare brain disorder known as progressive multifocal leukoencephalopathy (PML). Normally, the virus remains dormant in the kidney, but in HIV-infected individuals the HIV-encoded transactivator Tat acts as a transactivator of JCV leading to PML which progresses to death within 4 months after infection. Other important virus infections which emerge in AIDS patients are human herpesviruses (cytomegalovirus, herpes simplex viruses 1 and 2, varicella-zoster virus, and human herpesvirus 8, which causes Kaposi's sarcoma).

HIV is mainly spread through sexual activity between an infected and a noninfected person, and is most common in those who indulge in high-risk sexual behavior with multiple partners. It can also be transmitted by direct contact with infected blood, and is common in persons who indulge in intravenous drug use, particularly when needles, syringes, or equipment used to prepare drugs for injection are shared. It is therefore an example of a virus disease which is dependent on risky human behavior for its maintenance in the human population.

The ability of such new infections to spread in the population has been greatly enhanced by population growth and ease of movement as a result of rapid air travel. A dramatic recent example of this was the appearance of the coronavirus causing severe acute respiratory syndrome (SARS) in late 2002 which spread by air travel from a single infected Chinese physician who infected 12 persons in a Hong Kong hotel. These infected persons then traveled by air and spread the infection to more than 8000 individuals worldwide, 10% of whom died. The virus then apparently receded from the human population in July 2003. Only recently was it discovered that the SARS coronavirus has a natural reservoir in Chinese horseshoe bats (*Rhinolophus sinicus*). Some other species such as Himalayan palm civets and raccoon dogs from which the virus has been isolated may serve as amplification hosts.

Following the recognition of the human coronavirus SARS, research on coronaviruses intensified, and this led to the discovery in 2004 of two previously unrecognized human viruses, one found by Hong Kong University, called HKU1 virus, and another reported almost simultaneously from the Netherlands, called NL63, and from Yale University, called New Haven coronavirus. The latter viruses probably represent two isolates of the same virus species. They are clearly associated with lower respiratory tract infection in children, but initially it was claimed that New Haven coronavirus was also associated with Kawasaki disease in children. This intriguing claim was rapidly investigated and refuted by several different groups in Japan, Taiwan, and elsewhere, and the cause of Kawasaki disease, which has features resembling a virus infection, remains unknown.

In 2012, another novel human coronavirus was isolated from sputum from a fatal case of severe respiratory disease in the Arabian Peninsula. The virus, now called Middle East Respiratory Syndrome Coronavirus (MERS-COV), has since been detected in more than 187 patients with respiratory disease, 97 of whom died. In November 2013, the Ministry of Health in Saudi Arabia was notified of case of MERS-CoV infection in a 43 year-old male patient who had cared for ill dromedary camels in his herd of 9 animals starting in early October. The patient was confirmed with MERS-coronavirus by real-time PCR, and limited sequencing found the MERS-coronavirus in three of the dromedary camels. Other studies have detected MERS-coronavirus-infected dromedary camels in several other regions in Saudi Arabia. But it remains unclear whether camels are an important source of the virus from transmission to humans, and whether bats also play a role, as was the case with the SARS-coronavirus.

Zoonotic Diseases

A majority of recent emerging virus diseases have been zoonoses (i.e., diseases transmitted from animals to humans under natural conditions). Some of the more important of these include HIV-1, which was transmitted to humans from chimpanzees in Central Africa around 1931, and HIV-2, transmitted from sooty mangabeys to humans in West Africa around 1940.

Other important recent examples are the viruses of the genus *Henipavirus*. Hendra virus was first recognized through a disease outbreak in some horse stables in Hendra, Queensland, Australia, when 14 horses and their trainer died from pulmonary disease with hemorrhagic manifestations in 1994. The reservoir of the virus was found to be in large fruit-eating bats (*Pteropus* spp.), and one year later a horse farmer 600 miles away in Mackay, Queensland, died of encephalitis from the same virus. Then, in 1999, a related virus was discovered in Malaysia following a major outbreak of respiratory disease in pigs and neurological disease in humans in their close contact. More than 100 humans died, and in a successful effort to control the disease 1.1 million pigs were slaughtered. The causative virus was isolated from a fatal human case that had lived in Nipah River Village, and so was named Nipah virus. Hendra and Nipah viruses are clearly members of the family *Paramyxoviridae*, but have been placed in a separate genus as their RNA genome is about 19 kb in length, larger than that of any other paramyxovirus.

Nipah virus, like Hendra virus, was found to have a reservoir in *Pteropus* bats, and has since been identified in fatal human disease outbreaks in India in 2003 and Bangladesh in 2004.

Other new viruses which apparently have a reservoir in fruit bats include Menangle virus, a new paramyxovirus which emerged in a commercial piggery near Sydney, Australia, to cause stillbirths and abortion in pigs. Menangle virus also caused disease in two workers in the piggery. A new virus related to Menangle virus emerged during an investigation of urine samples from *Pteropid* bats collected on Tioman island, off the coast of Malaysia, in 2001, and was named Tioman virus. During the same investigation, a new orthoreovirus was isolated from *Pteropus hypomelanus* in 1999 and called Pulau virus, and more recently a related orthoreovirus called Melaka virus was isolated from a human case of acute respiratory disease in Melaka, Malaysia. Serological studies of sera collected from human volunteers on Tioman island showed that 13% had antibodies against both Pulau and Malaka viruses.

Another important group of zoonotic diseases are rodent-borne, and caused by members of the genus *Hantavirus* of the family *Bunyaviridae*. These viruses first emerged during the Korean War of 1950–52, when thousands of UN troops developed a mysterious disease with fever, headache, hemorrhage, and renal failure with a fatality rate of 5–10%. It was more than a quarter of a century before the causative virus was isolated from field mice in Korea, and named Hantaan virus, the cause of hemorrhagic fever with renal syndrome (HFRS) in humans.

Then, in 1993, a new hantavirus emerged in the Four Corners region of Southwestern USA as the cause of a severe acute respiratory disease syndrome, with a fatality rate close to 40%, and named Sin Nombre virus. This virus was shown to be transmitted to humans by inhalation of virus present in the urine, feces, or saliva of deer mice (*Peromyscus maniculatus*). It seems likely that this disease had existed for many years, and was only recognized in 1993 because of a clustering of human cases as a result of a regional upsurge in the rodent population resulting from climatic conditions causing increased availability of rodent food. Fortunately, in most of these infections, humans appear to be a dead-end host, and transmission between humans does not occur except with the Andes virus in South America.

Rodent-borne viruses of the family *Arenaviridae* also cause a number of serious zoonotic diseases in humans. The 'Old World' arenaviruses such as Lassa fever virus have been known for some time, but still cause thousands of fatal hemorrhagic fever cases every year in West Africa. However, 'New World' arenaviruses such as Junin virus causing Argentinian hemorrhagic fever and Machupo virus causing Bolivian hemorrhagic fever have long been recognized in South America. Recently, new arenaviruses have emerged, probably as a result of deforestation, which results in rodents seeking shelter in human habitation, and brings them into closer contact with people. These viruses include Guanarito virus that causes Venezuelan hemorrhagic fever with 36% mortality rate from confirmed cases, and Sabia virus isolated in 1990 that causes Brazilian hemorrhagic fever with a high fatality rate, including two laboratory acquired cases.

Rabies is a zoonotic disease of great antiquity that has mainly been associated with carnivores, such as dogs. The virus is excreted in the saliva of infected animals, and following infection it moves through the nervous system to attack the brain, causing aggressive behavior which results in the animal biting humans and animals with which it comes into contact and thereby spreading the virus infection. Fortunately, due to early work by Louis Pasteur, a vaccine was developed that protects humans or other animals from infection, and can also be given immediately post exposure, and the domestic dog population in the developed world is vaccinated and does not pose a risk to humans. However, in some developing countries, it is not uncommon for a rabid dog to bite and infect more than 25 people before it can be put down, and worldwide there are still some 30 000 human rabies deaths per year. Using molecular sequencing techniques, it is now possible to distinguish the genotypes of rabies viruses associated with different species of host, as the virus has become adapted through frequent transmission between members of the same host species. In the USA, there are six recognized terrestrial animal genotypes, in raccoons in eastern states, skunks in north-central states, skunks in south-central states, coyotes in southern Texas, red foxes in Alaska, gray foxes in Arizona, and several genotypes associated with particular species of bat. In fact, most fatal cases of human rabies in the USA can now be traced to bats, which are often not detected when the person is bitten; so rabies is not suspected and vaccination is not undertaken until the disease has taken hold.

Ecological Factors Favoring Virus Emergence

Many important virus diseases are spread by arthropods, and exposure to new arthropods and the viruses they carry is critical to the emergence of new virus diseases. Dengue hemorrhagic fever is caused by dengue virus which is transmitted mainly by the Asian mosquito (*Aedes albopictus*), and dengue fever is one of the most rapidly emerging diseases in tropical regions of the world. There are four serotypes of dengue virus, and it seems that consecutive infections with two antigenic types can lead to the more serious disease of dengue hemorrhagic fever with shock syndrome, which, if untreated, can result in up to 50% mortality. Unfortunately, through the importation of vehicle tires containing water from Korea, the Asian mosquito was introduced into the USA, and is now present in several regions of the Southern states. It can act as a vector not only for dengue virus, but also for California encephalitis virus.

In Europe, the emergence of two important animal diseases has occurred through the movement of arthropod vectors into the Iberian Peninsula. African horse sickness virus causes a disease that can be fatal to horses, mules, and donkeys, and is transmitted by nocturnal biting flies of the genus *Culicoides*. These were introduced inadvertently into Spain, and the disease is now endemic around Madrid and regions to the south. African swine fever virus is transmitted by ticks of the genus *Ornithodoros* and it causes a fatal disease resembling classical swine fever in domestic pigs. It first emerged in Portugal and Spain in 1957, France in 1964, Italy in 1967, and Cuba in 1971. Through slaughter of infected animals, the disease was eradicated from Europe, except Sardinia, by 1995.

The most recent dramatic example of the movement of a virus vector is provided by West Nile virus, a flavivirus first isolated in Uganda in 1937. This virus uses birds as a reservoir host, and is transmitted from birds to humans and other vertebrates by mosquitoes. In 1999, cases of encephalitis in New York were found to have been caused by a strain of West Nile virus that was phylogenetically similar to a virus isolated from geese in Israel.

At the same time, many birds, especially corvids, began dying in New York State. Since the introduction in 1999, West Nile virus has become well established throughout the USA and moved north into Canada and south into the Caribbean and into Mexico. It is not known how the virus moved from Israel to the USA, but the most reasonable explanation is that it was carried in an infected mosquito or possibly an infected bird in the hold of an aircraft. Transmission by an infected human seems less likely since the titer of virus in human blood is usually too low for efficient mosquito transmission.

It is clear, nevertheless, that once it arrived in North America, West Nile virus found an extremely favorable environment with abundant avian and arthropod hosts that facilitated its spread throughout the American continent.

Prospects

The emergence of new viruses is likely to continue as viruses evolve and find new ecological niches in the human and animal population. It is noticeable that most newly recognized viruses have been RNA viruses, perhaps since RNA evolves at a faster rate than DNA, for which host cells have developed efficient proofreading enzymes. It will be important in the future to detect new viruses before they can emerge to cause disease in the population. The SARS epidemic provides an excellent example. Before the epidemic, only two human coronaviruses were known, human coronaviruses 229E and OC43. Despite the fact that serious coronavirus diseases were well known in other vertebrates, such as feline infectious peritonitis and avian infectious bronchitis virus, it was not until the SARS epidemic that research on human coronaviruses led to the discovery of three new human coronaviruses – SARS, HKU1, and NL63/New Haven.

There are other genera of viruses that cause serious disease in animals but have not been adequately investigated in humans. An example is the genus *Arterivirus*, which has members causing serious disease in horses and pigs, but has not been reported at all in humans. This could be a worthwhile area for future investigation.

Another critical factor in the future control of emerging viruses is better vector control. When mosquito control was conducted using DDT, dengue fever virus was virtually eliminated from the Americas in the 1970s, but environmental concerns led to the

widespread banning of the use of DDT, so that since the 1980s there has been a considerable expansion of dengue fever in South America, with the appearance of dengue hemorrhagic fever there for the first time. There is a real need to improve mosquito control measures to control this disease. Although there are prospects for a dengue virus vaccine, this is so far not available.

In 2014, a new outbreak of Ebola virus occurred in West Africa, causing several thousand deaths in Liberia and Sierra Leone. The Ebola virus first emerged as the causative agent of viral hemorrhagic fever in two outbreaks occurring almost simultaneously along the Ebola river in the Democratic Republic of Congo in 1976. There were some 318 human cases in this episode with a mortality rate of 88%. Since that time, a number of smaller outbreaks of Ebola hemorrhagic fever affecting humans and monkeys were reported from West Africa, but although most infections have been associated with hunting and handling of great apes, the actual origin of the virus has never been ascertained. It is clear that transmission of the virus requires close contact with an infected individual, and does not involve airborne transmission. Ebola virions are usually long filamentous sometimes branched forms with a uniform diameter of about 80 nm, and varying in length up to 14000 nm, from which they are called filoviruses, belonging to the family filoviridae.

Finally, one of the most important viruses that continue to emerge in different antigenic forms is influenza virus. The main reservoir of influenza viruses is in birds, and over the past century several pandemics of influenza have emerged, the most serious of which was in 1918. Pandemic strains usually arise by a process of antigenic shift, where one of the genes encoding the hemagglutinin and/or the neuraminidase of influenza virus is replaced by one from birds. New pandemics occurred in 1918 (H1N1 subtype), 1957 (H2N2 subtype), and 1968 (H3N2 subtype). Since 1968, there have been no new pandemics, but it is widely expected that another will occur. At the time of writing, there is worldwide concern that a highly pathogenic avian influenza virus (H5N1 subtype), which has caused some human infections and deaths in persons in close contact with infected birds, might mutate or recombine to generate a virus which would be highly transmissible in the human population. Plans are being developed in many countries and by the WHO to try to prepare for such an event by generating possible vaccines against such a virus and stockpiling antiviral drugs.

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