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Zoonoses: A Stimulus for Wider Research and Consideration in Product Development

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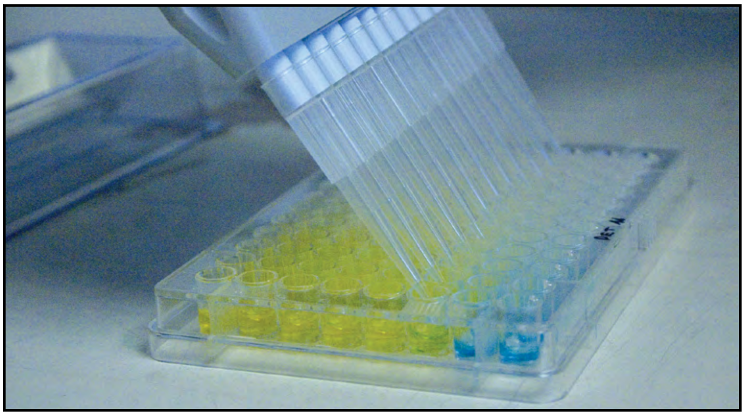
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The last decade witnessed remarkable scientific and technological advances in a number of scientific disciplines, including cell biology, microbiology, molecular biology, oncology, virology, infectious diseases, diagnostic technologies, analytical chemistry, instrumentation, and informatics. These advances have had a major impact on medicine, which has experienced fantastic progress in improving disease diagnosis, treatment, and overall patient care.

Despite the advances in developing ever more sophisticated technologies and increasing the understanding of disease, new maladies continue to emerge. This is especially true for infectious ailments. Despite great developments in epidemiology, diagnostics, and agent detection technologies, as well as a comprehensive understanding of the biology of many known infectious agents and their virulence factors, we also are witnessing a dramatic increase in the number of new agents and diseases. Many of these infectious agents originate in animals and can be transmitted to another member of the same species or to different animal species, including humans. These infections that originate in animals and spill over to humans are known as zoonoses and their respective infectious agents are described as zoonotic agents.

Zoonoses are caused by a wide range of microbial agents, including bacteria, protozoa, helminths, mycoplasma, chlamydia, rickettsia, viruses, and prions. Sarah Cleaveland, a research fellow at the Centre for Tropical Veterinary Medicine at the University of Edinburgh in Scotland, provides interesting insights into this subject. She carefully catalogued 1,415 known pathogens of humans, 616 known pathogens of domestic animal livestock, and 374 known pathogens of domestic carnivores. Of the human pathogens, 61.6% have a zoonotic origin. Of the 616 pathogens of domestic livestock, 77.3% are considered "multihost," while 90% of 374 carnivore pathogens are considered multihost.[1]

Many new and emerging microbial agents have been detected during the past decade. The emergence of these viral agents is in line with well-known concepts of co-evolution of animal viruses in contact with humans. This article provides an overview of some recent viral zoonotic agents that are important from the medical and biopharmaceutical perspective. It further discusses some significant factors responsible for the emergence of new agents and highlights potential opportunities for biotechnology applications that could be applied to address unmet medical needs in this field.

Lessons from the Past

Hendra Virus (Equine Morbillivirus)

In late September 1994, an outbreak of severe respiratory disease occurred among horses on a horse property near Brisbane, Australia. Within two weeks, 14 of 21 sick horses died or were killed after an acute illness characterized by high fever and severe respiratory insufficiency. Two people who had close contact with the dying horses became ill with a severe influenza-like illness; one died after six days.[2] Veterinary examination of the farm and virological laboratory investigations of tissues from the dead animals revealed a new virus morphologically resembling paramyxoviruses. The virus was later confirmed to be a completely new paramyxovirus of the genus morbillivirus, which was subsequently named Hendra virus after the town where the disease was first observed.

Equine morbillivirus (EMV) infections have been observed in cats and guinea pigs. An Australian fruit bat

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from the genus *Pteropus* (also known as the "flying fox") is a natural reservoir of the virus. Hendra virus is thought to have moved from flying foxes to horses, and then from horses to people.[2] There is a reasonably strong hypothesis for horse-to-human transmission of virus via nasal discharge, saliva, and/or urine. In contrast, there is no strong evidence for flying fox-to-human transmission. There is some evidence that the Australian paralysis tick, *Ixodes holocyclus*, a parasite of flying foxes, may transmit Hendra virus (and, perhaps, related viruses) from flying foxes to horses and other mammals. So far, the disease has not been described in Europe and North America.

Hendra virus grows rapidly on Vero, LLC-MK2, and MRC5 cells, producing visible focal syncytia. Nucleic acid amplification (NAA) technologies (e.g., RT-PCR), immunohistochemistry (IHC), and antibody detection assays (e.g., ELISA, IFA, SNT) are available for virus detection.[2,6]

Nipah Virus

Nipah virus, another novel paramyxovirus which is closely related to Hendra virus, emerged in northern Malaysia in 1998. The virus caused an outbreak of severe febrile encephalitis in humans with a high mortality rate.[7,8] In pigs it produces encephalitis and respiratory disease, but with a relatively low mortality rate. From Malaysia the outbreak spread south to Singapore due to the movement of infected pigs. In early 1999, a number of abattoir workers in Singapore were hospitalized with encephalitis and pneumonia. The illness was initially thought to be Japanese encephalitis, but virological, molecular, and electron microscopic studies at the Centers for Disease Control and Prevention (CDC) in the United States indicated that a completely new, Hendra-like paramyxovirus was associated in some cases. This new virus was named Nipah after the village where the disease was first observed.

Nipah and Hendra share about 80% homology at the nucleotide level. As in the case of Hendra virus, *Pteropid* fruit bats were identified as the natural reservoir of Nipah virus. Evidence suggested that climatic and anthropogenic-driven ecological changes (e.g., deforestation for pulpwood, slash-and-burn deforestation, drought driven by El Niño Southern Oscillation, reduction in the availability of flowering and fruiting forest trees for foraging by flying-foxes in their already shrinking wildlife habitat, migration of flying-foxes into cultivated fruit orchards) coupled with the location and design of the pig farms allowed the spillover of this novel paramyxovirus from its reservoir host into domestic pigs and ultimately to humans.[9] Nipah virus is spread mainly via air and close contact with infected individuals (respiratory and oropharyngeal secretions). The case fatality rate among infected human patients was 40%, and the course of the disease differed from the pattern seen in Japanese encephalitis. The virus has a narrow host range that is probably restricted to pigs and humans. Human-to-human transmissions of Nipah virus have not been documented.

Nipah virus has typical paramyxoviral morphology. It is an enveloped ssRNA virus 100–300 nm in size. The full sequence of the Nipah genome has been completed; it consists of 18,246 nucleotides, which is 12 nucleotides longer than the Hendra virus genome. Nipah virus is best detected by propagation in Vero cells where it produces a massive cytopathic effect, and by detection of viral nucleic acid using RT-PCR. Specific serum IgM antibodies can be confirmed in the paired serum samples from infected individuals (using ELISA and IFA).

This virus has not been described in Europe and the United States. However, the international movement of pigs, pig products, and porcine byproducts presents a risk of introduction of this virus into new geographical areas. Furthermore, the use of porcine-derived raw materials (e.g., trypsin, peptones) in biotechnology applications presents a risk of introduction of Nipah into manufacturing processes, possibly making its way to the final container.[7–11]

Menangle Virus

Australian investigators yet again surprised the world medical community in 1998 by reporting on another apparently new swine virus capable of infecting people. This time, the new disease was reported in an Australian pig property in New South Wales. The virus belongs in the family *Paramyxoviridae* and was isolated from stillborn piglets with deformities.[12] Workers associated with the infected pigs came down with a flu-like illness and reacted seropositive to the Menangle virus. An antibody survey among the local bat population in the affected area showed that the bat population was also seropositive for the newly detected virus, which implicated them as the source of the emerging disease.

Menangle virus is thought to have moved from flying foxes to pigs in the pig farm, and then from infected pigs to people. Direct contact with sick animals, body fluids, and air droplets are probably the primary routes of virus transmission.

The Menangle virus grows in a wide range of porcine and human cell types as well as the BHK21 cell line, producing a notable cytopathic effect. This virus has not been described in Europe and the United States. However, the international movement of pigs, pig products, and porcine byproducts presents a risk of introduction of Menangle into new geographical areas. Furthermore, the use of porcine-derived raw materials (e.g., trypsin, peptones, etc.) in biotechnology applications presents a risk of introduction of Menangle into manufacturing processes, potentially making its way to the final container.[12,13]

Porcine Hepatitis E Virus (HEV)

The end of the last century witnessed the discovery of yet another emerging zoonotic agent in pigs. Scientists from the United States, and later from other parts of the world, described a previously undetected viral agent of swine, named swine hepatitis E virus (HEV).[14] It was originally isolated from herd pigs in the midwestern United States. Subsequently, two cases of acute hepatitis E in human patients in the United States were found to be caused by a strain of virus that is genetically very similar to the swine HEV. These findings suggested that swine HEV may be involved in cross-species infection between swine and humans. Further studies in Taiwan, where the pig population is very dense, showed evidence of HEV circulation in herd pigs.[15] This fact helped to explain the high level of anti-HEV antibodies among humans, especially pig handlers, in areas where HEV is nonendemic. It was concluded that pigs serve as reservoirs for transmission of human HEV.

It was also demonstrated that domestic pigs could be experimentally infected with a human HEV isolate. Nucleotide similarity between the human and swine isolates in Taiwan is 80 percent; swine HEV is immunologically cross-reactive with human HEV. The virus is transmitted primarily by the fecal–oral route, which is reflective of low sanitary conditions. Although pigs are infected subclinically with no apparent symptoms, some human infections with HEV lead to acute hepatitis associated with a higher level of serum bilirubin and a higher mortality rate than acute hepatitis caused by the hepatitis A and B viruses. Swine HEV is endemic in many countries, and is ubiquitous in pigs from the midwestern United States. A recent serological survey of swine veterinarians, as well as non-veterinarians, suggested that veterinarians are at somewhat higher risk of HEV infection.[16]

Human HEV is a small, nonenveloped, ssRNA virus about 7.5 kb long. Although the virus was originally believed to be similar to caliciviruses, it now remains unclassified. The possibility that swine HEV may infect humans presents important public health concerns regarding zoonosis and xenozoonosis (the transmission of a disease to humans after the transplantation of an animal organ). Additionally, the use of porcine-derived raw materials (e.g., trypsin, peptones) in biotechnology applications may introduce HEV into manufacturing processes, potentially leading to product cross-contamination.

Hepatitis E virus can be detected by NAA technologies that target the conserved region of the viral RNA. An enzyme-linked immunosorbent assay (ELISA) test has been developed for specific HEV antibody detection in the serum of infected individuals.[14–16]

Porcine Endogenous Retrovirus (PERV)

The number of patients requiring organ or tissue transplantation far outweighs the availability of human donor organs. Xenotransplantation of non-human primate and pig organs is viewed as a means to alleviate this shortage of donor organs. While progress has been made on the immunological problems of xenotransplantation, the risks of infectious agent transmission from graft to recipient remains a very hot topic. The normal germline of many species contains sequences of endogenous retroviruses (ERVs), which might cause zoonotic disease if transmitted, even if they are not normally pathogenic in their natural host.

Endogenous retroviruses have been described in baboons, cats, pigs, chickens, and numerous rodent species. It has been shown that pig kidney cells (PK-15) and minipig kidney cells (MPK) in culture release porcine retroviral particles that could infect human embryonic kidney (HEK 293) cells.[17] It is estimated that the pig genome contains approximately 50 porcine endogenous retrovirus (PERV) copies per cell. Viral titer in the infected 293 cells can be up to 500 infectious units per milliliter of cell culture medium.[17] It remains unclear whether PERVs have potential to infect transplant recipients *in vivo* and, if so, whether they are pathogenic.

It is, therefore, essential to evaluate the risks and critical to use specific and sensitive screening technologies. For direct detection of PERV production, reverse transcriptase-based assays such as product enhanced reverse transcriptase (PERT) are used. Furthermore, PCR and quantitative PCR (qPCR) are used to detect the proviral DNA in the appropriately selected test sample. An immunoperoxidase assay (IPA) has been developed, which allows detection of viral proteins in infected cells.[18,19] This assay also is good for detection of specific circulating antibodies against PERV in the serum of an infected host. Therefore it may be very useful for the surveillance of preclinical and clinical experiments. This IPA is often employed in *in vitro* experiments for evaluation of the virus host range, virus titration, and antiviral properties of azidothymidine (AZT). Other test methods for PERVs include virus isolation by co-culture, ELISA, immunofluorescence assay (IFA), and Western blotting.[17–19]

Transmissible and Bovine Spongiform Encephalopathy (TSE/BSE)

Bovine spongiform encephalopathy (BSE) is a transmissible, fatal, neurodegenerative brain disease of cattle. The disease has a long incubation period of four to five years or more, but is ultimately fatal for cattle within weeks to months of its onset. This transmissible spongiform encephalopathy (TSE) first came to the attention of the scientific community in November 1986 with the appearance in cattle of a newly recognized form of neurological disease in the United Kingdom (UK).[29]

A similar disease in sheep, scrapie, has been endemic in the UK sheep population for more than 220 years. Epidemiological studies conducted in the UK suggest that the source of BSE was cattle feed prepared from inadequately treated tissues (such as brain and spinal cord) from sheep or other ruminants including cattle that were contaminated with the scrapie agent.

Bovine spongiform encephalopathy in cattle primarily targets the brain and spinal cord. These tissues, along with the tonsils and the optical nerve in the eye, contain the highest infectivity and are considered the highest risk tissues.

The nature of the BSE agent is still a matter of debate. According to the most widely accepted theory, the agent is composed largely, if not entirely, of a self-replicating protein referred to as a prion. Strong evidence collected over the past decade supports the prion theory, but the ability of the BSE agent to form multiple strains is more easily explained by a virus-like agent, although some explanations are consistent with the prion theory. The agent is highly stable and resists freezing, drying, and heating at normal cooking temperatures, even those used for pasteurization and sterilization.

Between November 1986 and November 2003, 182,253 cases of BSE were confirmed in the UK, and 3,913 cases were reported outside the UK. A single case of BSE was detected in the United States for the first time in December 2003.

The BSE agent has been linked with a newly recognized form of CJD, variant Creutzfeldt-Jakob disease (vCJD) in humans, which was first reported in March 1996 in the UK. In contrast to the classical forms of CJD, both sporadic and familial, which affects patients with an average age of 65, vCJD has affected younger patients, with an average age of 29. It has a longer duration of illness (a median of 14 months compared to 4.5 months in the classical form of CJD) and evidence strongly links it to BSE, probably through exposure to or consumption of certain types of beef tissues. Recent studies have confirmed that vCJD is distinct from sporadic and acquired CJD, and that it is clinically indistinguishable from the effects of the BSE agent. Similarities observed between the strain of the agent responsible for vCJD and that of BSE have been demonstrated experimentally, linking the emergence of new vCJD cases to the same agent that causes BSE in cattle.

From October 1996 to November 2003, 137 cases of vCJD were reported in the UK, six in France, and one each in Canada, Ireland, Italy, and the United States. There is insufficient information to make any precise prediction about the future number of vCJD cases, especially given that the disease may have a long latency period (10 to 20 years) before symptoms emerge.

Per World Health Organization (WHO) and Office International des Epizooties (OIE, the world organization for animal health) recommendations, all countries must prohibit the use of ruminant tissues in ruminant feed and must exclude tissues that are likely to contain the BSE agent from any animal or human food chain. Furthermore, all countries are encouraged to conduct risk assessments to determine if they are at risk for TSE in sheep and goats.

Human and veterinary vaccines prepared by using bovine- and human-derived raw materials in manufacturing process may carry the risk of transmission of TSE agents. Ideally, the pharmaceutical industry should avoid the use of bovine materials and materials from other animal species in which TSEs naturally occur. If absolutely necessary, bovine materials should be obtained from low risk tissues from countries which have a surveillance system for BSE in place and which have reported either zero or only sporadic cases of BSE.

Antemortem diagnosis of BSE is very difficult given the labor intensive nature of the most sensitive method, histopathology, and the low sensitivity of the easier and more rapid detection methods. The best detection is achieved by pathohistological examination of target tissues (e.g., brain and/or tonsil biopsy). However, postmortem brain histopathology provides the most definitive diagnosis. Several immunology-based assays are available for laboratory detection of prion agents including Western blotting, dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA), and enhanced chemiluminescence assay.[20–30]

West Nile Virus (WNV)

West Nile Virus is a member of the family *Flaviviridae*. It is an enveloped ssRNA virus, 40–50 nm in size. Serologically, it is a member of the Japanese encephalitis virus antigenic complex, which includes St. Louis, Japanese, Kunjin, and Murray Valley encephalitis viruses. The virus was first isolated in the West Nile province of Uganda in 1937. It can be detected using several different approaches: virus isolation in susceptible cell culture followed by direct fluorescence assay, detection of amplified nucleic acid regions by RT-PCR, and detection of specific antibodies by ELISA, IFA, hemagglutination inhibition (HI), or plaque reduction neutralization test (PRNT).[31–36]

Until 1999, West Nile virus (WNV) had never been detected in North America. It was an exotic disease confined to certain parts of Africa and several other temperate regions. In late summer 1999, the first domestically acquired human cases of West Nile (WN) encephalitis were documented in New York City. The discovery of virus-infected mosquitoes during the winter of 1999–2000 precipitated early-season vector control and disease surveillance in New York City and the surrounding areas.

These surveillance efforts were focused on detecting and identifying WNV infections in birds, mosquitoes, and equines as sentinel animals. The spread of WNV was tracked throughout much of the United States between 2000 and 2002. By the end of 2002, WNV was observed to be widespread and activity had been identified in 44 states and the District of Columbia. The 2002 WNV epidemic and epizootic resulted in 4,156 reported human cases of WN disease, 16,741 dead birds, 14,571 equine cases, and 6,064 infected mosquito pools. The 2002 WNV epidemic and epizootic was the largest recorded arboviral meningoencephalitis epidemic in the western hemisphere and the largest WN meningoencephalitis epidemic ever recorded. Significant human disease activity was observed in Canada for the first time, and the spread of WNV to the Caribbean basin and Mexico was also recorded. The human case-fatality rate in the United States has been around 7 percent overall, and among patients with encephalitis about 10 percent. Four new routes of virus transmission were observed: blood donation, organ transplantation, transplacental, and breastfeeding.[31,32]

West Nile virus is primarily transmitted in the United States by three species of *Culex* mosquito, which are its usual vectors. However, thirty-six species of mosquito have been shown to carry the virus, widening WNV's host range in the United States to about 27 susceptible mammalian species. The principal natural reservoirs of WNV are birds. Only birds seem to meet the criteria for a reservoir; they have a prolonged, high-titer viremia that enables them to serve as the source of infection for the mosquito vector. West Nile virus has been shown to infect 162 species of birds, with a high mortality rate. As a result, public health officials have been using bird mortality to effectively track the movement of WNV.

Vector mosquitoes become infected by feeding on the blood from viremic birds, then further transmit the infection by biting a susceptible host, either a bird or mammal. Mammalian hosts, primarily equines, sheep, cattle, and humans, are "accidental" hosts of WNV and do not appear to be primarily involved in the virus cycle because of the low-level viremia which is practically incapable of infecting a vector.[33,34]

Although it is still not certain when and how WNV was introduced into North America, international travel of infected persons to New York, importation of infected birds and mosquitoes, and bioterrorism are some possibilities. The disease usually occurs during summer when mosquitoes are most active and abundant. After a short incubation period, typically three to six days, WNV infection of human subjects usually produces either asymptomatic or subacute febrile illness, sometimes accompanied by rash. In a small percentage of patients it can cause severe, acute, and potentially fatal disease characterized by sudden onset accompanied by fever, cephalgia, muscular, ocular and articular pain, headache, myocarditis, meningitis, and encephalitis.

Approximately 8,470 human cases were reported to the CDC by November 2003, with about 180 deaths. In the mainland United States only two states, Oregon and Washington, still remain virus- and disease-free as of this writing.[35] Because the virus can be transmitted by blood and organ donations, donor screening has become routine. More than 2.5 million blood donations have been screened for WNV, and about 489 (0.2%) WNV viremic donors were identified during the screening process. Two cases of blood transfusion-related WNV cases were reported in 2003.

SARS

The beginning of 2003 was marked by another apparently new medical problem of potentially pandemic proportions known as severe acute respiratory syndrome (SARS). It was confirmed to be caused by a coronavirus, called SARS-associated coronavirus (SARS-CoV).[37] SARS was first reported in China, and soon afterward in Hong Kong and Singapore in February 2003. Over the next few months, the illness spread to more than 20 countries in North America, South America, Europe, and Asia.

According to the WHO, during the SARS outbreak of 2003, a total of 8,098 people worldwide became sick with SARS; of these, 774 (9.5%) died.[38] In the United States, there were 192 cases of SARS among people, all of whom recovered. Through July 2003, laboratory evidence of SARS-CoV infection had been detected in only eight U.S. cases. Most of the U.S. cases were among travelers returning from other parts of the world with SARS. There were very few U.S. cases among close contacts of travelers, including health-care workers and family members.

Stringent infection control precautions in health care institutions worldwide, broad isolation measures in affected communities, and international surveillance with barrier restrictions to travel led to termination of the epidemic. Although the SARS global outbreak of 2003 was contained, it is possible that the disease could re-emerge.

The SARS-CoV virus is believed to have its origin in wild animals, most likely civet cats in southern China. Its genome structure, gene expression pattern, and protein profiles are similar to those of other corona viruses. Although corona viruses are divided into three serogroups, phylogenetic analysis indicates that the SARS-CoV does not closely resemble any of these. The 29,751-bp genome of the Toronto strain (Tor2) isolate has been sequenced in record time and the results reveal that the virus is moderately related to the other two known human corona viruses, OC43 and 229E.

Distinct patterns of several open reading frames in the SARS virus genome may contribute to its severe virulence. Also, the potential mutability of the coronavirus genome may present problems in controlling future SARS outbreaks. Undoubtedly, the published genome sequence will aid in the accurate and rapid diagnosis of SARS, in the development of antivirals, and in the identification of putative epitopes for vaccine development.

Although it is likely that SARS-CoV is of animal origin, its animal reservoir is not yet confirmed with certainty. Its epidemiology and routes of transmission are also not fully understood. The main way that SARS seems to spread among people is by close person-to-person contact, most readily by respiratory droplets (droplet spread) produced when an infected person coughs or sneezes. Also, the virus can spread when a person touches a surface or object contaminated with infectious droplets and then most likely introduces it via the oral or respiratory route. In addition, it is possible that the SARS virus might spread more broadly through the air (airborne spread) or by other ways that are not yet known.

The mechanism of SARS pathogenesis may involve both direct viral cytocidal effects on the target cells and immune-mediated mechanisms. The virus induces symptoms of atypical pneumonia, which is clinically indistinguishable from influenza and other similar syndromes. Mortality rates of up to 15% can be expected among some cohorts. SARS begins with a high fever, lower respiratory symptoms, headache, cough, and other general symptoms resembling influenza. Ten to 20 percent of patients have diarrhea, which is not typically seen in influenza patients. No specific treatment has yet been identified as reliably successful.

The seasonal character of SARS during the winter period, coupled with clinical similarity to influenza and related syndromes, makes it difficult to clinically differentially diagnose the disease. Therefore, rapid laboratory confirmation is essential. Virus isolation in tissue culture (e.g, Vero cells), nucleic acid detection by RT-PCR, and antibody detection assays (e.g., ELISA, IFA) are available for specific virus detection.[37–43]

Monkeypox

Monkeypox is a rare smallpox-like viral disease that occurs mostly in central and western Africa. It is called "monkeypox" because it was first found in 1958 in laboratory monkeys. Subsequent blood screening of animals in Africa found that other types of animals probably had monkeypox. Animal antibody surveys in Africa suggested that squirrels play a major role as a reservoir of the virus and that humans are sporadically infected. Rats, mice, elephant shrews, rabbits, guinea pigs, and domestic pigs in Africa also showed neutralizing antibodies. Monkeypox was reported in humans for the first time in Africa in 1970. After smallpox eradication, surveillance for human monkeypox from 1981 to 1986 in the Democratic Republic of Congo (DRC) confirmed 338 human cases. The case-fatality rate was 9.8% for persons not vaccinated with vaccinia (smallpox) vaccine. One of the largest outbreaks of monkeypox was reported in 1996–1997 in the DRC.[44]

Like WNV, monkeypox had never been reported in the United States until recently. In early June 2003, monkeypox was reported among several people in the United States, most of whom got sick after contact with infected pet prairie dogs. This was the first recorded outbreak of monkeypox in the Western Hemisphere.

Monkeypox virus is in the family of *Poxviridae*. It belongs to a group of viruses that includes the smallpox virus (variola), the virus used in the smallpox vaccine (vaccinia), and the cowpox virus. The virus is transmitted via close contact with infected animals or by touching an animal's blood, body fluids, or its rash. Occasionally monkeypox virus is transmitted via bites by an infected animal. Also, the disease can spread from person to person through large respiratory droplets during long periods of face-to-face contact or by touching body fluids of a sick person or contaminated objects such as bedding or clothing.

In humans, the signs and symptoms of monkeypox resemble those of smallpox, but are much milder. Ten to 12 days after infection, patients develop fever, headache, muscle aches, backache, swollen lymph nodes, and fatigue. Skin rash develops usually within the first three days of the disease. This rash develops into raised bumps filled with fluid, often starting on the face and spreading to other parts of the body. The skin rashes go through several stages before they get crusty, scab over, and fall off. The illness usually lasts for two to four weeks.

Monkeypox virus can be detected by cultivation in human (HeLa) or monkey cell lines (Vero, LLCMK-2, or OMK), and is cytolytic for both. Nucleic acid amplification technology is used for nucleic acid detection; western blotting, HI, ELISA, and IFA are used for antibody detection.

To prevent the spread of monkeypox in the United States, the CDC and FDA issued an interim rule to amend their regulations to establish new restrictions and modify existing restrictions on the import, capture, transport, sale, barter, exchange, distribution, and release of African rodents, prairie dogs, and certain other animals.[45]

Can New Zoonoses be Predicted?

For many years, the concept of a so-called "species barrier" has been verified to provide a relative protection from certain infections to the individuals of certain species. However, sharing microbes that have multihost potential seems to be the norm. With so many pathogens in wildlife, the introduction of exotic species as pets to new habitats, and bringing various species in close proximity, we can expect additional pathogens to eventually emerge through co-evolution of these infectious agents with the human population and animals. As we continue to intermingle various animal species, we create the perfect environment for microbial interactions and adaptation by exchange of their genetic information through recombination, reassortment, and various mutations, hence giving rise to new agents and new diseases. Advances in nucleic acid diagnostic technologies will continue to make it possible to identify organisms that would otherwise escape traditional detection by conventional methods.

Today's lifestyle closely connects people and animals as it has for millennia. These connections are fluid, with lines constantly shifting due to factors such as: increasing numbers of pets, changes in natural habitats and ecosystems (e.g., global warming, droughts and floods, deforestation, and pollution), population increase, and the constant evolution of global trade. Moreover, as we continue to industrialize our world, undertake eco-tourism to view rare and endangered species in distant parts of the world, bring rare species to our households as pets, and import billions of tons of food from around the world, one conclusion remains almost certain: through co-evolution of humans and infectious agents, additional zoonotic diseases will continue to emerge.

Unmet Medical Needs

In the fluid world of animal and human cohabitation, we face the huge challenge of quickly recognizing and responding to each new emerging zoonotic disease. The need for one medicine is today more obvious than ever before. The phrase "one medicine" was coined by the U.S. epidemiologist Calvin Schwabe to focus attention on the concept that human and veterinary health interests should not be seen as separate, and that zoonotic diseases can be fought most effectively using a joint approach.[46,47] More than 150 years earlier, German pathologist Rudolf Virchow (1821–1901) strongly emphasized the importance of linking human and veterinary medicine. He reasoned that human and animal health should not be separated and that existing infrastructure for public health promotion could be more fully capitalized if public health and veterinary services were delivered together, especially in remote rural zones.[48,49] Medical and veterinary professionals, along with interdisciplinary scientific experts, must work together in a well-orchestrated cooperation in order to be able to fully respond to future zoonotic and biowarfare threats.

Awareness, preparedness, and the availability of adequate medical tools are essential factors needed to respond quickly to a new zoonotic emergency. The awareness is best achieved by mass education, and the preparedness can be addressed by resource and infrastructure planning and adequate training. The availability of medical supplies, however, is more problematic. Three important medical elements — diagnostic tests, specific treatments, and immunoprophylaxis — present a challenge and an opportunity for the biopharmaceutical industry to address these unmet medical needs.

Despite the viral cause of many devastating diseases known to humankind, today's medicine is rather ill equipped for the specific treatment of viral infections. Specific antiviral agents must be developed that can kill the viruses in a similar way to antibiotics' action in bacteria. A good antiviral should have potent killing abilities, be non-toxic to the host, and, ideally, work effectively against closely related viruses within the same family or within multiple families. Even viral immunoprophylaxis is problematic, as only a few human antiviral vaccines are commercially available. Incentives must be found to stimulate more rapid vaccine development against an increasingly wide range of zoonotic and other human and veterinary viral pathogens.

With the constant evolution and emergence of dangerous diseases, and the ever-present threat of biological warfare, we must ask, "Are government and industry ready and able to address these important areas of public health?"

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