Viruses and Bioterrorism☆

SA Morse, Centers for Disease Control and Prevention, Atlanta, GA, United States
RF Meyer

Introduction

Man has known that biological organisms and toxins were useful as weapons of war long before the germ theory of disease was understood. In fact, biological warfare has been used for centuries to sabotage and weaken the enemy (Geissler and van Courtland Moon, 1999). However, as the 20th century came to a close, the perceived difficulties in production, weaponization, and deployment of these biological agents, as well as a belief that moral restraints would preclude the use of these weapons, gave many a false sense of security. Subsequently, a number of events have served to focus attention on the overall threat of terrorism and, in particular, the potential use of biological, chemical, or nuclear weapons against the military, civilian populations, or agriculture for the purpose of causing illness, death, or economic loss. The possibility became a reality in October 2001 when someone sent letters containing spores of *Bacillus anthracis* through the United States mail to media companies in New York City and Boca Raton, Florida and to Senate offices in Washington, DC. This act of bioterrorism resulted in 22 cases of anthrax (11 inhalational and 11 cutaneous) and five deaths, caused considerable panic throughout the United States and other countries, and raised awareness of our vulnerability.

There are more than 1400 species of infectious organisms that are known to be pathogenic for humans; many additional organisms are capable of causing disease in animals and plants (Taylor *et al.*, 2001). Realistically, only a few of these infectious agents pose serious problems or are capable of affecting human, animal, or plant health on a large scale. Even fewer of these agents are viruses. Viruses that could be used as weapons against humans, animals, or plants generally possess such traits as ease of production and dissemination, transmissibility, environmental stability, and high morbidity and mortality rates. Some of these traits (eg, transmissibility, high mortality rates), limited accessibility, and the need for high levels of bio containment may dissuade terrorists from acquiring and producing large amounts of some of these viruses. However, an agroterrorism attack requires relatively little expertise or technology (Wheelis *et al.*, 2002) and terrorists can safely handle most of the causative agents with no risk to becoming infected themselves (Elbers and Knutsson, 2013).

Definitions

The nefarious use of biological agents is often characterized by the manner in which they are used. For the purposes of this article, we have defined terms as follows:

Agroterrorism: a subset of bioterrorism, defined as the deliberate introduction of animal or plant pests (eg, bacteria, viruses, fungi) with the goal of generating fear, causing economic damage, and/or undermining social stability (Keremidis *et al.*, 2013).
Biological warfare (synonymous with biowarfare): a specialized type of warfare involving the use of biological agents conducted by a government against a target (human, agriculture, or infrastructure) (Carus, 2002).
Bioterrorism: the threat or use of a biological agent (or toxin) against humans, animals, or plants by individuals or groups motivated by political, religious, ecological, or other ideological objectives (Carus, 2002).
Biocrime: the threat or use of a biological agent for murder, extortion, or revenge (Carus, 2002).

Historical Perspective

The use of viral agents for biological warfare has a long history, which predates their recognition and isolation by culture. Their early use is consistent with what, at the time, was known about infectious diseases, particularly smallpox. In the 16th century, the Spanish explorer Francisco Pizarro presented the indigenous peoples of South America with variola-contaminated clothing, which resulted in widespread epidemics of smallpox. During the French and Indian War (1745–67), Sir Jeffrey Amherst, commander of the British forces in North America, suggested the deliberate use of smallpox to "reduce" Native American tribes hostile to the British. One of Amherst's subordinates, Captain Ecuyer, fearing an attack on Ft. Pitt from Native Americans, acquired two variola-contaminated blankets and a handkerchief from a smallpox hospital and, in a gesture of "good will" distributed them to the Native Americans. As a result, several outbreaks of smallpox occurred in various tribes in the Ohio River valley (Hopkins, 1983).

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In 1775, during the Revolutionary War, the British attempted to spread smallpox among the Continental forces by inoculating (variolation) civilians fleeing Boston. In the South, there is evidence that the British were going to distribute slaves who had escaped during hostilities, and were sick with smallpox, back to the rebel plantations in order to spread the disease (Hopkins, 1983).

The use of viruses other than Variola major is a more recent phenomenon and reflects an increased knowledge of how to grow and stabilize viruses for delivery purposes. Allegations have been made by the government of Cuba that the CIA was responsible for the massive outbreaks of swine fever in 1971 and dengue fever in 1980 that ravaged the country. However, subsequent investigations have failed to find substantive proof of CIA involvement in these outbreaks (Zilinskas, 1999; Leitenberg, 2001). The Aum Shinrikyo, a religious cult responsible for the 1995 sarin gas release in the Tokyo subway system, was also involved in biological warfare activity and sent a team of 16 cult doctors and nurses to Zaire to acquire Ebola virus (Olson, 1999). In 1997, unknown farmers in New Zealand deliberately and illegally introduced rabbit hemorrhagic disease virus (a calicivirus) onto the South island as an animal control tool to kill feral rabbits (Carus, 2002). Furthermore, some farmers further propagated the disease by mixing homogenized organs from infected rabbits with carrots and oats and spreading the infected bait in areas heavily infested with rabbits (Carus, 2002).

For more than two decades, the human immunodeficiency virus (HIV) has been involved in a number of biocrimes. This most likely reflects the availability of HIV-contaminated blood as a source of this virus as well as widespread news reports concerning HIV and its association with the acquired immune deficiency syndrome (AIDS). For example, in 1990 Graham Farlow, an asymptomatic HIV-infected inmate at a prison in New South Wales, Australia, injected a guard with his HIV-contaminated blood. The guard became infected with HIV; Farlow subsequently died of AIDS (Carus, 2002). In 1992, Brian T. Stewart, a phlebotomist at a St. Louis, MO hospital, injected his 11-month-old son with HIV-contaminated blood during a fight over payment of child support (Carus, 2002). In 1993, Iwan E. injected his former girlfriend with 2.5 mL of HIV-contaminated blood after she broke up with him (Carus, 2002). In 1994, Dr. Richard J. Schmidt, a married Louisiana gastroenterologist, injected his former lover with HIV-contaminated blood (Carus, 2002). Molecular typing of the HIV strains demonstrated that she contracted the same strain of HIV as found in one of Dr. Schmidt's patients (Metzker et al., 2002). This case was the first time that phylogenetic evidence has been used as evidence in a United States criminal proceeding. In perhaps the most famous case, Dr. David Acer, a Florida dentist infected with HIV, transmitted the disease to six of his patients between 1987 and 1990 (Ou et al., 1992). The mechanism by which HIV was transmitted to these patients was not unambiguously determined and intentional infection remains a possibility (Ciesielski et al., 1994). However, an independent reanalysis of the available sequence data strongly supported dental transmission of HIV (Hillis and Huelsenbeck, 1994). In spite of these incidents and the public fear that HIV engendered, the virus was not included on lists of threat agents for public health bioterrorism preparedness (Rotz et al., 2002). However, others contend that HIV has great weapon potential if the goal is to destabilize a society (Casadevall and Pirofsky, 2004).

Viruses have also been involved in suspected incidents or hoaxes. In 1999, an article appeared suggesting that the CIA was investigating whether Iraq was responsible for causing the outbreak of West Nile fever in the New York City area (Preston, 1999). The story relied heavily on a previous story written by an Iraqi defector, claiming that Saddam Hussein planned to use West Nile virus strain SV 1417 to mount an attack. This was the first documented incidence of the West Nile virus in the Western hemisphere and raised concerns of an intentional introduction. However, an investigation revealed that the strain of West Nile virus responsible for this outbreak was the same strain that had been circulating in the Mediterranean region since 1998 (Lanciotti et al., 1999). A fictional "virus" was also responsible for one of the largest bioterrorism hoaxes in 2000. According to email messages widely circulated on the Internet, an organization known as the Klingerman Foundation was mailing blue envelopes containing sponges sealed in plastic contaminated with a fictional pathogen called the "Klingerman virus." According to the email alert, 23 people had been infected with the virus, including seven who died. This hoax was resurrected in the wake of the September 11, 2001 terrorist attacks on US and the subsequent anthrax attack, evidently because the specifics of the story resemble a germ warfare scenario. In 2011, a South African man was arrested for threatening to spread foot-and-mouth disease (FMD) virus in the US and Great Britain if he was not paid $4 million. If deployed, FMD virus would have caused the destruction of property and resulted in major economic losses. He was convicted of terrorist activity and money laundering even though he did not have FMD virus in his possession (Keremidis et al., 2013).

Viruses as Bioweapons

Advances in viral culture and virus stabilization made during the second half of the 20th century facilitated large-scale production of viral agents for aerosol dissemination. A report for the United Nations (World Health Organization, 1970) on chemical and biological weapons and the effects of their possible use gave estimates on the numbers of casualties resulting from a hypothetical biological attack (Table 1). Three viruses (Rift Valley fever virus, Tick-borne encephalitis virus, and Venezuelan Equine Encephalomyelitis (VEE) virus) were evaluated in a scenario in which 50 kg of the agent was released by aircraft along a 2 km line upwind of a population center of 500,000. The viral agents caused significantly fewer casualties and impacted a smaller area when compared to the bacterial agents (Francisella tularensis and B. anthracis) used in this hypothetical model. Factors that may impact the effectiveness of the aerosol delivery of viruses include: type of viral nucleic acid (Tseng and Li, 2005); temperature and relative

Table 1   Estimates of casualties produced by hypothetical biological attacks

Agent	Downwind Reach (km)	Dead	Incapacitated
Rift Valley fever	1	400	35,000
Tick-borne encephalitis	1	9,500	35,000
Venezuelan equine encephalomyelitis	1	200	19,800
Francisella tularensis	>20	30,000	125,000
Bacillus anthracis	>20	95,000	125,000

Note: These estimates are based on the following scenario: release of 50 kg of agent by aircraft along a 2 km line upwind of a population center of 500,000.

humidity (Tang, 2009; Lowen et al., 2007); and ultraviolet irradiation (Tseng and Li, 2005). Of note, smallpox was apparently not evaluated because it had not yet been eradicated and the level of vaccine-induced immunity in the population was high.

Viral agents were part of the biological weapons arsenal of both the Soviet Union and the United States (Leitenberg, 2001; Table 2). VEE virus was stockpiled by both countries as an incapacitating agent; Variola major and Marburg viruses were stockpiled as lethal agents by the Soviet Union. The Soviet Union reportedly conducted a live field test of Variola major virus on Vozrozhdeniye Island in the Aral Sea in the 1970s, in which 400 g of the virus was released into the atmosphere by explosion (Shoham and Wolfson, 2004; Enserink, 2002). Unfortunately, a laboratory technician who was collecting plankton samples from an oceanographic research vessel 15 km from the island became infected. It was reported that after returning home to Aralsk, she transmitted the infection to several people including children. All those infected died despite being immunized. It is unclear whether the strain of Variola major released was India-1967 (Enserink, 2002) or a highly virulent vaccine-resistant strain (Shoham and Wolfson, 2004). A number of other viruses that infect humans (eg, Ebola virus, Lassa fever virus, enterovirus 70, yellow fever virus) or livestock (eg, foot and mouth disease virus, rinderpest, Newcastle disease virus) have also been studied for their offensive capabilities or for the development of medical and veterinary countermeasures.

Today, with the increased level of concern, a number of viruses have been cited as possible weapons for use against humans or animals (Table 2). Requirements for an ideal biological warfare agent may include: availability; ease of production; stability after production; a susceptible population (human or animal); absence of specific treatment; ability to incapacitate or kill the host; appropriate particle size in aerosols so that the virus can be carried long distances by prevailing winds and inhaled deeply into the lungs of unsuspecting victims; ability to be disseminated via food or water; and, the availability of a vaccine to protect certain groups. Many of the viruses listed in Table 2 are Biosafety Level 4 (BSL-4) agents for which there is no available treatment (eg, Ebola and Marburg viruses). For those reasons, their production is likely to be restricted to national biological weapons programs. However, an agroterrorism attack would require relatively little expertise or the technology that is required to grow and disseminate many of the human viruses (Wheelis et al., 2002). Terrorists can safely handle many of the animal viruses with no risk of becoming infected themselves. Furthermore, terrorists do not necessarily have to acquire these agents from a laboratory and in theory, could acquire and use this kind of agent more easily than other biological agents that are pathogenic for humans (Keremidis et al., 2013). In addition, the risk of being caught in this kind of operation is low. Furthermore, the increased importation of food, global food trading, and transportation of animals, have made us more vulnerable to terrorist attacks (Polyak, 2004). Thus, other factors such as the economic impact of an attack on animal agriculture and the psychological impact on the population must also be considered.

Orthopoxviruses

Variola major (Table 2) is considered to be a major viral threat agent to civilian populations if used as a biological weapon (Henderson et al., 1999). Thus, considerable effort has been expended toward preparing the public health and medical communities for the possibility that this virus will be employed by a terrorist. Variola major is considered to be an ideal terrorist weapon because: it is highly transmissible by the aerosol route from infected to susceptible persons; the civilian populations of most countries contain a high proportion of susceptible persons; the disease is associated with a high morbidity and a mortality rate of 30% or more among unvaccinated persons and the absence of specific therapy; and initially, the diagnosis of a disease that has not been seen for more than 30 years would be difficult. In order to counteract the medical consequences of an attack with Variola major, the United States has stockpiled enough vaccine for every American (300 million doses), medical countermeasures to address the needs of individuals for whom the vaccine is contraindicated, and about 2 million doses of a new FDA approved antiviral, ST246 (Tecoviromat), against smallpox (Smith et al., 2009).

Alphaviruses

Alphaviruses (Table 2) are also of concern because they can be produced in large amounts using inexpensive and unsophisticated systems; they are relatively stable and aerosols are highly infectious for humans; and, strains are available that can produce

Table 2   Classification of viral agents that are considered to be of concern for bioterrorism and biowarfare and those that have been weaponized or studied for offensive or defensive purposes as part of former or current national biological weapons programs

Nucleic acid	Family	Genus	Species
Negative-sense single-stranded RNA	Arenaviridae	Arenaviruses	Lassa fever[a,b] (H)[k]
			Junin[a,b] (H)
			Machupo[a,b] (H)
			Sabia (H)
			Guanarito (H)
	Bunyaviridae	Phlebovirus	Rift valley fever[b] (R, H)
		Nairovirus	Crimean-Congo HF (H)
		Hantavirus	Hantaan and related viruses[b] (H)
			Sin Nombre (H)
	Filoviridae	Ebolavirus	Ebola[a](H)
		Marburgvirus	Marburg[c] (H)
	Orthomyxoviridae	Influenzaviruses	Influenza A[b] (H, P, S)
	Paramyxoviridae	Henipavirus	Hendra virus (H, E)
			Nipah virus (H, S)
		Morbillivirus	Peste des petits ruminants virus (R)
			Rinderpest[a,b,d,e,f] (R)
		Avulavirus	New Castle disease virus[b] (P)
	Rhabdoviridae	Lyssa virus	Rabies virus (H)
		Vesiculovirus	Vesiculostomatitis virus (R)
Positive-sense single-stranded RNA	Flaviviridae	Flavivirus	Dengue[b] (H)
			Japanese encephalitis virus[a] (H)
			Omsk hemorrhagic fever virus (H)
			Tick-borne encephalitis virus[a] (H)
			Yellow fever virus[a,b,d] (H)
		Pestivirus	Classical swine fever virus (H)
	Picornaviridae	Apthovirus	Foot and mouth disease virus[f,i] (R, S)
		Enterovirus	Enterovirus 70[h] (H)
			Swine vesicular disease virus (S)
		Hepatovirus	Hepatitis A virus (H)
		Teschovirus	Teschen disease virus (S)
	Togaviridae	Alphavirus	Venezuelan equine encephalomyelitis virus[c,g] (H, E)
			Eastern equine encephalomyelitis virus[b] (H, E)
			Western equine encephalomyelitis virus[b] (H. E)
			Chikungunya virus[b] (H)
Double-stranded DNA	Asfarviridae	Asfivirus	African swine fever virus[a] (S)
	Herpesviridae	Varicellovirus	Pseudorabies virus (S, R)
	Poxviridae	Capripoxvirus	Goat pox virus (R)
			Sheep pox virus
			Lumpy skin disease virus (R)
		Orthopoxvirus	Variola major[c,b,j] (H)
			Camelpox virus[h] (R)
		Parapoxvirus	Orf virus (R)
Double-stranded RNA	Reoviridae	Orbivirus	African horse sickness virus (E)
			Blue tongue virus (R)

[a]Studied by the Soviet Union BW program.
[b]Studied by the U.S. BW program.
[c]Weaponized by the Soviet Union BW program.
[d]Studied by the Canada BW program.
[e]Studied by the France BW program.
[f]Studied by the Germany BW program.
[g]Weaponized by the U.S. BW program.
[h]Studied by the Iraq BW program.
[i]Studied by the Iran BW program.
[j]Studied by the North Korea BW program.
[k]Potential target or animal affected: H, humans; R, ruminants; P, poultry; E, equines; S, swine.

incapacitating (eg, VEE) or lethal infections (EEE case fatality rates range from 50 to 75%) (Sidwell and Smee, 2003). Furthermore, the existence of multiple serotypes of VEE and EEE viruses, as well as the inherent difficulties of inducing efficient mucosal immunity, make defensive vaccine development difficult. Veterinary vaccines utilizing inactivated alphavirus preparations are available and in routine use to control infection in endemic areas (Barber et al., 1978). Unlicensed, investigational vaccines are also in use to protect at-risk laboratory workers (Burke et al., 1977). However, there are currently no vaccines licensed for general use in the United States for prevention or treatment of alphavirus infections, especially those acquired through the aerosol route (Spurgers and Glass, 2011).

Filoviruses and Arenaviruses

The filoviruses and arenaviruses that cause hemorrhagic fever (Table 2) have also been considered as agents that might be used by terrorists because of their high virulence and capacity for causing fear and anxiety (Rotz et al., 2002). The filoviruses, Ebola, and Marburg, can also be highly infectious by the airborne route. Humans are generally susceptible to infection with these viruses with fatality rates greater than 80%, and infection can be transmitted between humans through direct contact with virus-containing body fluids. There are five species of arenaviruses (Lassa fever, Junin, Machupo, Guanarito, and Sabia) that can cause viral hemorrhagic fevers with a case fatality rate of about 20% (Charrel and de Lamballerie, 2003). Large quantities of these viruses can be produced by propagation in cell culture. Infection occurs via the respiratory pathway suggesting that dissemination via aerosol might be used by a terrorist. Human-to-human transmission has also been reported with aerosol transmission the most likely route for at least some of the secondary cases. The filoviruses and arenaviruses discussed above are BSL-4 agents and widespread diagnostic capabilities for infections caused by these viruses are limited.

Apthovirus

Foot-and-mouth disease (FMD) is a severe, highly infectious viral disease of cattle, swine, sheep, goats, and other ruminant species; the virus is not a threat to human health. FMD is characterized by large blisters in the mouth, on the teats, and between the toes that burst to cause painful raw sores and even the loss of the hooves. Animals cannot eat, drink, or walk, nor can they be milked. FMD is rarely fatal, but the recovered animal usually loses its productivity of milk or meat (Breeze, 2004). Recovered animals may harbor and continue to shed infectious virus.

   FMD is the most infectious virus known; it is about 20-times more infectious than Variola major. There are seven different types and 70 subtypes of FMD. Thus, no single vaccine can be used to control this disease. About 15 different FMD vaccines manufactured throughout the world require the production and use of whole live virus. However, Federal law prevents research or manufacture using live FMD virus in any part of the US mainland because of the risks of contagion. Recently, a new molecular FMD vaccine was developed and granted conditional license for use in cattle by the USDA. This vaccine can be manufactured on the US mainland because it does not use live FMD virus.

   The last FMD outbreak in the US was in 1929; currently FMD is not found in North and Central America, The European Union, Australia, New Zealand, or Russia, but is present on a permanent or irregular basis in most of the rest of the world. Countries that are free of FMD maintain restrictions on imports of live animals and animal products that might carry the virus. Thus, the significance of FMD for the US is that our cattle, swine, sheep, and goats are totally susceptible to FMD and none have been vaccinated. These herds are the basis of a highly productive agribusiness, which is the largest in the world and a significant component of GDP and our foreign exports. The availability of FMD in endemic countries (eg, Afganistan, Pakistan, Iraq, Iran, and Syria) and the ease with which an animal can become infected make this virus the primary agent of concern for agroterrorism (Wheelis et al., 2002; Breeze, 2004). The economic loss from a FMD outbreak is considerable and is a major reason why this virus is important as an agent for agroterrorism. For example, in the 2001 FMD outbreak in the United Kingdom, which lasted 8 months, cost more than $6.9 billion with more than 6 million animals destroyed and 120,300 farms depopulated in FMD affected areas. It took 18 months to regain normalization of trade following eradication of the disease.

Impact of Biotechnology

Because the nucleic acid (DNA or RNA) of many viruses, including some that are currently not threats, can be manipulated in the laboratory, the potential for genetic engineering remains a serious threat. Biotechnology, which has had a tremendous impact on the development of medicines, vaccines, and in the technologies needed to counter the threat of naturally occurring disease, can also be used to modify viruses with unintended consequences or even for the development of novel biological agents. Several examples involving viruses are presented below.

Mousepox Virus

An Australian research group (Jackson et al., 2001) was investigating virally vectored immunocontraceptive vaccines based on ectromelia virus, the causative agent of the disease termed mousepox. The researchers created a recombinant virus, which expressed

the mouse cytokine IL-4 in order to enhance the antibody-mediated response to other recombinant antigens carried on the virus vector. Instead, the ectromelia virus vector expressing IL-4 altered the host's immune response to this virus resulting in lethal infections in normally genetically resistant mice (eg, C57BL/6). Moreover, this virus also caused lethal infections in mice previously immunized with ectromelia virus. The creation of this "supermousepox" led to speculation that similar genetic engineering could be performed on another pox virus (ie, Variola major) leading to a biological weapon that would be effective against an immunized population.

1918 Influenza Virus

The influenza pandemic of 1918–20, which followed World War I, was uniquely severe, causing an estimated 50 million deaths worldwide (Johnson and Mueller, 2002). This pandemic happened before the advent of viral culture and very little was known about the virus until the development of polymerase chain reaction (PCR) technology. Recently, the complete coding sequences of all eight viral RNA segments has been determined by using reverse transcription-PCR (RT-PCR) to amplify fragments of viral RNA retained in preserved tissues from individuals who died during this pandemic (Reid et al., 1999; Taubenberger et al., 1997, 2005). More recently, researchers reconstructed the 1918 Spanish influenza pandemic virus using reverse genetics and observed that this reconstructed virus exhibited exceptional virulence in the model systems examined and that the 1918 hemagglutinin and polymerase genes were essential for optimal virulence (Tumpey et al., 2005). The milestone achievement of reconstructing an "extinct" pandemic virus raised a number of questions including whether it was necessary or wise to recreate by molecular means a naturally extinct virus that caused one of the deadliest pandemics in human history (Taubenberger et al., 2012). Ultimately, senior US government scientists and officials at the Department of Health and Human Services concluded that research on this virus could play an important role in pandemic preparedness efforts as well as shed light on both the reasons for its extraordinary virulence and evolutionary origin.

Gain-of-Function Experiments

Influenza pandemics in humans arise from animal influenza viruses, yet the molecular changes that are required for an animal virus to be transmitted efficiently between humans is poorly understood. The viral hemagglutinin (HA) protein is a known host-range determinant as it mediates virus binding to host-specific cellular receptors. Highly pathogenic avian H5N1 influenza A viruses have circulated in poultry for more than 16 years. However, they only rarely cause human infections and do not transmit efficiently among humans. Two controversial studies were published in 2012 that described mutations leading to the airborne transmission of H5N1 between ferrets, a mammalian model of influenza. In the first study, Fouchier and his colleagues (Herfst et al., 2012) used a combination of genetic engineering and serial infection of ferrets to create a mutant H5N1 virus that could spread among ferrets without direct contact. They used reverse genetics to introduce four amino acid substitutions in the HA protein that have been shown in previous research to make H5N1 viruses more human-like in that the HA would bind preferentially to alpha 2,6-linked sialic acid receptors instead of alpha 2,3-linked receptors. They also introduced a mutation in another viral gene, PB2, the polymerase complex protein, which was linked to the ability to grow in the human respiratory tract, which is cooler than the intestinal tract of birds, where the viruses usually reside. Seeing that this mutant failed to achieve airborne transmission, the researchers serially passed this strain through a series of ferrets in an effort to force it to adapt to the mammalian respiratory tract. In the second study, Kawaoka and his colleagues (Imai et al., 2012) used a somewhat different approach in which they introduced four mutations in the HA gene from a H5N1 virus and then combined it with seven genes from a 2009 H1N1 virus, creating a hybrid virus that was able to spread by air between ferrets. On one hand, these studies provide further indication that a mammalian transmissible H5N1 influenza virus may arise naturally, and paves the way for improved influenza surveillance and pandemic preparedness. On the other hand, some scientists believe that the risks associated with these types of studies (particularly those by Fouchier and colleagues) outweigh the benefits, particularly with respect to the consequences of an accidental release (Roos, 2012).

Synthetic Genomes

A full-length poliovirus complementary DNA (cDNA) (c. 7500 bp) has been synthesized in the laboratory by assembling oligonucleotides of plus- and minus-strand polarity (Cello et al., 2002). The synthetic poliovirus cDNA was transcribed by RNA polymerase into viral RNA, which was translated and replicated in a cytoplasmic extract of uninfected HeLa S3 cells, resulting in the de novo synthesis of infectious poliovirus. The publication of this research raised concerns that more complicated viruses (eg, Variola major, Ebola, or Marburg) could be synthesized from scratch based on publically available sequences, or that viruses could be created that do not exist naturally.

Recognition, Response, and Deterrence

An effective defense requires a comprehensive approach that includes: prevention of access to viral stocks (Morse and Weirich, 2011); improved means of detecting deliberately induced disease outbreaks (Elbers and Knutsson, 2013; Khan and Pesik, 2011);

rapid medical recognition of specific syndromes (eg, hemorrhagic fever syndrome); rapid laboratory identification of viruses in human or animal specimens (Leijon and Belak, 2013); prevention of human-to-human or animal-to-animal transmission (Farsang et al., 2013); reliable decontamination procedures (Frentzel et al., 2013); development of effective vaccines (Spurgers and Glass, 2011; Lehrer and Holbrook, 2011; Marzi et al., 2011); and the development of effective antiviral therapies (Smith et al., 2009; Richardson et al., 2011).

Rapid and accurate detection of biothreat agents is the basis of an effective public health response to bioterrorism. In order to address this issue, CDC in collaboration with other partners established an international network of laboratories called the Laboratory Response Network (LRN) (Morse et al., 2003), which was provided with the tools to accomplish this mission. Rapid assays utilizing advanced molecular and immunological technology for detection of agents such as variola virus, as well as emerging public health threats such as SARS coronavirus and H5N1 influenza virus, were distributed to member laboratories. Equipment, training, and proficiency testing are elements of the LRN and contribute to a uniform operational plan. The importance of high-quality standardized testing for detection of these agents is exemplified by the rapid need for medical countermeasures to protect or treat civilian populations. Accurate laboratory analysis is a major element in the decision process for deployment of the Federal Government's Strategic National Stockpile (SNS) of medical countermeasures (Posid et al., 2011). A similar system was developed by the US Department of Agriculture and its partners to protect US agriculture and the food supply from a bioterrorist attack (Heintzelman, 2011).

As part of the effort to deter biological terrorism and strengthen the law enforcement investigative response to such an act, the US established a microbial forensic laboratory known as the National Bioforensic Analysis Center (Burans, 2011) that operates in partnership with the Federal Bureau of Investigation. Scientists are already developing methods for the forensic investigation of incidents involving viruses, including foot-and-mouth disease virus (Carrillo, 2011).

Summary

For the terrorist, the use of a viral agent would pose a challenge due to problems associated with acquisition, cultivation, and dissemination. The target for an attack with a viral agent can range from humans to animals and plants. Therefore, agricultural targets are a particular concern because a bioterrorist attack would require relatively little specialized expertise and technology, and can have very large economic consequences (Wheelis et al., 2002; Casagrande, 2002; Breeze, 2004). Nature has provided many challenges to combating viral diseases. Viral agents are much more prone to genetic variation and mutation, and can be manipulated or created in the laboratory to take on desired characteristics. With few exceptions, differentiating between natural and intentional viral disease outbreaks can be challenging. Unlike bacterial diseases, many of which are treatable, there are fewer medical countermeasures to employ when dealing with viral infections. For many viruses, laboratory diagnostic methods and reagents must continuously be refined to account for genetic changes and variants. Thus, the challenge of developing bioterrorism countermeasures is significant. Fortunately, this effort contributes to combatting natural disease events more effectively, which has global benefits.

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