Impact of Synthetic Genomics

on the Threat of Bioterrorism with Viral Agents

Marc S. Collett
ViroDefense Inc.

Cite as:

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Introduction

In 2002, a team of researchers at the State University of New York led by Eckard Wimmer assembled a DNA template for the RNA poliovirus using an internet-available nucleotide sequence and mail order synthetic oligonucleotides. Using a routine laboratory procedure, they then converted the DNA into RNA and produced an infectious, neurovirulent poliovirus capable of paralyzing and killing mice.[1]

This work demonstrated clearly for the first time the feasibility of chemically synthesizing a pathogen knowing only its nucleotide sequence. Some called the work "irresponsible," and there was widespread speculation in the press that bioterrorists might use the technology to create more virulent viruses, such as smallpox, from published gene sequences or create novel, more lethal viruses. Wimmer countered that "an evildoer would not use that very tedious method to synthesize a virus. That terrorist would rather use already existing viruses in nature." [2]

Indeed, all viruses, from the common cold to the deadliest, originate in nature, being identified and isolated from infected humans or animals or the virus's animal or insect vector. However, the rapidly advancing technology of whole genome assembly ("synthetic genomics") is making the chemical synthesis of viral genomes a much less tedious endeavor.[3]

This paper will explore the potential impact of synthetic genomics technology on the risks of a bioterrorist attack using viral pathogens. More succinctly: Does the ability to chemically synthesize and assemble a DNA copy of a pathogenic virus genome in the lab increase the risk of a bioterrorist attack using that pathogen? For the purposes of this

paper, it will be assumed that the synthetic technology is capable of preparing a DNA copy of any virus for which its nucleotide sequence is known.

While only "acquisition" of dangerous viruses will be considered here, it is important to maintain the present discussion in the context of the overall challenges of assessing the risks of a bioterrorism attack. The nature and sophistication of an attack can vary tremendously depending on the intent and capabilities of the would-be bioterrorist. If the desired impact of an attack is extensive human morbidity and mortality (a "high consequence event") such as with the widespread dissemination of smallpox virus several aspects of implementation, in addition to threat agent acquisition, must be executed successfully. These include the biological propagation and scale production of the threat agent, its packaging, storage and transport, and finally, its delivery or dissemination. There are numerous significant technical and logistical challenges at each step. In this case, the overall risk of an effective bioterrorism attack is the product of the probabilities of success of each component step. On the other hand, if the bioterrorist's objective is public fear or panic (societal or economic disruption), a simple isolated detection of an exotic pathogen may serve that end, and would require little technical or logistical expertise. For example, the detection of a broken blood sample tube in a New York City subway station containing infectious Marburg virus smuggled in from the recent outbreak in Angola, or a case of foot-and-mouth-disease in a Texas cattle feedlot caused by virus released from a vial stolen from a research lab in South America, would to the trick.

Synthetic genomics technologies could affect the availability of bioterrorism threat viruses, potentially providing a new or alternative means to obtain a pathogen. It might also facilitate the engineering of new or novel pathogens. This paper will review the viruses considered bioterrorism threat agents, their current sources and availability, and the potential impact of synthetic genomics on threat agent acquisition. The potential impact of synthetic technologies on the generation of new or novel viral pathogens will also be discussed briefly.

1. Viruses Considered Bioterrorism Threat Agents

The viral agents considered to pose severe threats to public health and safety are represented on various lists prepared by several government and international organizations. The three most widely-referenced lists are as follows:

1.1. The Centers for Disease Control and Prevention (CDC) established a list of high priority, exclusively human, "bioterrorism agents" ranked according to three categories.[4] Category A agents pose the most serious risk to national security because they can be easily disseminated or transmitted from person to person, are associated with high mortality rates, might cause public panic and social disruption, and require special action for public health preparedness. Category B agents include those that are relatively easy to disseminate; result in moderate morbidity rates and low mortality rates; and require specific diagnostic and surveillance enhancements. Finally, Category C agents comprise newly emerging pathogens, or pathogens that could be engineered for mass dissemination in the future because of their availability; ease of production and dissemination; and potential for high morbidity and mortality rates.

1.2. The National Institute of Allergy and Infectious Disease of the National Institutes of Health (NIAID, NIH) has developed a Category agents list of human pathogens quite similar, but not identical, to the CDC list.[5]

1.3. The Select Agents list, developed by CDC, the Department of Human and Health Services (HHS) and the United States Department of Agriculture (USDA), is a broader treatment and encompasses most Category A, B and C agents, additional human pathogens, and disease agents of livestock and plants.[6]

While there has been spirited debate on the constitution of the various pathogen lists, taken together, these lists provide the spectrum of virus pathogens that, if released in non-endemic areas or used in a bioterrorism attack, could cause a level of physical, economic and societal harm and disruption. Whether particular entries on the lists represent credible bioterrorism risks should be an area of continued review and assessment.

Table 1 is a compilation of the viruses from these lists, grouped alphabetically according to virus type (taxonomic family). Table 1 also indicates the government agency responsible for agent selection (HHS, CDC, NIAID, USDA), showing agency overlaps, non-overlaps, and for "Category" viruses, priority. The Table further provides the specific virus genome type, size, and infectivity.

There are three DNA virus families represented in Table 1, comprising the threat agents African swine fever virus (Asfarviridae), herpes B virus (Herpersviridae) and a number of poxviruses including smallpox virus (Poxviridae). These viruses have large double-stranded DNA genomes (150-205 kilobase pairs, kbp) and encode on the order of 100 gene products that are essential for virus replication, as well as another 100 "nonessential" gene products, many of which are involved in the modulation of host responses to virus infection and viral pathogenesis.

All remaining threat viruses, derived from 13 distinct virus families, are RNA viruses with genomes of positive (messenger) RNA sense (6 families), negative RNA sense (6 families) or double-stranded RNA (1 family). RNA virus genomes range in length from about 8 kb (Picornaviridae) to about 30 kb (Coronaviridae). Generally, most or all of the gene products encoded by RNA viruses are essential for virus replication. Some are also involved in the modulation of host responses to virus infection and viral pathogenesis.

2. Sources of Viral Threat Agents

All viruses listed in Table 1 were originally identified in and isolated from diseased humans, animals or, in one case, plants; or from animal or insect vectors of the respective pathogens. With the exception of smallpox (variola) virus, which was eradicated globally in 1977, and arguably the 1918 influenza virus, all viruses listed in Table 1 continue to circulate in nature. Therefore, nature represents a current and ongoing source of these viral pathogens.

All viruses listed in Table 1 also exist in numerous laboratories throughout the World, including academic research labs, diagnostic, hospital and nation state health labs, as well

as in biologics repositories (collectively, "laboratories"). For smallpox virus, the only known stocks remain in two high security laboratories. For all the other viruses, many research laboratories around the world have studied, and continue to study their structure, biology, molecular biology, genetics, immunology, pathogenesis and epidemiology.

Thus, nature and laboratories represent two current sources from which would-be bioterrorists could acquire viral threat agents. Since the complete genomic sequence for almost all viruses in Table 1 is known and publicly available, application of synthetic genomics represents a potential third source for any of these pathogens.

3. Does the Source of the Virus Matter?

Is the virus isolated from nature the same virus as the one found in laboratories, and would a synthetic replica of either be the same? Maybe, maybe not.

From a bioterrorism perspective, viruses isolated from nature are a sure bet. Their virulence and transmissibility are known. Their effect and impact can be predicted or calculated. Viruses isolated directly from diseased hosts (called "primary isolates") demonstrate clearly the consequences of their infection. Additionally, the virus's ability to survive, persist and spread in the environment and among susceptible hosts is generally known. Finally, pathogenic viruses isolated from diseased hosts are typically "hot" viruses; that is, primary isolates of pathogenic viruses tend to cause severe disease in their host.

When passaged in the laboratory (in either cell culture or lab animals), primary isolates often become attenuated. The attenuation is the result of adaptive genetic changes that the virus acquires in order to survive in its new environment. These genetic changes can be subtle (single nucleotide changes) or dramatic (genome deletions or rearrangements). Generally, the longer a virus is propagated in cell culture, or through non-natural animal hosts, the greater the attenuation. In fact, this is the basic methodology for the development of many live attenuated virus vaccines.

In order to retain as closely as possible the characteristics of the natural virus, many labs maintain low passage virus stocks. However, not all labs are so fastidious. Laboratory-adapted relatives of primary pathogenic virus isolates, while often well characterized for their *in vitro* attributes, may or may not have been characterized in a living host for infectivity, fitness, virulence and transmissibility, or compared to their primary isolate for these biological features. As a result, the degree of attenuation of laboratory-passaged viruses may or may not be known.

Most viral genome sequences deposited in databases are derived from laboratory-passaged viruses. While many sequences may be derived from low-passage viruses, and are therefore more likely to be close to their primary isolates, in some cases the passage history of the virus from which the sequence was derived is unclear, as are the biological attributes associated with that virus. Thus, there can in some cases be uncertainty regarding the biological attributes of a synthetic replica of a gene bank virus sequence.

That the biological attributes of a virus can be dramatically altered with very subtle genetic changes is exemplified by the first demonstration of viral synthetic genomics. For poliovirus, the introduction (for technical reasons) of several silent nucleotide changes into the virus genome resulted in a synthetic virus that was four orders of magnitude less virulent in mice than the natural virus.[1] Our understanding of the contribution of nucleotide sequence on genome structure, and in turn on biological attributes like virus replicative capacity, fitness, stability, and living host virulence and transmissibility are rudimentary at best.

The source of the virus may matter from a bioterrorism perspective.

4. From DNA Copy to Infectious Virus

There are descriptions of methodologies in the literature for the recovery of infectious virus from molecularly cloned DNA for member viruses from almost all the virus families listed in Table 1. These techniques are outlined generically below. Thus, in principle, it should be technically feasible to go from synthetic or recombinant DNA to infectious virus for any of the viral threat agents. However, in reality, for many of the

actual pathogens on the list, reverse genetics systems have not been demonstrated directly, and there may be unanticipated technical challenges for particular viruses. For example, a reverse genetics system has been demonstrated for Ebola virus, but has yet to be successful for the closely related Marburg virus. Therefore, while some of these methods are relatively straightforward, others require significant technical expertise and finesse.

Of the large dsDNA viruses, herpes B virus genomic DNA is itself infectious. African swine virus and poxvirus genomic DNAs are not infectious because of the requirement for activities of viral enzymes packaged within the virion. This requirement can be fulfilled for poxviruses, for example, by transfecting the viral genomic DNA into cells previously infected with another poxvirus. The resident "helper" virus provides the *trans*-acting systems needed to activate the transfected DNA and yield fully competent infectious virus.[7]

For the (+)ssRNA viruses, simply transfecting a DNA copy of the mRNA-sense genome into cells generally yields infectious virus.[8]
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For (–)ssRNA viruses, infectious virus can be recovered from cDNA designed with transcriptional promoters to yield full-length anti-genomic RNA upon transfection, either alone or together with plasmids encoding the expression of various viral proteins, into cells that provide the appropriate RNA polymerase. For segmented genomes, simultaneous transfection of multiple anti-genome plasmids is involved.[9, 10]

For the one dsRNA virus (Reoviridae), the system for the recovery of infectious virus directly from DNA has not been described. However, a reverse genetics system that involves the lipofection of cells with plus strand RNA transcripts or dsRNAs representing the 10 genomic segment of reovirus, together with a rabbit reticulocyte lysate in which ssRNA or melted dsRNA has been translated, can yield infectious virus after provision of a helper virus.[11]

5. Three Examples

Below, examples of three viral threat agents are considered to illustrate the opportunities for acquiring the pathogen from each of the sources mentioned above. The potential impact that synthetic genomics might have on the acquisition of each agent is then discussed. The three examples are:

1. <u>Smallpox (variola) virus</u>, a large double-stranded DNA virus,
2. <u>Ebola and Marburg</u> hemorrhagic fever filoviruses, negative-strand RNA viruses, and
3. <u>Foot-and-mouth disease virus</u>, a small positive-strand RNA virus.

5.1 Example 1 / Smallpox virus. Smallpox is caused by variola virus. Variola virus was declared eliminated from the world in 1979 by an aggressive global vaccination program. Once eradicated, immunization against smallpox ended, so individuals born after this time are immunologically naïve to the virus. Moreover, the level of immunity among persons who were vaccinated before eradication is uncertain but is likely low. Because of this high level of population susceptibility, smallpox (variola) virus is often considered the number one bioterrorism threat virus. Transmission of variola virus generally requires close contact with an infected individual. While this makes it possible to effectively interrupt chains of transmission by quarantine and restrictive movement methods, the average number of cases infected by a primary case is estimated at 3.5 to 6, indicating that an outbreak would produce a rapid rise in cases before control measures could be put in place. In addition to the significant morbidity associated with infection, death occurs in up to 30% of cases.

a) **Agent Source and Availability (Variola Virus)**

   i) **Nature**. Humans were the only host for variola virus. Once eradicated by global immunization, smallpox virus ceased to exist in nature.

   ii) **Research laboratories or repositories.** The only known stocks of variola virus have been retained in two World Health Organization

(WHO)-approved, high security laboratories: at the CDC in Atlanta and at the Russian State Center for Research on Virology and Biotechnology in Koltsovo, Novosibirsk Region, Russian Federation. However, some believe that secret caches of variola virus still remain undeclared or undetected and could be used by foreign governments or terrorist groups.[12]

iii) Potential impact of synthetic genomics. Since variola virus is not available from nature, and assuming there are no secret stores of the virus in covert laboratories, reconstruction of the dsDNA genome from the known variola virus nucleotide sequence may be the only path to the infectious agent available to bioterrorists. Consequently, while it would represent a considerable technical challenge for even a State-sponsored program, synthetic genomics technology could provide the means for the re-creation of variola virus, and therefore could affect the availability of this agent for malevolent use. Due to the large size of the poxvirus genome, however, it would be anticipated that well-established poxvirus recombination techniques would play a significant supportive or alternative role in producing an entire poxvirus genome. For example, smaller genome segments of a sequence derived from variola virus may be readily incorporated into a "base" monkeypox virus, resulting in chimeric orthopoxviruses with unknown and unpredictable biological characteristics.

5.2 Example 2 / Ebola and Marburg Hemorrhagic Fever Filoviruses. A number of distinct viral pathogens fall into the hemorrhagic fever virus group, including the arenaviruses (Lassa and South American hemorrhagic fever viruses), bunyaviruses (Rift Valley fever virus), flaviviruses (yellow fever virus) and filoviruses (Ebola and Marburg).   All are "Category A" bioweapons-bioterrorism agents. All are negative-strand RNA viruses. All are endemic in various parts of the world. All cause severe disease, characterized by fever,

multiple organ involvement with extensive vascular damage and bleeding diathesis, which in many cases is fatal.

For this example, attention will be limited to perhaps the best recognized of the hemorrhagic fever viruses, the filoviruses Ebola and Marburg. These viruses cause sporadic and recurrent disease in central Africa with case fatality rates ranging from 25% to 90%. Filoviruses are readily transmitted and disseminated by aerosol, droplets, and contact of oral mucosa or conjunctivae with any body fluids of the diseased. The world population is susceptible to infection by these viruses, and there are presently no vaccines and no specific treatments for Ebola or Marburg.

a) Agent Source and Availability (Ebola and Marburg Viruses)

    i)   **Nature**. While both diseases remain relatively rare, outbreaks have become more common since the mid-1990s. The 2005 outbreaks of Ebola in the Republic of the Congo and Marburg in Angola are recent examples. However, in the absence of an outbreak, filoviruses are hidden. Their reservoir in nature remains unknown. Therefore, to obtain these viruses from nature requires that it be done during an outbreak of human disease. Blood and other body fluids of infected individuals are rich sources of virus.

    ii) Research laboratories or repositories. Because of their virulence, filoviruses are handled in high containment laboratory facilities to prevent virus release into the environment, and also to protect those working with these highly pathogenic viruses. Consequently, the number of labs in possession of these viruses is limited, as is access to these labs. However, during outbreaks, unsecured local hospitals and medical field teams collect, hold and transport numerous infectious patient specimens. Additionally, there may be covert stores of virus outside known containment laboratories. Hemorrhagic fever viruses were the subject of biowarfare research in the former Soviet Union,

where weaponized Marburg virus was produced and research on Ebola was conducted. Upon the dissolution of the Soviet Union and these programs, the disposition of laboratory biological materials was not tracked.

iii) **Potential impact of synthetic genomics.** While Ebola and Marburg viruses may be readily obtained from diseased individuals, synthetic genomics technologies could provide an alternative source for these pathogens. Recovery of infectious virus from DNA has been demonstrated for Ebola virus, but not for Marburg virus.

5.3 Example 3 / Foot-and-Mouth Disease Virus. The potential for a terrorist attack against agricultural targets (agroterrorism) represents a daunting national security threat.[13-15] This reality is acknowledged by the inclusion of numerous livestock pathogens in the Select Agents list (Table 1). Deliberate introduction of an exotic animal or plant pathogen would elicit widespread public fear and would cause substantial economic loss and instability.

Foot-and-mouth-disease-virus (FMDV) is the most frequently mentioned disease agent of agroterrorism, and also the most likely terrorist threat. FMDV is extremely contagious and causes severe disease in cattle, swine, sheep, goats, and other cloven-hoofed animals. FMDV is not present in North America, and FMDV vaccination is not allowed. Consequently, the entire host animal population of North America is susceptible to infection and disease. The disease is "reportable" (i.e., subject to international quarantine) under rules of the Office International des Epizooties (OIE). So in addition to crippling national animal industries through lost production and mortality, an outbreak of FMD in the U.S. would suspend all exportation of meat and milk products until such time that disease (virus) eradication could be assured. For this example, the probability for catastrophic economic damage and social disruption is exceptionally high.

Unlike the previously discussed examples (smallpox and hemorrhagic fever viruses), where irrespective of how the pathogenic virus is sourced, there are considerable logistic challenges regarding the propagating and handling such hazardous human pathogens, none exist for FMDV. Humans are not susceptible to infection by FMDV; the virus is not a threat to human health or food safety.

a) **Agent Source and Availability (Foot-and-Mouth Disease Virus)**

   i) **Nature**. FMDV is endemic in large regions of Asia, Africa, the Middle East and South America, and consequent readily available for animal sources. In a recent, and typical, 18-month period, the OIE recorded FMD outbreaks in 15 countries. Sporadic outbreaks also occasionally occur in "disease-free" areas (e.g., Japan, 2000; United Kingdom, 2001).

   ii) **Research laboratories or repositories.** FMDV was first identified in 1898 and has been researched widely ever since. Suffice it to say, the number of academic and veterinary research facilities globally in possession of stocks of FMDV is quite large. The virus is readily available from these institutions. In endemic areas, there are typically no security measures employed in handling FMDV.

   iii) **Potential impact of synthetic genomics.** FMDV is a small (+)ssRNA virus within the same virus family as poliovirus. The total synthesis and recovery of infectious virus is without technological challenge. However, based on the ready availability of the virus in nature and from innumerable research labs, it is unlikely synthetic genomic technology would have any impact on the availability of FMDV for use in a bioterrorism attack.

6. The Dark Side – Making "Super-pathogens"

While nature has provided would-be bioterrorists an ample supply and selection of quite virulent viruses (Table 1), there is concern that genetic technologies will be used to modify these already pathogenic agents and create "super-pathogens", viruses that are

more lethal and disruptive than naturally occurring pathogens, and that are designed to evade vaccines or to be resistant to drugs.

The potential of this dark side was brightly illuminated in 2001 when Australian workers inadvertently created an unusually virulent mousepox (ectromelia) virus.[16] While trying to improve their experimental mouse contraceptive vaccine, they engineered the expression of cytokine IL-4 from ectromelia virus, hoping that infection with this recombinant poxvirus would enhance antibody production by their vaccine. It instead resulted in severe suppression of cellular immune responses in the mice, uncontrolled virus replication, and animal death. Even mice previously immunized against normal ectromelia virus [16] or treated with the antiviral drug cidofovir[17] were unable to survive ectromelia-IL-4 virus challenge. Although humans are not susceptible to ectromelia virus, there is clear concern that smallpox could be similarly modified to make it more deadly.

Indeed, there may be a number of ways to augment a viral bioweapon. Virus infectivity, virulence or transmissibility might be enhanced by, for example:

- Increasing the replicative capacity of the virus by modifying the viral polymerase or gene expression by optimizing for human codon usage,
- Changing the tropism of the virus by incorporating genes encoding particular cellular receptor binding proteins,
- Engineering drug-resistance determinants into the virus (should there exist antiviral drugs for the virus), or
- Compromising or overwhelming the host immune response to infection or vaccine-induced immunity by incorporating into the virus genes encoding human immune system antagonists (as with mouse genes in mousepox as mentioned above).

Additionally, random approaches, such as DNA shuffling (accelerated or directed molecular evolution) or combining genetic elements of distinct pathogenic viruses to create chimeric viruses, could be applied to bioweapons enhancement.

However, while all of these "pathogen enhancements" are theoretically possible, they require significant technical sophistication, and, importantly, the outcomes are not predictable. After their creation, putative super-pathogens would require characterization

of their infectivity, fitness and stability, and verification of their virulence and transmissibility in a living host to establish or confirm their super pathogenic powers. This would likely require some degree of physical containment during the agent's development (construction, propagation and production) and for animal studies, so as to prevent harm to the creators, as well as to maintain the covert nature of the operation. Finally, animal test systems may not be predictive of human pathogenicity, particularly if the enhancing modifications are designed to be human-specific (e.g., human codon optimization, human receptor binding proteins, human immune response antagonists).

Thus, for the high-tech bioterrorist embarking on a virus bioweapons enhancement strategy, there is a considerable level of complexity and risk, as well as uncertainty of the outcome and impact of an attack with such modified agents.

6.1 **Potential impact of synthetic genomics.** Does the availability of synthetic genomics technology influence the likelihood of super-pathogen construction? Certain of the approaches to bioweapons enhancement could be facilitated by the technology; for example human codon optimization. However, numerous other approaches involving gene insertions (as with the mousepox example above), genetic shuffling, mutagenesis, and recombination require only standard recombinant DNA procedures using readily available genetic materials and reagents. Synthetic genomics technology will likely have little impact on increasing the risk of the creation, or use, of a novel viral pathogen in a bioterrorism attack.

7. Summary Comments

All new technologies and technological advances have the potential to be used in malevolent ways. So when posed with the question: "Does the ability to chemically synthesize and assemble a pathogenic virus genome in the lab increase the risk of a bioterrorist attack using that virus?" The answer is: "Probably." The question then

becomes: "How much is the risk increased?" Here, the answer is: "Probably not substantially."

To expand on this opinion, and to provide a platform of further discussion, several component questions may be considered.

1. **Does the increased availability of a pathogenic virus increase the risk of its use in a bioterrorism attack?**

   Probably. If the pathogen is readily available, its use in an attack can be considered by the bioterrorist. Conversely, if you cannot obtain the virus, you cannot use it.

2. **Can synthetic genomics technology increase the availability of virus pathogens?**

   Clearly for one pathogen (variola virus), yes; for others, perhaps to some degree (e.g., Ebola/Marburg viruses), and for most, not at all (e.g., FMDV). Nature and laboratories already provide logistically easier, technologically less demanding, and sufficiently rich sources of all agents listed in Table 1 (except variola virus).

3. **If it were assumed that synthetic genomics technology does increase the availability of a particular pathogen, does that portend its greater likelihood of being used in a bioterrorism attack?**

   Probably not. Mere acquisition (by whatever means) of a viable infectious viral pathogen does not necessarily increase the threat of its use in an attack. Estimating the risk that a pathogen (once obtained) might be used in a bioterrorism attack involves a number of factors. Among these are: (1) the intent or desired outcome of the bioterrorist, which may vary from local panic to widespread dissemination with mass casualties, and (2) the level of technical sophistication and skill of the would-be terrorist to carry out an attack, which may range from little or no basic microbiology ability to biocontainment and weaponization capabilities. In view of these two factors, the risk (likelihood) of a bioterrorism attack must to be considered in the context of the overall risk at the "point of delivery." For example, if the intent of an attack is broad pathogen dissemination with high human mortality, there are many

steps that must be executed successfully after the acquisition of the threat agent. These include the propagation and scale production of a hazardous human pathogen, its packaging, storage, transport, and finally, its delivery or dissemination. In such a case, the contribution to the risk of an attack attributable to the pathogen acquisition step is quite low. If, on the other hand, the desired outcome of an attack is more modest, such as local panic and disruption, acquiring the suitable threat agent becomes more pivotal. In this situation, it would seem that simplicity and opportunism would dictate methods. It would be surprising if a technologically challenging, sophisticated approach such as whole genome synthesis was employed to acquire a pathogen for this purpose, particularly given the other available sources of the pathogen.

4. **Does synthetic genomics technology increase the likelihood of engineering a new or more virulent viral pathogen?**

Not substantially. Other currently available technologies are sufficient to undertake such a pathogen enhancement effort. Moreover, creating a new human pathogen, or a more lethal variant of a known pathogen, is associated with significant technical and logistic challenges as mentioned earlier. The availability of synthetic genomics technology does not remove or lessen these challenges.

8. **Closing Remarks**

- Bioterrorism using viral agents can readily proceed in the absence of synthetic genomics technology. Synthetic genomics is not an enabling technology in this context.

- A bioterrorism organism need not be extremely virulent, or virulent at all to humans (e.g., agroterrorism agents). But, it must be deliverable in order to be effective for its intended purpose.

- A bioterrorism attack employing either "low technology" (for example, natural FMDV) or "high technology" (for example, synthetic variola virus) has the capacity to be high consequence event.

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Table 1. Compilation of Viral Threat Agents[1]

Virus Name	Virus Genus / Family	Select Agent[2]	CDC (NIAID) Category[3]	Genome Type[4]	Genome Size (kb)[5]	Genome Infectivity[6]
Junín	Arenavirus / Arenaviridae	HHS	A(A)	2(−) ssRNA	10.5	No
Machupo	Arenavirus / Arenaviridae	HHS	A(A)		10.6	
Sabiá	Arenavirus / Arenaviridae	HHS	A(A)		10.5	
Guanarito	Arenavirus / Arenaviridae	HHS	A(A)		10.4	
Lassa	Arenavirus / Arenaviridae	HHS	A(A)		10.7	
Lymphocytic choriomeningitis	Arenavirus / Arenaviridae		(A)		10.1	
Flexal	Arenavirus / Arenaviridae	HHS			NR	
African swine fever	Asfivirus / Asfarviridae	USDA		dsDNA	170.1	No
Rift Valley fever	Phlebovirus / Bunyaviridae	HHS/USDA	A(A)	3(−) ssRNA	12.2	No
Hanta	Hantavirus / Bunyaviridae		C(A)	3(−) ssRNA	12.3	
Crimean-Congo	Nairovirus / Bunyaviridae	HHS	A(C)	3(−) ssRNA	18.9	
Akabane	Orthobunyavirus/ Bunyaviridae	USDA		3(−) ssRNA	NR	
La Crosse	Orthobunyavirus/ Bunyaviridae		(C)		12.5	
Swine vesicular disease	Vesivirus / Caliciviridae	USDA		(+) ssRNA	8.3	Yes

Virus Name	Virus Genus / Family	Select Agent[2]	CDC (NIAID) Category[3]	Genome Type[4]	Genome Size (kb)[5]	Genome Infectivity[6]
Japanese encephalitis	Flavivirus / Flaviviridae	USDA	(B)	(+) ssRNA	11.0	Yes
West Nile	Flavivirus / Flaviviridae		(B)		11.0	
Dengue	Flavivirus / Flaviviridae		(A)		10.7	
Yellow fever	Flavivirus / Flaviviridae		(C)		10.9	
Tick-borne encephalitis complex	Flavivirus / Flaviviridae	HHS	B(C)			
Omsk hemorrhagic fever					10.8	
Central European TBE					NR	
Far Eastern TBE					NR	
Russian spring summer					NR	
Kyasamur Forest					NR	
Classic swine fever	Pestivirus / Flaviviridae	USDA		(+) ssRNA	12.3	Yes
Cerceopithecine herpes (B virus)	Simplexvirus / Herpesviridae	HHS		dsDNA	156.8	Yes
SARS	Coronavirus / Coronaviridae		(C)	(+) ssRNA	29.8	Yes
Ebola	Ebolavirus / Filoviridae	HHS	A(A)	(–) ssRNA	19.0	No
Marburg	Marburgvirus / Filoviridae	HHS	A(A)	(–) ssRNA	19.1	

Virus Name	Virus Genus / Family	Select Agent[2]	CDC (NIAID) Category[3]	Genome Type[4]	Genome Size (kb)[5]	Genome Infectivity[6]
Avian influenza (HPAI)	Influenzavirus A / Orthomyxoviridae	USDA	(C)	8(–) ssRNA	13.5	No
Reconstructed 1918 influenza	Influenzavirus A / Orthomyxoviridae	HHS				
Hendra	Henipavirus / Paramyxoviridae	HHS/USDA	C(C)	(–) ssRNA	18.2	No
Nipah	Henipavirus / Paramyxoviridae	HHS/USDA	C(C)		18.2	
Newcastle disease	Avulavirus / Paramyxoviridae	USDA		(–) ssRNA	15.2	
Rinderpest	Avulavirus / Paramyxoviridae	USDA			15.9	
Peste Des Petits Ruminants	Rubilavirus / Paramyxoviridae	USDA		(–) ssRNA	16.0	
Menangle	Rubilavirus / Paramyxoviridae	USDA			NR	
Foot and mouth disease	Aphthovirus / Picornaviridae	USDA		(+) ssRNA	8.2	Yes
Variola major (Smallpox)	Orthopoxvirus / Poxviridae	HHS	A(A)	dsDNA	185.6	No
	Orthopoxvirus / Poxviridae	HHS			NR	
Variola minor (Alastrim)	Orthopoxvirus / Poxviridae	HHS			196.9	
Monkeypox	Orthopoxvirus / Poxviridae	USDA			205.7	
Camel pox						
Lumpy skin disease	Capripoxvirus / Poxviridae	USDA		dsDNA	150.8	
Goat pox	Capripoxvirus / Poxviridae	USDA			149.6	
Sheep pox	Capripoxvirus / Poxviridae	USDA			150.0	
African horse sickness	Orbivirus / Reoviridae	USDA		10 dsRNA	19.5	No
Bluetongue / catarrhal fever	Orbivirus / Reoviridae	USDA			19.2	
Rabies	Lyssavirus / Rhabdoviridae		(C)	(–) ssRNA	11.9	No
Vesicular stomatitis	Vesiculovirus / Rhabdoviridae	USDA		(–) ssRNA	11.2	
Eastern equine encephalitis	Alphavirus / Togaviridae	HHS/USDA	B(B)	(+) ssRNA	11.7	Yes
Western equine encephalitis	Alphavirus / Togaviridae		B(B)		11.5	
Venezuelan equine encephalitis	Alphavirus / Togaviridae	HHS/USDA	B(B)		11.4	
Chikungunya	Alphavirus / Togaviridae		(C)		11.8	

[1]According to documents accessed October 2007; http://www.cdc.gov/od/sap/docs/salist.pdf;
http://www.bt.cdc.gov/agent/agentlist-category.asp;
http://www3.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/PDF/Cat.pdf .

[2] Indicated is the agency responsible for entry on Select Agent List (HHS, USDA)

[3] Indicated are those agents listed on the CDC or NIAID (in parenthesis) Category A, B, and C lists

[4] All genomes are non-segmented (single molecules) except for those proceeded with a number, which indicates the number of genome segments. This is followed in parenthesis by the genome polarity (–, negative; +, positive), genome type (ss, single strand, ds, double strand; RNA or DNA).

[5] kb-kilobases, NR-not reported

[6] Infectivity of purified genomic nucleic acid