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An overview on the use of a viral pathogen as a bioterrorism agent: why smallpox?

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1. Introduction

Although the recent bioterrorist attacks in North America demonstrated the effectiveness of *Bacillus anthracis* as a biological weapon capable of severely disrupting the public health system (Jernigan et al., 2001), there are a number of viral pathogens that are candidates for use in a biological attack. These include the filoviruses, Ebola virus and Marburg virus, arenaviruses such as Lassa fever virus, and most important of all, variola virus which causes smallpox in humans. In 1999, a group of academic infectious disease experts, national public health experts, civilian and military intelligence experts and law enforcement officials met to review and comment on the threat potential of various agents to civilian populations in the USA (Rotz et al., 2002). They divided the potential threat agents into categories A, B, and C, category A containing those with the greatest potential for large scale dissemination or a heightened general public awareness that could cause mass public fear and civil disruption (Table 1). Smallpox (*Variola major*, Fig. 1) tops the list, and some viral pathogens which can cause severe hemorrhagic fever are also included.

The availability of these potential biological warfare agents varies widely. Smallpox is now an eradicated disease, and work in the two facilities where it exists, in America and Russia, is strictly controlled by the WHO. The filoviruses exist in an unknown reservoir species, but cause occasional dramatic outbreaks of disease with high (up to 80%) mortality in Africa. The outbreaks are frequently hospital based and originate from an unknown source, or from an infected non-human primate, but can be effectively controlled once the diagnosis is confirmed by isolating infected persons, and preventing spread of the disease by close contact between infected and susceptible individuals. The hemorrhagic disease caused by filoviruses is nevertheless very visible, and if introduced into North America would undoubtedly cause great public concern and panic, but once recognized it could be controlled. Marburg and Ebola virus are present in Africa, and could be obtained relatively easily by a terrorist group by sampling blood or other secretions from an infected person or non-human primate during an outbreak.

Much the same is true of Lassa fever, an arenavirus that causes up to 5000 deaths annually in West Africa. Although a milder disease than that caused by the filoviruses, Lassa fever is also a hemorrhagic disease with unpleasant sequelae in those who survive the infection (the mortality rate is generally less then 25%). However, the principal means of transmission of the infection is from contact with an infected field rat, *Mastomys natalensis*, or its excreta which may contain copious amounts of virus, and it is hard to envisage a successful bioterrorist attack using this virus.

Since no licensed vaccines or effective antiviral drugs exist to prevent or treat filovirus or arenavirus infections, these viruses are considered among the most dangerous, and can only be worked with safely in a biosafety level 4 facility. Currently, only two such facilities exist in the USA, CDC, Atlanta, GA and USAMRIID in Frederick, MD, although other facilities are planned to be built, for example in the Rocky Mountain Laboratories in Hamilton, Montana, and Galveston, TX. Thus although these viruses might conceivably be obtained from Africa, their availability in the USA is very restricted. Elsewhere in the world, the viruses are present in laboratories in Canada, England, Germany, Russia, and South Africa, so their availability is greater than that of smallpox (variola) virus. There remain many questions as to how best to regulate the possession and use of these potentially dangerous viruses. Whilst this is feasible within one nation such as the USA (Epstein, 2001), it is much more difficult to achieve internationally, and for this reason alone we need to be aware of the possibility that the filoviruses and arenaviruses may already be available to potential bioterrorists. Neither can we dismiss the possibility

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Table 1
Category A agents of bioterrorism (Rotz et al., 2002)

Agent	Disease
Variola major	Smallpox
Bacillus anthracis	Anthrax
Yersinia pestis	Plague
Clostridium botulinum (botulinum toxins)	Botulism
Francisella tularensis	Tularemia
Filoviruses and arenaviruses (e.g. Ebola virus, Lassa virus)	Viral hemorrhagic fevers

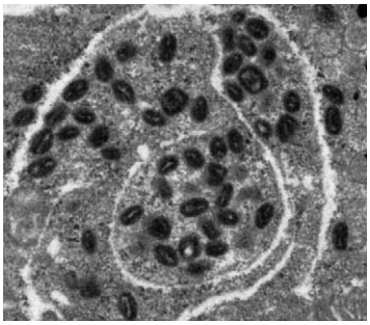

Fig. 1. Thin section electron micrograph of smallpox virions (*Variola major*) growing in BSC 40 cells. Magnification 56,000×.

that smallpox (variola) virus is deliberately being held for nefarious purposes elsewhere than in the two repositories known to WHO.

2. Smallpox (variola) virus

The last natural case of smallpox occurred in 1977 in Africa, and marked the end of the most successful public health campaign ever undertaken—the eradication of a devastating and highly communicable disease that had caused millions of deaths throughout the world for centuries (Fenner et al., 1988). The WHO then asked all laboratories that held stocks of variola virus to destroy them, or move them to one of two designated reference laboratories, at CDC in Atlanta or The Institute for Virus Preparations in Moscow, USSR. Unfortunately some research workers failed to comply with the WHO request in a timely fashion, and at a laboratory in Birmingham University, UK directed by Dr. Henry Bedson there was an accidental release of variola virus which infected a young woman working as a photographer at some distance from the smallpox laboratory. She subsequently died from smallpox, not long after Dr. Bedson, in despair over the tragedy caused by the release of the virus, had himself committed suicide.

This incident served as a sobering reminder of the danger of variola virus, but by 1980 the WHO was able to declare "smallpox is dead." Since that time the only known stocks of variola virus have been held in Atlanta, USA and Russia, where in 1984 the virus stocks were moved from Moscow to Novosibirsk, Siberia.

Nevertheless, variola virus is still considered as an ideal bioterrorist weapon for the following reasons:

1. It is highly transmissible by the aerosol route from infected to susceptible persons.
2. The civilian populations of most countries contain a high proportion of susceptible persons.
3. Smallpox is associated with high morbidity and about 30% mortality.
4. Initially, diagnosis of a disease that has not been seen for 20 years would be difficult.
5. At present, other than the vaccine, which may be effective in the first few days post-infection, there is no proven drug treatment available for clinical smallpox.

In a person infected with variola virus there is an incubation period of about 12 days, followed by an abrupt febrile prodrome associated with malaise, headache and muscle pain and a temperature which usually exceeds 101 °F. Two to three days after the onset of fever, a rash erupts first on the tongue and oropharyngeal mucosa. The lesions soon ulcerate in the mouth and high virus titres are present in the saliva. The skin rash then develops in stages and has a centrifugal distribution, most dense on the face, and more dense on the extremities than on the trunk, with lesions on the palms and soles of the feet. In all, the illness lasts about 21 days, and transmission occurs, mostly through inhalation of airborne variola virus by household contacts, from the first appearance of the rash until all the scabs separate. The spread of the disease can be interrupted by isolation of infected persons, locating and vaccinating all contacts, and isolation of any contacts who become ill. This strategy is of course dependent upon the availability of smallpox vaccine, and for this reason several western nations have begun building stockpiles of either the traditional vaccine prepared from calf skin (such as Dryvax) or modern tissue culture-derived vaccinia virus vaccines that remain to be tested for potency in the event of a smallpox outbreak (LeDuc and Jahrling, 2001; Rosenthal et al., 2001).

There is considerable debate concerning the transmission potential of smallpox in contemporary populations, where herd immunity from prior vaccination is estimated at no more than 18%, and is decreasing with time (Gani and Leach, 2001). There is little doubt, however, that a successful bioterrorist attack that initially infected 1000 persons, would be capable of spreading to the whole population of the earth within 180 days if there was no intervention (Meltzer et al., 2001). In addition, there is uncertainty as to the mortality caused by smallpox virus in the millions of people in the world who are immunocompromised as the result of HIV infection or treatment with immunosuppressive drugs—such large immunocompromised populations did not exist before 1980, when smallpox was eradicated.

The most favored response to a smallpox attack would employ a combination of ring vaccination and quarantine of all potentially infected individuals to reduce secondary transmission, and this was recommended in 2002 by the CDC's

Advisory Committee on Immunization Practises. However, the availability of an antiviral drug would be an invaluable adjunct to such a strategy, both by alleviating symptoms in infected persons as well as by reducing transmissibility.

For this reason a research program aimed at finding drugs that are suitable for treating human orthopoxvirus infections has been underway for some time. For the past 3 years a USAMRIID/NIAID collaboration has evaluated the activity of therapeutic drug candidates in in vitro assays and animal model systems using vaccinia, cowpox, and monkeypox viruses as a surrogate for variola virus. The culmination of this research, undertaken in the CDC maximum containment facility, is led by scientists from the US Army Research Institute for Infectious Diseases (Enserink and Stone, 2002). The ultimate aim is to identify an existing licensed drug which is not only inhibitory to smallpox virus replication in vitro, but can also be effective in a non-human primate model and humans, preferably following administration by the oral route. Initial work using cowpox virus infection of mice suggested that cidofovir (*S*-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine) was an effective drug when used at a dose of 100 mg/kg (Bray et al., 2000). In a more detailed in vitro study using camelpox, cowpox, monkeypox and vaccinia viruses grown in Vero cells, cidofovir was initially inhibitory, but with extended passage over time in the presence of the drug, resistant mutant viruses developed which were only inhibited by 8–27-fold higher concentrations. There was some evidence, however, that in mice the drug-resistant mutants were less virulent than the original wild-type viruses (Smee et al., 2002). In addition, the difficulty of developing resistant mutants in vitro suggests that naturally occurring resistance is unlikely to be a problem during acute treatment of smallpox.

3. Where would smallpox virus come from?

As mentioned previously, there are only two official stocks of smallpox (variola) virus held at the request of WHO in the USA and in Russia. However, Dr. Ken Alibek, a Russian scientist who was involved in the Bioweapons Program of the USSR from 1980 to 1990, and now resides in the USA, has claimed that smallpox virus was actively developed as a biological weapon during this 10-year period (Alibek, 1999). At that time, smallpox virus was believed only to be held in the official WHO repository in the Institute of Viral Preparations in Moscow. But according to Dr. Alibek it was being grown in large quantities in Zagorsk and at Vector, a biotechnology institute in Novosibirsk, Siberia. In support of this, it should be noted that genome sequences of the India 1967 strain of variola virus (the strain used for weaponization) were published from the Institute of Molecular Biology NPO Vector, Koltsovo, Novosibirsk as early as March 1993 (Shchelkunov et al., 1993a,b), well before publication of the first complete sequence of the Bangladesh 1975 strain of variola virus determined at CDC (Massung et al., 1993).

The possibility, therefore, exists that rogue organizations engaged in terrorist activities might have acquired smallpox virus from Russian scientist(s) during the 1980–1990 period, or even subsequently. Alternatively, it is possible that, despite the efforts of WHO, all smallpox virus samples were not destroyed or given to one of the two official repositories, and this would present another source from which the virus could get into the wrong hands. It seems inconceivable that anyone would want to reintroduce smallpox virus into the world, particularly a world where more than 80% of the population will have no prior immunity to the virus, but following the events in the USA on 11 September 2001, we can never say never to such an event, and so we must be prepared for it.

4. Smallpox vaccination

As a result of concerns that smallpox virus might be used in a bioterrorist attack, 1 year before the attacks of 11 September 2001 the CDC had entered into an agreement with a company in Cambridge, MA (then known as Oravax, but now part of Acambis Ltd., a UK company) to produce a new smallpox vaccine derived by growth in cell culture (LeDuc and Jahrling, 2001). The original vaccine used for the successful eradication of smallpox was based on live vaccinia virus produced on the skin of calves or sheep and known as vaccine lymph. However, the procedures involved made it virtually impossible to exclude bacterial contamination of the vaccine product, and this level of uncertainty as to the precise content of the vaccine would not be tolerated in a biological product approved for human injection use today. It was, therefore, decided to switch to vaccinia virus grown in human diploid cell culture for the production of stocks of vaccine to be held for use in the event of a smallpox attack.

In the absence of any human smallpox cases, considerable debate has centered around whether it is wise to "prevaccinate" members of the community who might become directly involved with smallpox cases (e.g. first responders, emergency services personnel, or doctors trained to administer the vaccine), but as yet no decision has been reached. The main difficulty is that vaccination itself is associated with a number of adverse side-effects, and these are likely to be more serious in immunocompromised persons, so the relatively straightforward strategy of vaccinating the whole population seems inadvisable in the absence of cases of smallpox. For example, HIV was not recognized and AIDS did not exist prior to 1977, when smallpox vaccination ceased. Now more than 40 million persons worldwide are infected with HIV. Currently, the proposed strategy involves the rapid identification of any smallpox case that presents itself followed by "ring vaccination" of contacts so as to provide a ring of immunity around each case. This should minimize the occurrence of adverse events, and also make the most efficient use of vaccine supplies. Priority groups for vaccination will be those known to have had

face-to-face contact with smallpox patients, persons exposed to an intentional release of smallpox virus, household members of contacts of cases, and finally persons involved with face-to-face evaluation, care or transportation of smallpox patients. The success of this strategy will depend upon the amount of vaccine available for use, personnel resources and readiness (e.g. ability to administer the vaccine, which requires effective use of a bifurcated needle), and the effective utilization of other control measures such as isolation and quarantine of patients.

So far as vaccine availability is concerned, in the USA the CDC has 15 million doses of the original freeze-dried calf lymph vaccine (Dryvax), plus an additional 85 million doses that were recently found and donated by Aventis-Pasteur. In a recent study, it was shown that the CDC stock of Dryvax could be diluted at least five-fold, to a titer as low as 10,000 pfu per dose, and still induce local viral replication and vesicle formation in more than 97% of persons, greatly expanding the potential numbers of doses in the original CDC stockpile (Frey et al., 2002). Delivery of stocks of the new vaccine from Acambis is expected to begin in September 2002 with 170 million doses, followed by a further 39 million doses by the end of 2002. The USA, at least, should be adequately prepared for a smallpox attack.

5. Other human viral pathogens

In addition to the viruses considered before, there are many viruses with high pathogenic potential that might be employed in a bioterrorist attack, though for the most part they would not cause the same high level of public concern and civil disruption. Probably the most important of these is influenza A virus. There are a number of considerations that make influenza virus a cause for concern at the present time.

First, the virus is highly transmissible in human populations, and has in the past caused severe pandemics involving high mortality when a new influenza virus is introduced that contains novel surface antigenic components to which the population has no prior immunity (antigenic shift). Such pandemics occurred in 1918, 1957, and 1968, and demonstrated the ability of influenza virus to cause extremely high levels of morbidity and mortality, and to disrupt the normal public healthcare infrastructure. For this reason, and also because antigenicity of currently circulating viruses is continuously subject to change (antigenic drift) influenza viruses are carefully monitored throughout the world, and annual vaccination is recommended for vulnerable groups in the population who are at high risk from infection (Cox and Kawaoka, 1998).

Second, new technology is now available that can be used to deliberately alter the genetic composition of influenza virus, and hence both the antigenic components and genes thought to be involved in virulence can be deliberately altered to make a virus with high potential for spread in the population (Neumann and Kawaoka, 2002). Fortunately, there are two new antiviral drugs that target the viral neuraminidase now available that could help to minimize an influenza pandemic; these were not available during previous pandemic years. Influenza viruses naturally infect avian species, and both the pandemics of 1957 and 1968 were caused by recombinant viruses containing a mixture of avian and human genes (Webster and Kawaoka, 1994). From the point of view of bioterrorism, one negative feature is that the extreme transmissibility of influenza virus would make it difficult to target a specific population, and a newly introduced virus would likely spread unpredictably.

Other viruses affecting humans that have been considered as possible bioterrorism agents include a group of neurotropic viruses spread by mosquitoes. Alphaviruses, especially Venezualan equine encephalitis (VEE), Eastern equine encephalitis (EEE) and Western equine encephalitis (WEE) all occur naturally, and may occasionally be transmitted to humans by mosquitoes. EEE in particular may cause high mortality (30–50%), with most survivors having paralytic or mental sequelae, but the mortality rates described for WEE (10%) and VEE (1%) are much lower. There is also a problem of how such mosquito-borne viruses could be used and disseminated in a bioterrorist attack.

Several other potentially lethal viral zoonoses are known, such as Hendra and Nipah viruses, and viruses causing hantavirus pulmonary syndrome, such as Sin Nombre virus in North America and Andes virus in South America, but in these cases too little is currently known about the acquisition of infection for them to be likely bioterrorist weapons. Nevertheless, there is certainly scope for research into the development of effective antiviral drugs against such viruses, that could be used in case of further disease outbreaks such as the epidemic of Nipah virus in Malaysia that killed more than 100 persons.

6. Viruses targeting economically important food animals

The recent outbreaks of foot-and-mouth disease in Taiwan and more recently in the UK provided a grim warning of the very serious economic consequences that can ensue when foot-and-mouth disease virus (FMDV) is introduced into a disease-free country. FMDV is believed to be the most highly contagious virus known, and in addition to it's ability to spread and cause disease among cloven-hoofed animals (e.g. cattle, sheep and pigs), it can survive and remain infectious for up to several years, for example in soil. For this reason, any serious consideration of viral bioterrorism must include the possibility of the deliberate introduction of viruses affecting farm animals as a means of attack. Candidate viruses in addition to FMDV, would include rinderpest (cattle plague), bluetongue, fowl plague (highly pathogenic influenza A virus) and African swine fever. Control of such virus infections would be possible by rapid diagnosis and slaughter of all animals on affected premises, but by far the

most serious of these is FMDV. Foot-and-mouth disease does not usually result in mortality, and most animals recover from the disease in 2 weeks, but there are lasting adverse effects on milk production and nutrition, and in particular the presence of the disease in a country results in an immediate embargo against animals and animal products with consequent trade losses that can amount to billions of dollars.

7. Conclusions

From this brief overview it is clear that smallpox virus is still the most likely candidate for a bioterrorist attack. It is not disputed that the former USSR put a great deal of effort into the creation of huge stocks of smallpox virus with a view to distributing them by aerosol over a large population (Alibek, 1999). The disruption that such a distribution could cause (O'Toole, 1999) far outweighs that of any other viral pathogen, and we must therefore make adequate preparation by having available stocks of vaccine as well as antiviral drugs so that the disease could be treated and once again stamped out should it ever reappear.

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