

Smallpox: an old disease but still a threat in the XXI century

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Throughout history, smallpox was a severe, highly contagious disease caused by the variola virus. The disease, which existed in a natural form until 1977, had great importance in the history of humanity, but has now been eradicated after a successful worldwide vaccination program. After the last naturally occurring case, in Somalia in 1977, routine vaccination against smallpox among the general public ceased because it was no longer necessary for prevention. The fact that we have returned to the study of smallpox, once eradicated, is that it is one of the main biological agents which can be used as a biological weapon of bioterrorism. The smallpox virus is included in the A category of classification of the US Centers for Disease Control and Prevention, because: it can be easily disseminated or transmitted from person to person; there are high mortality rates; the potential for a major public health impact is high; public panic may be caused; social disruption could follow and special action for public health preparedness would be required.

In this chapter, we analyze the characteristics of the virus and the possibility of its use in a bioterrorist attack. We describe the “table-top” exercises practiced since 2001 and the situation and the future of the cultures of virus stockpiled in the Federal State Research Center of Virology and Biotechnology (Vector) of Russia and the CDC of the United States, under supervision of the WHO. Finally we analyze the WHO resolution, taken in May 2007, to delay for at least four years any decision on when to destroy the world's last known stockpiles of smallpox.

Key words: smallpox, bioterrorism, biological weapon, vaccine.

1. Variola virus

According to the the International Committee of Taxonomy of Virus [1], smallpox viruses belong to the *Poxviridae* family. Among the virus of this family are the cowpox virus, the vaccinia virus (used in the vaccine), the myxomatosis virus of the rabbit, monkeypox, etc... Some of them (vaccinia, cowpox and monkeypox) may infect humans and have animal hosts. In contrast to the other poxviruses, there is no reservoir for variola virus.

These viruses, particularly the vaccinia virus, have contributed enormously to the development of general knowledge on infectious diseases and immunity. In addition, the vaccinia virus was the first animal virus observed microscopically, cultivated in tissue culture, titrated accurately, physically purified and chemically analyzed [2].

The investigation on poxvirus did not end when smallpox was eradicated. Recombinant DNA technology has led to important progress in the understanding of the virus replication. In addition, the virus vaccinia has been developed like a living vector of recombination, providing a new instrument for the expression of genes, and the production of vaccines against a variety of agents of infectious diseases. Negatively, it has also been studied for its use as a biological weapon.

Poxvirus is the most complex and largest animal virus that exists, 350x270 nm. The virions, seen through an electron microscope (Fig. 1), are generally brick-shaped or pleomorphic. They can be seen with a phase contrast microscope or in dyed preparations, although only as a point, without distinguishing morphology or structure, since they are at the limit of the power of resolution of the light

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microscope ($0.2 \mu\text{m} = 200 \text{ nm}$). They are also different from the majority of viruses because they are virus with double stranded DNA and because they replicate in the cytoplasm and have enzymes to synthesize messenger RNA. They have proteins that serve to evade mechanisms of immunity of the host.

The genome, fully sequenced, is not fragmented and contains a single molecule of linear double-stranded DNA with 191,636 nucleotides. The mol %GC of the DNA is 36. The viral genome encodes structural and non-structural proteins. The virion contains 4% lipids, which are located in the envelope. The composition of viral lipids and host cell membranes are similar. The lipids are host-derived and synthesized *de novo* (during the early phase of virus replication) and are derived from the plasma membrane. The viral membranes also include glycolipids.

Poxvirus has a special predilection for epithelial cells, in whose cytoplasm they reproduce, producing type A (cytoplasmic accumulation) and type B (viral factories) inclusion bodies. These may be observed in the epithelial cells at the base of the vesicles. A type bodies, with different designations, appear in other Poxvirus, not in the variola virus. In the latter, it only produces B type inclusion bodies, identified by hematoxylin-eosin stain with light microscopy as Guarnieri bodies (light basophilic granules) [3]. This fast diagnosis is important to know, since it is a simple way of determining, whether we are confronted with a case of smallpox, or severe chickenpox in an immunocompromised patient. In this second case, when there is an infection by herpesvirus, specifically the human Herpesvirus type 3, the epithelial cells will also be affected, but, unlike Poxvirus, the reproduction takes place in the nucleus, where the inclusion bodies may be observed with light microscopy.

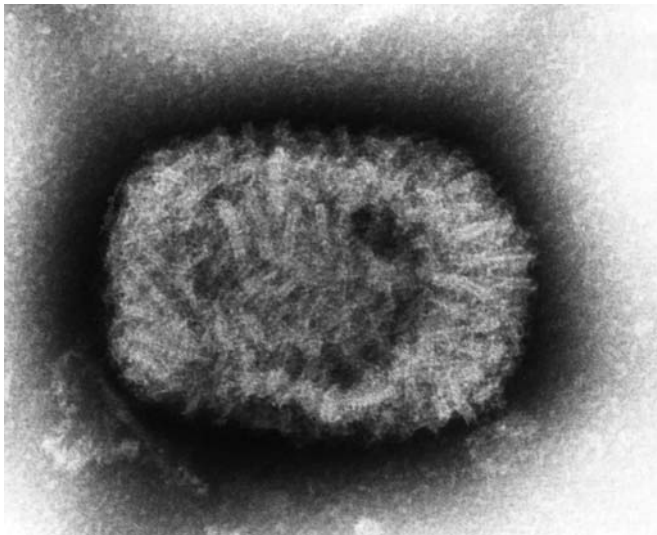


Fig. 1 Highly magnified at 310,000X (in the original), this negative-stained transmission electron micrograph depicted a smallpox (variola) virus particle. Courtesy of CDC Public Health Image Library

The variola virus is resistant to relatively high temperature (it must be heated to 55°C for at least 30 minutes to be inactivated), drying, cold and phenol or other common disinfectants [3]. The virus remain infectious after lyophilization and exposure to temperatures of -180°C . For that reason, the virus contained in exudates or scabs of the patients can almost remain viable one year at room temperature. Therefore, bedclothing, towels, objects, etc, that have been in contact with the patient, can be sources of infection.

2. Smallpox

Smallpox is a very severe, contagious, and sometimes fatal disease. There is no specific treatment for smallpox disease, and the only prevention is vaccination.

There are two clinical forms depending on the severity of the disease: *variola major* and *variola minor* [3, 4, 5].

- *Variola major* is the most common and also the most serious form of the disease, with a more extensive rash and higher fever. At its time, it was subdivided into four types: ordinary, modified, flat and hemorrhagic. The ordinary type, the most frequent (90% or more of cases) has a fatality rate of 30% (range 15-50%) in an unvaccinated population, and practically all the survivors have residual scars of the pustules on the face. The modified type (with a fatality rate of 3%), is less severe because it occurs in vaccinated people. The most severe types are the flat “malignant” smallpox and the hemorrhagic “fulminate” smallpox: both are very rare and usually fatal.

- *Variola minor* (so called modified smallpox, alastrim, amaas) is a less common presentation of smallpox, and a much less severe disease, with fatality rates of less than 1% and generally no scars on the face.

The disease is contracted via the respiratory tract and infects pulmonary macrophages during the first hour of the incubation period. The virus is transported from the lung to local lymph nodes, where it multiplies and is borne by blood. The virus also replicates in the spleen, bone marrow and lymph tissue. Finally, leucocytes with virus appear in dermal and mucosal vessels, resulting in characteristic skin and mucosal symptoms.

The clinical presentation of the disease proceeds in the following way:

Incubation period: After the infection, there is an incubation period during which there are no symptoms and the patient is not contagious for a period averaging 12 to 14 days, but which can also range from 7 to 17 days.

Prodrome: This phase, with a duration of 2 to 4 days, is the beginning of symptoms: fever, usually high (38 to 40°C), and chills, often accompanied by malaise, headache, lumbar pain, and less frequently by nausea, vomiting, abdominal pain and delirium. During these days the patient is unable to carry on normal activities. The patient may sometimes be contagious.

First rash: This phase has a duration of 4 days and begins with small red spots that appear on the tongue and in the mouth. These spots evolve to sores that break open and spread large amounts of the virus into the mouth and throat. At this time, the person becomes most contagious. A great dissemination of virus takes place inside the patient and, from this point to the exterior. At the same time when the sores are breaking open, an eruption appears on the skin beginning on the face and extending to the extremities, first the arms and legs, and soon after, the feet and hands. The eruption extends to the entire body in 24 hours. Usually the fever falls and the person may start to feel better. By the third day of the rash, the rash becomes raised bumps. The following day, these bumps fill with a thick, opaque fluid. The bumps present a depression in the center that looks like a navel. This form is a major distinguishing characteristic of smallpox. Fever will often rise again at this time and remain high until the next phase.

Pustular rash, lasting 5 days. The bumps evolve to sharply raised pustules, usually round and hard to the touch. The patient remains contagious.

During the next 5 days, the pustules begin to form a crust and then scab. The following 6 days, the scabs begin to fall off, leaving marks on the skin that eventually become pitted scars. Most scabs will have fallen off three weeks after the rash appears. The patient remain contagious until all of the scabs have fallen off.

Smallpox complications: Most patients have some degree of encephalopathy, ocular complications (10-20%), osteomyelitis variolosa (2-5% of children with symptomatic disease) and hemorrhagic smallpox (pregnant women have a propensity to develop this complication).

3. Smallpox and History

Smallpox is well-known as a serious disease that has produced a great impact on the history of humanity for thousands of years [6]. It is believed that the virus evolved from an Afrikaans rodent poxvirus 10,000 years ago [3]. The disease spread from Africa to India, where it became endemic [1]. From India, smallpox spread to China and Japan to the East, and to Europe and North of Africa to the West. The disease became endemic on the Iberian Peninsula and Mauritania in the year 700. From here, it

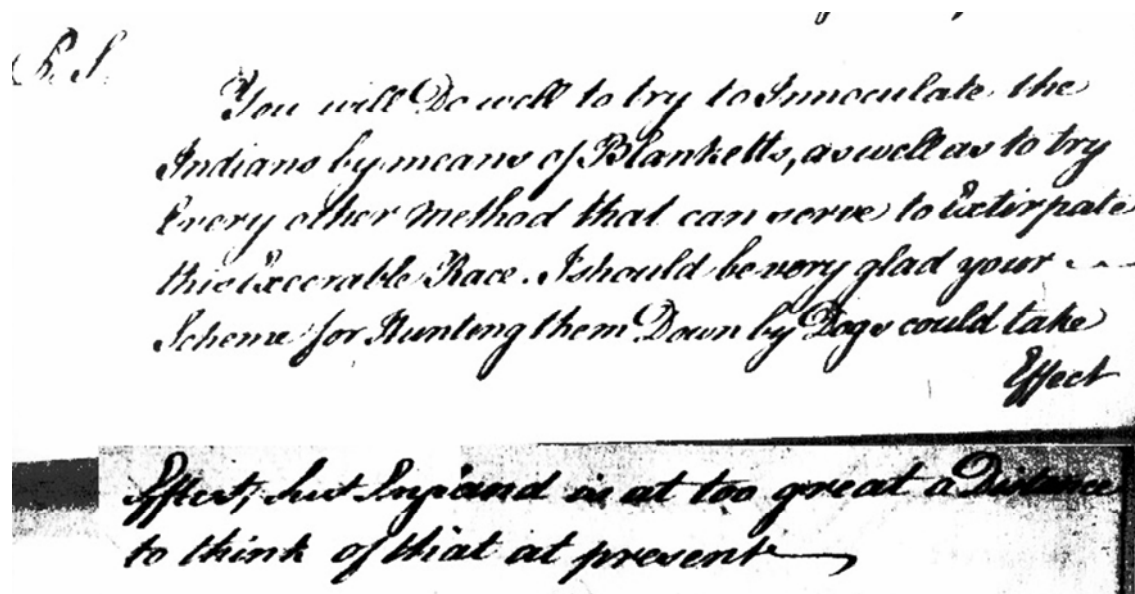
accompanied European adventurers in their exploration and colonization travels. Thus, it was introduced by the Spanish conquistadors in the New World by means of African slaves in 1518. Soon after arriving at La Española, now Dominican Republic and Haiti, a smallpox outbreak decimated the native population, who had never had contact with the disease and, therefore, lacked immunity. From this point, the disease reappeared in Mexico (1520) and Peru (1524). It was propagated from Africa to Brazil in 1555 in an independent form. The impact of these events had to be enormous, favoring the dominion of small groups of European explorers over the enormous populations that supposed great empires, the Aztecs and the Incas. It is possible that the introduction of a numerous African population, by means of the slave market, in lamentable conditions of hygiene, had decisive influence on the spread of the new disease on the continent. Numerous writings on the use of Biological War, the deliberate use of infectious diseases, in the conquest of America have been published.

They say that there are three moments in the American conquest when smallpox would have mainly been used to decrease the resistance of the native populations before the invading army: In the conquest of Mexico and the Azteca Empire (1520), in the conquest of Peru and the Inca Empire (1524) and in the French-Indian War (1754-1763) [7]. The last war is one of the historical episodes in which there is documentary evidence of deliberate use of microorganisms in the rebellion of the Pontiac Indians. This tribe declared war on the invaders of North America, the French and the English, although they were soon allied with the former.

On April 27 of 1763, an assembly of tribes, including the Pontiac, Ottawa, Chippewas, Shawnee, Mingo and Delaware, associated to fight against the British. By the end of May they had conquered several forts, and besieged forts Pitt and Detroit. Fort Pitt was under the command of captain Simeon Ecuyer, who received orders from Colonel Henry Bouquet in Philadelphia, who informed General Jeffrey Amherst. Captain Simeon Ecuyer had bought time by sending smallpox-infected blankets and handkerchiefs to the Indians surrounding the fort, starting an epidemic among them, an early example of biological warfare. Amherst, himself, had encouraged this tactic in a letter to Ecuyer.

In June 1763 cases of smallpox appeared among the besieged British. In an undated letter, General Jeffery Amherst asks colonel Bouquet if he could spread the smallpox to those Indian tribes. The answer, dated on July was: "I am going to try to inoculate them with some blankets that may fall in their power, taking care of of not contracting the disease myself ". Amherst's answer on July 16 was: *...you will do well to try to inoculate the Indians by means of blankets as well as to try every other method that can serve to extirpate this execrable race...*(Fig. 2). Before this official authorization for the use of biological war, there is a long series of episodes in which the systematic, deliberate propagation of smallpox has been demonstrated since 1755.

The subject of extermination of the American native populations has been dealt with many times in the last few years, when we speak about the bioterrorist threat. One of the reasons for the greater susceptibility of those populations in face of the disease was the lack of immunity. People had not had previous contact with those microorganisms.



P.S. You will do well to try to Inoculate the Indians by means of Blankets, as well as to try Every other Method that can serve to extirpate this execrable Race. I should be very glad your Scheme for Hunting them Down by Dogs could take Effect

Effect; but I find it is at too great a Distance to think of that at present

Fig. 2 Letter from General Amherst to Colonel Bouquet dated 16 July 1763. We can read:...you will do well to try to inoculate the Indians by means of blankets as well as to try every other method that can serve to extirpate this execrable race...

For that reason, Elizabeth Fenn, author of a book dealing with the action of smallpox in America in the 18th century, when speaking of the recent bioterrorism threats, describes all of us as Indians (“Today, we’re all Indians”) because as the disease was eradicated and the vaccinations were suspended, we lack immunity.

4. The vaccine

Edward Jenner (1749-1823), doctor of Gloucestershire (United Kingdom), observed how the people who worked milking cows and were infected with a species of benign smallpox in their hands, did not contract smallpox. Jenner’s observation was taken to experimental practice, in 1796, inoculating James Phipps, the 8 years-old son of one of his employees, with liquid coming from a pustule from the hand of Sarah Nelmes. This boy was protected against later inoculations, one month later, of authentic smallpox. Two years later, in 1798, Jenner repeated the inoculation to confer immunity to more children, including his own son. These and other results were published by Jenner in 1798, with the title “*An Inquiry into the Causes and Effects of the Variolae Vaccinae; a Disease Discovered in some of the Western Counties of England, Particularly Gloucestershire, and Known by the Name of The Cow Pox*”.

This technique of immunization extended all over the world, making an infectious disease disappear for the first time in the history of humanity. To this end, a concerted effort was required, mainly of the pioneers who acted in extreme conditions, like the Balmis Expedition of 1803, traveling around the world the vaccine to the Spanish colonies in the New World and Asia. Since cowpox could be inoculated from person to person, Balmis transported the vaccine through arm to arm vaccination of orphaned children on board their boats [8, 9].

The last vaccine used when the disease existed was Dryvax®, produced by Wyeth Laboratories, until 1982. It was a vaccine prepared from an attenuated strain of the virus, denominated New York City Board of Health strain. The live virus was prepared from bull calf lymph, which was purified, concentrated and dried by lyophilization.

The last case of smallpox in the United States was in 1949 in Texas, where 7 people were affected with one fatality. In Spain, the last outbreak was in Madrid, which affected two people who had traveled to India in 1961. One girl was hospitalized, and later 17 cases, broke out, 12 of which occurred in the hospital. The Provincial Headquarters of Health made the epidemiological study and proceeded to vaccinate practically the entire population of Madrid.

The last great worldwide campaign of vaccination sent by the WHO was developed in 1967. After this campaign, the last naturally occurring case of endemic variola major in the world was reported in Bangladesh in 1975, and the last case of endemic variola minor was seen in Somalia in 1977. The following year, there were still two deaths in Birmingham (United Kingdom) from a smallpox virus escaped from a research lab. One of the patients died. The director of the laboratory committed suicide. These were smallpox's last victims, although not of natural smallpox. It was laboratory accident [3].

Since 1980, WHO recommended cessation of routine vaccinations because no naturally occurring smallpox disease existed anywhere in the world [10]. All the virus stocks were to be destroyed, except for those kept by the two WHO collaborating Centers: the CDC in Atlanta (USA) and the Vector Laboratories (State Research Center for Virology and Biotechnology) in Novosibirsk, Russia.

5. The threat of biological war and terrorism

Bioterrorism has been defined by the CDC as “*the intentional release of bacteria, viruses or toxins for the purpose of harming or killing civilians*” [11]. This concise definition has been expanded to “*the intentional use of a pathogen or biological product to cause harm to a human, animal, plant or other living organism to influence the conduct of government and to intimidate or coerce a civilian population*” [12].

The CDC has created a list of critical biologic agents that have potential for use in bioterrorism. There are three categories (A, B and C), on the basis of their ability to cause victims, their capacity for dissemination, their ability to cause civil disruption and the special needs required for public health intervention [13]. One of the microorganisms historically used as a biological weapon has been the variola virus. In fact, this virus is included in the A category of classification of the CDC, because: it can easily be disseminated or transmitted from person to person; there are high mortality rates; the potential for a major public health impact is high; public panic may be caused; social disruption could follow and special action is required for public health preparedness.

In the meeting of the Ad Hoc Committee on Orthopoxvirus Infections [14] (Geneva, January 1999) a paper on the risk of the deliberate release of smallpox virus and its impact on virus destruction was presented. Smallpox virus was considered to be the primary candidate for use as a biological weapon. The infectious dose is likely to be small and there is documented evidence of ready aerosol spread of the virus which is further supported by the viability of the virus under various environmental conditions of temperature and humidity. A 30% case-fatality rate can be predicted in non-vaccinated individuals exposed to smallpox and the potential for secondary spread could result in ten secondary cases for each primary case.

Although the virus is not so easy to produce as bacteria, in this particular case, the variola virus is a microorganism that has been produced in great amounts. According to declarations of Kanatjan Alibekov, scientist of Kazajstan, residing in the USA since 1992, in the 1970s, the former USSR maintained a stockpile of 20 tons of variola virus in its biological weapons arsenal, in Zagorsk. This could produce from 80 to 100 annual tons of virus, violating, not only the Convention of 1972, but also the limitations that the WHO had imposed on the Vector Laboratories deposits. This massive production may be the possible origin of stocks to other nations or terrorist groups [15].

In fact, the variola virus has a moderate to high potential for large scale dissemination because it may be lyophilized and is relatively stable as an aerosol. In 1970, there was an airborne outbreak in a German hospital that demonstrated that only a few virions could be effectively disseminated through aerosolization and might serve to cause infection [16]. This seems far more likely than the people and media that speculate with the possibility that bioterrorists may infect themselves and circulate in airports

and other densely populated areas of high mobility. This is less probable because a person infected with smallpox virus, after the start of the prodrome, is too ill to circulate widely and disseminate the infection.

6. The “table-top” exercises

On 22–23 June 2001, the Johns Hopkins Center for Civilian Biodefense Strategies, in collaboration with the Center for Strategic and International Studies, the Analytic Services Institute for Homeland Security, and the Oklahoma National Memorial Institute for the Prevention of Terrorism, carried out a “table top” exercise entitled “Dark Winter”, which simulated a covert smallpox attack on the United States [17].

The 12 participants in Dark Winter portrayed members of the National Security Council (NSC). Among these, a former Senator from Georgia, played the President of the United States, the governor of Oklahoma, portrayed himself. The Director of the Central Intelligence Agency, the Secretary of Defense, the Secretary of Health and Human Services, the Secretary of State, the Attorney General, the Director of the Federal Emergency Management Agency, the Director of the Federal Bureau of Investigation, the White House Communications Director and the Press Secretary to the Governor were also represented. In addition, five journalists and approximately 50 people with current or former policy or operational responsibilities related to biological weapons preparedness observed the deliberations of the simulated NSC.

In Dark Winter 3,000 people were supposedly infected with the smallpox virus during 3 simultaneous attacks in 3 separate shopping areas in Oklahoma City, Philadelphia, and Atlanta. To cause these effects, only 30 g of weaponized smallpox would be sufficient to infect 3,000 people via an aerosol attack. The designers of the exercise considered that an attack resulting in 3,000 infections is scientifically plausible, because of the small infectious dose required to cause disease.

After the primary cases, the transmission rate for smallpox is a complex phenomenon contingent on multiple factors, not only biological, involving host and virus, but also other social and demographic factors. One important factor in the estimations of transmission rate is the level of susceptibility to smallpox virus infection. Currently, the susceptibility to variola virus is higher than it has ever been in recent history because of the absence of smallpox in the world and the absence of vaccination programs since the illness was eradicated. After the analysis of 34 instances of imported smallpox in Europe between 1958 and 1973, a 1 : 10 ratio for the transmission rate was used in the exercise. When the most closely parallel outbreaks were analyzed with the conditions and context of the exercise, the number of second-generation cases in those outbreaks ranged from 10 to 19, with an average of 13.3 secondary cases per initial case. This rate was in agreement with the last outbreak in Madrid, where as seen previously, a girl was hospitalized and, 12 more infected in the hospital [18].

Going back to the exercise, in a meeting of the NSC, on December 9 of 2002, the participants received the news that a smallpox outbreak was occurring in the United States: 20 cases had been confirmed by the CDC and 14 more suspected in Oklahoma. There were also suspect cases in Georgia and Pennsylvania. Given the incubation period of the illness, the initial infection occurred on or about 1 December. At that moment, the national stockpile of smallpox vaccine was 15.4 million doses, but it is estimated that this amount translates to approximately 12 million usable doses.

Three options of vaccine distribution are provided. Option 1 is a ring vaccination [19] (Fig. 2), in which enough vaccine would be distributed to each of the 3 affected states to vaccinate patient contacts and essential personnel, and 2.5 million doses would be set aside for the Department of Defense (DD). Option 2 is a combination of ring with mass vaccination, in which enough vaccine would be distributed to all residents of affected cities, as well as patient contacts and essential personnel, and 2.5 million doses for the DD. Finally, option 3 is a combination of ring with mass distribution policy, in which enough vaccine would also be distributed to each of the 3 affected states so that all residents of affected cities could be vaccinated, and 2.5 million doses for the DD. The remaining 47 unaffected states would immediately receive 125,000 doses of vaccine each, to use as necessary.

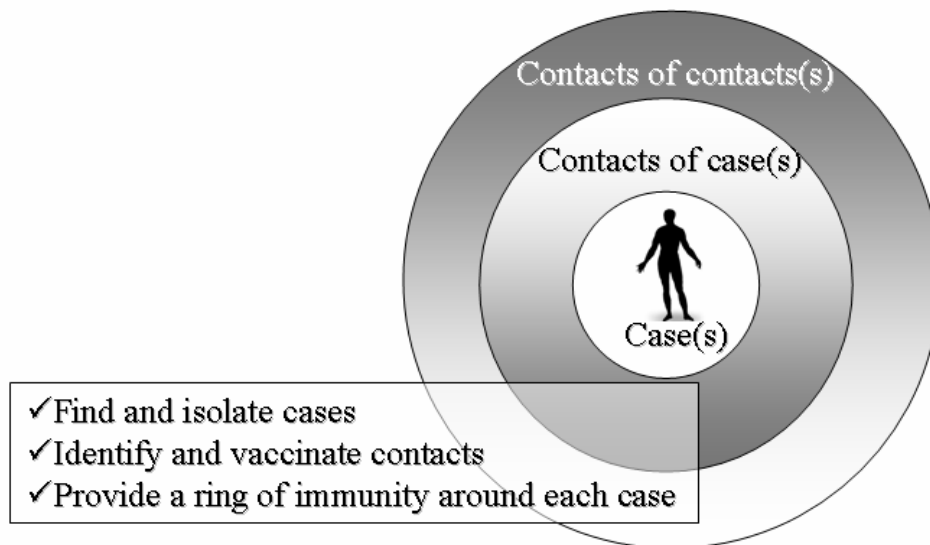


Fig. 3 Schematic representation of the *ring vaccination* policy to isolate the infected individuals with a *wall* of immune individuals. The strategy of containment is to identify contacts of the patient since he first became ill and to vaccinate them. A ring of immunity is created around each case by vaccinating contacts of contacts as well. (From Henderson *et al.*, 2003, modified.)

The NSC decided to use option 1, which is the ring vaccination policy, intended to focus and limit vaccination efforts to those at highest risk of contracting smallpox and preserving as much vaccine as possible for use as the epidemic unfolds. It also decided that the same directed vaccination strategy would be followed if additional new cases emerged in other places. In addition, the NSC decided to set aside sufficient doses of vaccine for the DD to meet its immediate needs.

In the second meeting, on 15 December 2002, the cumulative reported smallpox cases were 2,000, with 300 deaths. Related to the 3 initial outbreaks isolated cases appeared in Canada, Mexico, and the United Kingdom. In the third meeting, on 22 December 2002, a total of 16,000 smallpox cases were reported in 25 states and one thousand people died. The total number of second-generation victims was expected to reach 30,000 cases, 10,000 of whom were expected to die. In worst-case conditions, the third generation of cases could comprise 300,000 new cases and lead to 100,000 fatalities, and the fourth generation of cases could reach 3,000,000 cases and 1,000,000 deaths.

Due to the moment that this exercise took place, the participants verified the serious consequences that a bacteriological attack of that magnitude could have. The Dark Winter exercise offers instructive insights and lessons for those with responsibility for bioterrorism preparedness. Since the Dark Winter exercise, the country has suffered the events of 11 September, as well as anthrax attacks, so we can affirm that there will be more preparation of the defence against biological weapons.

A second “table-top” exercise, entitled *Atlantic Storm*, was carried out on January 14, 2005 by the Center for Biosecurity of the University of Pittsburgh Medical Center, the Center for Transatlantic Relations of the Johns Hopkins University, and the Transatlantic Biosecurity Network [20]. Some of the participants in the game were the same as in Dark Winter.

The game, which updates the existing conditions in Dark Winter, runs this way:
As in Dark Winter, the participants in the game portrayed members of European or American Governments and the Director General of the WHO. The President of the United States, was played by Madeleine Albright, former Secretary of State of the USA; the President of the European Commission, by Erika Mann, member of the European Parliament; the Prime Minister of Canada, by Barbara Mac Dougall, former Foreign Minister of Canada; the Director General of the WHO, by Gro Harlem, former

Director General of the WHO; the Prime Minister of Poland, by Jerzy Buzek, former Prime Minister of Poland, and so on. All were scheduled to meet for a Transatlantic Security Summit in Washington, DC, USA, to discuss the threat of international terrorism[21].

During the six hour meeting, the transatlantic leaders wrestled with the enormity and rapid pace of the emerging epidemics of smallpox. At 9:00 am they received the news that 51 cases had been detected in four European countries (25 in Germany, 15 in Turkey, 8 in Netherlands and 3 in Sweden. Just 4.5 hours later, at 1:30 pm, 3,320 cases were reported throughout Europe and North America, with projections indicating the possibility of 660,000 cases worldwide within 30 days. Ultimately, the outbreaks were discovered to be the result of covert attacks on transportation hubs and commercial centers in six cities: Istanbul, Rotterdam, Warsaw, Frankfurt, New York, and Los Angeles.

The difference in this case was that some countries were better prepared than in the first exercise. For example, the United States, Germany, the United Kingdom, France and the Netherlands had stockpiled sufficient smallpox vaccine to cover 100% of the population (estimates on January 14, 2005), whereas others, like Spain, Norway and Ireland, scarcely covered 15%.

Some of the characteristic differentials that can be established between smallpox and other microorganisms already used, as happened with the case of anthrax in the United States, are: first, smallpox is an infectious and contagious disease, whereas *Bacillus anthracis* is an agent of infectious and not contagious disease. Second, when the smallpox symptoms begin to appear, the second generation patients appears in places different from where an attack took place. It is very difficult to establish if it is a second generation of infected people or it is a new attack. Third, the capacity to stockpile vaccines and distribute the reserves, the great scale production, is insufficient in the face of an attack of such magnitude.

Atlantic Storm, like Dark Winter, was an occasion to instruct and to obtain experiences in the defence against bioterrorism in the medical, public health, political and national security fields. It is possible that these lessons help the resolution of the possible real cases that may be produced.

7. Toward the end of variola virus

If the disease in its natural form has disappeared, it is logical to think that the virus, which necessarily needs sensitive cells (human) for its propagation, has also disappeared from nature. However, many strains of the virus have been conserved, as stated, in the CDC and Vector. For a long time, the Special Committee of Orthopoxvirus of the WHO recommended that the reserves conserved in the two centers had to be destroyed [22]. Nevertheless, the destruction was not carried out, bearing in mind the preoccupations expressed by the scientific community in relation to public health and investigation.

In 1996, following a proposal to that effect by the Executive Board at its ninety-seventh session, the Health Assembly adopted resolution WHA49.10, recommending that destruction should take place on 30 June 1999, after a further decision had been taken by the Health Assembly. It was envisaged that the period between 1996 and 1999 would be used to achieve a broader consensus on the issue [23]. Since then, the virus has remained on death row because it was impossible to complete the investigations inside the deadlines that were set.

The recommendations of the WHO Advisory Committee to reach an agreement of destruction includes: the update on variola virus strains in the two virus repositories and the update on diagnostic assays, the sequence analysis of variola virus DNA; the study of biological properties of variola virus strains, to develop animal models; the update on vaccines and a review of candidate antiviral drugs [24].

On May 18, 2007, in its eighth plenary meeting, the Advisory Committee decided to include an item with the title "Smallpox eradication: destruction of variola virus stocks" in the Sixty-fourth World Health Assembly [24]. Finally, they made the following recommendations to Director General:

1. To undertake a major review of the results of the research carried out, currently underway, and the plans and requirements for further essential research in 2010
2. To continue the work of the WHO Advisory Committee on Variola Virus Research, and to disseminate its recommendations more widely to the scientific community

3. To review the membership of the WHO Advisory Committee and the representation of advisers and observers at meetings of this Committee, in order to ensure balanced geographical representation
4. To ensure that approved research proposals, research outcomes and the benefits of this research are made available to all Member States
5. To maintain biannual inspections of the two authorized repositories in order to ensure that conditions of storage of the virus and of research conducted in the laboratories meet the highest requirements for biosafety and biosecurity
6. To continually develop the operational framework for WHO's smallpox vaccine reserve
7. Annual reports on progress in the research program, biosafety, biosecurity and related issues to the Health Assembly through the Executive Board and implementation of the recommendations of the WHO Advisory Committee
8. To ensure that any research undertaken does not involve genetic engineering of the variola virus
9. To ensure that the two authorized repositories of live virus, and any other institution that has fragments of variola virus DNA, distribute such DNA only for purposes of research on diagnostics, treatment and vaccines
10. To submit an annual detailed report to the Health Assembly, through the Executive Board, on the research that has been completed, the results, research being undertaken, and research being planned at the two authorized repositories
11. To submit to the Sixty-first World Health Assembly a report on the legal status of the variola virus strains stored at the two repositories with respect to their ownership
12. To submit a report to the Sixty-first World Health Assembly, through the Executive Board, on measures that promote the widest and most equitable access possible to the outcomes of the research, including antiviral agents, vaccines and diagnostic tools in Member States.

As we see, until these matters are solved the stocks of virus will not be destroyed. Its necessary to have antiviral agents for smallpox and live variola virus will be needed to ensure efficacy testing in vitro. Finally, further refinement of the animal model might be needed to make it more suitable for efficacy testing of these agents and vaccines.

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