Smallpox as a Biological Weapon

Medical and Public Health Management

Donald A. Henderson, MD, MPH; Thomas V. Inglesby, MD; John G. Bartlett, MD; Michael S. Ascher, MD; Edward Eitzen, MD, MPH; Peter B. Jahrling, PhD; Jerome Hauer, MPH; Marcelle Layton, MD; Joseph McDade, PhD; Michael T. Osterholm, PhD, MPH; Tara O'Toole, MD, MPH; Gerald Parker, PhD, DVM; Trish Perl, MD, MSc; Philip K. Russell, MD; Kevin Tonat, PhD; for the Working Group on Civilian Biodefense

JAMA - Vol. 281 No. 22, June 9, 1999

Objective To develop consensus-based recommendations for measures to be taken by medical and public health professionals following the use of smallpox as a biological weapon against a civilian population.

Participants The working group included 21 representatives from staff of major medical centers and research, government, military, public health, and emergency management institutions and agencies.

Evidence The first author (D.A.H.) conducted a literature search in conjunction with the preparation of another publication on smallpox as well as this article. The literature identified was reviewed and opinions were sought from experts in the diagnosis and management of smallpox, including members of the working group.

Consensus Process The first draft of the consensus statement was a synthesis of information obtained in the evidence-gathering process. Members of the working group provided formal written comments that were incorporated into the second draft of the statement. The working group reviewed the second draft on October 30, 1998. No significant disagreements existed and comments were incorporated into a third draft. The fourth and final statement incorporates all relevant evidence obtained by the literature search in conjunction with final consensus recommendations supported by all working group members.

Conclusions Specific recommendations are made regarding smallpox vaccination, therapy, postexposure isolation and infection control, hospital epidemiology and infection control, home care, decontamination of the environment, and additional research needs. In the event of an actual release of smallpox and subsequent epidemic, early detection, isolation of infected individuals, surveillance of contacts, and a focused selective vaccination program will be the essential items of an effective control program.

JAMA. 1999;281:2127-2137

This is the second article in a series entitled *Medical and Public Health Management Following the Use of a Biological Weapon: Consensus Statements of the Working Group on Civilian Biodefense*.[1] The working group has identified a limited number of widely known organisms that could cause disease and deaths in sufficient numbers to cripple a city or region. Smallpox is one of the most serious of these diseases.

If used as a biological weapon, smallpox represents a serious threat to civilian populations because of its case-fatality rate of 30% or more among unvaccinated persons and the absence of specific therapy. Although smallpox has long been feared as the most devastating of all infectious diseases,[2] its potential for devastation today is far greater than at any previous time. Routine vaccination throughout the United States ceased more than 25 years ago. In a now highly susceptible, mobile population, smallpox would be able to spread widely and rapidly throughout this country and the world.

CONSENSUS METHODS

Members of the working group were selected by the chairman in consultation with principal agency heads in the Department of Health and Human Services (DHHS) and the US Army Medical Research Institute of Infectious Diseases (USAMRIID).

The first author (D.A.H.) conducted a literature search in conjunction with the preparation of another publication on smallpox[2] as well as this article. The literature was reviewed and opinions were sought from experts in the diagnosis and management of smallpox, including members of the working group.

The first draft of the working group's consensus statement was the result of synthesis of information obtained in the evidence-gathering process. Members of the working group were asked to make written comments on the first draft of the document in September 1998. Suggested revisions were incorporated into the second draft of the statement. The working group was convened to review the second draft of the statement on October 30, 1998. Consensus recommendations were made and no significant disagreements existed at the conclusion of this meeting. The third draft incorporated changes suggested at the conference and working group members had an additional opportunity to suggest final revisions. The final statement incorporates all relevant evidence obtained by the literature search in conjunction with final consensus recommendations supported by all working group members.

This article is intended to provide the scientific foundation and initial framework for the detailed planning that would follow a bioterrorist attack with smallpox. This planning must encompass coordinated systems approaches to bioterrorism, including public policies and consequence management by local and regional public and private institutions. The assessment and recommendations provided herein represent the best professional judgment of the working group at this time based on data and expertise currently available. The conclusions and recommendations need to be regularly reassessed as new information becomes available.

HISTORY AND POTENTIAL AS A BIOWEAPON

Smallpox probably was first used as a biological weapon during the French and Indian Wars (1754-1767) by British forces in North America.[3] Soldiers distributed blankets that had been used by smallpox patients with the intent of initiating outbreaks among American Indians. Epidemics occurred, killing more than 50% of many affected tribes. With Edward Jenner's demonstration in 1796 that an infection caused by cowpox protected against smallpox and the rapid diffusion worldwide of the practice of cowpox inoculation (ie, vaccination),[4] the potential threat of smallpox as a bioweapon was greatly diminished.

A global campaign, begun in 1967 under the aegis of the World Health Organization (WHO), succeeded in eradicating smallpox in 1977.[1] In 1980, the World Health Assembly recommended that all countries cease vaccination.[5] A WHO expert committee recommended that all laboratories destroy their stocks of variola virus or transfer them to 1 of 2 WHO reference laboratories—the Institute of Virus Preparations in Moscow, Russia, or the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. All countries reported compliance. The WHO committee later recommended that all virus stocks be destroyed in June 1999, and the 1996 World Health Assembly concurred.[6] In 1998, possible research uses for variola virus were reviewed by a committee of the Institute of Medicine (IOM).[7] The IOM committee concluded, as did the preceding WHO committee, that there were research questions

that might be addressed if the virus were to be retained. However, the IOM committee did not explore the costs or relative priority to be assigned to such an effort, and that committee was not asked to weigh the possible benefits resulting from such research activities contrasted with the possible benefits resulting from an international decision to destroy all virus stocks. These considerations will be weighed and decided by the 1999 World Health Assembly.

Recent allegations from Ken Alibek, a former deputy director of the Soviet Union's civilian bioweapons program, have heightened concern that smallpox might be used as a bioweapon. Alibek[8] reported that beginning in 1980, the Soviet government embarked on a successful program to produce the smallpox virus in large quantities and adapt it for use in bombs and intercontinental ballistic missiles; the program had an industrial capacity capable of producing many tons of smallpox virus annually. Furthermore, Alibek reports that Russia even now has a research program that seeks to produce more virulent and contagious recombinant strains. Because financial support for laboratories in Russia has sharply declined in recent years, there are increasing concerns that existing expertise and equipment might fall into non-Russian hands.

The deliberate reintroduction of smallpox as an epidemic disease would be an international crime of unprecedented proportions, but it is now regarded as a possibility. An aerosol release of variola virus would disseminate widely, given the considerable stability of the orthopoxviruses in aerosol form[9] and the likelihood that the infectious dose is very small.[10] Moreover, during the 1960s and 1970s in Europe, when smallpox was imported during the December to April period of high transmission, as many as 10 to 20 second-generation cases were often infected from a single case. Widespread concern and, sometimes, panic occurred, even with outbreaks of fewer than 100 cases, resulting in extensive emergency control measures.[2]

EPIDEMIOLOGY

Smallpox was once worldwide in scope, and before vaccination was practiced, almost everyone eventually contracted the disease. There were 2 principal forms of the disease, variola major and a much milder form, variola minor (or alastrim). Before eradication took place, these forms could be differentiated clinically only when occurring in outbreaks; virological differentiation is now possible.[11, 12] Through the end of the 19th century, variola major predominated throughout the world. However, at the turn of the century, variola minor was first detected in South Africa and later in Florida, from whence it spread across the United States and into Latin America and Europe.[13] Typical variola major epidemics such as those that occurred in Asia resulted in case-fatality rates of 30% or higher among the unvaccinated, whereas variola minor case-fatality rates were customarily 1% or less.[2]

Smallpox spreads from person to person,[10, 14] primarily by droplet nuclei or aerosols expelled from the oropharynx of infected persons and by direct contact. Contaminated clothing or bed linens can also spread the virus.[15] There are no known animal or insect reservoirs or vectors.

Historically, the rapidity of smallpox transmission throughout the population was generally slower than for such diseases as measles or chickenpox. Patients spread smallpox primarily to household members and friends; large outbreaks in schools, for example, were uncommon. This finding was accounted for in part by the fact that transmission of smallpox virus did not occur until onset of rash. By then, many patients had been confined to bed because of the high fever and malaise of the prodromal illness. Secondary cases were thus usually restricted to those who came into contact with patients, usually in the household or hospital.

The seasonal occurrence of smallpox was similar to that of chickenpox and measles—its incidence was highest during winter and early spring.[16] This pattern was consonant with the observation that the duration of survival of orthopoxviruses in the aerosolized form was inversely proportional to both

temperature and humidity.[9] Likewise, when imported cases occurred in Europe, large outbreaks sometimes developed during the winter months, rarely during the summer.[17]

The patient was most infectious from onset of rash through the first 7 to 10 days of rash (Figure 1).[17, 18] As scabs formed, infectivity waned rapidly. Although the scabs contained large amounts of viable virus, epidemiological and laboratory studies indicate that they were not especially infectious, presumably because the virions were bound tightly in the fibrin matrix.[19]

The age distribution of cases depended primarily on the degree of smallpox susceptibility in the population. In most areas, cases predominated among children because adults were protected by immunity induced by vaccination or previous smallpox infection. In rural areas that had seen little vaccination or smallpox, the age distribution of cases was similar to the age distribution of the population. The age distribution pattern of cases in the United States presumably would be such if smallpox were to occur now because vaccination immunity in the population has waned so substantially.

MICROBIOLOGY

Smallpox, a DNA virus, is a member of the genus orthopoxvirus.[20] The orthopoxviruses are among the largest and most complex of all viruses. The virion is characteristically a brick-shaped structure with a diameter of about 200 nm. Three other members of this genus (monkeypox, vaccinia, and cowpox) can also infect humans, causing cutaneous lesions, but only smallpox is readily transmitted from person to person.[2] Monkeypox, a zoonotic disease, presently is found only in tropical rain forest areas of central and western Africa and is not readily transmitted among humans.[21] Vaccinia and cowpox seldom spread from person to person.

PATHOGENESIS AND CLINICAL PRESENTATION

Natural infection occurs following implantation of the virus on the oropharyngeal or respiratory mucosa.[2] The infectious dose is unknown but is believed to be only a few virions.[10] After the migration of virus to and multiplication in regional lymph nodes, an asymptomatic viremia develops on about the third or fourth day, followed by multiplication of virus in the spleen, bone marrow, and lymph nodes. A secondary viremia begins on about the eighth day and is followed by fever and toxemia. The virus, contained in leukocytes, then localizes in small blood vessels of the dermis and beneath the oral and pharyngeal mucosa and subsequently infects adjacent cells.

At the end of the 12- to 14-day incubation period (range, 7-17 days), the patient typically experiences high fever, malaise, and prostration with headache and backache.[2] Severe abdominal pain and delirium are sometimes present. A maculopapular rash then appears on the mucosa of the mouth and pharynx, face, and forearms, and spreads to the trunk and legs (Figure 2).[2] Within 1 to 2 days, the rash becomes vesicular and, later, pustular. The pustules are characteristically round, tense, and deeply embedded in the dermis; crusts begin to form on about the eighth or ninth day of rash. As the patient recovers, the scabs separate and characteristic pitted scarring gradually develops. The scars are most evident on the face and result from the destruction of sebaceous glands followed by shrinking of granulation tissue and fibrosis.[2]

The lesions that first appear in the mouth and pharynx ulcerate quickly because of the absence of a stratum corneum, releasing large amounts of virus into the saliva.[22] Virus titers in saliva are highest during the first week of illness, corresponding with the period during which patients are most infectious. Although the virus in some instances can be detected in swabs taken from the oropharynx as many as 5 to 6 days before the rash develops,[22] transmission does not occur during this period.

Except for the lesions in the skin and mucous membranes and reticulum cell hyperplasia, other organs are seldom involved. Secondary bacterial infection is not common, and death, which usually occurs during the second week of illness, most likely results from the toxemia associated with circulating immune complexes and soluble variola antigens.[2] Encephalitis sometimes ensues that is indistinguishable from the acute perivascular demyelination observed as a complication of infection due to vaccinia, measles, or varicella.[23]

Neutralizing antibodies can be detected by the sixth day of rash and remain at high titers for many years.[24] Hemagglutinin-inhibiting antibodies can be detected on about the sixth day of rash, or about 21 days after infection, and complement-fixing antibodies appear approximately 2 days later. Within 5 years, hemagglutinin-inhibiting antibodies decline to low levels and complement-fixing antibodies rarely persist for longer than 6 months.[2]

Although at least 90% of smallpox cases are clinically characteristic and readily diagnosed in endemic areas, 2 other forms of smallpox are difficult to recognize—hemorrhagic and malignant. Hemorrhagic cases are uniformly fatal and occur among all ages and in both sexes, but pregnant women appear to be unusually susceptible. Illness usually begins with a somewhat shorter incubation period and is characterized by a severely prostrating prodromal illness with high fever and head, back, and abdominal pain. Soon thereafter, a dusky erythema develops, followed by petechiae and frank hemorrhages into the skin and mucous membranes. Death usually occurs by the fifth or sixth day after onset of rash.[23]

In the frequently fatal malignant form, the abrupt onset and prostrating constitutional symptoms are similar. The confluent lesions develop slowly, never progressing to the pustular stage but remaining soft, flattened, and velvety to the touch. The skin has the appearance of a fine-grained, reddish-colored crepe rubber, sometimes with hemorrhages. If the patient survives, the lesions gradually disappear without forming scabs or, in severe cases, large amounts of epidermis might peel away.[23]

The illness associated with variola minor is generally less severe, with fewer constitutional symptoms and a more sparse rash.[25] A milder form of disease is also seen among those who have residual immunity from previous vaccination. In partially immune persons, the rash tends to be atypical and more scant and the evolution of the lesions more rapid.[15]

There is little information about how individuals with different types of immune deficiency responded to natural smallpox infection. Smallpox was eradicated before human immunodeficiency virus (HIV) was identified and before suitable techniques became available for measuring cell-mediated immunity. However, it is probable that the underlying cause of some cases of malignant and hemorrhagic smallpox resulted from defective immune responses. Vaccination of immune-deficient persons sometimes resulted in a continually spreading primary lesion, persistent viremia, and secondary viral infection of many organs. One such case is documented to have occurred in a vaccinated soldier who had HIV infection.[26]

DIAGNOSIS

The discovery of a single suspected case of smallpox must be treated as an international health emergency and be brought immediately to the attention of national officials through local and state health authorities.

The majority of smallpox cases present with a characteristic rash that is centrifugal in distribution, ie, most dense on the face and extremities. The lesions appear during a 1- to 2-day period and evolve at the same rate. On any given part of the body, they are generally at the same stage of development. In varicella (chickenpox), the disease most frequently confused with smallpox, new lesions appear in crops every few days and lesions at very different stages of maturation (ie, vesicles, pustules, and scabs) are found in adjacent areas of skin. Varicella lesions are much more superficial and are almost never found on the palms and soles. The distribution of varicella lesions is centripetal, with a greater concentration of lesions on the trunk than on the face and extremities.

The signs and symptoms of both hemorrhagic and malignant smallpox were such that smallpox was seldom suspected until more typical cases were seen and it was recognized that a smallpox outbreak was in progress. Hemorrhagic cases were most often initially identified as meningococcemia or severe acute leukemia. Malignant cases likewise posed diagnostic problems, most often being mistaken for hemorrhagic chickenpox or prompting surgery because of severe abdominal pain.

Laboratory confirmation of the diagnosis in a smallpox outbreak is important. Specimens should be collected by someone who has recently been vaccinated (or is vaccinated that day) and who wears gloves and a mask. To obtain vesicular or pustular fluid, it is often necessary to open lesions with the blunt edge of a scalpel. The fluid can then be harvested on a cotton swab. Scabs can be picked off with forceps. Specimens should be deposited in a vacutainer tube that should be sealed with adhesive tape at the juncture of stopper and tube. This tube, in turn, should be enclosed in a second durable, watertight container. State or local health department laboratories should immediately be contacted regarding the shipping of specimens. Laboratory examination requires high-containment (BL-4) facilities and should be undertaken only in designated laboratories with the appropriate training and equipment. Once it is established that the epidemic is caused by smallpox virus, clinically typical cases would not require further laboratory confirmation.

Smallpox infection can be rapidly confirmed in the laboratory by electron microscopic examination of vesicular or pustular fluid or scabs. Although all orthopoxviruses exhibit identically appearing brick-shaped virions, history taking and clinical picture readily identify cowpox and vaccinia. Although smallpox and monkeypox virions may be indistinguishable, naturally occurring monkeypox is found only in tropical rain forest areas of Africa. Definitive laboratory identification and characterization of the virus involves growth of the virus in cell culture or on chorioallantoic egg membrane and characterization of strains by use of various biologic assays, including polymerase chain reaction techniques and restriction fragment-length polymorphisms.[27-29] The latter studies can be completed within a few hours.

PREEXPOSURE PREVENTIVE VACCINATION

Before 1972, smallpox vaccination was recommended for all US children at age 1 year. Most states required that each child be vaccinated before school entry. The only other requirement for vaccination was for military recruits and tourists visiting foreign countries. Most countries required that the individual be successfully vaccinated within a 3-year period prior to entering the country. Routine vaccination in the United States stopped in 1972 and since then, few persons younger than 27 years have been vaccinated. The US Census Bureau reported that in 1998, approximately 114 million persons, or 42% of the US population, were aged 29 years or younger.[30]

In addition, the immune status of those who were vaccinated more than 27 years ago is not clear. The duration of immunity, based on the experience of naturally exposed susceptible persons, has never been satisfactorily measured. Neutralizing antibodies are reported to reflect levels of protection, although this has not been validated in the field. These antibodies have been shown to decline substantially during a 5- to 10-year period.[24] Thus, even those who received the recommended single-dose vaccination as children do not have lifelong immunity. However, among a group who had been vaccinated at birth and at ages 8 and 18 years as part of a study, neutralizing antibody levels remained stable during a 30-year period.[31] Because comparatively few persons today have been successfully vaccinated on more than 1 occasion, it must be assumed that the population at large is highly susceptible to infection.

In the United States, a limited reserve supply of vaccine that was produced by Wyeth Laboratories, Lancaster, Pa, in the 1970s is in storage. This supply is believed to be sufficient to vaccinate between 6 and 7 million persons. This vaccine, now under the control of the CDC, consists of vaccine virus (New York Board of Health strain) grown on scarified calves. After purification, it was freeze-dried in rubber-stoppered vials that contain sufficient vaccine for at least 50 doses when a bifurcated needle is used. It is stored at -20°C (James LeDuc, PhD, oral communication, 1998). Although quantities of vaccine have also been retained by a number of other countries, none have reserves large enough to meet more than their own potential emergency needs. WHO has 500,000 doses.[32]

There are no manufacturers now equipped to produce smallpox vaccine in large quantities. The development and licensure of a tissue cell culture vaccine and the establishment of a new vaccine production facility is estimated to require at least 36 months (Thomas Monath, MD, unpublished data, 1999).

Because of the small amounts of vaccine available, a preventive vaccination program to protect individuals such as emergency and health care personnel is not an option at this time. When additional supplies of vaccine are procured, a decision to undertake preventive vaccination of some portion of the population will have to weigh the relative risk of vaccination complications against the threat of contracting smallpox.

A further deterrent to extensive vaccination is the fact that presently available supplies of vaccinia immune globulin (VIG), also maintained by the CDC, are very limited in quantity. The working group recommends VIG for the treatment of severe cutaneous reactions occurring as a complication of vaccination.[33, 34] Vaccinia immune globulin has also been given along with vaccination to protect those who needed vaccination but who were at risk of experiencing vaccine-related complications.[33] It has been estimated that if 1 million persons were vaccinated, as many as 250 persons would experience adverse reactions of a type that would require administration of VIG (James LeDuc, PhD, oral communication, 1998). How much VIG would be needed to administer with vaccine to those at risk is unknown.

POSTEXPOSURE THERAPY

At this time, the best that can be offered to the patient infected with smallpox is supportive therapy plus antibiotics as indicated for treatment of occasional secondary bacterial infections. No antiviral substances have yet proved effective for the treatment of smallpox, and the working group is not aware of any reports that suggest any antiviral product is therapeutic. Encouraging initial reports in the 1960s describing the therapeutic benefits of the thiosemicarbazones, cytosine arabinoside, and adenine arabinoside proved questionable on further study.[21, 35, 36]

Recent studies on tissue culture, mice, and a small number of monkeys have suggested the possibility that cidofovir, a nucleoside analog DNA polymerase inhibitor, might prove useful in preventing

smallpox infection if administered within 1 or 2 days after exposure (John Huggins, PhD, oral communication, 1998). At this time, there is no evidence that cidofovir is more effective than vaccination in this early period. Moreover, the potential utility of this drug is limited, given the fact that it must be administered intravenously and its use is often accompanied by serious renal toxicity.[37]

POSTEXPOSURE INFECTION CONTROL

A smallpox outbreak poses difficult public health problems because of the ability of the virus to continue to spread throughout the population unless checked by vaccination and/or isolation of patients and their close contacts.

A clandestine aerosol release of smallpox, even if it infected only 50 to 100 persons to produce the first generation of cases, would rapidly spread in a now highly susceptible population, expanding by a factor of 10 to 20 times or more with each generation of cases.[2, 10, 38] Between the time of an aerosol release of smallpox virus and diagnosis of the first cases, an interval as long as 2 weeks or more is apt to occur because of the average incubation period of 12 to 14 days and the lapse of several additional days before a rash was sufficiently distinct to suggest the diagnosis of smallpox. By that time, there would be no risk of further environmental exposure from the original aerosol release because the virus is fully inactivated within 2 days.

As soon as the diagnosis of smallpox is made, all individuals in whom smallpox is suspected should be isolated immediately and all household and other face-to-face contacts should be vaccinated and placed under surveillance. Because the widespread dissemination of smallpox virus by aerosol poses a serious threat in hospitals, patients should be isolated in the home or other nonhospital facility whenever possible. Home care for most patients is a reasonable approach, given the fact that little can be done for a patient other than to offer supportive therapy.

In the event of an aerosol release of smallpox and a subsequent outbreak, the rationale for vaccinating patients suspected to have smallpox at this time is to ensure that some with a mistaken diagnosis are not placed at risk of acquiring smallpox. Vaccination administered within the first few days after exposure and perhaps as late as 4 days may prevent or significantly ameliorate subsequent illness.[39] An emergency vaccination program is also indicated that would include all health care workers at clinics or hospitals that might receive patients; all other essential disaster response personnel, such as police, firefighters, transit workers, public health staff, and emergency management staff; and mortuary staff who might have to handle bodies. The working group recommends that all such personnel for whom vaccination is not contraindicated should be vaccinated immediately irrespective of prior vaccination status.

Vaccination administered within 4 days of first exposure has been shown to offer some protection against acquiring infection and significant protection against a fatal outcome.[15] Those who have been vaccinated at some time in the past will normally exhibit an accelerated immune response. Thus, it would be prudent, when possible, to assign those who had been previously vaccinated to duties involving close patient contact.

It is important that discretion be used in identifying contacts of patients to ensure, to the extent that is possible, that vaccination and adequate surveillance measures are focused on those at greatest risk. Specifically, it is recommended that *contacts* be defined as persons who have been in the same household as the infected individual or who have been in face-to-face contact with the patient after the onset of fever. Experience during the smallpox global eradication program showed that patients did not transmit infection until after the prodromal fever had given way to the rash stage of illness.[17, 18]

Isolation of all contacts of exposed patients would be logistically difficult and, in practice, should not be necessary. Because contacts, even if infected, are not contagious until onset of rash, a practical strategy calls for all contacts to have temperatures checked at least once each day, preferably in the evening. Any increase in temperature higher than 38°C (101°F) during the 17-day period following last exposure to the case would suggest the possible development of smallpox[2] and be cause for isolating the patient immediately, preferably at home, until it could be determined clinically and/or by laboratory examination whether the contact had smallpox. All close contacts of the patients should be promptly vaccinated.

Although cooperation by most patients and contacts in observing isolation could be ensured through counseling and persuasion, there may be some for whom forcible quarantine will be required. Some states and cities in the United States, but not all, confer broad discretionary powers on health authorities to ensure the safety of the public's health and, at one time, this included powers to quarantine. Under epidemic circumstances, this could be an important power to have. Thus, each state and city should review its statutes as part of its preparedness activities.

During the smallpox epidemics in the 1960s and 1970s in Europe, there was considerable public alarm whenever outbreaks occurred and, often, a demand for mass vaccination throughout a very widespread area, even when the vaccination coverage of the population was high.[2] In the United States, where few people now have protective levels of immunity, such levels of concern must be anticipated. However, the US vaccine supply is limited at present; thus, vaccine would have to be carefully conserved and used in conjunction with measures to implement rapid isolation of smallpox patients.

HOSPITAL EPIDEMIOLOGY AND INFECTION CONTROL

Smallpox transmission within hospitals has long been recognized as a serious problem. For this reason, separate hospitals for smallpox patients were used for more than 200 years. Throughout the 1970s, both England and Germany had fully equipped standby hospitals in case smallpox should be imported.[2] Infections acquired in hospitals may occur as the result of droplets spread from patients to staff and visitors in reasonably close contact or by a fine particle aerosol. In 1 such occurrence in Germany, a smallpox patient with a cough, although isolated in a single room, infected persons on 3 floors of a hospital.[10] Persons with the usually fatal hemorrhagic or malignant forms of the disease pose a special problem because they often remain undiagnosed until they are near death and extremely contagious. A number of outbreaks have occurred in laundry workers who handled linens and blankets used by patients.[15] The working group recommends that in an outbreak setting, all hospital employees as well as patients in the hospital be vaccinated. For individuals who are immunocompromised or for whom vaccination is otherwise contraindicated, VIG should be provided, if available. If it is not available, a judgment will have to be made regarding the relative risks of acquiring the disease in contrast with the risks associated with vaccination.

In the event of a limited outbreak with few cases, patients should be admitted to the hospital and confined to rooms that are under negative pressure and equipped with high-efficiency particulate air filtration. In larger outbreaks, home isolation and care should be the objective for most patients. However, not all will be able to be so accommodated and, to limit nosocomial infections, authorities should consider the possibility of designating a specific hospital or hospitals for smallpox care. All persons isolated as such and those caring for them should be immediately vaccinated. Employees for whom vaccination is contraindicated should be furloughed.

Standard precautions using gloves, gowns, and masks should be observed. All laundry and waste should be placed in biohazard bags and autoclaved before being laundered or incinerated. A special

protocol should be developed for decontaminating rooms after they are vacated by patients (see "Decontamination" section).

Laboratory examination requires high-containment (BL-4) facilities and should be undertaken only in designated laboratories with the appropriate trained personnel and equipment. Specific recommendations for safe specimen transport are described in the section on "Differential Diagnosis and Diagnostic Tests."

Protecting against the explosive spread of virus from the hemorrhagic or malignant case is difficult. Such cases occurring during the course of an outbreak may be detected if staff is alert to the possibility that any severe, acute, prostrating illness must be considered smallpox until proven otherwise.

Patients who die of smallpox should be cremated whenever possible and mortuary workers should be vaccinated.

VACCINE ADMINISTRATION AND COMPLICATIONS

Smallpox vaccine is currently approved by the US Food and Drug Administration (FDA) for use only in persons in special-risk categories, including laboratory workers directly involved with smallpox or closely related orthopoxviruses. Under epidemic circumstances, widespread vaccination would be indicated, as recommended by the working group.

Vaccination has been successfully and safely administered to persons of all ages, from birth onward.[40] However, there are certain groups for whom elective vaccination has not been recommended because of the risk of complications. Under epidemic circumstances, however, such contraindications will have to be weighed against the grave risks posed by smallpox. If available, VIG can be administered concomitantly with vaccination to minimize the risk of complications in these persons.

Vaccination is normally performed using the bifurcated needle (Figure 3). A sterile needle is inserted into an ampoule of reconstituted vaccine and, on withdrawal, a droplet of vaccine sufficient for vaccination is held by capillarity between the 2 tines. The needle is held at right angles to the skin; the wrist of the vaccinator rests against the arm. Fifteen perpendicular strokes of the needle are rapidly made in an area of about 5 mm in diameter.[41, 42] The strokes should be sufficiently vigorous so that a trace of blood appears at the vaccination site after 15 to 30 seconds. After vaccination, excess vaccine should be wiped from the site with gauze that should be discarded in a hazardous waste receptacle. The site should be covered with a loose, nonocclusive bandage to deter the individual from touching the site and perhaps transferring virus to other parts of the body.

After about 3 days, a red papule appears at the vaccination site and becomes vesicular on about the fifth day (Figure 4). By the seventh day, it becomes the typical Jennerian pustule—whitish, umbilicated, multilocular, containing turbid lymph and surrounded by an erythematous areola that may continue to expand for 3 more days. Regional lymphadenopathy and fever is not uncommon. As many as 70% of children have 1 or more days of temperature higher than 39°C (100°F) between days 4 and 14.[43] The pustule gradually dries, leaving a dark crust, which normally falls off after about 3 weeks.

A successful vaccination for those with partial immunity may manifest a gradient of responses. These range from what appears to be a primary take (as described herein) to an accelerated reaction in which there may be little more than a papule surrounded by erythema that reaches a peak between 3 and 7 days. A response that reaches a peak in erythema within 48 hours represents a hypersensitivity

reaction and does not signify that growth of the vaccinia virus has occurred.[2] Persons exhibiting such a reaction should be revaccinated.

Complications

The frequency of complications associated with use of the New York Board of Health strain (the strain used throughout the United States and Canada for vaccine) is the lowest for any established vaccinia virus strain, but the risks are not inconsequential.[44, 45] Data on complications gathered by the CDC in 1968 are shown in Table 1. Complications occurred most frequently among primary vaccinees.

Postvaccinial Encephalitis.
Postvaccinial encephalitis occurred at a rate of 1 case per 300,000 vaccinations and was observed only in primary vaccinees; one fourth of these cases were fatal and several had permanent neurological residua. Between 8 and 15 days after vaccination, encephalitic symptoms developed—fever, headache, vomiting, drowsiness, and, sometimes, spastic paralysis, meningitic signs, coma, and convulsions. Cerebrospinal fluid usually showed a pleocytosis. Recovery was either complete or associated with residual paralysis and other central nervous system symptoms and, sometimes, death. There was no treatment.

Progressive Vaccinia (Vaccinia Gangrenosa).
Cases of progressive vaccinia occurred both among primary vaccinees and revaccinees. It was a frequently fatal complication among those with immune deficiency disorders. The vaccinial lesion failed to heal and progressed to involve adjacent skin with necrosis of tissue, spreading to other parts of the skin, to bones, and to viscera. Vaccinia immune globulin was used for this problem.[34, 46] One case in a soldier with acquired immunodeficiency syndrome was successfully treated with VIG and ribavirin. These treatment strategies were off-label and would be considered experimental.[26]

Eczema Vaccinatum.
A sometimes serious complication, eczema vaccinatum occurred in some vaccinees and contacts with either active or healed eczema. Vaccinial skin lesions extended to cover all or most of the area once or currently afflicted with eczema. Vaccinia immune globulin was therapeutic.[46]

Generalized Vaccinia.
A secondary eruption almost always following primary vaccination, generalized vaccinia resulted from blood-borne dissemination of virus. Lesions emerged between 6 and 9 days after vaccination and were either few in number or generalized. This complication was usually self-limited. In severe cases, VIG was indicated.[46]

Inadvertent Inoculation.
Transmission to close contacts or autoinoculation to sites such as face, eyelid, mouth, and genitalia sometimes occurred. Most lesions healed without incident, although VIG was useful in some cases of periocular implantation.

Miscellaneous.
Many different rashes have been associated with vaccination. Most common are erythema multiforme and variously distributed urticarial, maculopapular, and blotchy erythematous eruptions, which normally clear without therapy.

Groups at Special Risk for Complications

Consensus recommendations for special-risk groups as set forth herein reflect the best clinical and science-based judgment of the working group and do not necessarily correspond to FDA-approved uses.

Five groups of persons are ordinarily considered at special risk of smallpox vaccine complications: (1) persons with eczema or other significant exfoliative skin conditions; (2) patients with leukemia, lymphoma, or generalized malignancy who are receiving therapy with alkylating agents, antimetabolites, radiation, or large doses of corticosteroids; (3) patients with HIV infection; (4) persons

with hereditary immune deficiency disorders; and (5) pregnant women. If persons with contraindications have been in close contact with a smallpox patient or the individual is at risk for occupational reasons, VIG, if available, may be given simultaneously with vaccination in a dose of 0.3 mL/kg of body weight to prevent complications. This does not alter vaccine efficacy. If VIG is not available, vaccine administration may still be warranted, given the far higher risk of an adverse outcome from smallpox infection than from vaccination.

VIG Therapy for Complications

Vaccinia immune globulin is valuable in treating patients with progressive vaccinia, eczema vaccinatum, severe generalized vaccinia, and periocular infections resulting from inadvertent inoculation. It is administered intramuscularly in a dose of 0.6 mL/kg of body weight. Because the dose is large (eg, 42 mL for a person weighing 70 kg), the product is given intramuscularly in divided doses over a 24- to 36-hour period and may be repeated, if necessary, after 2 to 3 days if improvement is not occurring.[47] Because the availability of VIG is so limited, its use should be reserved for the most serious cases. Vaccinia immune globulin, as well as vaccinia vaccine, is made available by the CDC through state health departments. Consultative assistance in the diagnosis and management of patients with complications can be obtained through state health departments.

DECONTAMINATION

Vaccinia virus, if released as an aerosol and not exposed to UV light, may persist for as long as 24 hours or somewhat longer under favorable conditions.[9] It is believed that variola virus would exhibit similar properties. However, by the time patients had become ill and it had been determined that an aerosol release of smallpox virus had occurred, there would be no viable smallpox virus in the environment. Vaccinia virus, if released as an aerosol, is almost completely destroyed within 6 hours in an atmosphere of high temperature (31°C-33°C) and humidity (80%) (Table 2).[9] In cooler temperatures (10°C-11°C) and lower humidity (20%), nearly two thirds of a vaccinia aerosol survives for as long as 24 hours.[9] It is believed that variola would behave similarly.

The occurrence of smallpox infection among personnel who handled laundry from infected patients is well documented[15] and it is believed that virus in such material remains viable for extended periods. Thus, special precautions need to be taken to ensure that all bedding and clothing of smallpox patients is autoclaved or laundered in hot water to which bleach has been added. Disinfectants that are used for standard hospital infection control, such as hypochlorite and quaternary ammonia, are effective for cleaning surfaces possibly contaminated with virus.

Virus in scabs is more durable. At a temperature of 35°C and 65% relative humidity, the virus has persisted for 3 weeks.[48] At cooler temperatures (26°C), the virus has survived for 8 weeks at high relative humidity and 12 weeks at a relative humidity less than 10%.[48] Dutch investigators demonstrated that it was possible to isolate variola virus from scabs that had been sitting on a shelf for 13 years.[49] It is unlikely, however, that the smallpox virus, bound in the fibrin matrix of a scab, is infectious in humans. This is borne out by studies conducted during the eradication program and by surveillance for cases in newly smallpox-free areas.[2] It was reasoned that if the virus were able to persist in nature and infect humans, there would be cases occurring for which no source could be identified. Cases of this type were not observed. Rather, when cases were found, there were antecedent human cases with whom they had direct contact.

RESEARCH

Priority should be directed to 3 areas of smallpox research: vaccines, immunotherapy and drugs, and diagnostics.

The working group recommends that an emergency stockpile of at least 40 million doses of vaccine and a standby manufacturing capacity to produce more is a critical need. At a minimum, this quantity of vaccine would be needed in the control of an epidemic during the first 4 to 8 weeks after an attack. Smallpox vaccine, contained in glass-sealed ampoules and stored at -20°C, retains its potency almost indefinitely. However, several steps are necessary before manufacturing can begin. The traditional method for producing vaccine on the scarified flank of a calf is no longer acceptable because the product inevitably contains some microbial contaminants, however stringent the purification measures. Contemporary vaccines require the use of tissue cell cultures. Thus, as a first step, the traditional New York Board of Health strain needs to be grown in a suitable tissue cell culture and comparative studies performed of the reactogenicity and immunogenicity of calf-derived and tissue cell culture vaccines. This should be a comparatively straightforward exercise. The cost of such a stockpile should be comparatively modest because the vaccine would be packaged in 50-dose rather than costly single-dose containers. In the mid-1970s, 40 million doses would have cost less than $5 million (D.A.H., unpublished data, 1975).

The frequency of vaccine complications is sufficiently great to recommend development, if possible, of a more attenuated strain that, hopefully, would retain full efficacy. Development of an entirely new, genetically engineered strain would be both costly and time consuming. Moreover, it would be difficult at this time to justify its use in large numbers of human subjects to evaluate safety. There is, however, a candidate attenuated strain that was developed and field tested in Japan in the mid-1970s (a Lister strain–derived vaccine[50] that has been produced in volume in rabbit kidney cell culture and has been given to more than 100,000 persons in Japan). Research showed no severe complications among the first 30,000 vaccinees.[51] The cutaneous responses to vaccination were much less severe and far fewer vaccinees developed fever. More than 95% developed a Jennerian pustule; immunogenicity, as measured by neutralizing antibody, was slightly lower than for nonattenuated strains.

Vaccinia immune globulin has been used for the treatment of vaccine complications and for administration with vaccine to those for whom vaccine is otherwise contraindicated. Production of VIG should be a high priority for research. An alternative to VIG is also needed because VIG is difficult to produce and cumbersome to administer. Immunotherapy using humanized monoclonal antibodies is an alternative that should be explored. Studies of antiviral agents or drugs, already approved or near approval for marketing for use in other viral diseases, have suggested that 1 or more such products might prove useful.

Finally, a simple, rapid diagnostic test to identify variola virus in the oropharynx during the prodrome or early in the exanthematous phase of illness would be of considerable help in triage of suspected patients during the course of an outbreak.

SUMMARY

The specter of resurgent smallpox is ominous, especially given the enormous efforts that have been made to eradicate what has been characterized as the most devastating of all the pestilential

diseases. Unfortunately, the threat of an aerosol release of smallpox is real and the potential for a catastrophic scenario is great unless effective control measures can quickly be brought to bear.

Early detection, isolation of infected individuals, surveillance of contacts, and a focused selective vaccination program are the essential items of a control program. Educating health care professionals about the diagnostic features of smallpox should permit early detection; advance regionwide planning for isolation and care of infected individuals in their homes as appropriate and in hospitals when home care is not an option will be critical to deter spread. Ultimately, success in controlling a burgeoning epidemic will depend on the availability of adequate supplies of vaccine and VIG. An adequate stockpile of those commodities would offer a relatively inexpensive safeguard against tragedy.

Author/Article Information

Author Affiliations: The Center for Civilian Biodefense Studies (Drs Henderson, Inglesby, Bartlett, O'Toole, Perl, and Russell), and the Schools of Public Health (Drs Henderson, O'Toole, and Russell) and Medicine (Drs Inglesby, Bartlett, and Perl), Johns Hopkins University, Baltimore, Md; Viral and Rickettsial Diseases, California Department of Health, Berkeley (Dr Ascher); US Army Medical Research Institute of Infectious Diseases, Frederick, Md (Drs Eitzen, Jahrling, and Parker); Office of Emergency Management (Mr Hauer) and Office of Communicable Disease, New York City Health Department (Dr Layton), New York, NY; Centers for Disease Control and Prevention, Atlanta, Ga (Dr McDade); Acute Disease Epidemiology, Minnesota Department of Health, Minneapolis (Dr Osterholm); and Office of Emergency Preparedness, Department of Health and Human Services, Rockville, Md (Dr Tonat).

Corresponding Author and Reprints: Donald A. Henderson, MD, MPH, Johns Hopkins Center for Civilian Biodefense Studies, Johns Hopkins University, Candler Bldg, Suite 850, 111 Market Pl, Baltimore, MD 21202 (e-mail: dahzero@aol.com).

Ex Officio Participants in the Working Group on Civilian Biodefense: George Curlin, MD, National Institutes of Health, Bethesda, Md; Margaret Hamburg, MD, and William Roub, PhD, Office of Assistant Secretary for Planning and Evaluation, DHHS, Washington, DC; Robert Knouss, MD, Office of Emergency Preparedness, DHHS, Rockville, Md; Marcelle Layton, MD, Office of Communicable Disease, New York City Health Department, New York, NY; and Brian Malkin and Stuart Nightingale, MD, FDA, Rockville, Md.

Funding/Support: Funding for this study primarily was provided by each participant's institution or agency. The Johns Hopkins Center for Civilian Biodefense Studies provided travel funds for 3 members of the group.

Disclaimers: In many cases, the indication and dosages and other information are not consistent with current FDA-approved labeling. The recommendations on the use of drugs and vaccine for uses not approved by the FDA do not represent the official views of the FDA or of any of the federal agencies whose scientists participated in these discussions. Unlabeled uses of the products recommended are noted in the sections of this article in which these products are discussed. Where unlabeled uses are indicated, information used as the basis for the recommendations is discussed.

The views, opinions, assertions, and findings contained herein are those of the authors and should not be construed as official US Department of Defense or US Department of Army positions, policies, or decisions unless so designated by other documentation.

Additional Articles: This article is second in a series entitled *Medical and Public Health Management Following the Use of a Biological Weapon: Consensus Statements of the Working Group on Civilian Biodefense*. See reference 1.

Acknowledgment: The working group wishes to thank Isao Arita, MD, Agency for Cooperation in International Health, Kumamoto, Japan; Joel Bremen, MD, DTPH, Fogarty International Center, Bethesda; Joseph Esposito, PhD, and Brian Mahy, PhD, ScD, CDC, Atlanta, Ga; Frank Fenner, MD, Australian National University, Canberra; and Ralph Henderson, MD, WHO, Geneva, Switzerland.

REFERENCES

1.
Inglesby TV, Henderson DA, Bartlett JG, et al.
Anthrax as a biological weapon: medical and public health management.
JAMA.
1999;281:1735-1745.
ABSTRACT | FULL TEXT | PDF | MEDLINE

2.
Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID.
Smallpox and Its Eradication.
Geneva, Switzerland: World Health Organization; 1988:1460.

3.
Stearn EW, Stearn AE.
The Effect of Smallpox on the Destiny of the Amerindian.
Boston, Mass: Bruce Humphries; 1945.

4.
Hopkins DR.
Princes and Peasants.
Chicago, Ill: University of Chicago Press; 1983.

5.
World Health Organization.
The Global Eradication of Smallpox: Final Report of the Global Commission for the Certification of Smallpox Eradication.
Geneva, Switzerland: World Health Organization; 1980.

6.
Breman JG, Henderson DA.
Poxvirus dilemmas: monkeypox, smallpox and biological terrorism.
N Engl J Med.
1998;339:556-559.
MEDLINE

7.
Institute of Medicine.
Assessment of Future Scientific Need for Live Variola Virus.
Washington, DC: National Academy Press; 1999.

8.
Alibek K.
Biohazard.
New York, NY: Random House Inc; 1999.

9.
Harper GJ.
Airborne micro-organisms: survival test with four viruses.
J Hyg.
1961;59:479-486.

10.
Wehrle PF, Posch J, Richter KH, Henderson DA.
An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe.
Bull World Health Organ.
1970;43:669-679.
MEDLINE

11.
Chapin CV, Smith J.
Permanency of the mild type of smallpox.
J Prev Med.
1932;1:1-29.

12.
Esposito JJ, Knight JC.
Orthopox DNA: a comparison of restriction profiles and maps.
Virology.
1985;143:230-251.
MEDLINE

13.
Chapin CV.
Variation in the type of infectious disease as shown by the history of smallpox in the United States, 1895-1912.
J Infect Dis.
1913;13:171-196.

14.
Anders W, Sosch J.
Die Pockenausbrucke 1961/61 in Nordrhein-Westfalen.
Bundesgesundheitsblatt.
1962;17:265-269.

15.
Dixon CW.
Smallpox.
London, England: J & A Churchill Ltd; 1962:1460.

16.
Joarder AK, Tarantola D, Tulloch J.
The Eradication of Smallpox From Bangladesh, New Delhi.
Geneva, Switzerland: WHO Regional Publications; 1980.

17.
Mack TM.
Smallpox in Europe, 1950-71.
J Infect Dis.
1972;125:161-169.
MEDLINE

18.
Mack TM, Thomas DB, Khan MM.

Epidemiology of smallpox in West Pakistan, II: determinants of intravillage spread other than acquired immunity.
Am J Epidemiol.
1972;95:157-168.
MEDLINE

19.
Rao AR.
Infected Inanimate Objects (Fomites) and Their Role in Transmission of Smallpox.
Geneva, Switzerland: World Health Organization; 1972. WHO/SE/72.40.

20.
Fenner F, Wittek R, Dumbell KR.
The Orthopoxviruses.
San Diego, Calif: Academic Press; 1988:432.

21.
Jezek Z, Fenner F.
Human Monkeypox.
Basel, Switzerland: S Karger; 1988.

22.
Sarkar JK, Mitra AC, Mukherjee MK, De SK.
Virus excretion in smallpox, 2: excretion in the throat of household contacts.
Bull World Health Organ.
1973;48:523-527.
MEDLINE

23.
Rao AR.
Smallpox.
Bombay, India: Kothari Book Depot; 1972.

24.
Downie AW, McCarthy K.
The antibody response in man following infection with viruses of the pox group, III: antibody response in smallpox.
J Hyg.
1958;56:479-487.

25.
Marsden JP.
Variola minor: a personal analysis of 13,686 cases.
Bull Hyg.
1948;23:735-746.

26.
Redfield RR, Wright CD, James WD, Jones ST, Brown C, Burke D.
Disseminated vaccinia in a military recruit with human immunodeficiency virus (HIV).
N Engl J Med.
1987;316:673-676.
MEDLINE

27.
Esposito JJ, Massung RF.
Poxvirus infections in humans.
In: Murray PR, Tenover F, Baron EJ, eds. *Clinical Microbiology.*Washington, DC: American Society of Microbiology; 1995:1131-1138.

28.
Knight JC, Massung RF, Esposito JJ.
Polymerase chain reaction identification of smallpox virus.
In: *PCR: Protocols for Diagnosis of Human and Animal Viral Disease*Heidelberg, Germany: Springer-Verlag; 1995:297-302.

29.
Ropp SL, Knight JC, Massung RF, Esposito JJ.
PCR strategy for identification and differentiation of smallpox and other orthopoxviruses.
J Clin Microbiol.
1995;33:2069-2076.
MEDLINE

30.
US Bureau of the Census.
Resident Population of the United States: Estimates, by Age and Sex.
Washington, DC: US Bureau of the Census; 1998.

31.
El-Ad R, Roth Y, Winder A.
The persistence of neutralizing antibodies after revaccination against smallpox.
J Infect Dis.
1990;161:446-448.
MEDLINE

32.
World Health Organization.
Smallpox vaccine and seed virus survey.
Working document for the meeting of the WHO Ad Hoc Expert Committee on Orthopoxvirus Infections; January 14-15, 1999; Geneva, Switzerland.

33.
Sharp JCM, Fletcher WB.
Experience of antivaccinia immunoglobulin in the United Kingdom.
Lancet.
1973;1:656-659.
MEDLINE

34.
Kempe CH.
Studies on smallpox and complications of smallpox vaccination.
Pediatrics.
1960;26:176-189.

35.
Koplan J, Monsur KA, Foster SO, et al.
Treatment of variola major with adenine arabinoside.
J Infect Dis.
1975;131:34-39.
MEDLINE

36.
Monsur KA, Hossain MS, Huq F, Rahaman MM, Haque MQ.
Treatment of variola major with cytosine arabinoside.
J Infect Dis.
1975;131:40-43.
MEDLINE

37.
Lalezari JP, Staagg RJ, Kuppermann BD, et al.

Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS: a randomized, controlled trial.
Ann Intern Med.
1997;126:257-263.
MEDLINE

38.
O'Toole T.
Smallpox: a case history.
Emerg Infect Dis.
In press.

39.
Dixon CW.
Smallpox in Tripolitania, 1946: an epidemiological and clinical study of 500 cases, including trials of penicillin treatment.
J Hyg.
1948;46:351-377.

40.
Centers for Disease Control and Prevention.
Vaccinia (smallpox) vaccine recommendations of the immunization practices advisory committee.
MMWR Morb Mortal Wkly Rep.
1990;40(RR-14):445-448.

41.
World Health Organization.
WHO Expert Committee on Smallpox Eradication.
Geneva, Switzerland: World Health Organization; 1972:493. WHO technical report series.

42.
Henderson DA, Arita I, Shafa E.
Studies of the bifurcated needle and recommendations for its use.
Geneva, Switzerland: World Health Organization; 1972. WHO Smallpox Eradications Paper SE/72.5.

43.
McIntosh K, Cherry JD, Benenson AS.
Standard percutaneous (smallpox) revaccination of children who received primary percutaneous vaccination.
J Infect Dis.
1990;161:445-448.

44.
Wyeth Smallpox Vaccine [package insert].
Lancaster, Pa: Wyeth Laboratories Inc; 1988.

45.
Lane JM, Ruben FL, Neff JM, Millar JD.
Complications of smallpox vaccination, 1968: national surveillance in the United States.
N Engl J Med.
1969;281:1201-1208.
MEDLINE

46.
Goldstein VA, Neff JM, Lande JM, Koplan J.
Smallpox vaccination reactions, prophylaxis and therapy of complications.
Pediatrics.
1975;55:342-347.
MEDLINE

47.
Centers for Disease Control and Prevention.
Vaccinia (smallpox) vaccine: recommendations of the Immunization Practices Advisory Committee.
MMWR Morb Mortal Wkly Rep.
1991;40:1-10.

48.
Huq F.
Effect of temperature and relative humidity on variola virus in crusts.
Bull World Health Organ.
1976;54:710-712.
MEDLINE

49.
Wolff HL, Croon JJ.
The survival of smallpox virus (variola minor) in natural circumstances.
Bull World Health Organ.
1968;38:492-493.
MEDLINE

50.
Hashizume S, Yoshizawa H, Morita M, Suzuki K.
Properties of attenuated mutant of vaccinia virus, LC16m8, derived from Lister strain.
In: Quinnan GV, ed. *Vaccine Virus as Vectors for Vaccine Antigens.*Amsterdam, the Netherlands: Elsevier Science Publishing; 1985:87-99.

51.
Hirayama M.
Smallpox vaccination in Japan.
In: Fukumi H, ed. *The Vaccination: Theory and Practice*Tokyo: International Medical Foundation of Japan; 1975:113-124.