Smallpox is a viral infection caused by the variola virus. It was declared eradicated worldwide by the World Health Organization in 1980 following a smallpox eradication campaign. Smallpox is seen as one of the viruses most likely to be used as a biological weapon. The variola virus exists legitimately only in two laboratories in the world. Any new case of smallpox would have to be the result of human accidental or deliberate release. The aerosol infectivity, high mortality, and stability of the variola virus make it a potential and dangerous threat in biological warfare. Early detection and diagnosis are important to limit the spread of the disease. Patients with smallpox must be isolated and managed, if possible, in a negative-pressure room until death or until all scabs have been shed. There is no established antiviral treatment for smallpox. The most effective prevention is vaccination before exposure.

**Introduction**
Smallpox is a viral infection caused by the variola virus which belongs to the family of Poxviridae. Smallpox was declared eradicated worldwide by the World Health Organization in 1980 following a smallpox-eradication campaign with the last case of endemic smallpox occurring in Somalia in 1977 [1]. The last fatal case was due to a laboratory acquired infection in the United Kingdom in 1978 [2].

**Smallpox and bioterrorism**
Smallpox is seen as one of the most likely viruses to be used as a biological weapon. Using this virus in warfare is an old concept: it was used with contaminated clothing in the 15th and 18th centuries. More recently, during the second world war, the Japanese military explored the weaponisation of smallpox in Mongolia and China. The variola virus exists legitimately only in two laboratories in the world: one in the Centers for Disease Control and Prevention in Atlanta, Georgia, USA, and the other in the State Research Centre of Virology and Biotechnology in Novosibirsk, Russia [3-9]. There is no documentation of clandestine stock of the virus. Any new case of smallpox would have to be the result of human accidental or deliberate release. The aerosol infectivity, high mortality, and stability of the variola virus make it a potential and dangerous threat in biological warfare. However, some authors claim that the virus would have limited potential as a biological weapon because a highly efficacious, easily administrable vaccine exists [7,10,11]. In addition, animal poxviruses such as monkeypox or a recombinant variant of smallpox could be developed as biological weapons. However, for the monkeypox virus, data indicate that it has limited potential for person-to-person transmission and furthermore, is not able to sustain an epidemic indefinitely in a community by human transmission only [12]. Nevertheless, we must keep in mind that these other poxviruses still have the potential of a biowarfare threat.

**Microbiological characteristics**
Smallpox is a member of the family Poxviridae, subfamily Chordopoxvirinae and genus orthopoxvirus which includes monkeypox virus, smallpox vaccine and cowpox virus [3]. It is a single, linear, double-stranded DNA virus and is characteristically a brick-shaped structure with a diameter of about 200 nm under the electron microscope.

Two different strains of variola virus are known and associated with two varieties of smallpox: variola major and variola minor or alastrim [7].

**Viral transmission**
Person-to-person contact remains the most common route of transmission but requires close contact [3,4,7]. Patients are not infectious during the asymptomatic incubation period (4 to 19 days; mean 10 to 12 days) before fever occurs. Smallpox is mostly contagious during the first week of rash, corresponding to the period when the lesions of the enanthem are ulcerated. At this stage, aerosol droplets from oropharyngeal lesions increase the likelihood of person-to-person transmission. After aerosol exposure, the virus infects the regional lymph nodes around the respiratory tract where replication occurs followed by viraemia. Multiplication of the virus may occur in other lymphoid tissues such as the spleen, liver, bone marrow, lung and lymph nodes.

After a second viraemia period, the virus localises in small blood vessels of the dermis and in the oral and pharyngeal mucosa and proceeds to infect adjacent cells. Viruses remain present in the lesions until all scabs have been shed following recovery. At this stage, when viruses are enclosed within hard dry scabs, the infectivity is lower than at the initial stage of the disease.

Close contact has been demonstrated to result in efficient transmission of smallpox. Historically, it has been estimated that approximately 30% of susceptible household members became infected at the time when smallpox was endemic [13]. Casual contact is much less likely to result in transmission. The virus is highly stable and remains infective for long periods of time outside the host. It has been estimated that variola can remain viable in certain conditions for up to a year in dust and cloth [14].

**Clinical features**

*Variola major*

The most virulent strain of variola virus causes variola major (classical smallpox). Five clinical forms of variola major, which differ in prognosis are described [3,4,7,15] (TABLES 1 and 2).

Ordinary-type smallpox is the most common form and occurs in 90% of cases. The prodromal phase (2 to 3 days) has an abrupt onset and is characterised by severe and generalised headache, fever (>40°C), extreme prostration, intense, ill-defined pain in the back, chest or joints, intense anxiety and sometimes abdominal pain. Children may have convulsions and some adults are delirious. The fever subsides over a period of 2 to 3 days (rarely 4 days).

Then enanthem in the form of minute red spots appears, a day before the exanthematous rash, over the tongue, palate, mouth, and oropharynx. At this time lesions can also occur in the respiratory tract.

The exanthema begins as a small reddish maculopapular rash on the face and forearms and spreads gradually with a centrifugal distribution to the trunk and legs and then to all parts of the body within 24 hours, including the palms of the hands and the soles of the feet. Within one to two days, the rash becomes vesicular, with a vesicle diameter of 2 to 5 mm, and later pustular. Pustules that are round (4 to 6 mm in diameter), tense, and deeply embedded in the dermis, remain for 5 to 8 days, followed by umbilication and crusting. The number of pustules can vary from a few to several thousand. These lesions can be confluent, semi-confluent or discrete.

A second, less pronounced, temperature spike might be noted 5 to 8 days after the onset of the rash. Lesions are generally synchronous in their stage of development, in distinct contrast to varicella. This characteristic also provides the main distinguishing feature from monkeypox. Monkeypox virus is also an orthopoxvirus, found in Africa. It is clinically indistinguishable from smallpox, with the exception of notable enlargement of cervical and inguinal lymph nodes. The disease occurs mainly in monkeys, but sporadic transmission to humans has been reported, as has limited human-to-human transmission [12,16].

Full blood count shows a lymphocytosis or at least a predominance of lymphocytes, with many atypical and activated mononuclear cells. In severe cases, early forms may be numerous, giving the picture of a leukaemoid reaction. The platelet count falls as haemorrhagic features develop. The chest X-ray is normal.

During the disease, the fever can remain elevated if secondary pyogenic infection of the skin occurs. Panophthalmitis and blindness, keratitis and corneal ulcers, osteomyelitis, arthritis, orbitis and encephalitis are possible complications (1%-5%). Bronchitis, pneumonitis, pulmonary oedema and associated bacterial pneumonia are not rare. Death may occur in the first 48 hours, before any feature of smallpox has appeared. Most fulminant cases die by the 4th or 5th day; many other malignant cases die between the 8th and 15th day. Death is ascribed to toxæmia, associated with immune complexes and to hypotension. The mortality rate is 30% in unvaccinated and 3% in vaccinated individuals.

Haemorrhagic-type smallpox is the most virulent form and occurs in 3% of patients. Haemorrhagic smallpox is characterised by haemorrhages into the skin and/or mucous membranes early in the course of the illness and intense toxæmia. Early and late haemorrhagic-type smallpox are described, defined by the occurrence of haemorrhages before or after the appearance of the rash. It causes death in 96% of unvaccinated and 94% of vaccinated patients, usually before the occurrence of the lesions.

The modified-type smallpox or milder type is more common in previously vaccinated populations (25%) but can be noted in unvaccinated persons (2%). The onset of the prodromal phase is also abrupt. Usually the lesions are fewer, smaller, more superficial and evolve more rapidly. Frequently, the pustular stage is absent.

Flat-type smallpox is defined by lesions that evolve more slowly than those of variola major and are coalescent. It is very rare in vaccinated subjects. It occurs in 2% to 5% of patients and is associated with severe systemic toxic affects. The enanthem is extensive and confluent. Vesicles contain very little fluid, are not multiloculated and do not show umbilication. Respiratory and abdominal complications are frequent. The mortality rate is 95% in unvaccinated and 66% in vaccinated individuals.

Variola sine eruptione occurs in previously vaccinated contacts or in infants with maternal antibodies. Patients are asymptomatic or have influenza-like symptoms with or without conjunctivitis, which can be the only clinical manifestation. No rash develops. Usually, diagnosis is performed retrospectively and serological confirmation is required.

Variola minor The strain of variola virus that is associated with variola minor is less virulent than those of variola major. The severity and the mortality rate (<1%) are lower.

The onset of the illness can be abrupt with fever (>40°C), headache and backache [7]. Toxaemia rarely occurs. The sequence of appearance, the distribution and the nature of the lesions are similar to those reported for variola major. But the evolution is usually more rapid. The skin lesions are smaller than those of variola major, are not confluent and not umbilicated. Haemorrhagic-type and variola sine eruptione are reported in this disease also.

Diagnosis
Case definitions of suspected or confirmed cases due to deliberate release are listed in Table 3.

A clinical diagnosis must be suspected in all cases even if many eruptive illnesses can be misdiagnosed as smallpox (e.g. chickenpox, monkeypox, Stevens-Johnson syndrome, measles, haemorrhagic fever viruses) [3]. For differentiation between the various orthopoxvirus, electron microscopy examination of vesicular or pustular fluid or scabs can be used. Poxviruses cannot be readily distinguished from one another except by PCR assay.

Definitive identification of strains is performed by PCR and/or restriction fragment-length polymorphism (RFLP) [3,4]. Definitive characterisation of the variola virus is made by culture in eggs and cell monolayers [3,4].

Treatment
Patients with smallpox must be isolated and managed, if possible, in a negative-pressure room until death or until all scabs have been shed (about 3 weeks) [3-8,17]. Nevertheless, isolation would be possible for a small number of infected patients only, due to the limited availability of such rooms. With a large number of cases, the creation of larger units similar to the smallpox hospitals of the past has been suggested.

There is no established antiviral treatment for smallpox. Cidofovir, an antiviral drug, is active in vitro on isolates of variola virus [18]. Obviously, no data are available for humans. This drug has been tested successfully for the treatment of cytomegalovirus, but is associated with serious renal side effects and must be administered intravenously. Antibiotics may be useful in the case of secondary bacterial infection.
The most effective prevention is vaccination before exposure [19,20]. The frequency of complications is low, but is higher than the most commonly used vaccines. In 1968 with over 14 million vaccinated persons in the United States, the more severe complications were postvaccinal encephalitis (n=16 with 4 deaths), progressive vaccinia (n=11 with 4 deaths), eczema vaccinatum (n=74) and 6 additional cases of eczema vaccinatum that occurred in contacts of vaccinated persons with one death) and generalised vaccinia (n=143) [21]. The death rate was less than one per million vaccinated (n=9). Vaccination is not recommended for pregnant women, patients with immunosuppression and patients with a history of severe eczema.

Vaccination can modify the course of the disease and reduce mortality if given immediately after exposure (mortality can be reduced by up to 100%) and up to four days after (mortality can be reduced by up to 50%).

The vaccine used to eradicate smallpox was highly efficacious; it was prepared using live animal's skin as a substrate. During the World Health Organization smallpox eradication programme (1977-1988) many countries produced and used such ‘first generation’ products despite its known reactogenicity, as it was accepted that the benefits outweighed the risks. Nowadays, quality requirements for vaccines have become much more stringent, leading to the development and testing of so-called ‘second generation’ vaccines. These are produced using the same vaccine strains as in the first generation vaccines on tissue culture substrate, allowing better production consistency and quality control testing (e.g. for adventitious agents). For Europe, the European Medicine Evaluation Agency (EMEA) has recently developed guidelines for development and production of second-generation vaccines [22]. While clinical data are at this moment not fully available at present, preliminary results from a 60-subject randomised double blind trial in adult volunteers indicate that the immunogenicity and safety profile does not differ significantly from the first generation vaccines [23]. There is a need therefore to develop a new (third) generation of smallpox vaccines, with an acceptable safety profile. This could be achieved by attenuation or genetic engineering (disabling) of vaccinia vaccine strains, while retaining their immunising properties [24].

In conclusion, smallpox must be considered to be a serious potential biological weapon. Early detection and diagnosis are important to limit the spread of the disease. There is no specific treatment for smallpox and studies are needed to evaluate new generations of safe smallpox vaccines.

References

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22. Note for guidance on the development and production of vaccinia virus based vaccines against smallpox. EMEA/CPMP/1100/02

* BICHAT, the European Commission's Task Force on Biological and Chemical Agent Threats, has developed this set of guidelines that may be the basis of national authorities' guidance, and may also be used directly by clinicians, general practitioners and specialists when confronted with patients infected by agents that may be due to deliberate release of biological agents. Ref. Bossi P, Van Loock F, Tegnell A, Gouvras G. Bichat clinical guidelines for bioterrorist agents. Euro Surveill. 2004; 9(12) http://www.eurosurveillance.org/erm/v09n12/0912-230.asp
Editorial note: These clinical guidelines were reviewed by the Task Force and by two experts designated by each Member State of the European Union. This review was completed at the end of February 2003. The revised guidelines were submitted to the Health Security Committee which approved them in April 2003 and agreed their publication in a widely disseminated journal so as to allow access to as large an audience as possible. The editorial process of Eurosurveillance also introduced modifications that improved the contents of these guidelines.
## Summary of clinical and diagnosis of smallpox

### Clinical features

Person-to-person contact is the most common route of infection but requires close contact. Smallpox is contagious during the prerruptive period and until all scabs have been shed. The maximum infectivity is during the first week of rash, when the lesions in the mouth and oropharynx are ulcerated.

### Variola major (classical smallpox)

**Ordinary-type smallpox** (90% of cases).
- Prodromal phase abrupt with severe headache, fever (>40°C), prostration, pain in the back, chest or joints, anxiety and abdominal pain. This subsides over a period of 2 to 3 days.
- Then, enanthem appears over the tongue, palate, mouth, and oropharynx a day before the exanthematous rash.
- The enanthem begins as a maculopapular rash on the face, forearms and spreads gradually with a centrifugal distribution to the trunk and legs and then all parts of the body within 24 hours including the palms of the hands and the soles of the feet.
- Within 1 to 2 days, the rash becomes vesicular (diameter of 2 to 5 mm) and later pustular. - Pustules which are round (4 to 6 mm), tense, and deeply embedded in the dermis, remain for 5 to 8 days, followed by umbilication and crusting.
- Lesions are generally synchronous in their stage of development.

**Haemorrhagic-type smallpox** is the most virulent form. It is characterised by haemorrhages into the skin and/or mucous membranes early in the course of the illness and intense toxæmia.

**The modified-type smallpox or milder type** is more common in previously vaccinated populations. The onset of the prodromal phase is abrupt. Usually the lesions are fewer, smaller, and more superficial and evolve more rapidly. Frequently, the pustular stage is absent.

**Flat-type smallpox** is defined by lesions that evolve more slowly than those of variola major. It is associated with severe systemic toxic affects. The enanthem is extensive and confluent. Respiratory and abdominal complications are frequent.

**Variola sine eruptione** occurs in previously vaccinated contacts or in infants with maternal antibodies. Patients are asymptomatic or have influenza-like symptoms with or without conjunctivitis, which can be the only clinical manifestation. No rash develops.

### Variola minor

The onset of the illness can be abrupt with fever (>40°C), headache and backache.

The sequence of appearance, the distribution and the nature of the lesions are similar to those reported for variola major. But the evolution is usually more rapid. The skin lesions are smaller than those of variola major, are not confluent and not umbilicated.

### Diagnosis

- Confirmed by electron microscopy examination of vesicular or pustular fluid, or scabs (identifies orthopoxviruses)
- Serologic tests do not differentiate between orthopoxvirus species
- DDefinitive identification and characterisation of the variola virus; culture in eggs and cell monolayers
- Characterisation of strains; PCR followed by sequencing and/or restriction fragment-length polymorphisms

### Treatment

- Isolation in a negative-pressure room until all scabs have been shed (3 weeks).
- Cidofovir?
- The most effective prevention is vaccination before exposure.
- Vaccination in the first four days of the incubation period can modify the course of the disease.
TABLE 2

Frequency and case-facility rates of different clinical types of variola major, according to vaccination status

<table>
<thead>
<tr>
<th>Clinical type</th>
<th>% of total cases</th>
<th>% of patients according to vaccination status</th>
<th>Case facility rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccinated</td>
<td>Unvaccinated</td>
<td>Vaccinated</td>
</tr>
<tr>
<td>Ordinary type</td>
<td>80</td>
<td>70</td>
<td>89</td>
</tr>
<tr>
<td>Modified type</td>
<td>13</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>Flat type</td>
<td>4</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Haemorrhagic type</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Total cases: n = 6942; Vaccinated patients: n = 3398; Unvaccinated patients: n = 3544
Source: from Rao during an outbreak in Madras-India 1972 in [7]

TABLE 3

Case definitions of suspected or confirmed cases due to deliberate release

<table>
<thead>
<tr>
<th>Clinical description</th>
</tr>
</thead>
<tbody>
<tr>
<td>An illness with acute onset of fever &gt;38°C, which is persistent, followed by a rash characterised by vesicles or firm pustules at the same stage of development without other apparent cause and with a predominantly centrifugal distribution. Atypical presentations may include haemorrhagic lesions or flat velvety lesions not appearing as typical vesicles nor progressing to pustules.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory criteria for diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation of smallpox (Variola) virus from a clinical specimen, or polymerase chain reaction (PCR) identification of variola DNA in a clinical specimen followed by sequencing</td>
</tr>
<tr>
<td>Negative stain electron microscopy (EM) identification of Variola virus in a clinical specimen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible:</td>
</tr>
<tr>
<td>- A clinically compatible case</td>
</tr>
<tr>
<td>Probable:</td>
</tr>
<tr>
<td>- A clinically compatible case with either identification of orthopox virus by EM or PCR, or an epidemiological link to other probable or confirmed cases</td>
</tr>
<tr>
<td>Confirmed:</td>
</tr>
<tr>
<td>- For initial cases, a case consistent with the clinical definition with laboratory confirmation by EM and PCR, followed by sequencing; During an outbreak, a clinically compatible case with an epidemiological link and, where possible, laboratory confirmation by either EM or PCR.</td>
</tr>
</tbody>
</table>

Source: [25,26]