



THE CONFIRMATION AND MAINTENANCE OF SMALLPOX ERADICATION

J. G. Breman<sup>a</sup> & I. Arita<sup>b</sup>



Introduction

The last smallpox patient in an endemic area was a 23 year old hospital cook, from Merka town, Somalia. He developed rash on 26 October 1977 and was discovered by a World Health Organization/Somali smallpox surveillance team on 31 October.<sup>1</sup> During the following two years, thousands of health staff searched house-by-house throughout Somalia and adjacent countries in an effort to discover additional cases. Two further cases occurred in Birmingham, England, in 1978, as a result of a laboratory accident but no other cases were found. On 9 December 1979, the WHO Global Commission for the Certification of Smallpox Eradication, an independent scientific group that had been evaluating smallpox eradication activities for more than two years concluded:

- "1. Smallpox eradication has been achieved throughout the world.
- "2. There is no evidence that smallpox will return as an endemic disease."<sup>2</sup>

The Commission made 19 recommendations.<sup>2</sup> These deal with future vaccination policy, maintenance of reserve stocks of vaccine, investigation of suspected cases, laboratories designated to retain variola virus, needed research on orthopoxviruses including human monkeypox infection and programme documentation. Proposed also are WHO activities to ensure enactment of the recommendations and measures to sustain public confidence that eradication has been achieved. In May 1980, the Thirty-third World Health-Assembly, composed of the Ministers of Health of all nations, endorsed the Commission's conclusions and recommendations. This action officially confirmed international acceptance that, for the first time in history, a disease had been eradicated.<sup>3</sup>

Confirmation of the eradication of smallpox, and the corollary recommendation to discontinue smallpox vaccination throughout the world, merits a review of the procedures used by WHO and the Global Commission in reaching these conclusions. The related scientific issues are also important to an understanding of recommended future measures and activities pertaining to variola, vaccinia and the closely related poxviruses.

Definition of Smallpox Eradication

Smallpox eradication was defined by a WHO Expert Committee in 1972<sup>4</sup>, "as elimination of clinical illness caused by variola virus. Since smallpox is transferred direct from man-to-man in a continuing chain of transmission, and since there is no human carrier state of epidemiological importance and no animal reservoir of the disease, the absence of clinically apparent cases in man may be assumed to signify the absence of naturally occurring smallpox.

<sup>a</sup> Medical Officer, Smallpox Eradication Unit, WHO, Geneva.

<sup>b</sup> Chief, Smallpox Eradication Unit, WHO, Geneva.

"In order to be able to confirm the interruption of smallpox transmission an effective surveillance is needed so that clinical infections can be detected. Recent experience indicates that, in all countries with a reasonably effective surveillance programme, residual foci can be detected within 12 months of apparent interruption. Thus, in countries with active surveillance programmes, at least two years should have elapsed after the last known case - excluding well-defined and contained importations - before it is considered probable that smallpox transmission has been interrupted."

#### The Global Smallpox Eradication Programme

In 1958, the Eleventh World Health Assembly requested the Director-General of WHO to study the financial, administrative and technical implications of a global smallpox eradication programme.<sup>2</sup> A resolution calling for the global eradication of smallpox was passed in 1959 at the Twelfth Assembly, but little progress was made during the next seven years. To provide impetus to the effort, the Nineteenth Assembly (1966) decided to intensify the activities through the provision of specially designated programme funds from its regular budget.

Conceptually, eradication was considered feasible because there is no animal reservoir; thus, for the virus to persist in nature, it was necessary that it be transmitted from human to human in a continuing chain of transmission. An individual, when infected, could transmit the virus to others only from the time of appearance of rash until the last scabs separated. Since chronic carriers did not exist, isolation of the patient and protection of those with whom he was in contact served to sever the chains of transmission. The fact that all infected persons capable of disseminating infection developed an illness with a distinctive rash greatly facilitated detection of disease. Other helpful features included the relatively short two to three week period of infectivity, the absence of second attacks and the fact that protection afforded by vaccination approached 100% for at least three years, with immunity waning relatively slowly up to 10 years or more after a successful vaccination. Development of a commercially practical method for freeze-drying smallpox vaccine rendered vaccinia virus stable at temperatures of 37°C or more for a month or longer, thus permitting wide distribution and use of viable vaccine even in the hottest climates.<sup>5</sup>

The fact that smallpox had been eliminated in a number of developing countries with limited health services provided a practical demonstration of the feasibility of the concept. However, many doubted the possibility of successfully undertaking a coordinated global effort which eventually would involve more than 50 developing countries, some of which were among the poorest.

In 1967, 33 countries had endemic smallpox and 14 others reported importations (Figure 1). The total population of the endemic countries was more than 1200 million persons. Most were developing countries in the tropics where surveillance systems then in existence are estimated to have been reporting perhaps 1% of the actual number of cases. Although 131 000 cases of smallpox were reported in 1967, as many as 10 to 15 million cases are believed to have occurred that year. Case fatality rates in different areas varied, depending on whether the disease was variola major or minor, but overall about 15-20% of all patients died.

In 1967 a smallpox eradication unit to provide overall coordination and direction was established at WHO headquarters in Geneva. A programme coordinator was designated in each of the four WHO regional offices in whose regions smallpox was endemic. WHO provided field epidemiologists and administrators to many national programmes for periods varying from a few months to several years. During the subsequent 12 years, 687 such individuals from 73 countries participated in the programme; there were never more than 150 WHO staff working on smallpox eradication at any one time.

The provision of adequate quantities of acceptable vaccine was a continuing challenge. At the beginning of the programme only 31% of the batches of vaccine intended for use in endemic countries and submitted to WHO for testing met WHO standards for potency and stability.<sup>6</sup> Overall, probably less than 15% of the vaccine in use in the endemic countries at that time met WHO standards. WHO sought contributions of heat stable vaccine, encouraged and supported production in a number of endemic countries and established quality control testing of vaccines in two WHO collaborating centres (Connaught Laboratories Limited, Toronto; Rijks Instituut voor de Volksgezondheid, Bilthoven, Netherlands).

Two other collaborating centres (Center for Disease Control, Atlanta, USA; Research Institute for Virus Preparations, Moscow, USSR) agreed to test specimens from suspected cases. In addition, WHO sponsored a wide variety of training courses and seminars, facilitated bilateral and multilateral support and was the central focus for receiving and disseminating information about the progress of the programme.<sup>7</sup> However, the programme itself was truly a cooperative international effort financed and executed mainly by the endemic countries themselves. They contributed approximately two-thirds of the estimated US\$ 315 000 000 which has been spent since 1967 in the eradication of smallpox and provided all but a minute proportion of the health manpower, numbering some 200 000 workers.

In May 1970, the first group of endemic countries - those in western and central Africa - became free of smallpox.<sup>8</sup> Here the US Agency for International Development and the Center for Disease Control supported a 20-country smallpox eradication and measles control programme. It was during the course of this programme that it became apparent that vigorous case detection and containment of smallpox outbreaks should be the primary strategy of the global campaign and should take precedence over nationwide mass vaccination programmes.<sup>9</sup> In the late 1960s the development of the bifurcated needle for administering vaccinations provided a simple but extremely useful advance.<sup>10</sup> This modification of a sewing needle design gave an instrument which allowed higher rates of successful vaccination while using lesser quantities of vaccine than traditional methods.

The Americas became free of smallpox in 1971 when Brazil recorded her last cases. The last case was recorded in Indonesia in 1972, and smallpox disappeared from eastern and southern Africa in 1973. That year the countries of southern Asia - Bangladesh, India, Nepal and Pakistan - greatly intensified their campaigns. In October 1975, Asia became free of smallpox when the last case of variola major, the most severe form of smallpox, was recorded in Bangladesh. In the Horn of Africa, where variola minor prevailed, Ethiopia reported her last cases in 1976 and Somalia and Kenya (imported cases) in 1977. The progressive decrease since 1958 in the number of countries reporting smallpox is shown in Figure 2. The number of cases of smallpox which were notified between 1958 and 1967 showed a more erratic pattern (Figure 3). Beginning in 1967, efforts were made to strengthen the surveillance system in all countries but some made more rapid progress than others. From 1967 through 1970, there was a steady decline in notified cases despite increasingly more complete case reporting from most areas. In 1971 Ethiopia began her eradication programme and that year reported over 25 000 cases compared to less than 1000 cases during preceding years. In 1973, intensive surveillance programmes began on the Indian subcontinent resulting in a sharply increased reported incidence both in 1973 and 1974. The increase in cases during these years reflected substantially improved reporting although an unknown fraction may have represented a real increase in the number of cases.

#### Confirmation of Smallpox Eradication

When transmission was believed to have been interrupted in each country, preparations were begun to document the absence of smallpox during a period of at least two years. In addition to an on-going routine programme of notification of cases by health units throughout the country, rigorous field studies were conducted in collaboration with WHO staff and consultants. Special investigations were undertaken in areas considered most likely to harbour persisting infection, e.g. where health units were sparse or where routine notification was incomplete, where the last cases had occurred, along borders with recently endemic countries, among special groups such as nomads, refugees and immigrant workers and in geographically remote areas and places which were relatively inaccessible due to security problems.

A great many specimens were collected from patients with fever and rash. Specimen collection was especially emphasized in those areas where variola minor had been prevalent because this form of the disease could be more easily confused with chickenpox or other eruptions and less frequently left facial scars typical of smallpox. In other countries, extensive surveys were conducted to determine the prevalence of facial pockmarks in different age groups. If pockmarks were found in children born since the last case was reported, or if any person reported that the scars had resulted from an illness which had occurred since the last known smallpox case, special investigations were initiated. To encourage the reporting of possible smallpox cases, a special reward was offered in many countries to the person who reported a subsequently confirmed case of smallpox and to the health worker who first investigated it. Tens of thousands of suspected cases were thus reported and investigated.

When the studies had been completed, each country was required to present a complete report of its programme of activities to an independent international commission convened by WHO. This report documented that the surveillance system and programme of special activities was adequate to detect cases in the country, if they had existed. Each commission then verified the reports through field visits of one to three weeks' duration.

Of concern throughout the programme was the question of how long smallpox could persist in a country without being detected. This related to the WHO Expert Committee's formulation that two years would need to elapse after the last known case, during which time active surveillance failed to detect further cases, before eradication could be considered. Four countries found active cases after periods of six to 36 weeks following presumed freedom from smallpox; these occurred in Nigeria in early 1970, in Brazil in 1971, in Indonesia in 1971, and in Botswana between 1972 and 1973. The reasons for the delay in detection of these outbreaks included uneven geographical coverage by the surveillance system, the suppression of reports by local staff or particular communities, and once because of lack of communication between the various levels involved in reporting. Despite these deficiencies, the existing active surveillance systems were, ultimately, able to detect the outbreaks long before the prescribed two year interval. On one occasion, in Malawi, investigation of a pockmarked person suggested that smallpox cases might have occurred in 1972, 19 months after the last reported case. However, the situation in Malawi differed from the other episodes in that an active surveillance programme was not developed there until 1974.

Since 1967, over 17 000 specimens from suspect cases were submitted to the two WHO collaborating laboratories (Figure 4). The specimens were screened for the presence of poxvirus particles by electron microscopy and cultured on the chorioallantoic membranes (CAM) of chicken eggs.<sup>11</sup> When smallpox was widespread in an area or country, few specimens were collected because the clinical diagnosis of smallpox was comparatively straightforward. Doubtful cases were dealt with as presumed smallpox. However, as the incidence decreased, at least one specimen was requested for testing from each outbreak. When transmission was thought to be interrupted, a specimen was taken from each patient with "fever and rash" even remotely suspected to have smallpox. Additionally, patients who had atypical or severe chickenpox, and those who were hospitalized or died with eruptive disease - especially those who had no smallpox vaccination scar - were investigated and specimens collected. In the Horn of Africa, particularly in the disputed Ogaden desert area, where some of the last outbreaks of variola minor occurred, an especially large number of specimens were collected prior to certification (Figure 5).

In January 1978, WHO established an international rumour register to record systematically suspect cases reported from all parts of the world. Through April 1980, 121 rumours were received from 49 countries (Table 1). Many of these reports were stimulated by the \$1000 reward offered by WHO during 1978 and 1979 to any person reporting a suspected smallpox case which was confirmed by laboratory results. Additionally, many hundreds of rumours continued to be investigated annually by national health authorities. Prompt and thorough investigation of each case and the reporting of results to informants, many of whom were private citizens, was of importance in sustaining the credibility that eradication had been achieved.

Since 1973, 79 countries have been certified as being free from smallpox by 21 international commissions and the Global Commission following special investigation (Figure 6).<sup>2</sup> Their total population is over 3000 million. These included 35 countries with a total population of 1200 million which experienced endemic disease between 1967 and 1977, and 44 others at special risk of importation and/or where the Global Commission considered that special reports and/or studies were required to judge whether or not smallpox had been eradicated. The remaining 121 countries and areas in the world submitted statements to WHO which attested to their smallpox freedom and cited the year of their last case. In these countries smallpox had not been endemic for at least ten years and surveillance systems were judged sufficiently sensitive to detect continuing smallpox transmission. Surveillance for suspected smallpox cases in all countries continued following certification; in no instance was continuing smallpox transmission detected.

#### Smallpox Infections from Laboratory Sources

The threat that smallpox might again be reestablished through laboratory infection was emphasized by the occurrence of laboratory-associated outbreaks in London in 1973 and in Birmingham, United Kingdom, in 1978. In London, two persons died from smallpox after being infected by a laboratory worker who had been infected while working in a laboratory.<sup>12</sup> In Birmingham a medical photographer working on the floor above a laboratory where studies on variola virus were in progress contracted the disease, presumably from the virus passing through the ducting system. She died and her mother developed mild smallpox.<sup>13</sup> This latter outbreak, which occurred in a population estimated to be less than 40% vaccinated against smallpox, was rapidly stopped by prompt identification, vaccination and surveillance of 341 persons in contact with the patients. Vaccination was offered to other selected groups but a mass city- or nation-wide campaign was not mounted.

To identify laboratories which might have stocks of variola virus, WHO, in 1975, contacted all countries and territories to request a list of all laboratories which then maintained stocks of variola virus. Additional laboratories were identified through a search of the world's medical literature since 1950 to identify those which had published articles on poxviruses. Finally contact was made with all laboratories which were registered as having the capability to undertake smallpox diagnosis. Seventy-six laboratories with stocks of variola virus were identified.

Of note is that the Expert Committee defined smallpox eradication in terms of the interruption of person-to-person transmission. Some had urged that eradication be defined as total elimination of the virus. However, the Committee recognized that it would be impractical, if not impossible, to assure that all laboratory cold storage facilities were properly searched and all specimens of virus destroyed, whatever the merits of so doing.

The Thirtieth World Health Assembly (1977) recommended that variola virus be retained only by WHO collaborating centres under conditions assuring maximum safety. In response to this request, 70 laboratories voluntarily destroyed or transferred their stocks. Six laboratories currently retain variola virus as shown in Table 2. The present goal of WHO is to reduce the current number to no more than four, as recommended by the Global Commission.

Strict physical and administrative security measures for such laboratories were formulated in 1977 by a scientific group convened by WHO (Report of a Workshop Meeting on Safety Measures in Laboratories Retaining Variola Virus, unpublished WHO document SME/77.2). The guidelines were further revised in 1979 (unpublished WHO document SME/77.2, rev. 1). Included among its provisions are structural requirements such as imperviously sealed walls, floors and ceilings; contiguous clothing change and shower rooms; double door autoclaves sealed to the laboratory barrier wall; self-contained bio-waste treatment system; separate ventilation systems to maintain negative air pressure and directional air flow into the laboratory; passage of air into and out of the laboratory through "high efficiency particulate air" filters; and biological safety cabinets and sealed centrifuge buckets. Administrative recommendations include: the requirement that authorization to maintain variola virus strains be given by national authorities; a plan for special guarding of the

facility; yearly vaccination of personnel who enter the laboratory; and special procedures for reporting and dealing with laboratory accidents and absences of laboratory personnel. Beginning in 1978, WHO teams composed of consultant biosafety experts, virologists and epidemiologists inspected each laboratory to determine compliance with recommendations. All laboratories had been visited by July 1979; some have been inspected more than once. These visits will continue periodically.

In April 1979 the directors of these laboratories, and the national public health authorities under whose jurisdiction they operate, met in Geneva to review the established guidelines. They exchanged information on methods used to assure containment, reaffirmed the responsibility of national authorities to maintain adequate containment and supported regular WHO inspections.

Some have speculated on the possible use of variola virus as a biological warfare agent. Since 1972, 87 nations have signed the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction.<sup>14</sup> This Convention specifically outlaws the use of biological warfare. Abhorrent as such an act would be, the remote possibility that variola virus might be released as an act of war or terrorism cannot be dismissed. The potential harm would increase as the immunity of the population waned. In addition to illness and death, there would be psychological and possibly socio-economic damage. This risk, however, should not be exaggerated. Many other agents possess characteristics of transmission, severity of illness and persistence which could cause greater damage.<sup>15</sup> For many of these no protective vaccine is available. Should the worst occur and an aerosol of variola virus be released, some of the exposed would develop infection and perhaps 20% would die. A contact group of susceptibles would undoubtedly be exposed before the diagnosis was made. However, smallpox, with a 10 to 12 day incubation period, usually spreads slowly. Intensive selective containment vaccination programmes initiated immediately after diagnosis should be able to prevent all but a handful of patients in a third generation and to terminate spread within four to six weeks. Such has been the experience following recent importations into Europe.

The Global Commission considered these factors and stated, "Unless the public health services had completely broken down, the outbreaks that followed could be readily contained and the virus then eliminated. The existence of such a possibility nevertheless emphasizes the need for the security of laboratories holding variola virus stocks, for provision for the maintenance of vaccine reserves, and for the epidemiological and laboratory expertise for diagnosis and control. The remote risk of such an act does not constitute a ground for the continuation of smallpox vaccination of the general public."<sup>2</sup>

As part of the post-eradication era "insurance policy", WHO is stockpiling enough vaccine to vaccinate 200 million persons. This will be kept in two locations, Geneva and New Delhi. In addition, many countries are developing their own national vaccine reserves. The capability for clinical and laboratory diagnosis of smallpox is also being assured by WHO.

#### Variola Virus on Inanimate Objects, Reactivation and Latency

Some have been concerned that viable variola virus might persist on inanimate objects. In fact, it was reported in 1968 that viable virus could be detected in scabs from a smallpox patient kept at room temperature on a shelf in a European laboratory for over 13 years.<sup>15</sup> More recent experiments, measuring virus decay under tropical conditions, showed that infected scabs initially containing  $2.8 \times 10^8$  pock-forming units (PFU)/gm became negative on culture at nine weeks when kept at room temperature (25.8 - 26.4°C) and 85-90% relative humidity.<sup>17</sup> However, at least since 1967 no cases of smallpox have been detected that could not be epidemiologically linked to other human cases or to a laboratory accident. Taking into account the millions of cases which have occurred over recent decades, and the undoubted extensive contamination of fomites, it would be surprising not to have detected some apparently "spontaneous" cases if indeed this represented a real risk.

Use of stored scabs for variolation represents another hypothetical risk. Variolation is the centuries-old practice of taking material from skin lesions of smallpox patients and inoculating it into the skin of healthy persons. Those inoculated develop a less severe form of smallpox than those infected by the normal respiratory route. Variolation was extensively practised by traditional variolators in Afghanistan and Ethiopia during the intensified eradication programme and some were variolated in other countries as well. To evaluate this risk, WHO and national staff collected samples of variolators' stocks in Afghanistan, Ethiopia and Pakistan between 1969 and 1976. Of 45 specimens, 41 failed to yield viable variola virus on culture, although 14 of 39 (36%) specimens tested showed poxvirus particles in electron microscopy (Table 3). Of the four specimens from which the virus was isolated, two had been collected from patients within the preceding nine months and the time interval was unknown for two others. Moreover, interviews with variolators in Afghanistan and Ethiopia all revealed that they rarely found it possible to retain viable material for more than a year and none considered material collected more than two years previously to be effective. The fact that no further cases of smallpox were detected which had occurred in these countries after smallpox transmission had been interrupted provides further evidence that this is a highly unlikely source of infection in the future.

Other possible mechanisms which, it has been hypothesized, might result in a recurrence of variola virus include transformation and reactivation. Earlier views held that vaccinia virus evolved from variola virus through animal passage or arm-to-arm transfer. If this were so, the possibility for reverse transformation was perhaps conceivable. However, when rigid precautions were taken to exclude vaccinia virus from the experimental system transformation of variola virus into vaccinia virus did not occur.<sup>18</sup> Moreover, genome analysis clearly indicates that variola and vaccinia viruses are separate and distinct species of orthopoxvirus<sup>19,20</sup> and one could not be expected to evolve through transformation into the other. Heat-inactivated poxviruses, including variola virus, can be reactivated by co-infection of cells with an active poxvirus.<sup>21</sup> However, such reactivation only occurs under specially planned laboratory conditions and would appear to have no relevance to a natural system.

The possibility has been suggested that variola virus might remain latent, as occurs with varicella virus, and become manifest at a later date as active infection, perhaps in an immunodepressed patient. There is no evidence for recurrence of smallpox in man, or recurrence of other orthopoxvirus infections in animals, due to this phenomenon. Furthermore, epidemiological observations have not revealed apparently "spontaneous" outbreaks which could not be traced to known recent infections. If such did occur one would expect these "spontaneous" outbreaks to originate from persons who had smallpox previously and who developed immunologic dysfunction due to malignancy or other causes. Unquestionably, there have been numerous opportunities for this to occur.

#### Monkeypox

von Magnus first reported the isolation of a simian poxvirus in 1959.<sup>22</sup> The strain came from an outbreak in 1958 of a pox disease amongst laboratory cynomolgus monkeys shipped from Singapore to Copenhagen and hence the name monkeypox. To determine the frequency of monkeypox outbreaks in captive monkey colonies a survey of 27 laboratories was conducted in 1970. All laboratories had used large numbers of monkeys in their work. Ten outbreaks caused by monkeypox virus affecting non-human primates in captivity were documented; <sup>23,24</sup> the last occurred in 1968. No human infections were associated with these outbreaks.

Monkeypox virus is a separate species of orthopoxvirus with biological properties and a genome map which is distinct from those of all other orthopoxvirus species including variola virus (Table 4). No human cases were documented until 1970 when the first human infection caused by this virus was discovered in Basankusu Province, Equateur Region, Zaire.<sup>25,26</sup> Until the virus isolate had been identified as monkeypox, the case caused great concern, since the signs and symptoms were indistinguishable from smallpox and the area where the case occurred had been free of smallpox for two years.

From 1970 through April 1980, a total of 50 cases of human monkeypox had been reported from tropical rainforest areas of west and central Africa. Details on 48 of these cases have been reported previously.<sup>27</sup> Forty cases have occurred in Zaire. Most cases, like the first, have resembled smallpox. Eight persons (16%) have died, a case-fatality ratio similar to that caused by variola major in this area. Only four patients (8%) had a vaccination scar, all having been vaccinated five or more years before. Vaccination against smallpox provides protection of man against monkeypox. This is substantiated by the low vaccination scar rate of patients, vaccination scar surveys indicating that over 50% of the population in areas where monkeypox cases occurred had been vaccinated against smallpox and from serological evidence showing that monkeypox shares antigens with variola and vaccinia. Protection of monkeys from monkeypox infection by prior inoculation with vaccinia virus has also been demonstrated.<sup>28</sup>

On five occasions cases have occurred among close contacts at intervals ranging from nine to 17 days, suggesting that secondary transmission may have occurred. Assuming that all five represented person-to-person transmission, the secondary attack rate among susceptible, close family members was 9.8% (4/41) and among all susceptible contacts, 4.0% (5/124). This is much lower than the secondary attack rate among close contacts observed in smallpox outbreaks, usually 25 to 40%,<sup>29,30</sup> but on occasion reaching close to 90%.<sup>31</sup> Possible spread to a third generation has not been observed. Because of the difficulty of inter-human transmission of monkeypox, with the observation that milder illness appears to occur in some of the second generation patients,<sup>27</sup> it seems highly unlikely that the disease could become endemic by person-to-person transfer. However, special surveillance activities in west and central Africa have been established to monitor the situation closely over the next five years, a period during which vaccination immunity in the population will be waning steadily.

Although efforts have been made to recover monkeypox virus from animals captured in the wild, no strains have been isolated. Hence, the natural reservoir is yet unknown. Serological surveys of primates and other mammals captured in west and central Africa near areas where human monkeypox cases have occurred have demonstrated haemagglutination-inhibition and neutralizing antibodies to orthopoxviruses.<sup>32-36</sup> A major impediment to defining the presence of monkeypox antibodies in human and animal populations has been the antigenic cross-reactivity of members of the orthopoxviruses. Hence, the presence of serum antibody indicates only that the animal has been infected previously with one of the orthopoxviruses. Species-specific fluorescent antibody<sup>34</sup> and radioimmunoassay tests<sup>37</sup> have been recently developed to identify antibodies to variola, vaccinia and monkeypox viruses, but they are not easy to apply to animal sera. Recently, more comprehensive studies of various animal species have begun in Zaire to measure the prevalence of orthopoxviruses, and monkeypox virus in particular, by serological and virological testing.

#### Whitepox Virus and Possible Animal Reservoirs of Variola Virus

##### Whitepox virus

Although attempts so far have failed to isolate monkeypox virus from animals captured near human monkeypox cases, four virus strains, termed "whitepox" virus, were isolated in Moscow on CAM infected with aliquots of organs of animals captured in the wild. These "wild whitepox" strains came from kidney tissues of one chimpanzee, one monkey and two rodents captured in the forest in Zaire between 1971 and 1975.<sup>38,39,40</sup> Orthopoxvirus antibodies were present in the sera of three of the four animals and the virus was re-isolated from the tissues of two animals. Two strains of whitepox virus had been isolated previously. Twice in a period of two weeks in September 1964, the virus was recovered from the kidney tissue of apparently healthy cynomolgus monkeys shipped from Malaysia to a laboratory in Utrecht.<sup>41</sup>



Whitepox virus is indistinguishable from variola virus by all laboratory tests including DNA analysis with restriction endonucleases, although whether these strains could infect humans is unknown. If it is assumed that these are naturally occurring variola virus strains one would expect to observe apparently "spontaneous" outbreaks of smallpox in these areas which could not be traced to prior known human infections. Although the surveillance system in Zaire and elsewhere in west and central Africa should be sensitive enough to detect such cases (since 50 human monkeypox cases have been detected, mostly from remote small villages) no infections with a variola-like virus have occurred in Zaire during the nine-year period since the last smallpox case was recorded, or in other ecologically similar areas where smallpox eradication has been achieved. The apparently contradictory evidence must be weighed with the knowledge that variola virus was being handled in both laboratories where the isolations were made at about the time that the whitepox virus strains were isolated. It is impossible in either case to rule out the possibility that the isolates were contaminants. Nevertheless, the observations are such as to merit continued laboratory and field investigations and these are in progress.

#### White variants from monkeypox virus

Monkeypox virus produces haemorrhagic pox on CAM of embryonated chickens. Mutants, which do not produce haemorrhages but are white, and so resemble variola, do occur. Contradictory observations have been reported on the properties of white pox variants of monkeypox virus. One group has reported that five white variant isolates which they had characterized were indistinguishable from variola virus in their biological properties,<sup>42,43</sup> and this resemblance was confirmed by restriction endonuclease analysis of their genomes. Other workers, after careful follow-up study of these findings, have found that all of 21 white variants from monkeypox virus can be distinguished from variola virus in its biological properties or by DNA analysis (J. J. Esposito, J. F. Obijeski, J. H. Nakano, personal communication, 1980; K. R. Dumbell, personal communication, 1980). These white pox variants were a heterogeneous group. Bearing in mind the distinctive nature of the genome maps of different species of orthopoxvirus<sup>19,20</sup> (including variola, vaccinia and monkeypox viruses), it would appear highly unlikely that variola virus could have been derived from monkeypox virus by one or even several sequential mutational steps. Further, DNA analysis of two stock strains of "monkeypox virus" from which the first group recovered variola-like white variants showed that there were fragments of variola DNA in the digested products (J. J. Esposito, J. F. Obijeski, J. H. Nakano, personal communication, 1980).

Laboratory evaluation and epidemiological evidence from the field do not support the presence of an animal reservoir for smallpox;<sup>44</sup> but, like other negative propositions, this one is impossible to disprove.

#### Surveillance and Vaccination

Although the global smallpox eradication programme has now concluded, surveillance for poxvirus infections will continue, especially where human monkeypox cases and whitepox viruses have been found. Special surveillance began in Zaire in 1971 to define further the epidemiology and ecology of monkeypox and other animal poxviruses. A WHO programme devoted to monkeypox surveillance in a wide area of west and central Africa will continue at least until the end of 1985. The WHO international rumour register of suspected cases of smallpox will be maintained and all suspected cases will be promptly investigated.

The capability for laboratory diagnosis of poxvirus infections will be maintained by WHO collaborating laboratories. Further research, involving the comparison of variola viruses and monkeypox viruses, the possible differentiation of variola and whitepox viruses and the preparation of species-specific monoclonal antibodies for variola and monkeypox viruses, is of importance and will be promoted by WHO. The research and surveillance programme will be appraised periodically over the next few years.

Complications associated with smallpox vaccination are infrequent but do occur, and death following post-vaccinal encephalitis occurs about once in one million primary vaccinations.<sup>45</sup> Now that worldwide eradication of smallpox has been accepted by the World Health Assembly, smallpox vaccination is justified only for investigators at special risk. Hence, the Global Commission and WHO have recommended the cessation of smallpox vaccination (except for investigators) and discontinuation of requests from travellers for international vaccination certificates for smallpox. This recommendation also applies to areas where human monkeypox has occurred, as even here the overall current risk of vaccination complications appears greater than the protection afforded against monkeypox.

To provide protection against presently unforeseen problems, WHO will ensure retention of vaccinia seed strains and a supply of freeze-dried smallpox vaccine sufficient to vaccinate 200 million persons. A number of countries have reported that they plan to maintain vaccinia seed strains and national stockpiles of smallpox vaccine.

As of 30 May 1980, 73 countries have stopped routine vaccination and all but four of the 200 countries and areas of the world have stopped requesting an international certificate of smallpox vaccination from travellers.<sup>46</sup>

### Conclusions

The intensified WHO smallpox eradication programme, which began in 1967, reached its goal in 1977 when the last case of endemic smallpox occurred in Somalia. From 1973 to 1979 certification activities confirmed that smallpox no longer exists in countries where it was recently endemic or in those at risk of importation of cases. Independent international commissions and the Global Commission certified 79 countries free from smallpox. In 1979, the Global Commission, after review of all scientific data, confirmed that smallpox eradication has been achieved. The Thirty-third World Health Assembly, in 1980, voted unanimously to accept the Global Commission report and recommendations. Laboratories remain the sole source of variola virus and adequate security must be maintained to prevent its escape into an unprotected population. If such an escape did occur, however, the outbreak should be able to be rapidly controlled by application of vigorous case finding and containment methods used successfully during the global programme. Although human monkeypox resembles smallpox clinically it does not spread easily from person-to-person and is considered to constitute no threat. There is no evidence of an animal reservoir of smallpox virus. Field and laboratory studies of monkeypox virus and other poxviruses are continuing, however, as further assurance.

For the first time, man has deliberately set out to eradicate a disease and has succeeded. WHO is now undertaking a complete review of smallpox and its eradication. Part of this review will include an analysis of the smallpox experience as it might relate to the control and eradication of other diseases.

TABLE 1  
RUMOURS OF SMALLPOX REPORTED TO WHO: JANUARY 1978-APRIL 1980

WHO Region where smallpox claimed to exist	Number of countries	Number of rumours	Final diagnosis					
			Smallpox or healed smallpox	Monkeypox	Chickenpox	Measles	Other skin diseases	Erroneous report
Americas	9	10	-	-	6	-	-	4
Africa	16	31	-	1	6	2	7	15
Eastern Mediterranean	8	16	1 <sup>a</sup>	-	3	-	3	9
Europe	7	10	2 <sup>b</sup>	-	4	1	2	1
South-East Asia	7	48	2 <sup>a</sup>	-	20	10	9	7
Western Pacific	2	6	-	-	3	-	-	3
Total	49	121	5	1	42	13	21	39

<sup>a</sup> Disease occurred prior to 1977.

<sup>b</sup> Laboratory associated cases in the United Kingdom, 1978.

TABLE 2  
LABORATORIES RETAINING VARIOLA VIRUS (28 MAY 1980)

Laboratory	Location
1. Center for Disease Control <sup>a</sup>	Atlanta, Georgia, USA
2. Institute for Control of Drugs and Biological Products	Beijing, China
3. National Institute of Virology	Sandringham, South Africa
4. Research Institute of Virus Preparations <sup>a</sup>	Moscow, USSR
5. Rijks Instituut voor de Volksgezondheid <sup>a</sup>	Bilthoven, Netherlands
6. Centre for Applied Microbiology and Research <sup>a</sup>	Porton Down, Salisbury, UK

<sup>a</sup> WHO Collaborating Centre.

TABLE 3  
SUMMARY OF INFORMATION FROM SPECIMENS OBTAINED FROM VARIOLATORS

Country	Laboratory results				Year collected	Interval from time materials taken from patients to when tested
	Number tested	EM positive for poxvirus particles	Agar gel positive for poxvirus antigen	Variola virus isolated		
Afghanistan	4	ND	ND	4	1969-1970	4 months and 9 months <sup>c</sup> 6-10 years <sup>c</sup>
	5	4	4	0	1972-1976	
Ethiopia	1	ND	ND	0	1974	Unknown <sup>d</sup> 3-7 years <sup>d</sup>
	24	1	1	0	1976-1979	
Pakistan	1	ND	ND	0 <sup>b</sup>	1975	1 year 2-8 years <sup>c</sup>
	10	9 <sup>a</sup>	7	0 <sup>b</sup>	1975-1976	
Total	45	14/39	12/39	4/45		

EM - electron microscopic examination

ND - not done

<sup>a</sup> one other specimen showed herpes-varicella virus.

<sup>b</sup> one specimen grew vaccinia virus.

<sup>c</sup> interval unknown for 2 specimens.

<sup>d</sup> interval unknown for 20 specimens.

TABLE 4  
BIOLOGICAL AND CHEMICAL CHARACTERS OF SOME ORTHOPOXVIRUSES

Characters	Variola	"Whitepox" <sup>a</sup>	Monkeypox	Monkeypox white pock mutants <sup>a</sup>	Vaccinia
Isolated from	Man	Ape, monkey, rodent	Man, monkey anteater	Laboratory	Man; for vaccine production, origin unknown
Pocks on CAM <sup>b</sup>	small, white	small, white	small, pink	small, white	large, white to grey
Ceiling temperature on CAM	37.5-38.5°C	38.5°C	39.5°C	38.5-39.5°C	41.0°C
Growth on PEK cells <sup>c</sup>	+	+	-	-	- or +
Growth in rabbit skin	-	-	++	- to ++	+ or ++
Pathogenicity for baby mice	low	low	high	ND <sup>d</sup>	high
Antigens specific to:	vaccinia	-	-	-	+
variola	+	+	-	-	+
monkeypox	-	-	+	+	-
Polypeptide pattern:	vaccinia	-	-	-	+
variola	-	-	+	+	-
monkeypox	-	-	+	+	-
DNA pattern	vaccinia	-	-	-	+
variola	+	+	-	-	-
monkeypox	-	-	+	+	-

<sup>a</sup> Monkeypox virus white pock variants isolated in four different laboratories have the characters indicated (R. Gispen & B. Brand-Saathof 129:289-295; L. Harper, H. S. Bedson & A. Buchan 1979; 93:435-444; J. J. Esposito, J. F. Obijeski & J. H. Nakano, 1980, personal communication) but one group of scientists<sup>42,43</sup> has reported that all the white pock variants have the same biological characters as "whitepox" virus.

<sup>b</sup> CAM- chorioallantoic membrane.

<sup>c</sup> PEK - pig embryo kidney.

<sup>d</sup> ND - not done



FIG. 2 SMALLPOX REPORTED IN THE WORLD, COUNTRIES, 1958-1979

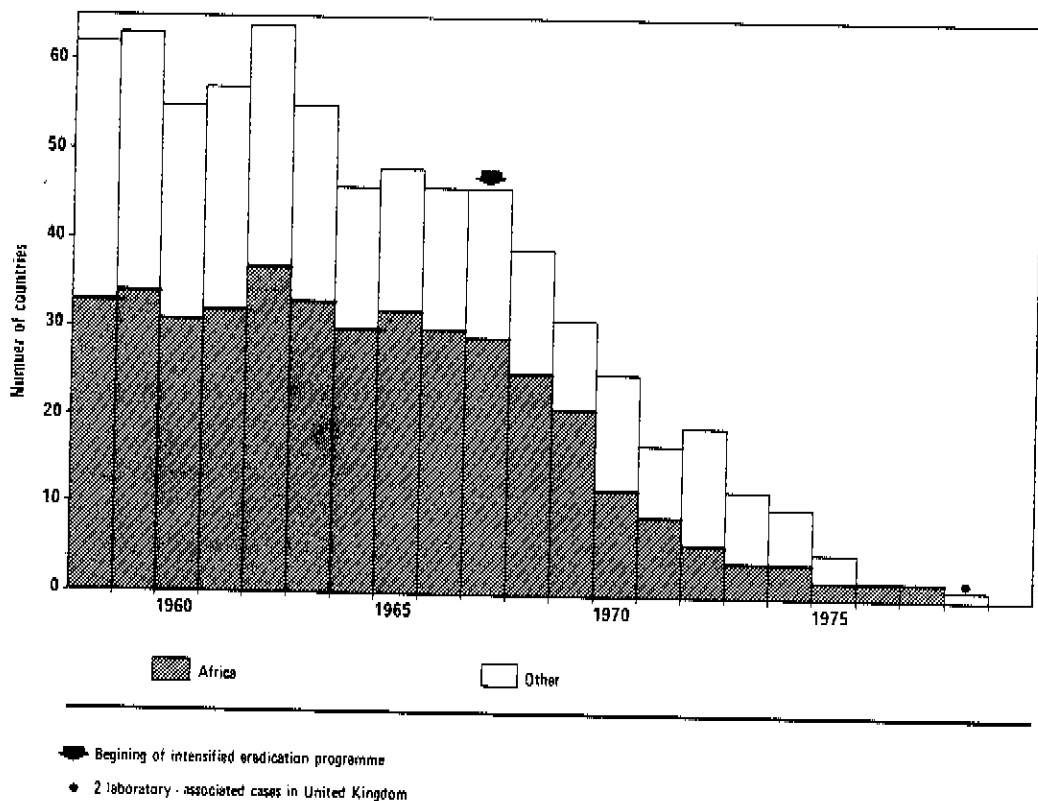


FIG. 3 SMALLPOX REPORTED IN THE WORLD, CASES, 1958-1979

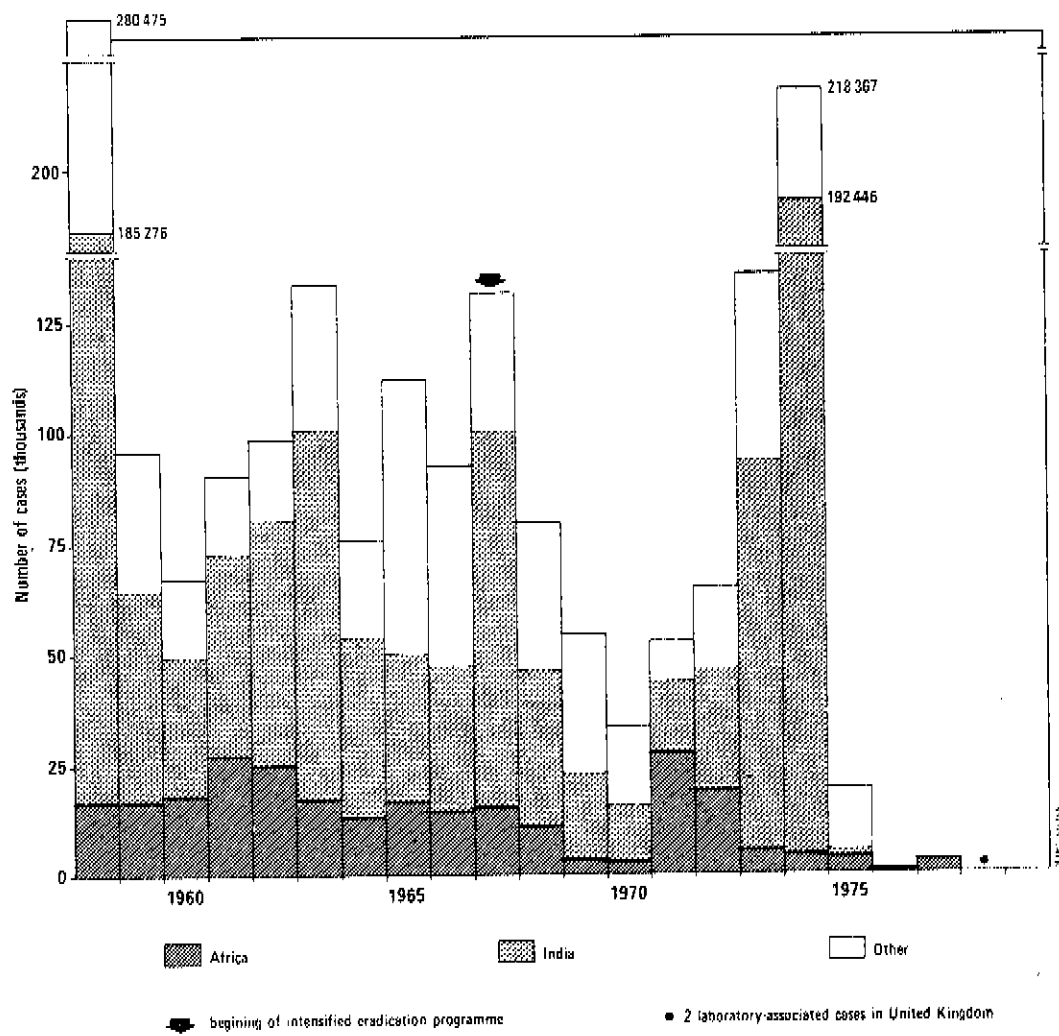




FIG. 4 SPECIMENS TESTED FOR VARIOLA VIRUS BY WHO COLLABORATING CENTRES, 1967-1979

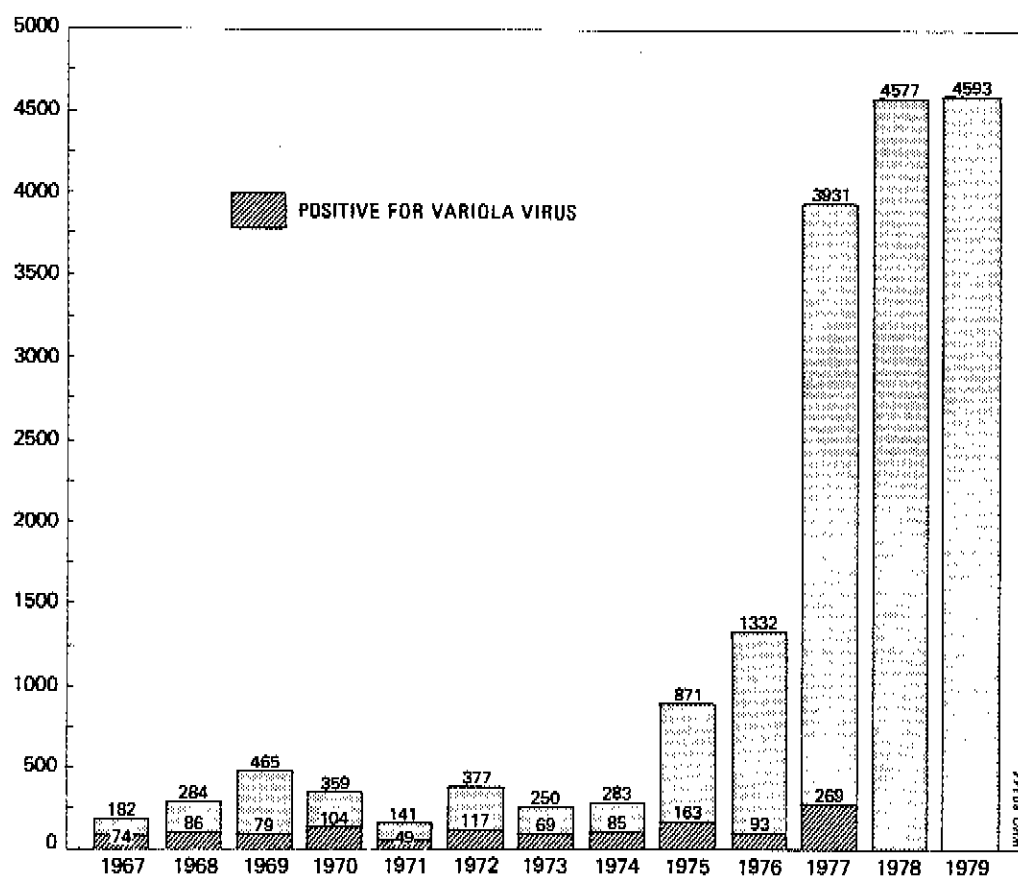




FIG. 6

79 COUNTRIES WHICH REQUIRED SPECIAL PROCEDURES FOR THE CERTIFICATION OF SMALLPOX ERADICATION SHOWING YEAR OF LAST REPORTED SMALLPOX CASE AND YEAR OF CERTIFICATION

	Year of last case*	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979
1 ARGENTINA		•			•									
2 BOLIVIA														
3 BRAZIL	1964													
4 CHILE	1963													
5 COLOMBIA	1968													
6 ECUADOR	1964													
7 FRENCH GUIANA			•											
8 GUYANA	1951													
9 PARAGUAY	1968													
10 PERU	1966													
11 SURINAME	1920													
12 URUGUAY			•	•										
13 VENEZUELA	1962													
14 INDONESIA														
15 BENIN														
16 GAMBIA	1966													
17 GHANA														
18 GUINEA														
19 GUINEA-BISSAU	1962													
20 IVORY COAST		•												
21 LIBERIA														
22 MALI														
23 MAURITANIA	1968													
24 NIGER														
25 NIGERIA														
26 SENEGAL		•												
27 SIERRA LEONE														
28 TOGO														
29 UPPER VOLTA														
30 AFGHANISTAN								•						
31 PAKISTAN														
32 NEPAL														
33 BHUTAN		•	•					•	•					
34 INDIA														
35 BURUNDI														
36 CENTRAL AFRICAN REPUBLIC	1963													
37 CHAD		•	•											
38 CONGO	1965													
39 EQUATORIAL GUINEA	1960													
40 GABON	1965													
41 RWANDA														
42 UNITED REPUBLIC OF CAMEROON														
43 ZAIRE														
44 BURMA		•	•	•										
45 BANGLADESH														
46 MALAWI														
47 MOZAMBIQUE														
48 UNITED REP. OF TANZANIA														
49 ZAMBIA					•									
50 UGANDA								•	•					
51 SUDAN														
52 BAHRAIN	1957													
53 IRAN								•	•					
54 KUWAIT		•												
55 LAO PEOPLE'S DEM. REP.	1963													
56 NAMIBIA	1965													
57 OMAN	1962													
58 QATAR	1961													
59 SAUDI ARABIA														
60 SOUTHERN RHODESIA														
61 SYRIAN ARAB REPUBLIC														
62 THAILAND	1962													
63 UNITED ARAB EMIRATES		•	•											
64 VIETNAM	1962													
65 IRAQ														
66 SOUTH AFRICA														
67 MADAGASCAR	1934													
68 ANGOLA	1968													
69 BOTSWANA		•												
70 LESOTHO		•												
71 SWAZILAND														
72 DEMOCRATIC YEMEN	1961		•											
73 YEMEN														
74 DJIBOUTI														
75 ETHIOPIA								•	•	•				
76 KENYA														
77 SOMALIA														
78 CHINA	1960													
79 DEMOCRATIC KAMPUCHEA	1959													

\* If occurred before 1967

 Considered endemic and cases reported  
 Considered endemic but no cases reported  
 Cases considered to be importations  
 Year of certification of smallpox eradication

WHO 80.156

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