



SMALLPOX under Control



Throughout the history of humanity, we have managed to eradicate only one viral infection that took hundreds of millions of lives, i. e., smallpox. This event of the 20th century clearly demonstrated what the international community is capable of when it joins forces in pursuit of a noble goal. It is worth noting that the campaign for mass vaccination against smallpox was initiated by the Soviet Union in the times of global confrontation between the blocs of states with different social and economic systems. Nevertheless, the campaign was a success. But the fight against smallpox and smallpox-like diseases did not stop. We had to gain control over the deadly pathogen and protect ourselves from it, which is why we required cutting-edge molecular genetic methods to study not only the smallpox virus but also its potentially dangerous “relatives,” i. e., orthopoxviruses. This task was solved through international cooperation, which has now acquired a scientific dimension. One of the main roles in this joint effort belonged to specialists from the VECTOR State Research Center of Virology and Biotechnology. In those days, a motto was popular among Siberian scientists: “For the first time in the world; for the second time – in Siberia.” The author and his colleagues became the world’s pioneers in deciphering the entire genomes of both the smallpox (variola) virus and other orthopoxvirus pathogens obtained from sick people. This knowledge laid the foundation for the development of modern test systems for rapid diagnosis of these infections and the production of the world’s first new-generation preventive smallpox vaccine, which is already being used in practice

On the left is a smallpox patient with characteristic skin manifestations. Photo from Svetlana S. Marennikova’s archive

Key words: smallpox, variola virus, cowpox virus, monkeypox virus, vaccinia virus, orthopoxviruses, genome sequencing, vaccination, SRC VB VECTOR

Smallpox (or *variola*) belongs to the class of especially dangerous infectious diseases of humans. In the past, smallpox epidemics took the lives of up to 30–40% of those infected; many of the survivors went blind. In the 20th century alone, in less than 80 years (before the end of mass vaccination against smallpox), at least 300 million people died from this infection!

In 1958, at the 11th session of the World Health Assembly, the USSR delegation initiated a campaign for the complete eradication of smallpox. The program for the global elimination of this disease, which operated under the auspices of the WHO, was successfully completed by 1978 through mass vaccination and strict anti-epidemic surveillance.

Thus, smallpox, which is often called the scourge of humanity, became not only the first infection against which we invented a vaccine but also the first one we managed to defeat. So far, this victory remains the only example of global eradication of an especially dangerous infectious human disease by the world community.

This event was immensely important also because of what followed. When, on May 8, 1980, the 33rd World Health Assembly solemnly announced the victory of the peoples of the Earth over smallpox, all countries were strongly advised to stop smallpox vaccination. As a result, in almost half a century that passed since then, a huge part of humanity (primarily those under 45 years of age) were left without immune protection not only from smallpox itself but also from other smallpox-like infections, whose pathogens remain “dormant” in nature and are, in principle, capable of infecting humans.

Can smallpox make a comeback?

A distinctive feature of the *smallpox virus* (or *variola virus* as it is usually called) is that it reproduces in the human body only. But there are other, closely related viruses, such as *monkeypox* and *cowpox viruses*, which act as causative agents of the so-called *natural focal infections* and whose reservoirs, i.e., the main habitats and hosts, are various species of wild animals. From these carriers, directly or through domestic animals, monkeypox and cowpox viruses can spread to humans.

Of greatest concern for specialists has always been the monkeypox virus. When infecting humans, it caused the development of an infection with clinical manifestations reminiscent of smallpox with a mortality rate as high as 10% during outbreaks in Central Africa. However, for the time being, this infection occurred only in the tropical rainforests of Africa and spread from person to person with very low efficiency.

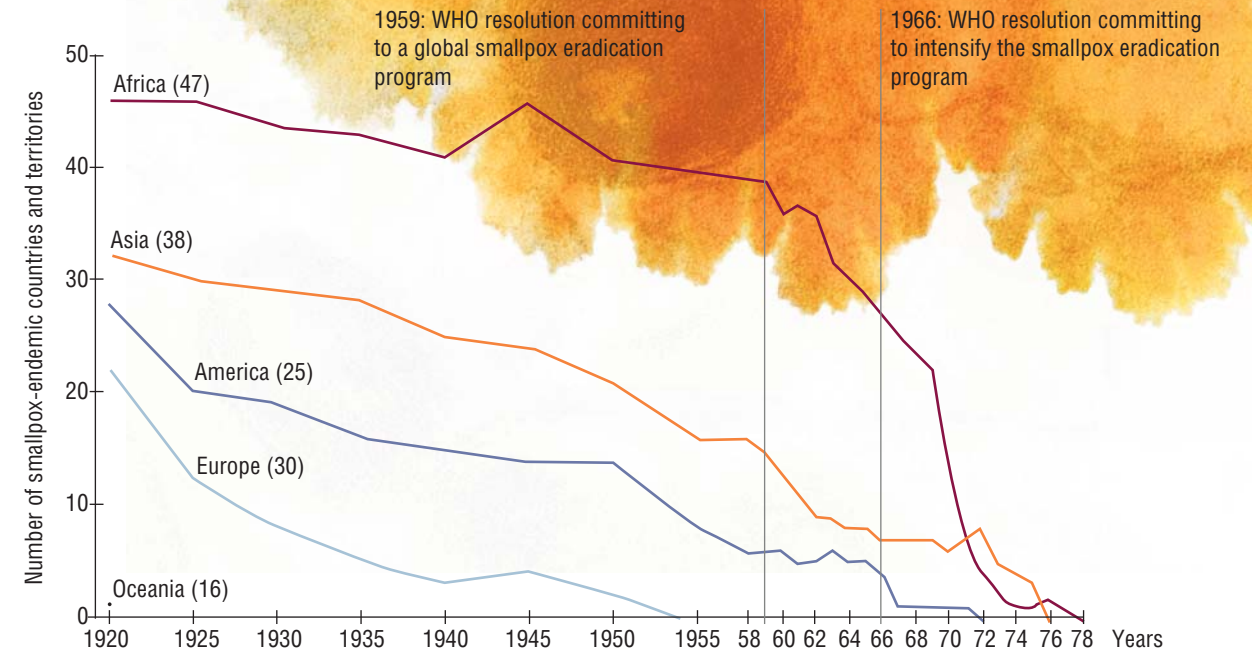
All this gave hope that the monkeypox virus would not reach beyond the African continent. However, evolution



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The smallpox virus belongs to the genus *Orthopoxvirus* of the family *Poxviridae*, i.e., the most large-sized and complex DNA-containing mammalian viruses with a complicated reproductive cycle. In the early stages of infection, they synthesize dozens of different proteins that suppress the activity of the key regulatory proteins of the host's innate and adaptive immunity. The genus *Orthopoxvirus* includes such species as *variola* (smallpox), *cowpox*, *monkeypox*, and *vaccinia viruses*, the latter being the characteristic virus of the genus

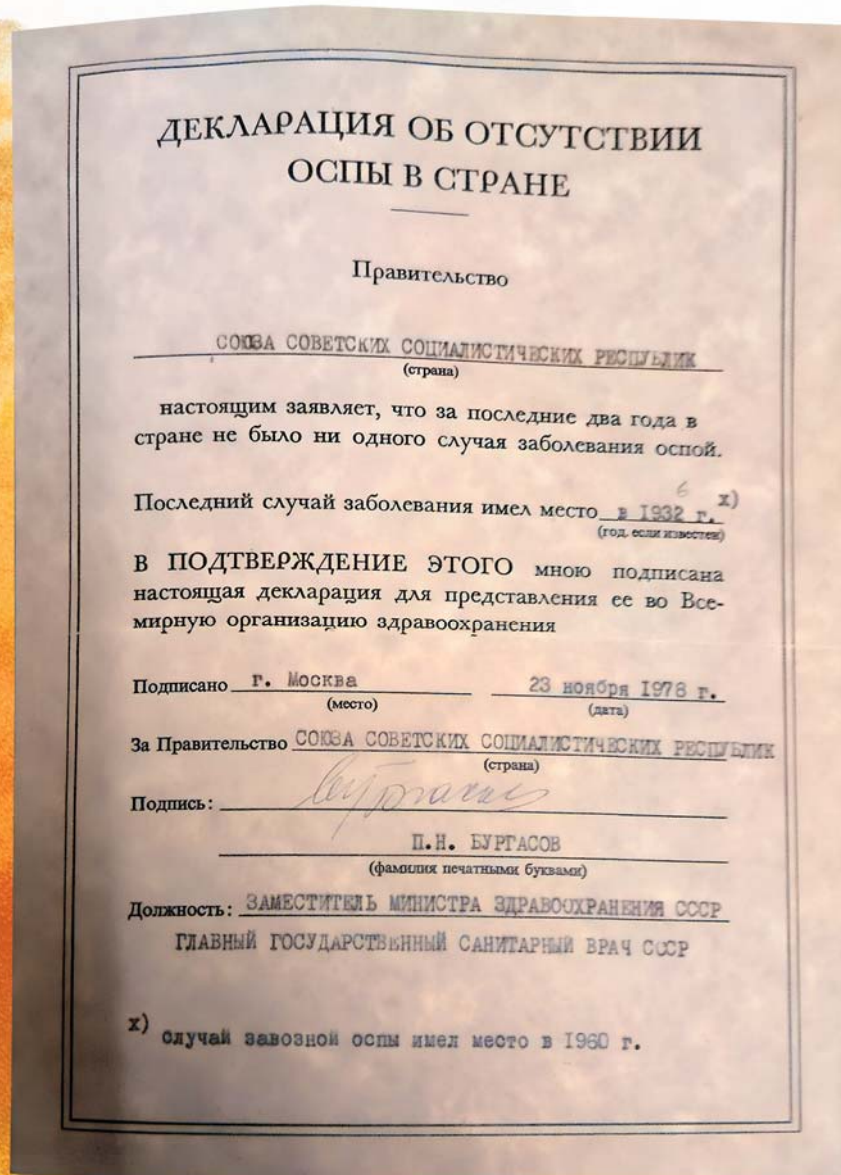
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Epidemiological situation for smallpox in 1920–1978 on different continents where the disease remained endemic. The numbers in brackets indicate the number of countries and territories included in the study. Adapted from: (Fenner et al., 1988)

Vaccination during a smallpox epidemic in Palestine. Photo album, c. a. 1922. Public Domain Mark/Wellcome Collection





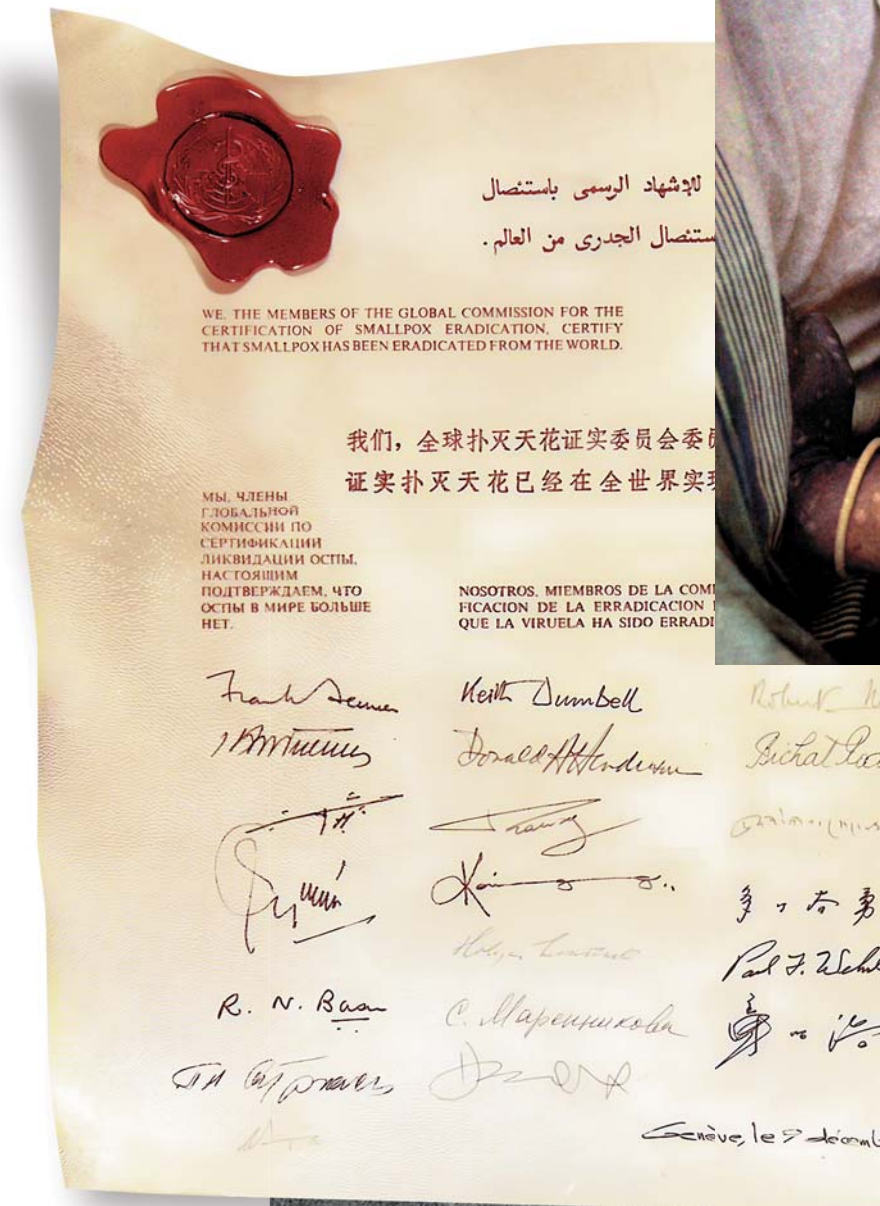
The last outbreak of smallpox in the Soviet Union occurred in Moscow in 1960. The pathogen was brought in from India at the end of December 1959. A total of 46 people were infected; three of them died. The last case of the disease was recorded on February 3, 1960. The period from the time of bringing the infection into the country till the end of the epidemic was 44 days, and that from the beginning of the emergency response was 19 days. In less than two weeks in January 1960, over 8 million people were vaccinated in Moscow and the Moscow region, which was an unprecedented campaign both in scale and time

Document certifying that the USSR was smallpox-free, from the WHO archive in Geneva.
Photo by the author

One of them was located in the Soviet Union, at the Research Institute of Viral Preparations (Moscow), and the other in the United States, with the Centers for Disease Control and Prevention (CDC, Atlanta).

However, even the presence of two WHO-controlled repositories of viable smallpox virus strains was considered a possible biological threat. Therefore, in March 1986, at a meeting of the WHO Ad hoc (Special) Committee on Orthopoxvirus Infections, it was decided to preserve information about the genome of this virus in a biologically safe form. To this end, it was proposed to clone fragments of the viral genome in bacterial plasmids, i. e., small autonomous circular DNA molecules, which are incapable of existing outside the cell. The collections of the virus itself were to be destroyed at a later date.

Destroy and forget. Nothing could possibly be simpler, couldn't it? But one could not be absolutely sure that the disease would not return. Smallpox was eradicated before the rapid development of molecular biology and experimental virology, so its pathogen, as well as its close "relatives," remained poorly understood. To get prepared for the possible return of this especially dangerous infection, one had to carry out a large complex of scientific studies using the latest methods.



This three-year-old girl, Rahima Banu, from Bhola Island in Bangladesh, is believed to be the world's last known case of smallpox major (*variola major*) with a mortality rate of 5–40%. She has a maculopapular rash on her skin, typical of the disease. 1975. Public Domain/ CDC/ World Health Organization; Stanley O. Foster

"We, the members of the Global Commission for the Certification of Smallpox Eradication, declare the world free of smallpox."
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goes by its own rules and patterns. Contacts between different countries and continents are becoming increasingly intense, so the likelihood of emergence of a new, more deadly virus, easily transmitted from person to person, is not zero.

The opportunity to lift the curtain on the evolutionary patterns of orthopoxviruses arose due to advances in molecular biology and the development of new molecular genetic research methods. It became clear that in order to gain control over infectious diseases caused by these pathogens, scientists ought to develop effective methods of express diagnostics and forge new-generation preventive measures.

One also had to prevent an accidental leakage of the smallpox virus from laboratories that kept the samples. In 1975, 75 such laboratories existed in different countries, but by 1981, their number dropped to four (in the Soviet Union, United States, United Kingdom, and South Africa).

By 1984, only two such laboratories remained worldwide, which had received the status of WHO Collaborating Centers for Smallpox and Related Infections.

VECTOR: from vaccinia to smallpox virus

First and foremost, one had to ensure reliable and biologically safe conservation of genetic material for different isolates of the smallpox pathogen. Furthermore, the WHO committee also considered it a necessary and urgent task to decipher the nucleotide sequence (i.e., carry out the sequencing) and analyze the genome organization of this unique virus.

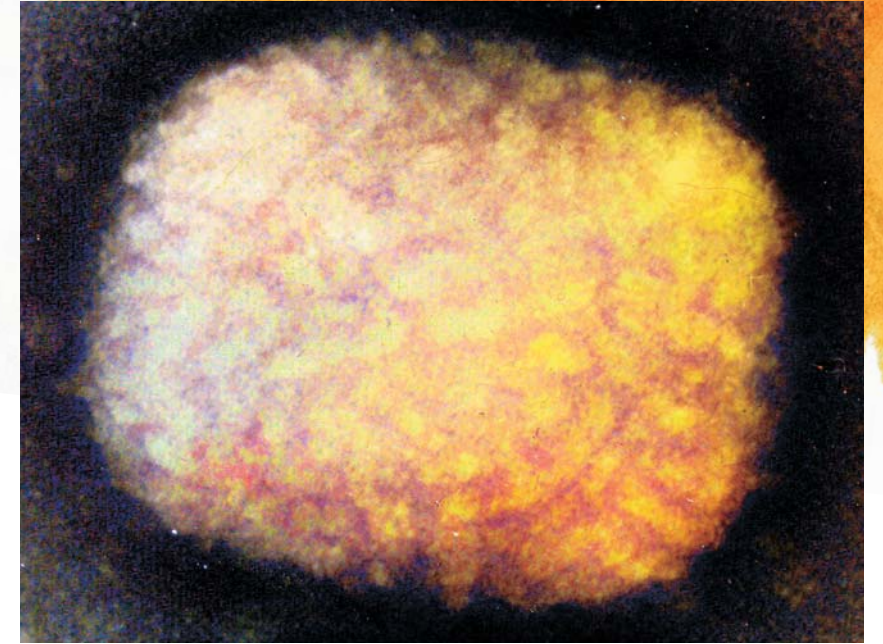
The All-Union Research Institute of Molecular Biology (the core institute of what would subsequently become first the NPO Vector and then the VECTOR State Research Center of Virology and Biotechnology) launched the first research efforts related to the molecular biological study of orthopoxviruses in the first half of the 1980s.

In those years, one of the tasks set for Siberian virologists was to obtain a *subunit protein vaccine* against orthopoxvirus infections. These vaccines, which are essentially a mixture of purified proteins of viral particles, stand out for their stability and provide greater safety as they cause fewer undesirable side effects for the human body.



Smallpox (variola) virus virions are large in size, so after special staining, one can see them in a cell using a light microscope, which is impossible for other viruses. The photo shows mature smallpox virions of the India-3a strain in cell cultures. Electron transmission microscopy. Photo by E. Ryabchikova. Adapted from: (Shchelkunov, 2012)

Transmission electron micrograph of a smallpox virus particle. 1974. © CC BY-SA 4.0 / Dr Graham Beards



Smallpox vaccines had long been prepared using the so-called *vaccinia virus*, whose origin remains unknown. The particle of this virus (*virion*) contains about a hundred different proteins. Therefore, it was a very complicated task to make a vaccine containing, instead of live viruses, their immunologically active proteins, considering that scientists did not know which of the genes encoded the proteins.

The virology department was producing samples of purified vaccinia virus in preparative quantities; then the protein department was “disassembling” the virions into individual proteins in an attempt to make vaccine preparations from different protein combinations. This “classical” approach demanded too much time and effort and yielded too little effect. However, in the course of this work, researchers obtained preparations of antibodies against several individual virion proteins, and these antibodies enabled the mapping of the corresponding genes in the viral genome.

At about the same time, the department I headed began cloning fragments of the vaccinia virus genome in bacterial plasmids and mapping the genes that encoded individual viral proteins. Then the genes were sequenced, and the data obtained were analyzed using special computer programs to determine the amino acid sequence of the proteins.

In 1990, VECTOR received a visit from Prof. Svetlana S. Marennikova, head of the WHO Collaborating Centre for Smallpox and Related Infections in Moscow, and we learned that our colleagues in Moscow possessed a variety of vaccinia strains and a unique collection of cowpox virus isolates. The first outcome of this meeting was an agreement on cooperation in studying the genome organization of these viruses.

The second outcome concerned a more ambitious project. It was planned to discuss a program for sequencing the smallpox virus genome at a meeting of the WHO *Ad hoc* Committee on Orthopoxvirus Infections in December 1990. The Moscow laboratory had in its possession one of the world’s two official collections of smallpox virus strains. We, in turn, had experience in obtaining bacterial plasmids containing fragments of the orthopoxvirus genome, and we also had a group of researchers proficient in DNA sequencing methods. The stars aligned, and the National Program for the Conservation of Genetic Material of the National Collection of Smallpox Virus Strains was born, which was presented and approved at that meeting of the WHO Special Committee.

At the same meeting, a national program was approved for research into the genome of the highly virulent Bangladesh 1975 smallpox virus strain, which was proposed by the United States, and a WHO Technical Committee was established to evaluate the results of all the sequencing projects.

Siberians: the first to cross the finish line

Thus, for the first time, we were faced with the task of sequencing a complete viral genome of about 200,000 nucleotide pairs. In those years, it was a fairly complicated task since we used to decode nucleotide sequences manually, mostly by the Maxam–Gilbert sequencing. This method, which is based on chemical degradation of DNA, required the use of radioactive isotopes as labels and had a relatively low productivity; i.e., we were able to decode a sequence no longer than 500 nucleotides in one experiment.



The smallpox virus genome is a linear double-stranded DNA molecule containing 187,000 nucleotide pairs. The molecule has covalently closed hairpin structures at both ends. The genome contains about 200 genes, a huge number compared with the majority of viruses of other families. These genes encode numerous structural virion proteins as well as enzymes necessary for virus reproduction in the host cell

Participants in the First Meeting of the WHO Technical Committee on the Analysis of Nucleotide Sequences of Variola Virus Genomes (the author is third from the left in the top row). December 11, 1992. CDC, Atlanta, USA. Photo from the author's archive

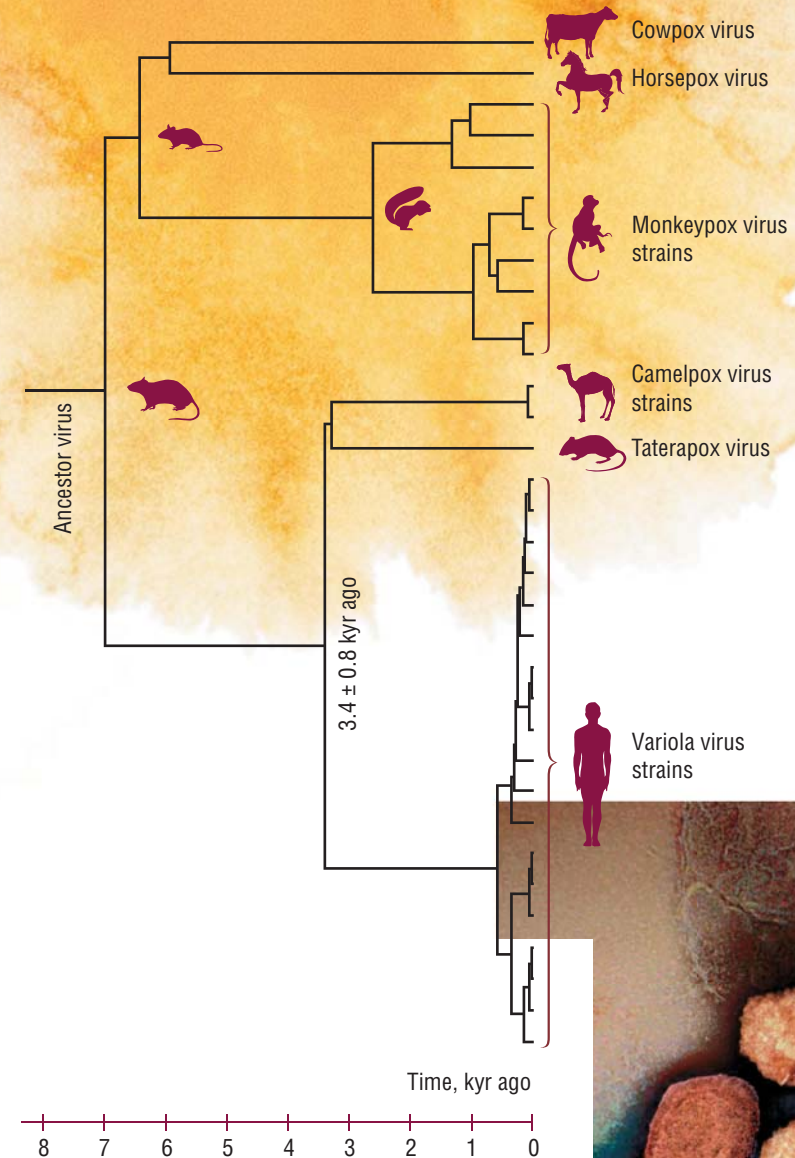
Nevertheless, through the painstaking efforts of all the program participants, we completed by the summer of 1992 the sequencing of the genome of the highly virulent India-1967 strain of the smallpox virus from the Moscow collection.

The results of this work were first presented in September of the same year at the International Conference on Poxviruses and Iridoviruses in Switzerland and then at the International Conference "100 Years of Virology" in Leningrad (now St. Petersburg). The report on the achievements of Siberian virologists made a great impression on one of the organizers of the Leningrad conference Dr. Brian Mahy, director of the CDC Division of Viral and Rickettsial Diseases, who actually headed the American program for sequencing the smallpox virus genome. As a result, after discussing the report, an idea emerged to organize a joint Russian–American project to sequence the DNA of another smallpox virus strain, i.e., the weakly virulent Garcia-1966, which was isolated by the Americans from a patient in Brazil.

When the first meeting of the WHO Technical Committee on the Analysis of Nucleotide Sequences of Variola Virus Genomes was held in Atlanta in December 1992, it turned out that the Russian group was the only one to have completed deciphering the entire coding DNA sequence for its strain.

At the meeting, the Committee recommended additional sequencing of the entire genome of the Garcia-1966 strain, emphasizing the need to find ways to financially support the work. As a result, NPO Vector was awarded a WHO grant to sequence 2/3 of the viral genome, and the rest of the work was to be done by the Americans. This was the first international grant received by our organization, and this support was very important since the financial state of Russian science was literally deplorable at that time.

We did our part of the work in the joint project by the beginning of 1994. By that time, our American colleagues had finished sequencing their Bangladesh-1975 strain, and in 1995, they sequenced the remaining part of the genome of Garcia-1966. So the work on sequencing the entire genome of two highly virulent and one weakly virulent smallpox virus strains was done.



By analyzing the central conservative region of the genome, 101,000 nucleotide pairs in size, we were able to construct the phylogenetic tree of the variola (smallpox) virus and other orthopoxviruses and estimate the times of divergence of these species. Adapted from: (Babkin and Shchelkunov, 2008)

Micrograph of poxvirus particles obtained at the Comprehensive Research Center, National Institute of Allergy and Infectious Diseases, Fort Detrick, USA. Scanning electron microscopy. © CC BY 2.0/NIAID

In 1994, we also carried out a joint detailed analysis of the sequencing data for the strains under study. The results of this work were published in several papers in leading international scientific journals, where VECTOR was likely mentioned for the first time in its new status as a state scientific center.

In view of the potential danger of working with the live pathogen in Moscow, the collection of smallpox virus strains was transferred to VECTOR in late September 1994. In June 1997, following an inspection, the WHO officially registered the establishment of a VECTOR-based WHO Collaborating Centre for Orthopoxvirus Diagnosis and Repository for Variola Virus Strains and DNA.





Genetic dossier on pathogens

In addition to the smallpox virus, whose transmission in nature had been fully stopped by 1978, there also are zoonotic orthopoxvirus infections, as we said above. For example, the cowpox virus sporadically causes infection in humans, mostly not lethal. But as far as the more dangerous monkeypox virus is concerned, some experts believe that it could have been an evolutionary predecessor of the human smallpox virus.

It was only genome sequencing that could answer the question about the evolutionary relationships between the different types of human pathogenic orthopoxviruses. Therefore, on our own initiative, we began studying the gene structure of the GRI-90 cowpox virus strain isolated by Moscow scientists from a child who contracted the virus from a sick mole.

This study, which was supported by domestic grants, was completed in 1997; we managed to sequence the long terminal regions of the viral DNA, the ones that determined all the species-specific properties of the virus.

Thus, our group became the first one in the world to identify the main differences between the genome of the cowpox virus and those of its “relatives.” Based on the data obtained, we also predicted the functions of several genes absent in other species of orthopoxviruses. We also came to the conclusion that the cowpox virus had the most complete genome in its genus and could, therefore, have been the “progenitor” of the smallpox virus and other

Participants of the Second Meeting of the WHO Technical Committee on the Analysis of Nucleotide Sequences of Variola Virus Genomes: B. Moss, S. Shchelkunov, and J. Esposito. January 28, 1994. WHO, Geneva, Switzerland. *Photo from the author's archive*

smallpox-like infections in humans (Marennikova and Shchelkunov, 1998).

In December 1996, a Working Meeting on Emerging and Re-emerging Infectious Diseases took place in St. Petersburg (Russia). Its main goal was to coordinate the positions of Russian scientists and their potential collaborators from the US National Institutes of Health (NIH) to prepare applications for joint projects for a competition organized by the US Civilian Research and Development Foundation (CRDF). At this meeting, we reached an agreement with Dr. Bernard Moss, a recognized leader in orthopoxvirus research, and his colleague Joseph Esposito from the CDC on a joint project to sequence the genome of the monkeypox virus strain Zaire-96-I-16. This virus was isolated from a person in Zaire (now the Democratic Republic of the Congo) in 1996 during an unusually large outbreak of this disease in humans. In this regard, the question arose with renewed vigor whether the monkeypox virus could adapt to humans in the course of its evolution and become closer to the smallpox virus in terms of pathogenicity and the effectiveness of person-to-person transmission.

Pathogen species	Strain	Number of potential genes	Organization	Sequencing year
Vaccinia virus	Copenhagen	198	Virogenetics, USA	1990
Variola virus	India-1967	199	VECTOR, Russia	1992
Variola virus	Bangladesh-1975	196	CDC, США	1993
Variola virus	Garcia-1966	206	VECTOR, Russia, CDC, USA	1995
Cowpox virus	GRI-90	212	VECTOR, Russia	1997
Vaccinia virus	Ankara	157	Biomedical Research Center, Austria	1998
Monkeypox virus	Zaire-96-I-16	191	VECTOR, Russia	2001

The most obvious way to answer this question was to sequence the pathogen’s genome and compare it with the previously studied smallpox virus genomes. Due to the limited funding, it was decided to sequence only the left half of the virus genome within the two-year CRDF project.

Fortunately, at the end of 1996, our center was visited by a delegation from the US National Academy of Sciences, which proposed to consider small (in terms of time and funding) cooperation projects with US scientists with the prospect of funding through the International Science and Technology Center (ISTC). Our project, together with Joseph Esposito and Peter Jahrling (Fort Detrick), on sequencing and analyzing the right half of the monkeypox virus genome was supported.

Thus, in August 1997, we launched the ISTC/NAS project on the monkeypox virus genome and, two months later, the CRDF/NIH project on sequencing and analysis of the human pathogenic monkeypox virus genome. By 2001, the genome of the strain Zaire-96-I-16 was fully decoded. An in-depth computer analysis showed that the monkeypox virus was not capable of transforming into the smallpox virus although it could well become more pathogenic for humans.

Multidiagnostics: four in one

As a result of these works, VECTOR became the holder of the world’s only database of DNA nucleotide sequences for different species of human pathogenic orthopoxviruses. Many years of experience in comparative analysis of the organization of both viral genomes and individual genes allowed us to formulate a qualitatively new agenda for further scientific research and practical applications in diagnostics and vaccine prevention of orthopoxviruses and related infections (Shchelkunov *et al.*, 2005).

The first sequenced genomes of orthopoxviruses





One of these applications is the rapid and accurate identification of the pathogen in the first cases of a local infection outbreak, which is extremely important as it allows one to take timely measures and prevent an epidemic. Although orthopoxvirus infections have characteristic external manifestations in the form of skin lesions, experience shows that clinical diagnostics based on these symptoms may often be erroneous.

The development of the *polymerase chain reaction* (PCR) method, which allows detecting a pathogen in a sample even in trace amounts, led to the introduction of modern procedures for detecting and identifying microorganisms with high specificity and in a short time. Importantly, this method does not require manipulations with especially dangerous live infectious agents such as pathogenic orthopoxviruses.

Speaking about the PCR identification of these viruses, the greatest interest here lies with test systems that enable one to simultaneously detect not only the genus but also the species of the pathogen. Once we obtained information on the whole genomes of all human pathogenic orthopoxviruses, we managed to identify species-specific differences between them and became the world's pioneers in designing test systems based on the so-called *multiplex PCR*. This analysis allows for simultaneous identification of smallpox, monkeypox, cowpox, and vaccinia viruses in one sample (Gavrilova *et al.*, 2003; Shchelkunov *et al.*, 2011). These test systems were registered in Russia for medical use.

Owing to the rapid development of next-generation sequencing technologies, today we have the opportunity to quickly obtain information about the entire nucleotide sequence for the genome of an object under study. This is why an increasing number of scientific reports on new cases of orthopoxvirus infections are accompanied by data on the whole-genome sequencing of viral isolates. The results of these studies show the need for further improvement of laboratory diagnostics of these dangerous infections and for strict epidemiological surveillance.

Fourth-generation "immune armor"

It is still believed that the most reliable way to prevent any viral disease is to infect a person or animal with a low-infectious variant of the pathogen or a low-pathogenic closely related virus. Smallpox vaccinations became the first historical example of this kind of protection against an infectious disease. It is worth noting that the kingdom of viruses itself was discovered only a century after smallpox vaccinations were introduced into clinical practice.

Different species of orthopoxviruses are antigenically and immunologically close to one another; they demonstrate *cross-serological reactions* (i.e., interactions between the antigen and the corresponding specific antibody) and, as a result, immune protection. It is these properties that gave rise to the reliable method for protecting people from smallpox, first by vaccinations with cowpox and horsepox viruses and then with the vaccinia virus, which has high immunogenicity and yet produces less pronounced side effects during vaccination.

However, in rare cases, vaccinations with the vaccinia virus led to serious health consequences, including death, which is why the vaccination of the population was stopped after the global eradication of smallpox in 1980. As a result, zoonotic orthopoxviruses got an opportunity to circulate



During mass vaccination against smallpox, it was a common practice to use an injector gun, with which one could safely vaccinate about a thousand people per hour.
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in the human population, which, in turn, may have affected their ecology and the range of potential hosts.

Thus, due to a long-term absence of vaccinations and considerably more frequent infection of humans, the monkeypox virus may acquire properties characteristic of the smallpox virus. If this happens, humanity will be faced with a much grimmer problem than the eradication of smallpox, primarily because this pathogen has a natural reservoir in the numerous African rodents (Shchelkunova and Shchelkunov, 2023).

To prevent infection outbreaks from developing into widespread epidemics and thereby reduce the risk of emergence of a highly human pathogenic orthopoxvirus, it is necessary to develop a new-generation of safe live vaccines based on the vaccinia virus. These vaccines have no pronounced species-specificity and can be used to immunize humans and animals during outbreaks caused by any species of orthopoxviruses.

The *first-generation* live smallpox vaccine was essentially a preparation of the vaccinia virus obtained by propagating the virus on the skin of calves or other animals. Its use for mass vaccination is currently limited due to the growing number of people with immunodeficiencies (HIV-infected, transplant patients, etc.), which increases the risk of severe complications.

Nowadays, smallpox vaccine strains are obtained on mammalian cell cultures; these preparations are referred to as *second-generation* smallpox vaccines. The most studied and massively applied vaccine is ACAM2000, licensed for use in the United States in 2007. However, second-generation smallpox vaccines, too, may lead to serious side effects and have a limited application range.

Attenuated third-generation smallpox vaccines are obtained through multiple successive cultivations of a particular strain in animal cells, such as chicken or rabbit cells. The most studied third-generation vaccine, MVA, was

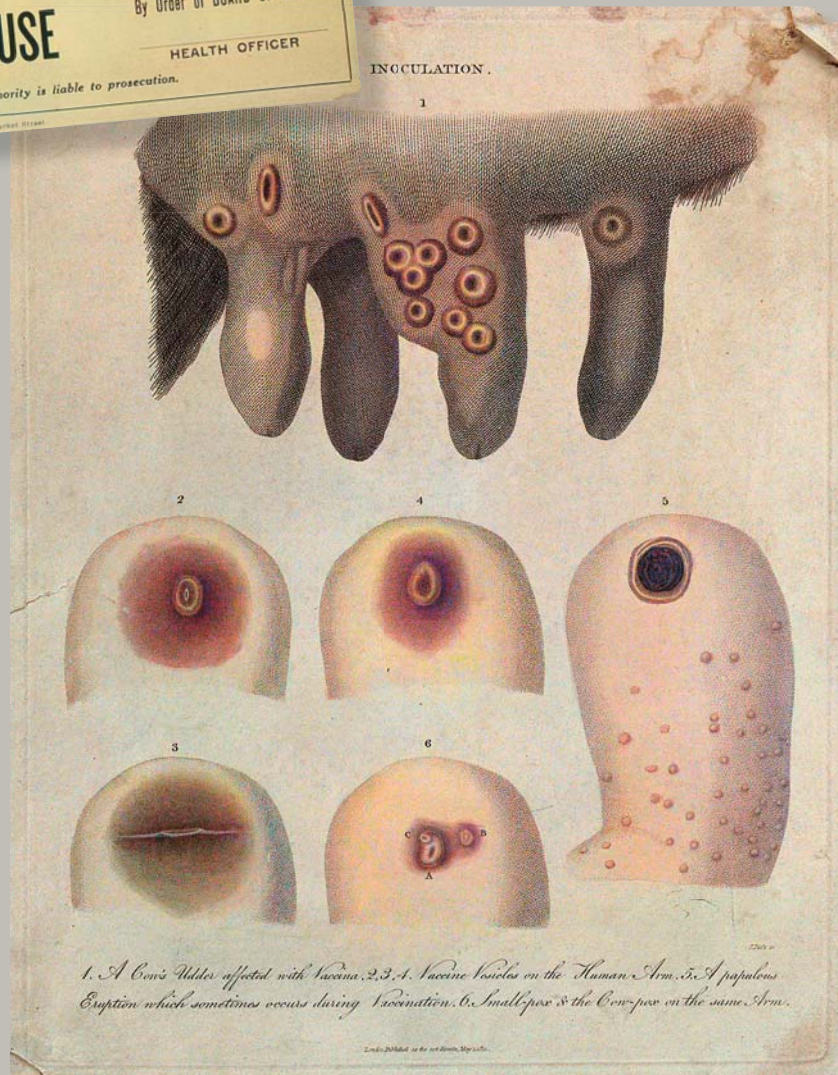


FROM VARIOLATION TO VACCINATION

A smallpox survivor is easily identified by characteristic scars (the so-called *pockmarked face*). It was long noticed that such people were protected from this disease during subsequent epidemics. These observations appear to have underpinned a procedure long practiced in India and China, when healthy people received material from the skin lesions of smallpox patients by rubbing it into cuts in their skin (usually on the arm). A person who recovered from the disease would develop a characteristic scar at the injection site, and he or she would become resistant to smallpox or tolerated it in a milder form. This method of protection against smallpox was called *variolation* (from the Latin word *'variola'*, meaning smallpox). However, it did not become widely popular since the mortality rate for this procedure reached 0.5–2%. However, against the background of smallpox epidemics with a mortality rate of 30–40%, this was an acceptable result.

The 18th century saw a revolution in protection against smallpox, made by a young British doctor, Edward Jenner. At that time, cattle and horses in rural England often fell ill with a disease called cow pox, which was accompanied by rashes on the mucous membranes and udders. People who had contact with sick animals became infected through abrasions or microcracks on their hands or arms, but they tolerated the disease relatively easily. In places where the infectious agent penetrated the skin,

the patients got a scar resembling that after variolation. Moreover, people with such scars did not fall ill during smallpox epidemics. Having summarized these observations, Jenner conducted several experiments on people, administering the contents drawn from pustules of pox-infected cows into cuts in the skin on their arms. After recovery and the formation of a skin scar, Jenner carried out variolation, i. e., infected the subjects with smallpox to prove that they had become immune to the infection.



This color engraving shows skin lesions characteristic of orthopoxvirus infections. At the top is a cow's udder with pustules typical of cowpox virus infection. Below is a human arm with cowpox and smallpox pustules. 1811. Engraving by J. Pass. Public Domain Mark/ Wellcome Collection

The first vaccination with cowpox virus taken from a sick person was made on May 14, 1796 to James Phipps, an eight-year-old boy, who developed resistance to smallpox infection as a result of the procedure. Subsequently, the grateful Jenner built a house for Phipps and even planted roses in his garden himself. Jenner named the material he used in his experiments in Latin as *variolae vaccinae* (which literally means 'cow pox'), and he called the corresponding method of protection against smallpox *vaccine inoculation*, which was later shortened to *vaccination*. In 1881, at the International Medical Congress in London, the famous French microbiologist Louis Pasteur proposed to use this term for all immunization procedures against any infectious agents. In the 20th century, the virus used for smallpox vaccinations, which was already different in several characteristics from the cowpox virus, was classified as a separate species, i. e., *Vaccinia virus*

obtained through 572 passages of the Ankara vaccinia strain in a culture of chicken *fibroblasts* (i. e., connective tissue cells). As a result, the genome of the MVA strain contained multiple mutations and extensive *deletions* (i. e., losses of DNA sections) that were not present in the original strain. The virus lost the ability to reproduce in most mammalian cells, including human cells.

Free smallpox vaccination in the grand foyer of the Parisian daily newspaper *Le Petit Journal*. 1905. France. © CC BY-ND 2.0 LME Press



The MVA-BN vaccine, produced by Bavarian Nordic (Denmark), passed numerous clinical trials, including on atopic dermatitis patients and HIV-infected individuals, and was licensed in the EU countries, Canada, and the United States. Initially, this vaccine was approved for immunization against smallpox and, subsequently, against monkeypox. MVA-BN is used for primary vaccination of patients with contraindications to first- and second-generation smallpox vaccines. The main disadvantage of this vaccine is its relatively low immunogenicity, which is why it has to be administered in high concentrations and at least twice.

A new approach to obtaining attenuated smallpox vaccines involves the use of genetic engineering methods to produce targeted deletions and insertions, which disrupt the genes that control the body's defenses against viral infection but do not affect the genes whose products are important for the virus to reproduce in the human body. However, the first well-studied variant of this vaccinia virus, i.e., the NYVAC strain, gave noticeably lower immunity against smallpox compared with the classic vaccine and thus found no practical application.



To determine the concentration (titer) of the vaccinia virus in a vaccine preparation obtained after multiplication in the African green monkey kidney cell culture, the preparation is successively diluted tenfold, each time applying a certain volume of the solution to the cell monolayer in the plate wells. The plates are then placed in an incubator thermostat. After 48 hours, in those places of the wells where the virus multiplies, one can see the emergence of the so-called plaques, which are stained and then counted. On the right are the results of the vaccinia virus titration in two repetitions. Photo from VECTOR's archive



Smallpox vaccine preparation is produced in cell culture using modern equipment, packaged in ampoules, and freeze-dried. Below is the facility for obtaining the vaccine in preparative quantities. Photo from VECTOR's archive



The Shchelkunovs, Sergei and Galina, at Edward Jenner's House Museum in Berkeley (UK). September 2004. Photo from the author's archive





OrthopoxVac live vaccine against orthopoxvirus infections, including smallpox, was created by Siberian virologists and licensed in Russia in 2022.

Photo from VECTOR's archive

In 2009, within the federal target program “The National System of Chemical and Biological Safety of the Russian Federation,” we began working to produce a safe *replicating* (i. e., multiplying in human tissues locally at the injection site) fourth-generation vaccine against smallpox and other orthopoxvirus infections (Yakubitskiy *et al.*, 2015).

As a result of sequential introduction of targeted deletions/insertions into six genes of the LIVP vaccinia strain, which serves as a basis for the Russian first-generation smallpox vaccine, we obtained the VACdelta6 strain, which is highly immunogenic yet substantially less *reactogenic* (i. e., able to cause adverse reactions).

The vaccine based on this strain, called OrthopoxVac, underwent full cycles of preclinical studies and clinical trials on volunteers. It proved to be no less

effective than the Russian first-generation vaccine while causing far fewer undesirable health consequences for those vaccinated.

The live culture vaccine OrthopoxVac was licensed in Russia in November 2022. It became the world's first fourth-generation smallpox vaccine approved for medical use, i. e., for immunization against smallpox and other dangerous orthopoxvirus infections.



Thus, VECTOR specialists became the first to decode the entire genomes of smallpox, monkeypox, and cowpox viruses isolated from sick humans. The studies on the organization of the orthopoxvirus genome, which began about four decades ago, made it possible to devise methods for multiplex PCR identification of these viruses, leading to the development of the first diagnostic test systems certified for medical practice. It took more than a decade to devise the first fourth-generation genetically engineered vaccinia-based live vaccine, which is no less effective than Dr. Jenner's smallpox vaccine yet incomparably safer.

As for the question whether smallpox could return, one cannot answer it definitely even today. There remains a potential danger of a smallpox “rebirth” since large areas in Eurasia, Central Africa, and South America still contain natural foci of human pathogenic orthopoxviruses, and infection outbreaks occur there from time to time, involving thousands of animals and hundreds of people.

In this regard, a major concern is monkeypox, especially after the 2022–2023 human epidemic, which affected many countries on all continents. In August 2024, the WHO declared the current outbreak in Africa a “public health emergency of international concern.” With the increase of these outbreaks, one of the zoonotic orthopoxviruses may, in theory, fully adapt to the human body.

Therefore, all local outbreaks of orthopoxvirus infections in humans must be controlled using modern methods of species-specific express diagnostics. To prevent them from becoming epidemics, one must apply quarantine measures and vaccinations. If all of us, especially medical agencies, act wisely and have enough organization, smallpox will not return.



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Photo from the author's archive

Sergei Shchelkunov in the house of Edward Jenner, the creator of the first smallpox vaccine. United Kingdom. 2004. Photo from the author's archive