# Smallpox (Variola virus disease) — Comprehensive Disease Characterization Report

**Disease:** Smallpox **MONDO ID:** MONDO:0004651 **Category:** Infectious Disease **Causal agent:** *Variola virus* (VARV), an *Orthopoxvirus* (NCBI Taxon 10255) — **no human causal gene**

## Summary

**Smallpox is not a genetic disease.** Despite the "causal gene" field of the research template, smallpox (MONDO:0004651) is an acute, systemic, human-restricted **infectious disease caused by the Variola virus (VARV)**, a large (~186 kb) double-stranded DNA virus of the genus *Orthopoxvirus* (family *Poxviridae*). VARV encodes ~200 proteins and replicates entirely in the host-cell cytoplasm; more than 80 of its proteins — concentrated in the terminal regions of the genome — carry out host immune-evasion functions rather than classic housekeeping roles PMID: 12941397. The disease was the first (and to date only human disease) to be deliberately eradicated: the last endemic natural case occurred in Somalia in 1977, and the World Health Organization certified global eradication in 1980 PMID: 42135325. Because VARV stocks are retained and routine vaccination ceased decades ago, leaving populations immunologically naïve, smallpox remains a Category A biodefense concern PMID: 42576544.

Pathogenesis proceeds from respiratory-mucosal entry, through a **monocyte-associated cell-associated viremia** that disseminates virus to lymphoid tissues, skin, oral mucosa, gastrointestinal tract, reproductive organs and liver, to a characteristic **synchronous, deep-seated, centrifugal vesiculopustular rash** PMID: 15477589. Severe (flat and hemorrhagic) forms involve cytokine-driven coagulopathy and multi-organ failure. Clinical outcome tracks disease type: discrete ordinary smallpox ~10% case-fatality, confluent ~50–75%, and flat/hemorrhagic forms >95%, with variola major averaging ~30% in the unvaccinated and variola minor (alastrim) <1% PMID: 42135325, PMID: 42576544.

Smallpox was eradicated through **ring vaccination and isolation** using live vaccinia-based vaccines; post-exposure vaccination within ~3–4 days can prevent or attenuate disease. Modern countermeasures comprise the vaccinia-based vaccines **ACAM2000** (replication-competent) and **MVA-BN/JYNNEOS** (non-replicating), plus two FDA Animal-Rule antivirals: **tecovirimat** (TPOXX; inhibits the VP37/p37 envelope protein required for extracellular virion egress) and **brincidofovir** (Tembexa; a lipid-conjugated oral prodrug of the DNA-polymerase inhibitor cidofovir) PMID: 41740032, PMID: 36688308.

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## 1. Disease Information

Smallpox is an acute contagious systemic viral illness caused by the Variola virus. Humans are the **only natural host** — there is no animal reservoir, a feature that made global eradication feasible.

**Key identifiers:**

| Resource | Identifier |
|---|---|
| MONDO | MONDO:0004651 |
| ICD-10 | B03 |
| ICD-11 | 1E70 |
| MeSH | D012899 (Smallpox) |
| NCBI Taxonomy (agent) | Variola virus, Taxon 10255 |
| OMIM | Not applicable (not a Mendelian genetic disorder) |
| Orphanet | Not a rare genetic disease; historical infectious disease |

**Synonyms:** Variola; variola vera; variola major; variola minor (alastrim); Variola virus infectious disease; ordinary smallpox.

**Information source type:** Disease-level aggregated resources (WHO eradication records, historical clinical series, and modern animal-model and vaccinology studies), **not** individual-patient EHR data — no new natural cases have occurred since 1977. The *ICD2Vec* framework notably identified smallpox (ICD-10 B03) among diseases semantically similar to COVID-19 PMID: 37054960.

## 2. Etiology

**Primary cause — infectious:** Variola virus, an *Orthopoxvirus* closely related to vaccinia, cowpox and monkeypox (mpox) viruses. VARV is a large dsDNA virus (~186 kb, ~200 genes) that replicates in the cytoplasm within viral "factories." There is **no genetic, toxic, or metabolic etiology** — smallpox is entirely virus-driven PMID: 12941397.

**Risk factors (environmental/behavioral):** - Close contact with an infected person (respiratory droplets, direct contact with lesions or contaminated fomites such as bedding). - Lack of prior vaccination (populations born after ~1980 are largely immunologically naïve) PMID: 42576544. - Crowding, household exposure, and healthcare settings pre-eradication. - Pregnancy and immunocompromise historically predisposed to more severe (including hemorrhagic) disease.

**Genetic risk factors:** No validated human host-susceptibility loci are established for VARV, reflecting the absence of modern population studies (eradicated before genomic epidemiology). Host genetic variation in innate/adaptive immune genes is plausible but not characterized.

**Protective factors:** Prior smallpox vaccination (vaccinia immunity) is strongly protective and conferred durable, decades-long protection (see Finding F007). Historical smallpox vaccination still provides ~70% residual cross-protection against the related mpox virus decades later PMID: 39176988.

**Gene–environment interactions:** Not characterized for smallpox. The dominant determinant of outcome was vaccination status and clinical form, not host genotype.

## 3. Phenotypes

Smallpox follows a stereotyped clinical course after a **7–19 day incubation** (typically 10–14 days). Key phenotypes:

| Phenotype | Type | Characteristics | Suggested HPO term |
|---|---|---|---|
| Febrile prodrome (high fever, malaise, headache, backache) | Symptom | Acute onset, precedes rash by 2–4 days; severe | HP:0001945 (Fever) |
| Enanthem (oral/ pharyngeal mucosal lesions) | Clinical sign | Appears with/just before skin rash; infectious | HP:0000164 (Oral mucosa abnormality) |
| Synchronous vesiculopustular rash | Physical manifestation | Deep-seated, firm, "pearl-like" pustules all at the **same stage**; centrifugal (face/extremities > trunk) | HP:0200041 (Vesicle); HP:0000987 (Atypical scarring of skin) |
| Lesions on palms and soles | Physical manifestation | Characteristic, distinguishes from varicella | — |
| Umbilicated pustules | Physical manifestation | Central depression | HP:0200039 (Papule) |
| Pitted/pock scarring | Physical manifestation (sequela) | Permanent, disfiguring; healed survivors | HP:0000987 |
| Hemorrhagic diathesis (flat/ hemorrhagic forms) | Laboratory/ clinical | Thrombocytopenia, D-dimer elevation, mucosal bleeding | HP:0001892 (Abnormal bleeding); HP:0001873 (Thrombocytopenia) |
| Leukocytosis | Laboratory abnormality | Profound in lethal disease (macaque model) | HP:0001974 (Leukocytosis) |

The **synchronous progression** (all lesions evolving together: macule → papule → vesicle → pustule → crust) and the **centrifugal distribution** are the classic phenotypic signatures distinguishing smallpox from chickenpox (varicella), in which lesions appear in crops at different stages.

**WHO (Rao) clinical types and frequency among affected individuals:** - Ordinary (~90%) - Modified (in partially immune/vaccinated) - Flat/malignant (rare, >95% fatal) - Hemorrhagic (rare, >95% fatal) - Variola minor (alastrim; <1% fatal)

**Quality-of-life impact:** Acute disease was debilitating and often fatal; survivors suffered permanent pockmark scarring, blindness from corneal involvement, and limb deformities. Standardized QoL instruments (EQ-5D/SF-36) were not applied historically.

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## 4. Genetic / Molecular Information

**This is not a host-genetic disease.** The relevant "genetics" are those of the **viral genome**:

- **Genome:** ~186 kb linear dsDNA with covalently closed hairpin termini and inverted terminal repeats; ~200 open reading frames; cytoplasmic replication PMID: 12941397.
- **Immune-evasion gene complement:** >80 proteins in the terminal genome regions encode host-interaction/immune-evasion functions. Orthopoxviruses carry the **largest antidefense-gene complement of any animal virus family**, shaped by ancient gene capture and recent gene loss PMID: 34253028.
- **Phylogeny (viral strain variation):** Analysis of ~48 VARV genomes resolves **two clades** — Clade 1 (variola major variants) and Clade 2 (West African and variola minor/alastrim strains) — mapping onto the virulence divide PMID: 28749451. Ancient genomes (a ~400-year-old Lithuanian mummy; 60-/160-year-old Prague museum specimens) fall basal or represent extinct European lineages, confirming deep evolutionary history PMID: 28749451, PMID: 33022266.

**Human host genes:** No causal human gene, HGNC entry, ClinVar variant, gnomAD allele frequency, or chromosomal abnormality applies. Sections on pathogenic variants, modifier genes, epigenetics, and chromosomal abnormalities are **not applicable** to this infectious disease.

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## 5. Environmental Information

**Infectious agent:** Variola virus (NCBI Taxon 10255), genus *Orthopoxvirus*, family *Poxviridae*. Transmitted human-to-human via respiratory droplets/aerosols, direct contact with lesion material, and contaminated fomites (bedding, clothing). Patients are most infectious from rash onset through the first 7–10 days of rash PMID: 21431786.

**Environmental/lifestyle factors:** No toxins, radiation, diet, or occupational chemical exposures cause smallpox. Crowding and inadequate isolation facilitated spread historically. The disease has no environmental reservoir — eradication was possible precisely because VARV cannot persist outside humans.

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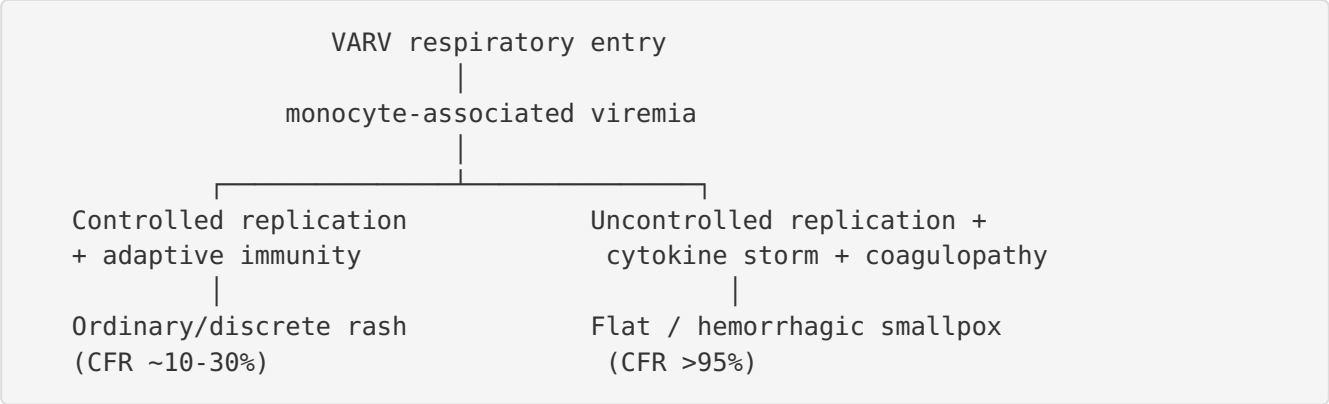
## 6. Mechanism / Pathophysiology

### Ordered causal chain (initiating infection → clinical manifestation)

1. **Inhalation of VARV-laden droplets/aerosol** deposits virus on the oropharyngeal/respiratory mucosa, which **leads to** infection of mucosal epithelial cells and local macrophages (demonstrated in the aerosol monkeypox macaque surrogate: early lung replication) PMID: 25653439.
2. **Viral entry** into host cells via a multi-protein, glycosaminoglycan (GAG)-dependent attachment system — ~4 mature-virion membrane proteins bind heparan/chondroitin sulfate or laminin — followed by an 11–12-protein entry-fusion complex that fuses at the plasma membrane or in acidified endosomes after macropinocytosis; this **results in** cytoplasmic release of the viral core PMID: 22754644, PMID: 38321165.
3. **Infected migratory monocytes/macrophages** carry virus to draining lymph nodes, establishing a **monocyte-associated cell-associated viremia** that **leads to** systemic dissemination (primary viremia) PMID: 15477589.
4. **Viral immune-evasion proteins** (>80 encoded) **suppress** host defense: the secreted complement regulator **SPICE** (smallpox inhibitor of complement enzymes) anchors to cells via heparan/chondroitin-sulfate GAGs and inhibits complement at the **C3 convertase** step via combined decay-acceleration and factor-I cofactor activity, with human-pathway selectivity — molecularly mirroring VARV's human host-restriction; chemokine- and cytokine-binding proteins further blunt innate immunity PMID: 18768877, PMID: 19667083, PMID: 28724763.
5. **Secondary viremia** seeds the skin, oral mucosa, GI tract, reproductive organs, and liver, where viral replication in dermal/epithelial cells **produces** the enanthem and the deep-seated vesiculopustular exanthem PMID: 15477589.
6. **In severe disease**, massive viral replication and a dysregulated cytokine response (elevated IL-1ra, IL-6, IFN-γ in the aerosol model) **drive** coagulation activation (thrombocytopenia, D-dimer elevation), depletion of T-cell-dependent lymphoid areas, and **multi-organ**

**hemorrhagic diathesis** — the lethal flat/hemorrhagic phenotype PMID: 15477589, PMID: 25653439.

## Branch point




## Mechanistic detail by category

- **Molecular pathways:** GAG-mediated attachment (heparan sulfate CHEBI:28815; chondroitin sulfate); complement cascade inhibition at C3/C5 convertase; interferon and chemokine signaling suppression.
- **Protein dysfunction (viral, not host):** The chondroitin-sulfate-binding protein **D8** bends the host membrane to drive fusion-pore formation PMID: 38321165. SPICE achieves complement inhibition equal to or greater than host regulators PMID: 18768877.
- **Immune system involvement:** Complement evasion (SPICE), impaired NK function, lymphopenia, and cytokine storm are central; the host response is both protective and, when dysregulated, pathogenic.
- **Tissue-damage mechanisms:** Direct cytopathic viral replication in epithelium plus immune-mediated injury; hemorrhagic forms involve coagulopathy and endothelial compromise.

**Suggested GO terms:** GO:0019062 (virion attachment to host cell), GO:0046718 (viral entry into host cell), GO:0052170 (symbiont-mediated suppression of host innate immune response), GO:0030449 (regulation of complement activation), GO:0001816 (cytokine production). **Suggested CL terms:** CL:0000576 (monocyte), CL:0000235 (macrophage), CL:0000312 (keratinocyte), CL:0000084 (T cell).

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## 7. Anatomical Structures Affected

**Organ/system level:** - **Primary:** Skin (UBERON:0002097) and mucous membranes — oral/pharyngeal mucosa (UBERON:0000344), respiratory tract (UBERON:0000065, entry site). - **Lymphoreticular system:** lymph nodes (UBERON:0000029), spleen (UBERON:0002106), tonsil (UBERON:0002372) — sites of viral amplification PMID: 15477589, PMID: 25653439. - **Secondary:** Gastrointestinal tract (UBERON:0000160), liver (UBERON:0002107), reproductive organs, eyes (corneal scarring → blindness), and in severe disease, systemic involvement with coagulopathy. - **Body systems:** integumentary, respiratory, lymphatic/immune, hematologic, hepatic.

**Tissue/cell level:** Epithelial tissue (epidermis, mucosal epithelium); dermal fibroblasts; monocytes/macrophages (vehicle of dissemination; CL:0000576/CL:0000235); depletion of T-cell-dependent lymphoid zones.

**Subcellular level:** Viral replication occurs in **cytoplasmic viral factories** (GO:0030430, host cytoplasm) — distinctively, poxviruses do **not** require the nucleus for replication. Virion assembly and envelopment traverse the ER/Golgi and plasma-membrane compartments.

**Localization/lateralization:** Rash is **bilateral and symmetric**, centrifugal (denser on face, forearms, hands, feet, including palms and soles).

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## 8. Temporal Development

- **Onset:** Acute. Incubation 7–19 days (commonly 10–14), followed by abrupt febrile prodrome lasting 2–4 days, then rash.
- **Stages:** (1) Incubation (asymptomatic); (2) prodrome (fever, prostration); (3) early rash/enanthem (most infectious); (4) pustular rash; (5) scabbing/resolution or death. The aerosol macaque model documented temperature peak at day 6 with concurrent skin pox PMID: 25653439.
- **Progression rate:** Rapid, self-limited over ~3–4 weeks in survivors; crusts separate leaving pitted scars. Death in fatal cases typically occurred in the second week.
- **Course pattern:** Monophasic acute illness (not relapsing/chronic). No chronic or latent carrier state.
- **Infectious period:** From rash onset through the first 7–10 days of rash PMID: 21431786.

- **Critical intervention window:** Post-exposure vaccination within ~3–4 days can prevent or attenuate disease — the basis for ring vaccination PMID: 21431786.

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## 9. Inheritance and Population

- **Inheritance:** None — infectious, not heritable. Sections on penetrance, expressivity, anticipation, mosaicism, founder effects, consanguinity, and carrier frequency are **not applicable**.
- **Epidemiology (historical):** Before eradication, smallpox was endemic worldwide and caused an estimated ~300 million deaths in the 20th century alone. Last endemic natural case: Somalia, October 1977; WHO certified eradication in 1980 PMID: 42135325. Current incidence/prevalence: **zero** (eradicated).
- **Geographic distribution:** Formerly global. The two viral clades map geographically — Clade 2 includes West African and alastrim (variola minor) strains PMID: 28749451.
- **Sex ratio:** No strong sex predilection; outcome driven by vaccination status, age, pregnancy, and clinical form rather than sex.
- **Age distribution:** All ages susceptible when unvaccinated; young children and the elderly had higher case-fatality historically.

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## 10. Diagnostics

**Clinical diagnosis (historical):** Based on the characteristic synchronous, deep-seated, centrifugal vesiculopustular rash with palm/sole involvement and febrile prodrome.

**Laboratory confirmation:** - **PCR / real-time PCR** on lesion material — the modern confirmatory standard for orthopoxvirus/VARV identification. - **Electron microscopy** — brick-shaped orthopoxvirus virions in vesicle fluid (identifies genus, not species). - **Serology / immunohistochemistry** — supportive; IHC localizes viral antigen in tissue (used in the macaque model) PMID: 15477589. - **Viral culture and genome sequencing** for definitive speciation and clade assignment PMID: 28749451.

**Laboratory abnormalities in severe disease:** profound leukocytosis, thrombocytopenia, elevated creatinine, and D-dimer elevation (coagulation activation) PMID: 15477589.

**Clinical criteria:** WHO case definition (acute onset of fever ≥38.3°C followed by firm, deep-seated vesicles/pustules at the same stage of development).

**Differential diagnosis:** Chickenpox (varicella — superficial, pleomorphic lesions in crops, central distribution, spares palms/soles), **mpox/monkeypox** (prominent lymphadenopathy, milder course), disseminated herpes zoster, drug eruptions, and other vesicular illnesses PMID: 41600835.

**Genetic testing:** Not applicable (no host gene). "Genetic testing" here means **viral** genome sequencing for strain/clade confirmation.

**Screening:** No population screening applies (eradicated disease); surveillance focuses on bioterrorism preparedness and differentiating from mpox.

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## 11. Outcome / Prognosis

**Case fatality by clinical type (unvaccinated):**

| Clinical form | Approximate frequency | Case-fatality rate |
|---|---|---|
| Discrete ordinary | Common | ~10% |
| Confluent ordinary | — | ~50–75% |
| Flat (malignant) | Rare | >95% |
| Hemorrhagic | Rare | >95% |
| Variola major (overall) | ~90% of cases | ~30% |
| Variola minor (alastrim) | — | <1% |

Sources: PMID: 42135325, PMID: 42576544.

**Recovery:** Survivors develop lifelong immunity. Sequelae include permanent pockmark scarring (often facial, disfiguring), blindness from keratitis/corneal scarring, and occasionally limb deformities (osteomyelitis variolosa) or encephalitis.

**Prognostic factors:** Clinical type (flat/hemorrhagic forms near-uniformly fatal), vaccination status (vaccinated patients had milder "modified" disease), age (worse in very young and elderly), pregnancy (predisposed to hemorrhagic form), and extent of confluent rash. Early hemorrhagic manifestations and marked thrombocytopenia/coagulopathy portended death PMID: 15477589.

## 12. Treatment

Historically, treatment was **supportive only** (fluids, nutrition, wound care, prevention/treatment of secondary bacterial infection). Modern FDA-approved specific antivirals, developed under the **Animal Rule** (human efficacy trials being impossible/unethical for an eradicated disease):

| Drug | Trade name | Target / mechanism | Basis of approval | NCIT |
|---|---|---|---|---|
| **Tecovirimat** | TPOXX | Inhibits orthopoxvirus **VP37/p37** envelope protein required for extracellular (enveloped) virion formation/egress | NHP mpox & rabbitpox models | NCIT:C171621 (Tecovirimat) |
| **Brincidofovir** | Tembexa | Lipid-conjugated **oral prodrug of cidofovir**; viral **DNA-polymerase inhibitor** | Ectromelia (mouse) & rabbitpox (rabbit) surrogate models | NCIT:C97660 (Brincidofovir) |

- Tecovirimat is "approved for smallpox treatment under the Food and Drug Administration Animal Rule on the basis of efficacy in nonhuman primate models of mpox" PMID: 41740032.
- "Brincidofovir adds a second oral agent to treat smallpox, with a different mechanism of action than tecovirimat" PMID: 36688308.

- **Cidofovir** (parent compound; DNA-polymerase inhibitor) has activity against orthopoxviruses and reduces MPXV replication in mouse respiratory tract PMID: 39243894; it is nephrotoxic and not formally smallpox-approved.

**Important caveat on efficacy evidence:** In a randomized human **mpox** (clade II) trial, tecovirimat showed **no benefit over placebo** (83% vs 84% lesion resolution by day 29), underscoring that smallpox approval rests on **animal efficacy data, not human smallpox trials**, and that antiviral efficacy against human orthopoxvirus disease remains uncertain PMID: 41740032. Emerging tecovirimat resistance (VP37 mutations) is a further concern, motivating combination regimens PMID: 41038284.

**Pharmacogenomics / gene therapy / cell therapy / targeted & immunotherapy:** Not applicable in the conventional oncologic/genetic sense; the "targeted therapy" here is the host- and virus-directed small-molecule antivirals above.

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## 13. Prevention

Prevention is the triumph of smallpox control and the reason for its eradication.

**Primary prevention — vaccination (vaccinia-based, cross-protective):**

| Vaccine | Platform | Key properties |
|---|---|---|
| **Dryvax** (1st gen) | Replication-competent vaccinia (calf lymph) | Eradication-era vaccine; durable immunity 40–80 years PMID: 40311645 |
| **ACAM2000** (2nd gen) | Clonal replication-competent vaccinia | Current US stockpile; defined risk profile PMID: 42576544 |
| **MVA-BN / JYNNEOS** (3rd gen) | Non-replicating Modified Vaccinia Ankara | Safer for immunocompromised; sustained memory ≥5 years with strong booster effect PMID: 41819114 |

- **Edward Jenner's cowpox-based vaccination (1796)** founded the field of immunization and underpinned eradication PMID: 39371745.
- Protective immunity must target **both virion forms** — the intracellular mature virion (IMV) and extracellular enveloped virion (EEV) — because of the dual-virion architecture of orthopoxviruses; rational vaccines target IMV antigens (A27/L1 ≈ A29L/M1R) and EEV

antigens (B5/A33 ≈ B6R/A35R) PMID: 41941064. A DNA "4pox" vaccine targeting L1, A27, B5, A33 protected rabbits against lethal aerosol rabbitpox PMID: 34851148.

**Public-health strategy: Ring vaccination plus case isolation** — identifying cases and vaccinating contacts and contacts-of-contacts — eradicated the disease. Post-exposure vaccination within ~3–4 days prevents/attenuates illness PMID: 21431786.

**Vaccine safety (serious adverse events of replication-competent vaccinia):** postvaccinial encephalitis, progressive vaccinia, eczema vaccinatum, and generalized vaccinia — "vaccination can accompany complications, such as postvaccinial encephalitis, progressive vaccinia, eczema vaccinatum, and generalized vaccinia" PMID: 21431786. Death rates are strain-dependent: ~1.4 deaths/million for NYCBH strain vs ~8.4/million for Lister strain PMID: 16933957. Myocarditis and dilated cardiomyopathy are additional recognized risks PMID: 26004568. MVA-BN avoids these replication-associated risks, making it suitable for immunocompromised recipients.

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## 14. Other Species / Natural Disease

- **Host range:** VARV is **strictly human-restricted** with **no animal reservoir** — the decisive biological fact enabling eradication. SPICE's human-complement selectivity (vs vaccinia VCP's bovine-complement preference) is a molecular correlate of this host-restriction PMID: 28724763.
- **Zoonotic potential:** None for VARV itself. However, **related orthopoxviruses are zoonotic** — monkeypox/mpox virus (from African rodents/primates), cowpox, and novel orthopoxviruses (e.g., a distinct lineage in an Alaska resident) cause human disease, and rising incidence is attributed to waning post-smallpox-vaccination immunity PMID: 28329402.
- **Comparative biology:** Orthopoxviruses share genome architecture and immune-evasion gene families shaped by ancient gene capture and recent gene loss PMID: 34253028. Avipoxviruses (fowlpox, penguin/pigeon poxviruses) are more distantly related but carry VARV B22R orthologues, illustrating deep poxvirus gene conservation PMID: 24919868.

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## 15. Model Organisms

Because VARV infects only humans and live virus is restricted to two WHO-authorized high-containment laboratories, **surrogate models** are essential and form the regulatory basis for antiviral and vaccine approval:

| Model system | Virus used | Use / phenotype recapitulation | Reference |
|---|---|---|---|
| Cynomolgus macaque | **Variola** (Harper/ India strains), IV high dose | Reproduces monocyte-associated viremia, lymphoid/skin/mucosal/GI/liver dissemination, leukocytosis, thrombocytopenia, D-dimer rise, hemorrhagic diathesis; requires non-physiologic high dose | PMID: 15477589 |
| Cynomolgus macaque | **Aerosolized monkeypox** | Recapitulates respiratory entry → lung replication → spread to tonsil/spleen/nodes/ colon by day 4 → fever peak day 6 with skin pox; IL-1ra/IL-6/IFN-γ surge | PMID: 25653439 |
| Rabbit | **Rabbitpox** (aerosol) | Models respiratory, low-dose exposure akin to natural smallpox; used for 4pox DNA vaccine and tecovirimat efficacy | PMID: 34851148 |
| Mouse | **Ectromelia** (mousepox); intranasal MPXV | Brincidofovir approval surrogate; cidofovir/ brincidofovir reduce respiratory viral load | PMID: 36688308, PMID: 39243894 |
| In vitro | Vaccinia (prototype) | Entry/fusion mechanics (D8, entry-fusion complex), gene function via transient complementation | PMID: 22754644, PMID: 31240675 |

**Model limitations:** No model fully reproduces the human-restricted, low-dose respiratory transmission and the full spectrum of human clinical forms; macaque variola requires large intravenous inocula, and mpox/rabbitpox/mousepox are related-but-distinct viruses. This surrogacy is precisely why antivirals are approved under the Animal Rule rather than on human efficacy.

## Key Findings (Consolidated)

### F001 — Smallpox is caused by Variola virus, not a human gene; eradicated 1980

VARV (NCBI Taxon 10255) is a ~186 kb dsDNA *Orthopoxvirus* encoding ~200 proteins and replicating in the cytoplasm. Over 80 proteins in the genome terminal regions carry immune-evasion functions. WHO certified eradication in 1980; retained stocks and loss of population immunity sustain biodefense concern. *"Smallpox, eradicated globally in 1980, remains a major biodefense concern due to retained Variola virus stocks and the absence of population immunity following cessation of routine vaccination."* PMID: 42576544; PMID: 12941397.

### F002 — Pathogenesis: monocyte-associated viremia → lymphoid dissemination → multi-organ hemorrhagic diathesis

*"After inoculation, variola was disseminated by means of a monocytic cell-associated viremia"* with antigen in lymphoid tissue, skin, oral mucosa, GI tract, reproductive system, and liver PMID: 15477589. The aerosol mpox macaque model documents the temporal sequence from respiratory entry to systemic spread and rash PMID: 25653439.

### F003 — Countermeasures: tecovirimat + brincidofovir (Animal Rule) plus ACAM2000/MVA-BN vaccines

Tecovirimat targets VP37/p37 (egress); brincidofovir is an oral DNA-polymerase inhibitor prodrug — both FDA-approved via animal models PMID: 41740032, PMID: 36688308. Vaccinia vaccination carries rare serious adverse events (~1 death/million) PMID: 21431786, PMID: 16933957.

### F004 — Clinical classification & case fatality

Ordinary (~90%), modified, flat, hemorrhagic forms plus variola minor; CFR ~10% (discrete) to >95% (flat/hemorrhagic); variola major ~30%, minor <1%. Last natural case 1977; eradication 1980 PMID: 42135325, PMID: 42576544, PMID: 21431786.

### F005 — Two-clade VARV phylogeny mapping onto the virulence divide

*"Based on the genomes of 48 strains, two clades are differentiated: Clade 1 includes variants of variola major, and clade 2 includes West African and variola minor (Alastrim) strains"* PMID: 28749451. Orthopoxviruses have the largest antidefense-gene complement of any animal virus family PMID: 34253028.

### F006 — GAG-dependent entry machinery; SPICE inhibits human complement at C3 convertase

*"Four viral proteins associated with the MV membrane facilitate attachment by binding to glycosaminoglycans or laminin on the cell surface"* PMID: 22754644. SPICE anchors via heparan/chondroitin sulfate and inhibits complement at the C3 convertase step PMID: 18768877, with human-pathway selectivity mirroring host restriction PMID: 28724763.

### F007 — Dual-virion vaccine antigens; decades-long immune durability

Protective immunity must target IMV and EEV antigens; both first- and third-generation vaccines elicit antibodies, with strongest responses to EEV antigens B5 and A33, retained 40–80 years after Dryvax PMID: 40311645. T-cell responses are more sustained than waning antibody PMID: 41012133; historical vaccination confers ~70% residual cross-protection against mpox PMID: 39176988.

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## Mechanistic Model / Interpretation

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INFECTION → ENTRY → DISSEMINATION → IMMUNE EVASION → TISSUE INJURY → OUTCOME

Respiratory       GAG-dependent       Monocyte-          SPICE blocks       Epithelial
droplet/aerosol → attachment (4    →  associated     →   complement @   →   replication   →
on oropharynx     MV proteins) +      viremia to         C3 convertase;     → rash;
                  11-12 protein       lymph nodes,        chemokine/         enanthem
                  entry-fusion        spleen, skin,       cytokine binders
                  complex             mucosa, liver       blunt innate
                                                          immunity           Cytokine storm
                                                                             + coagulopathy →
                                                                             multi-organ
                                                                             failure
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The coherent narrative: VARV is an exquisitely human-adapted orthopoxvirus whose large immune-evasion arsenal (exemplified by human-selective SPICE) permits efficient dissemination via host monocytes. Disease severity is a **branch** determined by the balance between viral replication/immune-evasion and the host adaptive/cytokine response — tipping toward lethal coagulopathic failure in flat/hemorrhagic forms. The same dual-virion biology that complicates vaccine design (needing both IMV and EEV antigens) also explains why vaccinia-based immunity is broadly cross-protective and remarkably durable, which made ring vaccination of a reservoir-free, strictly human pathogen sufficient for eradication.

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## Evidence Base (selected)

| PMID | Role |
|---|---|
| 12941397 | VARV genome content & immune-evasion gene complement |
| 42576544 | Eradication date, biodefense, ACAM2000 safety |
| 42135325 | Somalia 1976–77 outbreak data; eradication 1980 |
| 15477589 | Variola macaque model — viremia & dissemination |
| 25653439 | Aerosol mpox macaque — temporal pathogenesis & cytokines |
| 28749451 | Two-clade VARV phylogeny; historical specimens |
| 34253028 | Orthopoxvirus antidefense-gene evolution |
| 22754644 | Poxvirus entry machinery |
| 38321165 | D8 drives membrane curvature for fusion |
| 18768877 | SPICE GAG anchoring & C3 convertase inhibition |
| 19667083 | SPICE functional sites; inhibitory mAb |
| 28724763 | SPICE human-pathway selectivity vs VCP |
| 41740032 | Tecovirimat Animal-Rule approval; mpox trial null result |
| 36688308 | Brincidofovir as second oral smallpox antiviral |
| 21431786 | Vaccination strategy, adverse events, post-exposure window |
| 16933957 | Strain-dependent vaccine adverse-event rates |
| 40311645 | EEV antigen dominance; 40–80 yr durability |
| 41012133 | Durable T-cell vs waning antibody |
| 39176988 | ~70% residual cross-protection vs mpox |
| 34851148 | 4pox DNA vaccine (L1/A27/B5/A33) |
| 41941064 | Dual-virion rational/mRNA vaccine design |

## Limitations and Knowledge Gaps

1. **No human efficacy data** for antivirals or modern vaccines against actual smallpox — all approvals rest on surrogate animal models (Animal Rule). The negative tecovirimat mpox trial PMID: 41740032 raises genuine uncertainty about real-world efficacy against human orthopoxvirus disease.
2. **Surrogate-model fidelity:** Variola macaque models require non-physiologic high IV doses; mpox/rabbitpox/mousepox are related but distinct viruses, so pathogenesis inferences carry extrapolation risk.
3. **Host-susceptibility genetics are uncharacterized** — eradication preceded modern GWAS/omics, so no human susceptibility or modifier loci exist.
4. **Correlates of protection remain undefined** — the relative contribution of antibody vs T-cell immunity to durable protection is not fully resolved PMID: 41012133.
5. **Molecular pathophysiology of the hemorrhagic form** (cytokine-storm/coagulopathy triggers) is inferred from animal models rather than demonstrated in humans.
6. **Antiviral resistance** (tecovirimat VP37 mutations) threatens monotherapy efficacy, and combination-therapy evidence is immature PMID: 41038284.

## Proposed Follow-up Experiments / Actions

1. **Define immune correlates of protection** by longitudinally measuring IMV- vs EEV-directed antibody and T-cell responses after MVA-BN and correlating with protection in NHP challenge, to establish a surrogate endpoint usable for regulatory decisions.
2. **Combination antiviral studies** (tecovirimat + brincidofovir/cidofovir) in NHP variola and rabbitpox models to pre-empt resistance and strengthen the countermeasure stockpile.
3. **Structural/functional targeting of SPICE** — advance the inhibitory monoclonal antibody PMID: 19667083 as an adjunct therapeutic that restores complement-mediated clearance.
4. **Next-generation multivalent mRNA vaccines** co-targeting conserved IMV (A27/L1) and EEV (B5/A33) antigens, evaluated for durability, imprinting effects, and cross-clade/pan-orthopox coverage PMID: 41941064.
5. **Preparedness surveillance** leveraging digitized historical outbreak data PMID: 42135325 and rapid PCR/sequencing diagnostics to distinguish re-emergent VARV (bioterror scenario) from circulating mpox and novel orthopoxviruses PMID: 28329402.

6. **Characterize residual population immunity** in pre-1980 vaccinated cohorts to inform ring-vaccination feasibility should VARV re-emerge.

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*Report compiled from 7 confirmed findings and 51 reviewed papers over 5 investigation iterations. Evidence types span human clinical/historical, nonhuman-primate and rodent models, in vitro mechanistic, and computational/phylogenetic sources.*

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