Cellular Pathophysiology in Zoonotic Transmission of Orthopoxviruses (OPXVs) from Animal Host to Human

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Abstract

Orthopoxviruses (OPXVs), belonging to the Poxviridae family, are large, double-stranded DNA viruses known for their zoonotic potential and impact on human and animal health. This review explores the transmission dynamics of OPXVs from animal hosts to humans and the underlying cellular pathophysiological mechanisms.
Animal hosts such as rodents, primates, and livestock are central in the ecology of OPXVs, with transmission typically occurring through direct contact or exposure to contaminated materials. Clinical manifestations in animals range from skin lesions and fever to respiratory and gastrointestinal symptoms, reflecting the diverse tissue tropism of these viruses. Humans primarily acquire OPXVs through contact with infected animals or their products, highlighting the zoonotic risk posed by these viruses.

At the cellular level, OPXV infections involve complex interactions between viral proteins and host cell receptors, triggering robust immune responses characterized by cytokine release and inflammation. The viruses replicate within the cytoplasm without accessing the host cell nucleus, evading detection by nuclear sensors and exploiting cellular machinery for viral assembly.

A comprehensive grasp of OPXV transmission dynamics and cellular pathophysiology is requisite to devise effective prevention and control strategies. Insights into host immune responses and viral replication mechanisms provide a foundation for antiviral drug development and vaccine strategies. This review synthesizes current knowledge on OPXV ecology, transmission, and cellular interactions, emphasizing their significance in veterinary, medical, and public health contexts.

**Keywords:** Animal-to-Human Transmission, Ecology, Pattern Recognition Receptors, Skin Rashes, Zoonotic.

**Abbreviations:** CNS: Central Nervous System; CPXV: Cowpox Virus; EEV: Extracellular Enveloped Virus; HPA: Hypothalamic-Pituitary-Adrenal; IFNs: Interferons; IL-1: Interleukin-1; IL-6: Interleukin-6; MPXV: Monkeypox Virus; OPXV: Orthopoxvirus; PCR: Polymerase Chain Reaction; PRRs: Pattern Recognition Receptors; TLRs: Toll-Like Receptors; TNF-alpha: Tumor Necrosis Factor-Alpha; VACV: Vaccinia Virus; VARV: Variola Virus; VIGIV: Vaccinia Immune Globulin Intravenous.


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**Introduction**

Orthopoxvirus (OPXV) infections encompass several clinically significant diseases, including smallpox (*Variola* virus [VARV]), localized skin infections from the *Vaccinia* virus (VACV), cowpox (*Cowpox* virus [CPXV]), and monkeypox (*Monkeypox* virus [MPXV]). Each infection has distinct characteristics, historical contexts, epidemiological patterns, and impacts on individual quality of life, society, and healthcare systems.

**Smallpox (*Variola* Virus)**

Smallpox, caused by the VARV, is a highly contagious and often fatal disease characterized by high fever, malaise, and a distinctive progressive skin rash. The rash develops from macules to papules, vesicles, pustules, and scabs, leaving severe scarring (MacNeill, 2022).

Smallpox is considered to have emerged over 3,000 years ago, with evidence found in Egyptian mummies. The disease caused numerous epidemics throughout history, including the decimation of indigenous populations in the Americas following European colonization (MacNeill, 2022). Notable figures such as Edward Jenner made considerable strides in combating smallpox by developing the first successful smallpox vaccine in 1796 using the CPXV. In 1980, the World Health Organization declared smallpox
eradicated after a successful global vaccination campaign (MacNeill, 2022; Shchelkunova & Shchelkunov, 2022).

Before its eradication, smallpox was endemic in many parts of the world, causing millions of deaths annually. The global prevalence varied, with high incidence rates in densely populated regions. The last known natural case occurred in Somalia in 1977. Since eradication, smallpox remains a potential bioterrorism threat, leading to ongoing research and preparedness efforts (Breman, 2021; MacNeill, 2022).

Smallpox had a devastating effect on individuals’ quality of life, often resulting in severe disfigurement, blindness, and high mortality rates. The societal consequences included widespread fear, social disruption, and considerable economic burden due to healthcare costs and loss of productivity. The eradication of smallpox alleviated these burdens, although costs associated with surveillance and potential outbreak preparedness persist (MacNeill, 2022).

Localized Skin Infections (Vaccinia Virus)

VACV, used in the smallpox vaccine, can cause localized skin infections characterized by pustular lesions at the inoculation site. These infections are generally mild but can lead to complications, particularly in immunocompromised individuals (Shchelkunova & Shchelkunov, 2022; Walsh & Dolin, 2011).

The use of VACV in the smallpox vaccine dates back to Edward Jenner’s work in the late 18th century. The widespread use of this vaccine was instrumental in the eradication of smallpox. Research continues to explore the use of VACV in other vaccines and therapeutic applications (MacNeill, 2022; Shchelkunova & Shchelkunov, 2022).

Localized skin infections from the VACV are primarily linked to vaccination practices. Following the cessation of routine smallpox vaccination after eradication, the incidence of such infections has drastically decreased. However, laboratory workers and military personnel may still encounter these infections due to continued vaccine use in specific settings (Lu et al., 2019).

While generally mild, VACV infections can cause discomfort and require medical attention, particularly in cases with complications. The impact on quality of life is usually limited, but severe cases can lead to marked morbidity. Healthcare costs are relatively low compared to other conditions, though complications can increase expenses. The societal and economic burden is minimal, given the rarity of these infections in the general population today (MacNeill, 2022).

Cowpox (Cowpox Virus)

CPXV infection in humans typically presents with localized pustular lesions, primarily on the hands and face, following contact with infected animals. The disease is usually self-limiting but can be painful and unsightly (Andreani et al., 2019; Fashina, Huang, Thomas, Conrady, & Yeh, 2022).

Cowpox has been recognized for centuries, notably in its role in Edward Jenner’s smallpox vaccine development. Jenner observed that milkmaids who had contracted CPXV were immune to smallpox, leading to the first use of CPXV in vaccination (Cherry & Johnston, 2009; MacNeill, 2022).

Cowpox is currently rare, primarily occurring in rural areas where humans may contact infected animals, particularly rodents and domestic cats. Human cases are sporadic and typically linked to occupational exposure (Diaz, 2021; Fashina et al., 2022; MacNeill, 2022).

CPXV infections generally cause localized pain and discomfort, impacting an individual's quality of life during the acute phase. Due to the rarity of the disease, the societal and economic burden is minimal. Healthcare costs are primarily associated with managing the lesions and preventing secondary infections. The impact on work hours and productivity is limited, given the self-limiting nature of the infection (Fashina et al., 2022; MacNeill, 2022).
Monkeypox (*Monkeypox Virus*)

MPXV infection presents with fever, headache, muscle aches, and a rash that evolves from macules to pustules. The disease is similar to smallpox but generally less severe, though it can lead to complications such as secondary bacterial infections and respiratory distress (MacNeill, 2022).

Monkeypox was first identified in laboratory monkeys in 1958 and humans 1970 in the Democratic Republic of Congo. It primarily affects regions in Central and West Africa, with periodic outbreaks in other countries due to travel and trade (Hirani et al., 2023; MacNeill, 2022; Shchelkunova & Shchelkunov, 2022).

Monkeypox remains endemic in regions of Central and West Africa, with occasional outbreaks reported in other areas (Hirani et al., 2023). The prevalence has increased due to lessened smallpox vaccination rates, which previously provided some cross-protection against MPXV (Akter et al., 2023).

MPXV can notably impact the quality of life due to the painful and disfiguring rash and the potential for severe complications. The societal impact includes the strain on healthcare systems during outbreaks and the economic burden of managing and containing the disease. Healthcare costs are substantial, particularly during outbreaks, due to the need for isolation, supportive care, and treatment of complications. The disease can result in considerable loss of work hours and productivity, particularly in affected regions with limited healthcare resources (Ahmed et al., 2022; MacNeill, 2022). Smallpox, caused by the VARV, was a highly contagious and often fatal disease, eradicated in 1980 following a global vaccination campaign initiated by Edward Jenner’s discovery in 1796. The VACV, used in the smallpox vaccine, can cause mild localized skin infections, primarily affecting individuals in specific occupational settings. CPXV infection presents with localized pustular lesions after contact with infected animals and was crucial in developing the smallpox vaccine. Monkeypox, first identified in humans in 1970, remains endemic in parts of Central and West Africa, causing a smallpox-like disease that is generally less severe. Each infection impacts the individual’s quality of life, society, and healthcare systems differently, with smallpox historically causing widespread dread and economic burden. In contrast, the other infections have more localized and limited societal impacts (MacNeill, 2022; Shchelkunova & Shchelkunov, 2022).

**Discussion**

*Orthopoxvirus* (OPXV) is a genus of viruses within the family Poxviridae. These viruses are large, brick-shaped or ovoid, and contain double-stranded DNA. The OPXV genus includes several species that are pathogenic to humans and animals (Silva, De Oliveira, Kroon, De Souza Trindade, & Drumond, 2020).

OPXVs are known for their ability to induce immunity against other OPXVs. This ability has been utilized in vaccination strategies. These viruses replicate in the cytoplasm of host cells and have complex lifecycles, requiring both early and late gene expressions for successful replication and assembly (Xiang & Lane, 2021).

**Contraction of OPXVs by Animal Hosts**

OPXVs have a broad range of animal hosts. *Cowpox* virus (CPXV) primarily infects rodents, such as voles and mice, which are considered its natural reservoir. These rodents contract the virus through contact with contaminated environments or bites and scratches from infected animals. Cows and other domestic animals can contract the virus by contacting contaminated soil or bedding, whereas the *Monkeypox* virus (MPXV) has been found in various wild animals, including primates and rodents. These animals contract the virus through bites, scratches, or consumption of infected animal carcasses. *Vaccinia* virus (VACV), used in the smallpox vaccine, can infect various livestock species and laboratory animals, often through...
experimental inoculation or accidental exposure (MacNeill, 2022; Shchelkunova & Shchelkunov, 2022; Silva et al., 2020).

Table 1 summarizes the information on OPXVs, their animal hosts, and modes of transmission.

<table>
<thead>
<tr>
<th>Animal Hosts</th>
<th>OPXVs</th>
<th>Mode of Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodents</td>
<td>CPXV, MPXV</td>
<td>Direct contact with contaminated environments, bites and scratches from infected animals</td>
</tr>
<tr>
<td>Domestic Animals</td>
<td>CPXV</td>
<td>Contact with contaminated soil or bedding, direct contact with infected rodents</td>
</tr>
<tr>
<td>Non-human Primates</td>
<td>MPXV</td>
<td>Bites, scratches, or consumption of infected animal carcasses</td>
</tr>
<tr>
<td>Various Livestock Species and Laboratory Animals</td>
<td>VACV</td>
<td>Experimental inoculation or accidental exposure</td>
</tr>
</tbody>
</table>

Animals infected with OPXVs often show signs and symptoms of the infection. The clinical presentation can vary depending on the specific OPXVs and the animal species affected.

**Signs and Symptoms of OPXV Infection in Animal Hosts**

Common signs and symptoms in animals infected with OPXVs (MacNeill, 2022; Shchelkunova & Shchelkunov, 2022; Silva et al., 2020):

1. **Skin Lesions**: Similar to humans, many animals develop skin lesions, including macules, papules, vesicles, pustules, and crusts. These lesions may appear on various body parts, often in exposed areas.
2. **Fever**: Infected animals may exhibit elevated body temperatures as part of the systemic response to the infection.
3. **Lymphadenopathy**: Swollen lymph nodes are common in infected animals, reflecting the body’s immune response to the virus.
4. **Respiratory Symptoms**: Some animals may develop respiratory signs such as nasal discharge, coughing, or difficulty breathing, especially if the respiratory tract is involved.
5. **Gastrointestinal Symptoms**: In some instances, infected animals may show signs of gastrointestinal distress, including vomiting or diarrhea.
6. **Behavioral Changes**: Infected animals might exhibit lethargy, decreased appetite, or other behavioral changes indicative of illness.

Specific examples (MacNeill, 2022; Shchelkunova & Shchelkunov, 2022; Silva et al., 2020):

- **VACV**: Used in vaccines, it can cause localized lesions at the inoculation site in various animals, including livestock and laboratory animals.
- **CPXV**: Infected cattle may develop localized lesions, particularly on teats and udders, while domestic cats can show more widespread lesions and systemic illness, especially if immunocompromised.
- **MPXV**: Infected primates and rodents often exhibit a rash, fever, and lymphadenopathy. Severe cases can lead to more extensive systemic involvement.

Monitoring and diagnosing OPXV infections in animals involve:

- Clinical observation
• Laboratory testing (such as polymerase chain reaction [PCR] for viral DNA)
• Sometimes, histopathological examination of lesions (Burrell, Howard, & Murphy, 2017)

Animals do not contract the *Variola* virus (VARV). VARV is a human-specific virus with no known animal reservoir or intermediate host. Transmission occurs exclusively between humans. Thus, there are no naturally occurring animal models for VARV infection (Shchelkunova & Shchelkunov, 2022). However, certain animals, such as non-human primates, have been used in experimental settings to study smallpox pathogenesis and evaluate potential treatments. In these experimental models, infected animals typically exhibit high fever, widespread rash, and systemic illness, closely mimicking the disease presentation seen in humans (Delaune & Iseni, 2020; Schmitt, Mätz-Rensing, & Kaup, 2014).

**Transmission from Animal Hosts to Humans**

Transmission of OPXVs from animal hosts to humans typically occurs through direct contact with infected animals or contaminated materials.

CPXV can be transmitted to humans by handling infected cattle or rodents, often resulting in localized skin infections at the contact site. MPXV transmission to humans can occur through bites or scratches from infected animals, handling bushmeat, or direct contact with body fluids or lesion material from infected animals. There is also evidence of secondary human-to-human transmission of MPXV through respiratory droplets, direct contact with bodily fluids, or contaminated materials (Gieryńska et al., 2023). VACV can be transmitted to humans through accidental inoculation, particularly among laboratory workers or during vaccination procedures (Tack et al., 2013). VARV primarily spreads through respiratory droplets or direct contact with infectious bodily fluids from infected individuals (Gieryńska et al., 2023).

Table 2 summarizes how OPXVs can be transmitted from animal hosts to humans through various vectors.

<table>
<thead>
<tr>
<th>Transmission Vector</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Contact</td>
<td>Handling infected animals or contact with their bodily fluids, lesions, or scabs; contaminated materials</td>
<td>CPXV from infected cattle, domestic cats, or rodents to humans, often causing localized skin infections at the contact site</td>
</tr>
<tr>
<td>Aerosol Transmission</td>
<td>Respiratory droplets from infected animals; secondary human-to-human transmission; common route for VARV</td>
<td>VARV spread primarily between humans, potentially involving animal reservoirs; MPXV through respiratory droplets</td>
</tr>
<tr>
<td>Bites and Scratches</td>
<td>Infected animals biting or scratching humans; handling bushmeat</td>
<td>MPXV from non-human primates through bites or scratches, direct contact with body fluids, or lesion material</td>
</tr>
<tr>
<td>Accidental Inoculation</td>
<td>Accidental exposure during laboratory work or vaccination procedures</td>
<td>VACV transmission to laboratory workers or during vaccination procedures</td>
</tr>
</tbody>
</table>

**Human Manifestations of OPXV Infection**

The clinical manifestations of OPXV infections in humans vary depending on the specific virus involved:

• **Smallpox (VARV):** Characterized by high fever, malaise, and a distinctive progressive skin rash (primarily pustules evolved from macules). Smallpox was highly contagious and often fatal, causing widespread epidemics before its eradication (Meyer, Ehmann, & Smith, 2020).
• **Localized Skin Infections (VACV):** Typically results in localized pustular lesions at the inoculation site, such as from vaccination. While generally mild, the virus can cause complications in immunocompromised individuals (MacNeill, 2022).

• **Cowpox (CPXV):** Presents with localized pustular lesions, primarily on the hands and face, following contact with infected animals. The lesions are usually self-limiting but can be painful and unsightly (Fashina et al., 2022).

• **Monkeypox (MPXV):** Symptoms include fever, headache, muscle aches, and a rash that evolves from macules to pustules, similar to smallpox but generally less severe. MPXV can lead to complications such as secondary bacterial infections, respiratory distress, and, in severe cases, death (Khattak et al., 2023).

OPXVs remain consequential in medical research and public health due to their potential for zoonotic transmission and the historical impact of diseases like smallpox. Their ability to induce cross-immunity has been pivotal in developing effective vaccines, exemplifying their dual role as both a threat to human health and a tool in infectious disease management (Xiang & Lane, 2021).

Common Cellular Pathophysiological Mechanisms of OPXVs

OPXV infections in humans involve intricate cellular interactions triggering robust immune responses, leading to characteristic symptoms including high fever, malaise, and pustular formation (Fashina et al., 2022; MacNeill, 2022; Shchelkunova & Shchelkunov, 2022).

**Cellular Interactions and Immune Response in OPXV Infections**

Upon infection, OPXVs interact with host cells primarily through pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), which recognize viral components like glycoproteins. (TLRs are a type of PRR on immune cells that detect specific components of pathogens and activate immune responses.) This recognition triggers intracellular signaling cascades that activate transcription factors and produce pro-inflammatory cytokines and chemokines (Perdiguero et al., 2023).

Cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and interferons (IFNs) are crucial in the immune response against OPXV infections. IL-1 and TNF-alpha initiate local inflammation and activate endothelial cells to express adhesion molecules, facilitating immune cell recruitment (Brown et al., 2023; Thompson, Kaminski, Kurt-Jones, & Fitzgerald, 2011). IL-6 amplifies the acute phase response, stimulating hepatocytes to produce acute-phase proteins. IFNs induce an antiviral state in neighboring cells, inhibiting viral replication and spread (Brown et al., 2023; Thompson et al., 2011).

At the cellular level, OPXVs typically enter host cells through endocytosis or direct fusion with the cell membrane. Once inside the cytoplasm, the virus replicates and assembles virions without accessing the host cell nucleus. This strategy allows OPXVs to evade detection by nuclear sensors and exploit host cell machinery for their replication (Schmidt, Bleck, & Mercer, 2012).

Basal keratinocytes in the skin serve as primary targets for several OPXVs. Infected keratinocytes undergo cytopathic effects, leading to cell death and the formation of characteristic skin lesions such as pustules (Hatmal et al., 2022). These lesions are sites of intense viral replication and host immune responses, characterized by the infiltration of neutrophils, macrophages, and lymphocytes (MacLachlan & Dubovi, 2011).

Also, OPXV infections can affect the central nervous system (CNS), particularly in severe cases. The viruses may disseminate hematogenously or via neuronal pathways, causing encephalitis or other neurological manifestations. This involvement is mediated by the interaction between viral proteins and
receptors on neuronal cells, leading to neuronal dysfunction and inflammation (MacLachlan & Dubovi, 2011).

The hypothalamic-pituitary-adrenal (HPA) axis regulates the systemic response to OPXV infections. Elevated levels of cytokines, particularly IL-1 and TNF-alpha, signal to the hypothalamus, leading to the production of corticotropin-releasing hormone and subsequent activation of the HPA axis (Bailey, Engler, Hunzeker, & Sheridan, 2003; Webster & Sternberg, 2004). This activation releases cortisol from the adrenal glands, which modulates immune responses and helps regulate the fever response in these infections.

OPXV infections in humans involve complex interactions between viral proteins and host cell receptors, triggering vigorous immune responses characterized by cytokine release, chemokine recruitment of immune cells, and activation of the HPA axis.

**High Fever**

The events leading to high fever begin when OPXV activates TLRs and other PRRs on infected and nearby immune cells, triggering the secretion of pro-inflammatory cytokines, including IL-1, IL-6, TNF-alpha, and IFNs. These cytokines provoke the systemic inflammatory response typical of viral infections (Thompson et al., 2011).

IL-1 and IL-6, among other cytokines, transmit signals to the hypothalamus (the brain region regulating body temperature). This action resets the body’s thermoregulatory set point, causing an elevation in core body temperature. Fever is a host defense mechanism that impedes viral replication and bolsters immune function (El-Radhi, 2019).

During fever development, physiological adjustments such as heightened metabolic rate, peripheral vasoconstriction, and shivering promote heat production and conservation. (Elevated body temperature enhances immune cell activity and antibody production, inhibiting viral replication within infected cells.) However, prolonged or excessively high fever may lead to complications such as dehydration, metabolic imbalances, and neurological symptoms (El-Radhi, 2019).

**Malaise**

Upon infection, OPXV targets respiratory epithelial cells and spreads to lymphoid tissues, undergoing extensive replication. This viral replication triggers an acute systemic inflammatory response, marked by the release of pro-inflammatory cytokines such as IL-1, IL-6, TNF-alpha, and IFNs. These cytokines are vital in mediating the symptoms associated with viral infections, including malaise (El-Radhi, 2019; Rijsbergen, Van Dijk, Engel, De Vries, & De Swart, 2021).

Cytokine release into the bloodstream leads to systemic circulation and interaction with various organs and tissues. In particular, cytokines act on the CNS by crossing the blood-brain barrier or signaling through the vagus nerve (Roberto, Patel, & Bajo, 2018). Within the CNS, cytokines influence the hypothalamus and other brain regions that regulate energy balance, mood, and behavior. This interaction results in the activation of the HPA axis and the subsequent release of stress hormones such as cortisol (Roberto et al., 2018).

Cytokines and stress hormones affect neurotransmitter systems, including serotonin, dopamine, and norepinephrine (critical for maintaining normal mood and cognitive function). The disruption of these neurotransmitter systems contributes to malaise, characterized by a general feeling of discomfort, fatigue, and lack of energy (Miller, Haroon, Raison, & Felger, 2013). Also, cytokine-induced metabolic changes increase protein and energy catabolism, further exacerbating feelings of weakness and tiredness (Krapić, Kavazović, & Wensveen, 2021).
The inflammatory response also causes alterations in muscle function and metabolism. Cytokines promote muscle protein breakdown and reduce muscle synthesis, leading to muscle wasting and fatigue (Bouredji, Argaw, & Frenette, 2022). The combined effects of CNS changes, neurotransmitter disruption, and muscle metabolism contribute to the pervasive malaise experienced during OPXV infection.

**Pustular Formation**

VARV, VACV, CPXV, and MPXV cause pustular lesions through similar cellular mechanisms involving infection of epithelial cells, viral replication, cytopathic effects, and inflammatory response. Annexure 1 highlights the similarities and differences in pustular formation among the specific OPXVs.

Upon infection, OPXVs initially attach to specific receptors on host cells, whether in the skin or respiratory epithelium, facilitating entry through endocytosis or direct membrane fusion. In skin infections, basal keratinocytes serve as primary targets, where viral replication leads to cytopathic effects such as inclusion body formation and cellular damage (Lei, Petty, Atwater, Wolfe, & MacLeod, 2020). This process results in vesicle formation progressing to pustules on the skin surface.

Conversely, viral entry into respiratory epithelial cells triggers similar replication cycles within the cytoplasm in respiratory infections like the VARV, bypassing the need for nuclear access. The infected cells undergo morphological changes and cytopathic effects akin to those seen in cutaneous infections, ultimately contributing to systemic dissemination and multi-organ involvement (Lei et al., 2020; Louten, 2016; Sobhy, 2017).

Regardless of the site of primary infection, both skin and respiratory epithelial cells release viral antigens that elicit local inflammation characterized by the secretion of pro-inflammatory cytokines (e.g., IL-1, IL-6, TNF-alpha) and chemokines. These mediators attract immune cells—neutrophils, macrophages, and lymphocytes—to the site of infection, fostering an inflammatory milieu conducive to pustular formation (MacLachlan & Dubovi, 2011; Megha, Joseph, Akhil, & Mohanan, 2021).

Neutrophils contribute to early immune response by phagocytosing viral particles and infected cells, culminating in pus-filled lesion formation as a manifestation of tissue damage. Subsequent recruitment and activation of macrophages and lymphocytes further intensify the immune response, promoting tissue repair processes amidst ongoing viral replication (Chan et al., 2022).

The convergence of viral replication, cytopathic effects, and immune activation within affected tissues culminates in the characteristic pustular lesions observed in OPXV infections (MacNeill, 2022). These lesions are factorial for viral transmission and diagnostic recognition.

**Management and Treatment for OPXV Infection**

OPXVs cause distinct medical conditions in humans, each requiring specific treatment strategies. Below are the management and medical treatments for conditions caused by VARV, VACV, CPXV, and MPXV.

Table 3 outlines the treatment approaches for various OPXVs, focusing on supportive care measures and specific treatments such as antiviral agents where applicable.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Treatment Approach</th>
<th>Specific Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variola (Breman, 2021; Breman &amp; Henderson, 2002; Centers for Disease Control and Prevention [CDC], n.d.-b)</td>
<td>Supportive care focusing on hydration, nutrition, pain management, and fever reduction</td>
<td>- Hydration and nutrition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Pain management</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Antipyretics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Antibiotics for secondary bacterial infections</td>
</tr>
</tbody>
</table>
The treatment of OPXV infections in humans varies based on the specific virus and the severity of the disease. Supportive care is fundamental across all conditions, while specific antiviral agents and immune therapies are reserved for severe or complicated cases. Proper wound care and management of secondary infections are critical components of the treatment strategy for OPXV infections.

### Cellular Mechanisms of OPXV Antiviral Medications

The cellular pharmacological actions and mechanisms of OPXV antiviral medications, such as tecovirimat (ST-246), cidofovir, and brincidofovir, are designed to disrupt various stages of the viral replication cycle, thereby inhibiting viral proliferation and mitigating infection.

**Tecovirimat (ST-246):** Tecovirimat is a small-molecule antiviral that targets the VP37 protein of OPXVs, a critical component in forming and releasing viral particles. By binding to VP37, tecovirimat inhibits the virus's ability to wrap itself in a host-derived membrane, a process necessary for producing extracellular enveloped virus (EEV) forms that facilitate cell-to-cell spread. Without the formation of EEV, the virus is restricted to intracellular accumulation and cannot efficiently disseminate within the host. This mode of action effectively reduces viral load and limits the spread of infection (Russo et al., 2020).

**Cidofovir:** Cidofovir is a nucleoside analog that exerts antiviral effects by inhibiting viral DNA polymerase. Once inside the host cell, cidofovir is phosphorylated to its active diphosphate form by...
cellular kinases. This activated form competes with deoxycytidine triphosphate, the natural substrate of viral DNA polymerase, and is incorporated into the viral DNA chain. This incorporation results in premature chain termination and the inhibition of viral DNA synthesis. Because cidofovir targets a key aspect of viral replication, it exhibits broad-spectrum antiviral activity against various OPXVs. Its primary use is in treating severe infections, especially in cases where other antivirals may be less effective (Bottino et al., 2023).

**Brincidofovir**: Brincidofovir, a lipid conjugate of cidofovir, enhances the pharmacokinetic properties of its parent compound, allowing for improved cellular uptake and bioavailability. After administration, brincidofovir is converted into cidofovir diphosphate within host cells, exerting similar inhibitory effects on viral DNA polymerase as cidofovir. The lipid moiety of brincidofovir facilitates better cellular entry and prolonged intracellular retention, leading to sustained antiviral activity with potentially fewer side effects. This property makes brincidofovir a potent antiviral agent with enhanced efficacy in inhibiting OPXV replication (Alvarez-Cardona, Whited, & Chemaly, 2020).

Collectively, these antiviral medications target distinct stages of the OPXV replication cycle. Tecovirimat interferes with viral egress and spread, while cidofovir and brincidofovir inhibit viral DNA synthesis. Combining these mechanisms provides a multifaceted approach to combating OPXV infections, helping reduce viral load, limit disease severity, and improve patient outcomes.

**Differential Diagnosis of Conditions Associated with OPXVs**

Differential diagnosis of conditions caused by OPXV infections requires a systematic approach encompassing patient history, clinical presentation, and diagnostic testing. Below are the detailed steps for distinguishing between smallpox (VARV; Breman & Henderson, 2002), localized skin infections (VACV; Kelly et al., 2004; Walsh & Dolin, 2011), cowpox (CPXV; Andreani et al., 2019; Bruneau et al., 2023), and monkeypox (MPXV; Altindis, Puca, & Shapo, 2022; Flores, Kerna et al., 2023).

**Smallpox (Variola Virus)**

1. **History:**
   - Inquiry about potential exposure to infected individuals
   - Review of vaccination history for smallpox
   - Assessment of recent travel to areas with known OPXV activity
2. **Clinical Presentation:**
   - High fever, severe malaise, and fatigue
   - Progressive skin rash starts as macules, evolving into papules, vesicles, pustules, and crust
   - Rash distribution typically includes the face, arms, legs, and trunk, with synchronous lesions
3. **Diagnostic Tools:**
   - **Visual Observation**: Identification of the characteristic rash and lesion stages
   - **Laboratory Work**: PCR for VARV DNA
   - **Swab Culture**: Viral culture and electron microscopy to confirm virus identity
   - **Serology**: Detection of VARV-specific antibodies

**Localized Skin Infections (Vaccinia Virus)**

1. **History:**
Recent vaccination with the smallpox vaccine or exposure to infected laboratory animals
Assessment of immunocompromised status or skin conditions like eczema
2. Clinical Presentation:
Localized pustular lesions at the inoculation site
Possible satellite lesions and regional lymphadenopathy
Generally mild systemic symptoms, but may cause severe complications in immunocompromised individuals
3. Diagnostic Tools:
Visual Observation: Assessment of lesion characteristics and location
Laboratory Work: PCR for VARV DNA
Swab Culture: Viral culture is used to confirm the VACV
Serology: Detection of VARV-specific antibodies

Cowpox (Cowpox Virus)
1. History:
Recent contact with infected animals, especially rodents, cats, or cows
Occupational history, including veterinary or farming work
2. Clinical Presentation:
Localized pustular lesions, primarily on the hands and face
Lesions are painful, often with associated swelling and erythema
Generally mild systemic symptoms, such as low-grade fever and malaise
3. Diagnostic Tools:
Visual Observation: Identification of lesion appearance and distribution
Laboratory Work: PCR for CPXV DNA
Swab Culture: Viral culture is used to confirm the CPXV
Serology: Detection of CPXV-specific antibodies

Monkeypox (Monkeypox Virus)
1. History:
Recent travel to endemic regions in Central or West Africa
Contact with wild animals or bushmeat
History of close contact with infected individuals
2. Clinical Presentation:
Fever, headache, muscle aches, and lymphadenopathy
• Rash progression from macules to papules, vesicles, pustules, and crusts, often with lesions in various stages
• Potential for respiratory distress and secondary bacterial infections

3. Diagnostic Tools:
• **Visual Observation:** Rash assessment and recognition of systemic symptoms
• **Laboratory Work:** PCR for MPXV DNA
• **Swab Culture:** Viral culture and electron microscopy to confirm virus identity
• **Serology:** Detection of MPXV-specific antibodies

**Summary Steps for Differential Diagnosis**

1. **Patient History:**
   • Compile a detailed history of exposure, travel, vaccination, and underlying health conditions

2. **Clinical Presentation:**
   • Evaluate symptoms and lesion characteristics, noting distribution and progression

3. **Diagnostic Testing:**
   • Perform PCR to identify specific viral DNA
   • Conduct swab cultures for viral isolation and confirmation
   • Utilize serology to detect virus-specific antibodies

4. **Additional Diagnostic Tools:**
   • Employ electron microscopy for visual confirmation
   • Use immunohistochemistry for antigen detection in tissue samples

By following these steps, clinicians can effectively differentiate between the conditions caused by OPXV infections, ensuring accurate diagnosis and appropriate management. Annexure 2 outlines the signs and symptoms, visual observations, and diagnostic tools (laboratory work, swab culture, serology) used to identify and confirm infections caused by VARV, VACV, CPXV, and MPXV.

**Conclusion**

Orthopoxviruses (OPXVs) are a genus of viruses within the Poxviridae family, characterized by their large, double-stranded DNA and complex replication cycle in host cell cytoplasm. Notable OPXVs, including *Variola* (VARV), *Vaccinia* (VACV), *Cowpox* (CPXV), and *Monkeypox* (MPXV), demonstrate a substantial ability to induce cross-immunity, which has been integral in vaccine development, especially the smallpox vaccine.

OPXVs have a broad range of animal hosts. Transmission occurs through direct contact with infected animals or contaminated materials, resulting in various clinical manifestations in animals and humans. Animal hosts, such as rodents for CPXV and primates for Monkeypox, are pernicious in the zoonotic transmission of these viruses. Human infection pathways include direct contact with infected animals, handling bushmeat, and exposure to contaminated materials.
The clinical manifestations in humans vary by virus. Smallpox, caused by the VARV, is characterized by high fever, malaise, and a progressive pustular rash, contributing to significant morbidity and mortality before its eradication. VACV, used in vaccination, typically causes localized pustular lesions. CPXV results in painful localized lesions, commonly on the hands and face. MPXV presents with fever, headache, muscle aches, lymphadenopathy, and a rash similar to smallpox but generally less severe.

OPXV infections comprise involved cellular interactions between viral proteins and host cell receptors, triggering vigorous immune responses. These responses include the release of cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and interferons (INFs), leading to inflammation, fever, malaise, and pustular formation. Antiviral medications like tecovirimat, cidofovir, and brincidofovir target various stages of the viral replication cycle, offering therapeutic options for managing OPXV infections.

Differential diagnosis of OPXV infections requires a comprehensive approach involving patient history, clinical presentation, and diagnostic testing such as polymerase chain reaction (PCR), viral culture, and serology. Effective management of OPXV infections combines supportive care, specific antiviral treatments, and preventive measures, particularly vaccination, to control and mitigate the impact of these pathogens.

Conflict of Interest Statement

The authors declare that this paper was written without any commercial or financial relationship that could be construed as a potential conflict of interest.

References


Annexures

Annexure 1. Similarities and Differences in Pustular Formation by Specific OPXVs

The following table highlights the similarities and differences in pustular formation among specific OPXVs (*Variola*, *Vaccinia*, *Cowpox*, and *Monkeypox*).

<table>
<thead>
<tr>
<th>Aspect</th>
<th><em>Variola</em> (VARV)</th>
<th><em>Vaccinia</em> (VACV)</th>
<th><em>Cowpox</em> Virus (CPXV)</th>
<th><em>Monkeypox</em> Virus (MPXV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Infection and Viral Entry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entry via viral glycoproteins and cellular receptors</td>
<td>Infects keratinocytes</td>
<td>Infects keratinocytes</td>
<td>Entry through specific interactions with cell surface receptors</td>
<td>Attachment to specific epithelial cell receptors</td>
</tr>
<tr>
<td>Membrane fusion releases viral genetic material into cytoplasm</td>
<td>Membrane fusion releases viral genetic material into cytoplasm</td>
<td>Viral genetic material (double-stranded DNA) released into infected cell cytoplasm</td>
<td>Genetic material (double-stranded DNA) delivered into infected cell cytoplasm</td>
<td></td>
</tr>
<tr>
<td><strong>Viral Replication</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utilizes host cell machinery for transcription and translation</td>
<td>Utilizes host cell machinery for transcription and translation</td>
<td>Utilizes host cell machinery for viral replication</td>
<td>Replicates genetic material, produces viral proteins</td>
<td></td>
</tr>
<tr>
<td>Replication occurs entirely in the cytoplasm</td>
<td>Replication occurs entirely in the cytoplasm</td>
<td>Viral genes transcribed and translated for new virion assembly</td>
<td>Accumulation of viral particles disrupts cellular functions</td>
<td></td>
</tr>
<tr>
<td><strong>Cytopathic Effects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant: cell swelling, rounding, membrane blebbing</td>
<td>Significant: cell swelling, rounding, membrane blebbing</td>
<td>Infected cells undergo cytopathic effects</td>
<td>Cell swelling, membrane blebbing, cell death</td>
<td></td>
</tr>
<tr>
<td>Accumulation of viral particles causes cellular damage</td>
<td>Accumulation of viral particles causes cellular damage</td>
<td>Substantial alterations in host cell morphology and function</td>
<td>Structural changes, disruption of cellular functions</td>
<td></td>
</tr>
<tr>
<td><strong>Inflammatory Response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triggers robust inflammatory response (IL-1, IL-6, TNF-alpha)</td>
<td>Triggers vigorous inflammatory response (IL-1, IL-6, TNF-alpha)</td>
<td>Pro-inflammatory cytokines and chemokines released</td>
<td>Pro-inflammatory cytokines and chemokines released</td>
<td></td>
</tr>
<tr>
<td>Recruitment of immune cells: neutrophils, macrophages, lymphocytes</td>
<td>Recruitment of immune cells: neutrophils, macrophages, lymphocytes</td>
<td>Immune cells recruited to site of infection</td>
<td>Neutrophils, macrophages recruited to infection site</td>
<td></td>
</tr>
<tr>
<td>Formation of Pustules</td>
<td>Influx of immune cells leads to pustule formation</td>
<td>Influx of immune cells leads to pustule formation</td>
<td>Formation of pus-filled lesions on skin surface</td>
<td>Pustules form due to immune response and viral replication</td>
</tr>
<tr>
<td>----------------------</td>
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<td>-------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Neutrophils phagocytose infected cells, release enzymes and reactive oxygen species</td>
<td>Neutrophils phagocytose infected cells, release enzymes and reactive oxygen species</td>
<td>Neutrophils crucial in phagocytosis and tissue damage</td>
<td>Tissue damage, formation of characteristic pustules</td>
</tr>
</tbody>
</table>

| Disease Severity and Progression | Severe systemic disease, widespread pustules | Localized skin infections, pustules at inoculation site | Localized inflammatory reaction, pustular lesions primarily on hands and face | Less severe than smallpox, localized tissue damage |

| Immune Evasion Strategies | Inhibits apoptosis, modulates cytokine production | Less virulent, self-limiting infections | Moderate virulence, potential severity in anergy | Modulates host immune response |

| Clinical Manifestations | Extensive viral replication, systemic symptoms | Localized pustules, mild systemic effects | Localized pustular lesions, mild to moderate symptoms | Pustular lesions, localized inflammation |

| Systemic Symptoms | High fever: >101°F (38.3°C), severe malaise | High fever, malaise | High fever, malaise | High fever, malaise, headache, muscle aches |

| — | — | Feeling unwell, local symptoms at infection site | Fatigue, body aches, general sense of illness, headache, muscle aches |

Sources: Bailey et al., 2003; Bouredji et al., 2022; Brown et al., 2023; Chan et al., 2022; El-Radhi, 2019; Fashina et al., 2022; Hatmal et al., 2022; Krapić et al., 2021; Lei et al., 2020; Louten, 2016; MacLachlan & Dubovi, 2011; MacNeill, 2022; Megha et al., 2021; Miller et al., 2013; Rijsbergen et al., 2021; Roberto et al., 2018; Schmidt et al., 2012; Shchelkunova & Shchelkunov, 2022; Thompson et al., 2011; Webster & Sternberg, 2004

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References


**Annexure 2. Diagnostic Tools for Specific OPXVs**

This table outlines the signs and symptoms, visual observations, and diagnostic tools (laboratory work, swab culture, serology) used to identify and confirm infections caused by *Variola, Vaccinia, Cowpox*, and *Monkeypox* viruses.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Signs and Symptoms</th>
<th>Visual Observation</th>
<th>Laboratory Work</th>
<th>Swab Culture</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variola (VARV)</td>
<td>High fever, severe malaise, fatigue, progressive skin rash (macules, papules, vesicles, pustules, crusts)</td>
<td>✔</td>
<td>PCR for VARV DNA</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Rash distribution includes face, arms, legs, trunk</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vaccinia (VACV)</td>
<td>Pustular lesions at inoculation site, satellite lesions, lymphadenopathy</td>
<td>✔</td>
<td>PCR for VACV DNA</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Cowpox (CPXV)</td>
<td>Localized pustular lesions on hands and face, pain, swelling, erythema</td>
<td>✔</td>
<td>PCR for CPXV DNA</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Mild systemic symptoms (low-grade fever, malaise)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkeypox (MPXV)</td>
<td>Fever, headache, muscle aches, lymphadenopathy, rash (macules, papules, vesicles, pustules, crusts)</td>
<td>✔</td>
<td>PCR for MPXV DNA</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Respiratory distress, potential for secondary bacterial infections</td>
<td></td>
<td>Viral culture, electron microscopy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** Checkmarks indicate the presence of specific signs and symptoms, visual observations, and diagnostic tests applicable to each OPXV.

**Sources:** Altindis et al., 2022; Andreani et al., 2019; Breman & Henderson, 2002; Bruneau et al., 2023; Kelly et al., 2004; Walsh & Dolin, 2011
The table provides a quick reference for the diagnostic approach for each virus based on clinical presentation and laboratory investigation.

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**References**


