SMALLPOX

Pathogen
Poxviruses: Variola major (causes the severe form of the disease) and Variola minor (less common, causes a mild form of the disease).

Size of poxviruses: 200 x 250 - 300 x 250 nm³

The lesions of smallpox are largely limited to the skin and oropharyngeal mucosa (localized disease), but in some cases, a disseminated form of the disease can develop. “Each infected cell produces 2 different kinds of virions. The majority (intracellular mature virions) remain within necrotic cells and are shed in skin debris or saliva droplets, where they serve as sources of infection. A small percentage acquire an additional membrane and are transported to the cell surface. These extracellular enveloped virions are responsible for cell-to-cell spread and may participate in systemic dissemination. A number of factors determine whether an orthopoxvirus causes localized or disseminated infection in a given host. The route of infection is important, because variola causes severe systemic illness when inhaled, but it usually produces milder disease when inoculated into the skin. Host immune status is critical, because vaccinia virus can cause diffuse infection in persons with atopic dermatitis and progressive disease in those with cell-mediated immunodeficiency.”

Portal of entry into the host: skin, respiratory tract, oral route (minor)

The variola virus was recovered from many different tissues of infected patients, infection of squamous epithelium appears to be essential to the virus’s “survival strategy.”

Progression: “The mousepox model suggests that replication at the point of entry was followed by infection of mononuclear phagocytic cells in regional lymph nodes, possibly with further spread through the bloodstream to similar cells in the liver, spleen, and other tissues. The incubation period ended when the release of inflammatory mediators from infected cells caused fever and other symptoms, and the spread of virus— either within infected monocytes or as free virions—to capillaries in the skin and mucous membranes initiated the rash. After reaching the skin, the virus spread cell-to-cell through its mid- and basal layers, causing expanding zones of necrosis that formed vesicles.”

Stages of the disease:
1. Incubation period: 10-14 days
2. Flu-like prodrome: 2-3 days
3. Appearance of centrifugally distributed lesions (papular rash) on skin (more numerous on the extremities than on the trunk – probably because the virus replicates best at 35°C)

PATHOGEN LOAD

Data from monkeys infected with two variola strains:
- Peripheral blood white blood cell count increased 20%
- Viral load in peripheral blood monocytes: $10^2 - 10^6$ PFU (plaque forming units) per $10^6$ cells (on second day after inoculation) and increased with time. No viruses in the cell-free plasma.

- Evaluation of infectious viral burdens in organs of monkeys at necropsy revealed viral titers in vast excess of the inoculum, ranging from $10^5$ pfu/g of brain to $10^9$ pfu/g in adrenal, kidney, spleen, and liver. A detailed report on the pathological findings will be submitted separately (M.J.M., L.E.H., J.W.H., W. Shieh, S. Zaki, and P.B.J., unpublished work).

Zaucha et al. report the viral load in monkeys that died 9 to 17 days after aerosol exposure to monkeypox virus in lung, spleen, liver, kidney and adrenal (Table 3)⁵.

<table>
<thead>
<tr>
<th>Avg. viral titer in animals with detectable titers (log 10 PFU/g)</th>
<th>Lung</th>
<th>Spleen</th>
<th>Liver</th>
<th>Kidney</th>
<th>Adrenal</th>
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<tr>
<td></td>
<td>8.0</td>
<td>5.1</td>
<td>6.2</td>
<td>4.3</td>
<td>4.8</td>
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To calculate the viral load in the blood, need to know the number of monocytes in the blood. Using the normal ranges for human white blood cell counts of $4.3 - 10.8 \times 10^6$/L⁶:

Load range = $(10^2 - 10^6 \text{ PFU/10}^6 \text{ monocytes}) \times (4.3-10.8 \times 10^6 \text{ WBC/ml blood}) \times (1-10\% \text{ monocytes}) = 4.3- 1.8\times10^6 \text{ PFU/ml blood}

Avg. load ≈ 4200 PFU/ml blood.

According to Donaldson et al., virus load in the oropharyngeal or respiratory mucosa (naso-oropharynx) ranges from $10^6$ to $10^8$ pfu/ml⁷.

Sarkar and colleagues report the following values for viral loads⁸:

- Throat swabs:
  - $10^3$-$10^5$ pock-forming units/ml on days 1-4
  - $10^3$-$10^5$ pock-forming units/ml on days 5-7
  - $0-10^3$ pock-forming units/ml on days 8-14

- Conjunctival swabs:
  - $5-7\times10^4$ on day 3
  - $4\times10^2 - 9\times10^4$ on days 4-7
  - $0-7\times10^2$ on days 8-11

- Urine:
  - $10^2 - 10^5$ days 3-4
  - $10-10^4$ on days 5-7
  - $0-10^2$ on days 8-14

Viral load in skin:

- Mice infected with ectromelia (mousepox, mouse model of smallpox): $10^7$ particles/g (on day 9)³.
- Human monkeypox: a 10% suspension of scab material contains up to $10^6$ to $10^7$ PFU/mL (Jezek and Fenner, page 27)⁹.
Viral load/titer in spleen and liver of mice infected with ectromelia on day 7\(^{10}\):
- Spleen: 4.5 log10 units/spleen
- Liver: 4 log10 units/liver

Number of lymphocytes in the spleen of mice\(^{10}\):
- healthy: 1.3x10\(^8\) lymphocytes/spleen
- infected with ectromelia virus: 2.35x10\(^8\) lymphocytes/spleen

Mousepox is a model of human smallpox. Ectromelia virus (ECTV) is a genetically and antigenically very similar to variola virus. Xu et al. reported the viral titers in spleen, liver and draining lymph nodes of mice infected with ectromelia on days 1,3,4, and 7 after infection \(^{11}\):

Relevant values for BioDMET on naïve mice (based on Figure 3) \(^{11}\):
- Draining lymph node:
  - Stage 1: 1.3 log10 PFU/lymph node
  - Stage 2: 6.5-7.2 log10 PFU/lymph node
- Spleen, stage 2: 4-8 log10 PFU
- Liver, stage 2: 3.3-7.7 log10 PFU

References

11. Xu RH, Fang M, Klein-Szanto A, Sigal LJ. Memory CD8+ T cells are gatekeepers of the lymph node draining the site of viral infection. Proc Natl Acad Sci U S A 2007;104(26):10992-10997.