The role of Squanto is complicated.

Tisquantum, known as Squanto, did play a large role in helping the Pilgrims, as American children are taught. His people, the Patuxet, a band of the Wampanoag tribe, had lived on the site where the Pilgrims settled. When they arrived, he became a translator for them in diplomacy and trade with other native people, and showed them the most effective method for planting corn and the best locations to fish, Ms. Sheehan said.

That’s usually where the lesson ends, but that’s just a fraction of his story. He was captured by the English in 1614 and later sold into slavery in Spain. He spent several years in England, where he learned English. He returned to New England in 1619, only to find his entire Patuxet tribe dead from smallpox. He met the Pilgrims in March 1621.

Family: Poxviruses
subfamily: Chordopoxivirus
genus: Orthopoxvirus
species: Variola Major

F. A. Murphy, UC-Davis.

Pox Americana: The Great Smallpox Epidemic of 1775-82. By Elizabeth Fenn

http://whqlibdoc.who.int/smallpox/9241561106.pdf
Small pox is an ancient disease of humans

Brought to India and China by 100 A.D., probably long before

Endemic in east Asia by 1000 A.D.

Large epidemics in Europe 1200-1600 AD

Huge epidemics in South America 1507-1524 (3.5 million Aztecs died in 1520-22)

Estimated to have killed >500 million people in the last 700 years

Daniel Hopkins, The Greatest Killer: Smallpox in History

The Chordopox subfamily is widely distributed in animals

<table>
<thead>
<tr>
<th>Orthopoxvirus</th>
<th>Variola, major and minor</th>
<th>strictly Humans—smallpox.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthopoxvirus</td>
<td>Monkeypox</td>
<td>West Africa and Congo basin. unknown reservoir. wide tropism. Zoonosis to humans</td>
</tr>
<tr>
<td>Orthopoxvirus</td>
<td>Vaccinia</td>
<td>Used as smallpox vaccine, origins not clear, wide tropism.</td>
</tr>
<tr>
<td>Leporipoxvirus</td>
<td>Myxoma</td>
<td>Rabbits, S. America—lethal to old world rabbits</td>
</tr>
<tr>
<td>Avipoxvirus</td>
<td>Fowlpox</td>
<td>birds, many species-specific viruses</td>
</tr>
<tr>
<td>Capripoxvirus</td>
<td>Sheepox</td>
<td>Sheep (goats)</td>
</tr>
<tr>
<td>Suipoxvirus</td>
<td>Swinepox</td>
<td>pigs</td>
</tr>
<tr>
<td>Molluscipoxvirus</td>
<td>Molluscum contagiosum</td>
<td>strictly Humans, usually benign self-limiting skin tumor. can be lethal in AIDS patients</td>
</tr>
<tr>
<td>Yatapoxvirus</td>
<td>Yaba-like disease virus</td>
<td>old world primates (rare zoonosis to humans)</td>
</tr>
</tbody>
</table>

The Entomopox subfamily is very divergent and infects a wide range of insects
Orthopoxvirus diversity

- Despite their genetic diversity, infection with one orthopoxvirus confers protective immunity against another
  - amount of protection depends somewhat on the degree of replication

- Cross-species transmissions occur
  - e.g. cowpox from cows to humans, monkeypox from primates to humans, buffalopox from humans to water buffaloes
  - However, limited spread within the species after such transmissions
Some characteristics of pox viruses

- Virions can be seen with light microscopy

- Large dsDNA genome (~150-300 Kbp depends on virus)
  - encodes many immunomodulatory genes
  - tightly packed genome, but orfs are not overlapping, and no introns
  - >150 open reading frames

- Replicates in the cytoplasm
  - needs to encode enzymes usually associated with the nucleus.
  - mRNAs are capped and polyadenylated by viral enzymes
  - No mRNA splicing

- RNA polymerase encapsidated into virion
  - Virion DNA is not infectious

Pox virus genome structure

Essentially a single-stranded DNA circle with complementary base pairing

---

*Ends of terminal inverted repeats are not perfectly complementary*
VARV genome architecture

**Variola virus genome**

- **Concatamer resolution sequences**
- **Palindromic end**
- **HindIII map (BSH75 banu)**
- **Tandem repeat (TR) arrays**
- **Coding region sequences**

**NOT drawn to scale**

- **Genome ends are not free**
- **Central coding region encodes genes conserved between poxviruses such as replication enzymes and virion proteins**
- **Terminal coding regions at both ends encode genes that are highly variable between pox viruses (virulence genes)**
- **Many viral antagonists of host responses and immunomodulatory genes**

**Pox virus DNA replication**

1. **DNA is nicked and generates a free 3’ -OH**
2. **Self-priming at terminus**
3. **Self-primed extension**
4. **Hairpin formation after end duplication**

Red arrow indicates DNA 3’ ends.

...continued
DNA replication by concatemer formation and resolution

Displacement synthesis from 3’ end

Concatemer formation from continued synthesis

DNA cut to resolve concatemers

Poxvirus replication cycle

1. Entry
2. Early mRNA
3. Uncoating
4. DNA Replication
5. Intermediate mRNA
6. Late mRNA
7. Translation
8. Assembly
9. Concatemer resolution
10. DNA Packaging
11. Maturation
12. Golgi wrapping
13. Exit

early mRNA occurs within virion core
products of early mRNA necessary for release of viral DNA from core and DNA replication
Immune defense genes are also expressed early
Intermediate and late mRNA made in viral-induced membranes

Virion-associated enzymes
- RNA polymerase
- Transcription factors
- Capping enzyme
- Poly(A) polymerase

Early mRNA occurs within virion core
Temporal transcription of pox virus genes

Early transcripts are synthesized within the core particle. About 50% of transcripts including replication genes depend on early genes and DNA replication. The 5' end of the mRNAs contain a non-templated poly(A) “head”. Promoters also use nuclear factor (YY1) recruited to the cytoplasm.

All 3 classes are transcribed by virus-encoded RNA polymerases.

Viral replication takes place in “nucleus-like” virally induced cell organelles derived from the ER.

Yellow represents viral DNA.
Vaccinia DNA factories

Generation of different membrane forms of virus

Membranes form around virion components and DNA is incorporated

DNA stained blue; viral DNA binding protein (I3) stained red

Traffic 6, p839-846 (2005)
Three different types of infectious particles

intracellular mature virion

“wrapped” virion

extracellular virion

membranes: 2
released by cell lysis
major species

4
longer range
infections

3

Smallpox transmission/pathology

• $R_0 = \sim 5$. Mostly face-to-face contacts and spread between family members
• Spread by aerosol/inhalation and less so by contact with skin pustules

It was very important to distinguish smallpox from chickenpox infections
Impressions of Smallpox in Bombay in 1958

“The majority of patients had fully developed smallpox in the suppurative stage, with confluent pustules covering the entire body. The head was usually covered by what appeared to be a single pustule; the nose and the lips were glued together. When the tightly filled vesicles burst, the pus soaked through the bedsheets, became smeared on the blanket and formed thick, yellowish scabs and crusts on the skin. When the pulse was taken tags of skin remained stuck to the fingers... When secondary haemorrhage appeared, the affected area of skin formed a single black mass.

“All the gravely ill patients were also tortured by mucosal symptoms. The tongue was more or less swollen and misshapen and hindered breathing through the mouth. The voice was hoarse and faltering. Swallowing was so painful that the patients refused all nourishment and, in spite of agonizing thirst, often also refused all fluids. We saw patients with deep invasion of the respiratory passages... Wails and groans filled the rooms. The patients were conscious to their last breath.

“Some... just lay there, dull and unresponsive. They no longer shook off the flies which sat on purulent eyelids, on the openings of mouth and nose, and in swarms on the inflamed areas of the skin. But they were still alive, and with touching gestures they lifted their hands and begged for help.” (Translated from Herrlich, 1958.)
Smallpox case fatality depends on strain

- 10-30% case fatality Variola Major
- Everyone infected either died or recovered and had life-long immunity (many survivors have facial scars)

- 1-10% case fatality for Variola Minor (alastrim and amaas) which appeared in South America and Africa at the turn of the 20th century [from the Portuguese alastra, something which "burns like tinder, scatters, spreads from place to place"


Phylogenetic relationships of Variola Major to Variola Minor

Case fatality rates cluster with phylogeny
Early History of Small Pox Vaccines

- “Variolation” was the practice of subcutaneous inoculation of dried pus from a smallpox case into naïve patients.
- **Used wild-type Variola Major virus**
- More localized infection, reduced case fatality from ~20% to 1%, provided life-long immunity to survivors.
- However, inoculated individuals remained infectious for ~2 weeks and had to be quarantined.
- Practiced from at least the 10th century, in China and India.
- Widespread in China by 17th century and in Europe by the 18th century.


Edward Jenner

1796—Jenner took pus from the hand of a milkmaid, Sarah Nelmes

“Grease” was a disease of the hooves of horses that was repeatedly transmitted to cows by milking, and subsequently to human who milked them. Jenner described cases of farm workers who had been infected and years later protected from smallpox.

and inoculated a 8 year old boy, James Phipps on the arm

Jenner then challenged the boy by Variolation 2 months later

Phipps was resistant to smallpox

“Vaccination” from Vacca = “cow” in Latin
Why Variola could be globally eradicated

- Humans were the sole source—no animal reservoir
- All infections result in easily visible symptoms (could begin quarantine and “ring” vaccinations early)
- All infections were resolved (no prolonged shedding)
- One serotype only.
- Vaccine was cheap, could be stored without refrigeration
- Vaccination could be done by community workers and did not require injections
- There was widespread political support

Which of these conditions might preclude eradication of some the other viruses that we have studied in this course?

WHO Resolution for the Eradication of Smallpox, 1959

Legend:
- Red = endemic
- Pink = re-introduced
- Green = imported
- Brown = transmission interrupted

What happened next?

1972 routine childhood vaccination in US was halted

1978: Medical photographer working one floor above a lab in England that studied Variola was infected. Her father was infected and died of smallpox.

The head of the lab committed suicide shortly after

1979: All Variola strains were transferred to either the CDC in Atlanta (450 strains) or to the former Soviet Union, now transferred to Novossibirsk (150 strains)

1990: US military no longer routinely vaccinated
What happened after that?

1990’s remaining stocks of Variola were recommended to be destroyed, but continued debate on issue

They have not been destroyed.

2001: perceived threat of bioterrorism

2002-3: resumption of vaccinations to military and some health care workers.

The US has stockpiles of 100 million doses of vaccine that can be diluted 1:5.

2011: WHO decided to delay destruction of remaining stocks for at least 3 more years. 2014 and 2016; they again decided to defer a decision, this time until 2019

Monkeypox outbreak in the US due to importation of exotic animals from Ghana, 2003

MMWR June 13, 2003 / 52(23):537-540
Increase in monkeypox incidence in the 30 years since the last smallpox vaccination campaign

Comparison of average annual cumulative incidence of human monkeypox by age group Kole Health Zone, Demographic Republic of Congo: 1981–86 vs. 2006–7. *,

What you should know about Poxviruses

- Relationships between different poxvirus genera and diversity of orthopoxviruses
- Characteristics of poxviruses
- Genome structure, replication
- Replication strategy including temporal RNA and generation of different membrane forms
- History of smallpox vaccine and eradication
- The possibility of other poxviruses adapting to humans
- No discussion paper. Work on your final project.