Variola virus & smallpox: Past, present, or future tense?

- Orthopoxvirus Laboratory (DVRD/NCID/CDC)
- World Health Organization Reference Center for Orthopoxviruses

Inger Damon: Lab Director
Yu Li, Richard Kline
Russell Regnery
Poxviruses (I)

- Large complex “brick shaped” virions
- Double stranded DNA
- Cytoplasm of host cells is the ONLY environment permissive for growth
Poxviruses (II)

2 Subfamilies:

**Chordopoxvirinae (vertebrate poxviruses)**
- Orthopoxvirus (variola, cowpox, vaccinia, monkeypox, raccoonpox, camelpox, skunkpox, volepox, ectromelia, taterapox)
- Parapoxvirus (orf, pseudocowpox, …)
- Avipoxvirus (canarypox, fowlpox…)
- Capripoxvirus (goatpox, lumpy skin disease…)
- Leporipoxvirus (myxoma, fibroma…)
- Molluscipoxvirus (molluscum contagiosum)
- Yatapoxvirus (tanapox, Yaba monkey tumor)
- **Entomopoxvirinae (insect poxviruses)**

**Entomopoxvirinae (insect poxviruses)**
Orthopoxviruses:
Spectrum of human disease in normal host

- **Localized infections**: vaccinia, cowpox
- **Systemic illness**: monkeypox, variola
vaccinia vs. variola

- 96% nucleotide identity
- Essential virion proteins with >98% AA identity
- Envelope (glyco)proteins important in humoral recognition over 93% AA identity
- Proteins predicted to, or demonstrated to be involved in immune evasion or host range demonstrate a greater range of homology
  - Variola encodes 24 ORFS whose Vaccinia homologs are truncated or absent
  - Vaccinia encodes 7 ORFS whose Variola homologs are truncated
Patient with Acute, Generalized Vesicular or Pustular Rash Illness

- Institute Airborne & Contact Precautions
- Alert Infection Control on Admission

Low Risk for Smallpox (see criteria below)
- History and Exam: Highly Suggestive of Varicella
- Varicella Testing: Optional

Moderate Risk of Smallpox (see criteria below)
- Diagnosis: Uncertain
- Test for VZV and Other Conditions as indicated
- Non-Smallpox Diagnosis Confirmed Report Results to Infection Control
- ID and/or Derm Consultation
- VZV +/- Other Lab Testing as indicated
- No Diagnosis Made
  - Ensure Adequacy of Specimen
  - ID or Derm Consultant Re-evaluates Patient
  - Appropriate Treatment for Varicella/Other Conditions as Clinically Indicated

High Risk for Smallpox (see criteria below)
- Diagnosis: Uncertain
- ID and/or Derm Consultation
  - Alert Infection Control & Local and State Health Depts

- Patient with Acute, Generalized Vesicular or Pustular Rash Illness
- Institute Airborne & Contact Precautions
  - Alert Infection Control on Admission
- Not Smallpox
  - Further Testing
  - SMALLPOX
- Testing at CDC
- Response Team Advises on Management & Specimen Collection
Diagnostic aims and goals

- Mitigate generation of false-positive results
- Provide laboratory capacity to confirm causes of febrile, vesicular, rash illness, including smallpox
- What combination of diagnostic(s) confers adequate sensitivity and specificity?
- How best to determine what is appropriate at the various levels (under different scenarios)?
  - “pre-event”, probability of smallpox is remote
  - “post-event”, vaccination would be implemented
Overview: laboratory methods for confirmation of Orthopoxvirus dx

- Virus culture
- Immunohistochemistry
- Electron microscopy
- Various PCR
- Serology
  - Antigen detection (IFA, EIA ag capture)
  - IgM capture
  - Neutralization antibodies **
  - IgG ELISA **

What about the Past? and Future??
Virus Culture: The measure of infectious virus

- The gold standard to which all measures of sensitivity are measured.
- Important source of reference material for analysis (e.g., detailed DNA analysis)
- If it isn’t infectious, it isn’t infectious (hoax scenario implications)
- N.B. Issues associated with culture as a diagnostic (facilities and treaty).
PCR strategies

- Essential, conserved genes (E9L, A25R)
  - Difficulty to discriminate amongst species of orthopoxviruses (i.e. vaccinia and variola): typically species generic
  - Unlikely to be manipulated

- Nonessential, variable genes (HA, ATI, crmB)
  - Able to discriminate amongst species of orthopoxviruses: species specific
  - Sources of potential manipulation
HA locus PCR: Variola scabs/crusts

Sensitivity: 8/8  
Correlates with > day 10 rash
PCR: Species generic
HA locus (5-6 hrs)

+ enzyme: TaqI
7-8 hours total
VAC MPX VAR

RFLP: Species specific
Extend-PCR/RFLP variola Bangladesh 1975

PCR Amplicon

BstU I digest patterns

MW
15 kb
10 kb
5 kb

7087 bp
1605 bp
517 bp
222 bp
36 bp
Real time PCR of essential orthopoxvirus genetic loci: Rapid, high throughput screening test: NOT SPECIES SPECIFIC

Melt Curve analysis of PCR products
Real time PCR “TaqMAN” assay development

Essential gene target: E9L (DNA polymerase)

Variola SPECIFIC

monkey blood samples

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Controls: monkeypox (10 to 10x10^6 copies negative)

varicella (negative)
Negative stain electron microscopy

variola vs. varicella

variola  variola  varicella
Immuno electron microscopy: orthopoxvirus (not species-specific)

Virus: vaccinia               monkeypox
Primary: 1:5000 rabbit anti-variola  1:2000 mouse anti variola HMAF
Secondary: 12 nm colloidal gold conjugated , species specific
Day 4 rash
Speciation of clinical sample by single gene PCR-RFLP analysis: ATI gene

Case: v01-I-02

Specimens: vesicular fluid (v), skin roof from vesicle (sk)

RFLP (BglII)

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PCR amplified product yield: skin > vesicular fluid samples

1/8 amplicons sufficient yield for speciation by RFLP: Vaccinia
Extend-PCR RFLP variola Bangladesh 1975

L  2 46 8 10 12 1 4 16 1 8
1  3   5  7 9  11  1 3  15  17 R
DP O C E K H M L I F N A J B GSRQ

PCR Amplicon

MW
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BstU I digest patterns

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E-PCR RFLP evidence for multiple crossover events to produce Patient-02 isolate from two common lab vaccinia strains

*BstUI* digestion of amplicons 1-18
E-PCR RFLP evidence for multiple crossover events to produce Patient-02 isolate from two common lab vaccinia strains

\[ BstUI \text{ digestion of amplicons 1-18,} \]

6 common band patterns not presented
Febrile vesicular rash illness example: disseminated vaccinia lessons summarized

- DFA-VZV negative
- EM – no viral particles seen:
  - Optimize specimen collection: utilize grid to lesion method
- TaqMAN: correct answers in our lab
  - Need to standardize species specific assays, better characterize their sensitivity and specificity
  - Potential utility to screen for orthopoxvirus
- Orthopoxvirus IgM + at day 4 rash (first specimen)
- Culture positive <24 hours
- Interesting, intelligent answers possible
Opportunities for pessimism
“Dark optimism”:

- Smallpox was once an EID event (probably zoonotic) that subsequently benefited from thousands of years to evolve very clever mechanisms to optimize transmission and survival in a limited, host-specific, human context (prevaccination).

- Reminder of the need to be responsive to future possible EID poxvirus events (perhaps not completely unlike possible BT events).
- Febrile Vesicular Rash Algorithm is a creative way to enhance good medicine by better identifying smallpox look-alike diseases, and focus reference diagnostic resources on finite suspect smallpox cases.
- A proven strategy for smallpox control exists (just in case).
- Considerable immune cross-reactivity (the basis of a proven vaccine). Implications beyond vaccines.
- Strategies for vaccines with less adverse rx’s over the horizon.
- Increasingly “intelligent” analytic tests.
- Increasingly sophisticated understanding of basic orthopoxvirus virology leads to potential vulnerabilities.