The challenge of sero-epidemiology for monkeypox

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Human Monkeypox

- Monkeypox (MPX) – caused by MPXV infection
- Fever, swollen lymph nodes, lesions
- Two clades – West African / Congo Basin
- *Poxviridae, Chordopoxvirinae, Orthopoxvirus* genus
- Large dsDNA virus, ~200 kB genome, encodes ~200 ORFs
- OPXV genus
  - Variola virus, vaccinia virus, cowpox virus
  - Proteins encoded by OPXVs exhibit high percent identity
  - Ab developed against one OPXV - broadly reactive
  - Cross-protection provided by smallpox vaccine (Vaccinia virus)
  - Ab cross-reactivity challenges species-specific detection
Serology

- Focus particularly on humoral response
- Generation of immune response after infection - clearance of infection, resolution of lesions
- Detection of Abs - not a diagnostic test
- Serology is important to determine the extent of spread in a population
- Secondary cases – asymptomatic / pausi-symptomatic group
- Anti-OPXV results confounded by prior vaccination / exposure
- Childhood smallpox vaccination – long lasting Ab response
- Vaccination status, age of patients / contacts - important factors to interpret the results
Serology

➢ Types of Abs
  ➢ Neutralizing Antibodies
    ➢ PRNT – Plaque reduction neutralization test
    ➢ Reporter based assays
      ➢ GFP – Flow cytometry/Microscopy
      ➢ Luciferase
  ➢ Binding Antibodies – Ab isotype specific detection
    ➢ ELISA
      ➢ Virus particles
      ➢ Proteins
      ➢ Peptides
    ➢ IFA – indirect fluorescent antibody test
      ➢ Low-throughput
    ➢ Protein microarray

1. Antigen is fixed to a surface.
2. Patient serum is added; if antibodies are present, they bind to the antigen.
3. Secondary antibody (with fluorescent label) is added; if patient antibodies are present, the secondary antibody binds to the patient antibodies.
Serology

- Neutralizing antibodies
  - Two infectious forms of virions – Mature Virus and Extracellular Virus
  - Virus entry is mediated by a large (11-proteins) entry/fusion complex present on the MV membrane
  - Different Ab requirements for MV vs EV neutralization
  - PRNT or reporter-based assays focus mainly on MV neutralization
  - Disadvantages of PRNT
    - Live OPXV
    - Takes 2-3 days

EV membrane, 6 proteins

MV, 30 proteins
Serology

- **Binding antibodies - ELISA**
  - IgM – 1:50 dilutions of serum
  - IgG – 1:100 dilutions of serum
    - End-point titer
  - Correlation with PRNT
    - Endpoint vs PRNT titers better correlation
  - Inactivated virus / proteins / peptides as an antigen
  - IgM positive
    - Indicates recent infection by an OPXV
    - Important, with Epi and Clinical data – probable case
Serology

- Anti-OPXV IgM assay developed during the 2003 US MPX outbreak
  - Capture based assay
Serology

- Anti-OPXV IgM assay
  - Capture based assay

Karem et al, Clin Diagn Lab Immunol, 2005
Serology

- Anti-OPXV IgM assay
  - Capture based assay
  - 2003 US outbreak – 94% IgM pos
    - IgM Pos in previously vaccinated – antigenic differences
  - 2007-11 DRC Kole MPX study – 94% IgM pos
    - All MPX positive cases pos for IgG
- IgM – detected 4-56 days post-rash onset
- IgG – detected 3-7 days following IgM response (in case of primary exposure)

Karem et al, Clin Diagn Lab Immunol, 2005
Pittman et al. medRxiv, 2022
Serology in MPX cases

➢ Sero-positivity in MPX positive cases
  ➢ Both IgM/IgG positive
    ➢ ROC, 2017
    ➢ Sierra Leone, 2017 – MPX positive case after 44 years
    ➢ Nigeria, 2017/18 – MPX positive case after 39 years

➢ 2021 travel related MPX cases in the US
  ➢ Both cases positive for IgM

➢ 2022 MPX outbreak
  ➢ One case tested is positive for IgM positive

Doshi et al., Emer Inf Dis, 2019
Reynolds et al, Emer Inf Dis, 2019
Yinka-Ogunleye et al., Lancet Inf Dis, 2019
Unpublished
Anti-OPXV sero-surveillance

- Cross-sectional sero-surveys to determine prevalence of anti-OPXV Abs
- Uganda, 2004/5; 2011 – 60/20% IgG pos (n=3246)
Anti-OPXV sero-surveillance

- High sero-positivity rate demonstrate potential OPXV circulation
  - Ghana, 2004 – 53 / 36 % IgG pos (n=172) (total / naïve population)
  - Republic of Congo, 2003 – 57 / 49 % IgG pos (n=994)
  - Sierra Leone, 2007 – 10 / 1 % IgG pos (n=1596)
  - Cote d’Ivoire – 51 / 19 % (n=737)
  - DRC – 60 / 26 % (n=267)

Reynolds et al., Am J Trop Med Hyg, 2010
MacNeil et al., BMC Res Notes, 2011
Leendertz et al., Viruses, 2017
Serology assay – additional developments

- Species specific assay
  - Peptide assay – monkeypox specific
    - High sensitivity and specificity
  - Protein microarray based
    - VACV / VARV proteome – Ab response against individual proteins

Dubois et al., Vector-Borne and Zoo Dis, 2012
Davies et al., J. Virol, 2008
Serology assay – additional developments

- Species specific assay
  - Peptide assay – monkeypox specific
    - High sensitivity and specificity
  - Protein microarray based
    - VACV / VARV proteome
    - With Epi/Ecology data – non-VACV Ab detection
  - Retrospective case – Akhmeta virus, Georgia

Townsend et al., J Inf Dis, 2017
Serology assay availability

- Orthopoxvirus specific Ab detection
  - ELISA or other tests are not widely available
  - USA, Germany, Italy, Brazil, UK
  - Commercial ELISA
    - www.myBiosource.com
      - Pox ELISA kit: Rabbit poxvirus
      - No assay details, but potential to cross-react and identify anti-MPXV response
      - Will explore to validate the assay
Serology assay @ CDC

- CDC routinely performs anti-OPXV serology assays – IgG/IgM
  - CLIA approved / ISO17025

- CDC has been reached out by few groups for serological testing

- Research Collaboration Agreement and Simple Letter Agreement available

- Please reach out to us if you are interested
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  - spanayampalli@cdc.gov

- https://www.cdc.gov/poxvirus/monkeypox/index.html

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