Electron Microscopy in Diagnosis of Infectious Diseases

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D
D. Real cases
   1. Examples of organisms diagnosed from patients
   2. Quiz for fun
Cases
Branching bacteria inside vessel in brain
Case Referred from NCSLPH

- Parent noticed facial lesion on child
- School nurse sent her home
- MD suspected anthrax (hx feeding goats); notified HD
- HD notified NCSLPH; took digital pictures
- NCSLPH discounted anthrax; suggested impetigo
- Patient started on antibiotics
- NCSLPH suspected orf virus (goat contact)
- Tissue scraping sent to Duke EM
- Fluid negative, tissue sections positive
Differential Diagnosis

Anthrax
Impetigo
Orf virus
Herpesvirus
Differential Diagnosis: Anthrax
Differential Diagnosis: Impetigo

- Impetigo contagiosa
- Bullus impetigo
- Ecthema

Staphylococcus aureus ("staph")
Streptococcus pyogenes ("strep")
Differential Diagnosis: Orf virus

United States Department of Agriculture Animal and Plant Health Inspection Service National Animal Health Monitoring System (USDA APHIS NAHMS) 2001 sheep survey: 40% of U.S. operations reported sore mouth infecting their flocks in the previous 3 yrs.

Photo courtesy Edie Lederman, MD

Sore mouth in sheep

Sore mouth in goat kid

CDC. Division of Viral and Rickettsial Diseases National Center for Zoonotic, Vector-Borne, and Enteric Diseases
Differential Diagnosis: Herpesvirus

Prof. Dr. Fartasch, Dermatology Department
Universitätsklinik Erlangen, Germany
Herpesvirus
EM Frequently a Last Resort

- Patient was ill for 6 years with a chronic wasting disease
- All tests for infectious agents were negative
- Upon autopsy, an organism was found in heart, liver, intestine, brain by EM
Clinical History

- 59-year-old woman with refractory CLL
- Multiple erythematous nodules on bilateral proximal upper and lower extremities
- No diarrhea, abdominal pain, cough, or fevers
H & E of Skin Biopsy
EM of Skin Biopsy
Centers for Disease Control and Prevention (CDC)

- **Culture**
  - Bone marrow, urine, stool: Negative
- **DNA sequencing**
  - Skin: Positive for microsporidia
- **Polymerase chain reaction (PCR)**
  - Skin: Negative
- **Immunofluorescence assay**
  - Skin: Positive for *Encephalitozoon species*
Results from CDC & EMDV Laboratory

Two species of microsporidia

CDC:
• *Encephalitozoon*
• 2.0-2.5 x 1.0-1.5 µm, polar tube in one row of 6 turns

Duke EM:
• 3 x 2 µm, polar tube in one row of 6 turns
• Consistent with *Encephalitozoon*
• PLUS 3.3 x 1.3 µm, polar tube in one row of 10 turns
Unidentifiable Microsporidia

Poor preservation
Potential Solutions for Soft Blocks

• Rebake (95 °C, hours; 60 °C days)
• Cut thicker sections
• Cut slower
• Pick up sections on grids with a support membrane (carbon-coated Formvar)

• Get more tissue
• Longer infiltration times (days)
• Use microwave processing
Immunosuppressed Child: Parainfluenza Virus Positive Culture During Life

Parainfluenza Virus Immunostain of autopsy tissue

Giant cells, lung parenchyma  αpflu+ Bronch, Trach Epith  αpflu+ Bronch Mucus Glds
αpflu+ Pancr Acinar Cells  αpflu+ Kid Tub Epith Cells  Giant Cells/αpflu+ Bladder Ep

Paramyxovirus

Conventional Fixative

Tissue retrieved from a paraffin block
Trichodysplasia Spinulosa: Clinical History

• 44-year-old man; type I diabetes mellitus
• Kidney-pancreas transplant
• Triple-drug immunosuppressive therapy
• 3 years post-transplant, developed alopecia, which began with his eyebrows and progressed to involve most of his body
• Small, friable, white spines projected from follicular orifices in the affected areas

Invest Dermatol Symp Proc 4:268; 1999
Friable spines in hair follicles
Of Kidney Transplant Patient

Punch biopsy
Trichodysplasia Spinulosa Studies

- **EM**
  - Polyomavirus identified
- **Polymerase chain reaction (PCR)**
  - Negative for HPV subtypes 6/11, 16, 18, 31/33/35/39, 40/42/53/54, 51/52/55/58, 45/56
- **Immunoperoxidase staining**
  - Negative for HPV (broadly crossreacting and several subtype-specific mAb)
  - Negative for BK polyomavirus
  - Weakly positive with broad-spectrum mAb raised against SV40
H&E of Unusual Skin

Thin Section of Polyomavirus in Hair Follicle
Location of Small and or Focal Pathology

Vibrating Tissue Slicer
Selection of Tissue for EM Exam
By Confocal Microscopy

Fix tissue slab 1x1x0.5 cm) in glutaraldehyde
Cut thick sections (100-200) on vibrating microtome
Stain thick sections with propidium iodide

Embed tissue flat surface parallel to eventual plane of thin section
Excise area of interest
Examine thick sections with confocal microscope to identify area of interest

Focal Pathology Identified by Confocal Microscopy of Wet Tissue

Miller et al.
Quiz: What Izzit?
What Izzit?

Infant Aye Aye. Duke University Lemur Center
References for Protocols:

Negative Staining Electron Microscopic Protocol for Rash Illness.
http://www.bt.cdc.gov/labissues/
Then click on title above.

Electron Microscopy for Rapid Diagnosis of Emerging Infectious Agents.

Bioterrorism and electron microscopic differentiation of poxviruses from herpesviruses: dos and don’ts.
Ultrastruc Pathol. 2003;27:133-140.

Modern uses of electron microscopy for detection of viruses.