Electron Microscopy in Diagnosis of Infectious Diseases

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Duke Medicine

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Society for Ultrastructural Pathology

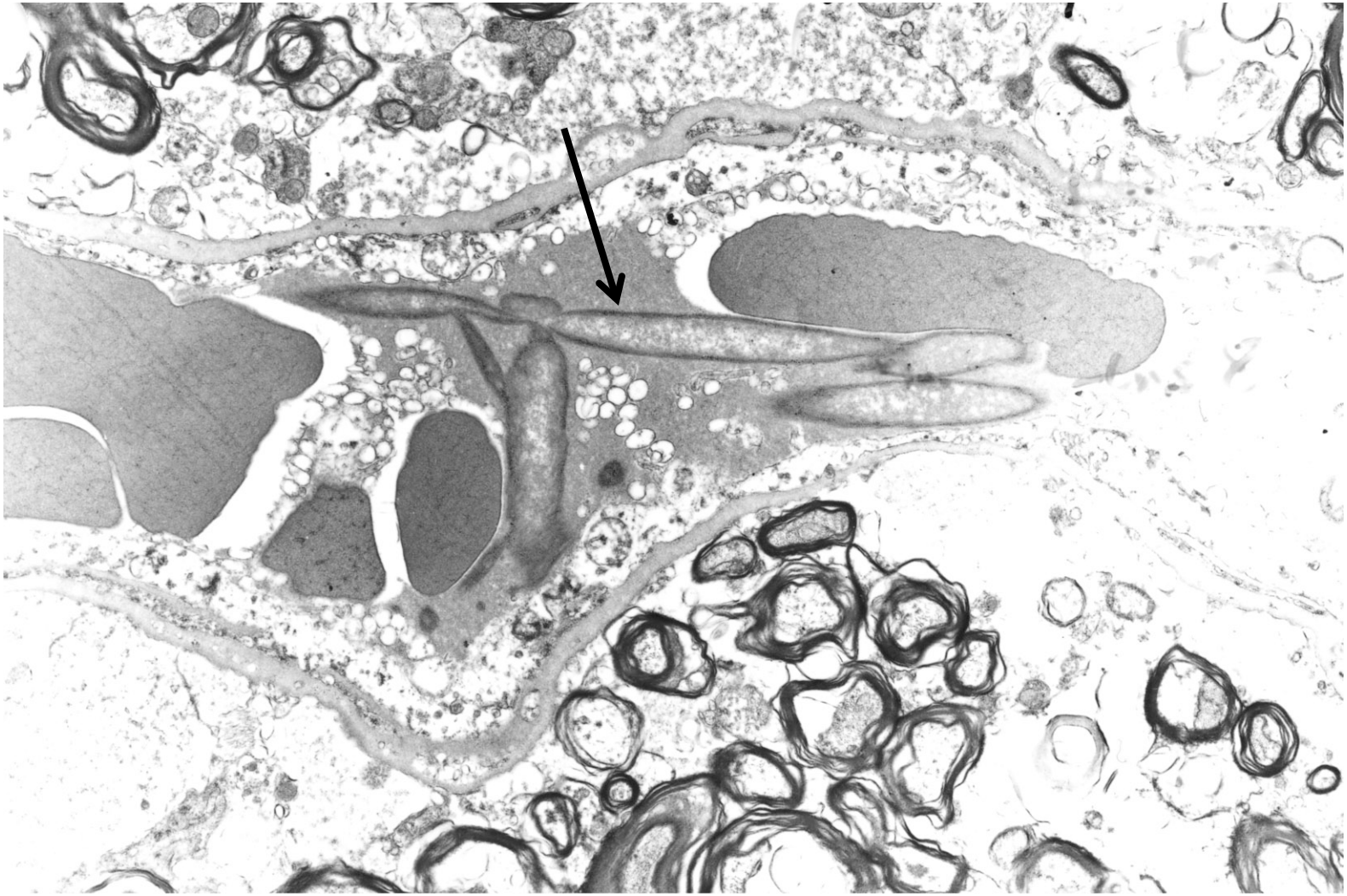
Outline Part 2

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

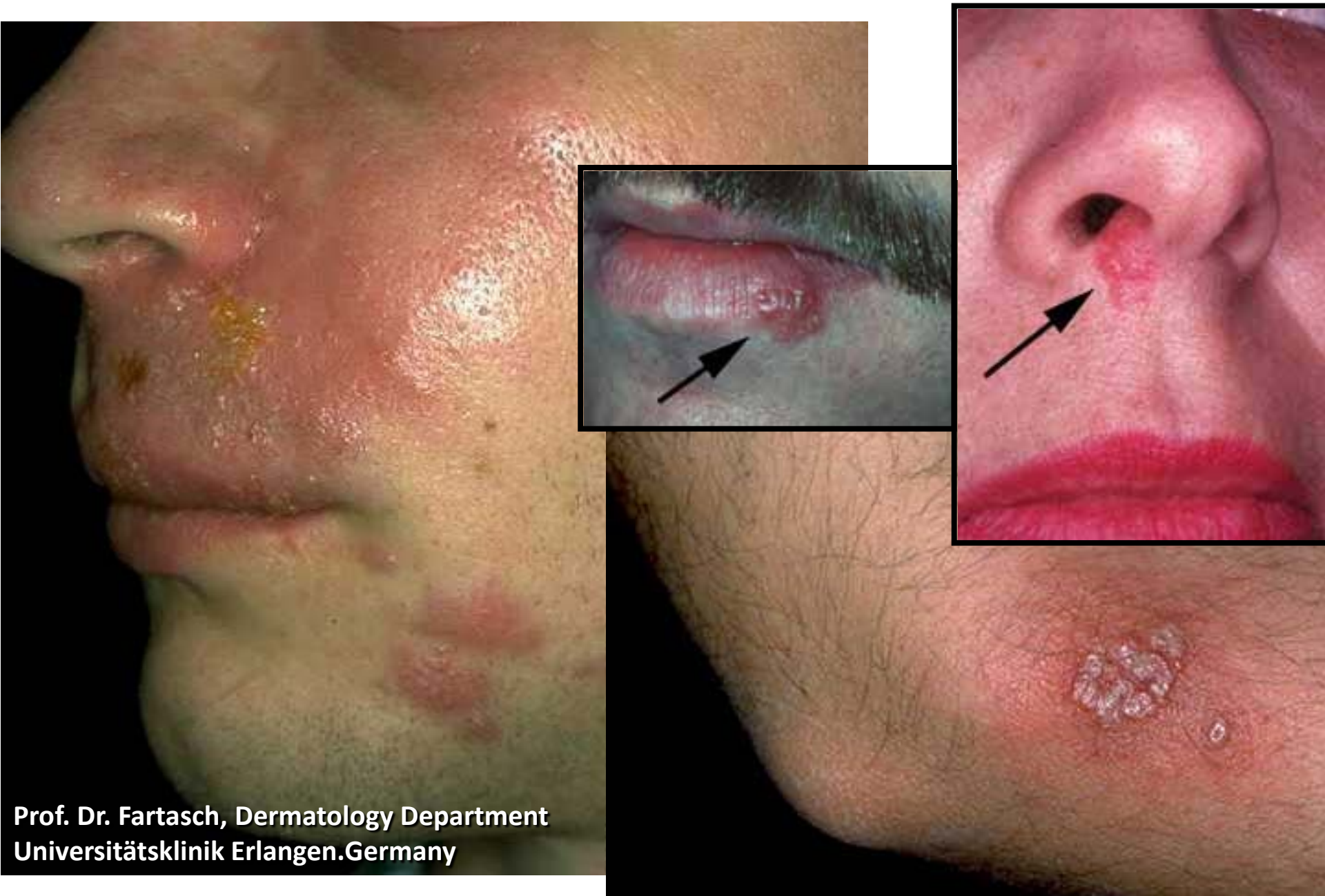
Differential Diagnosis: Impetigo

Staphylococcus aureus **(“staph”)**
Streptococcus pyogenes **(“strep”)**

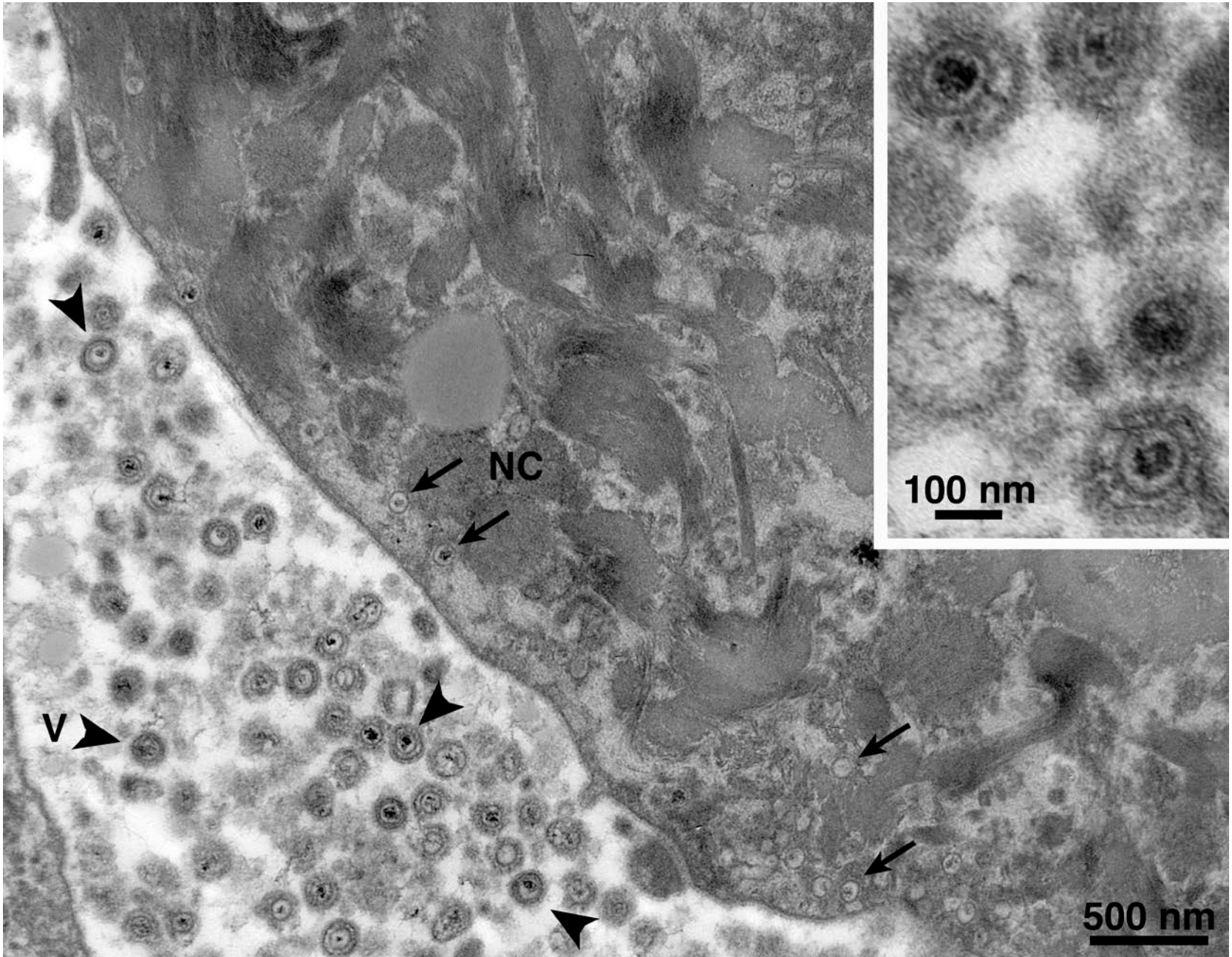
Differential Diagnosis: Orf virus

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.

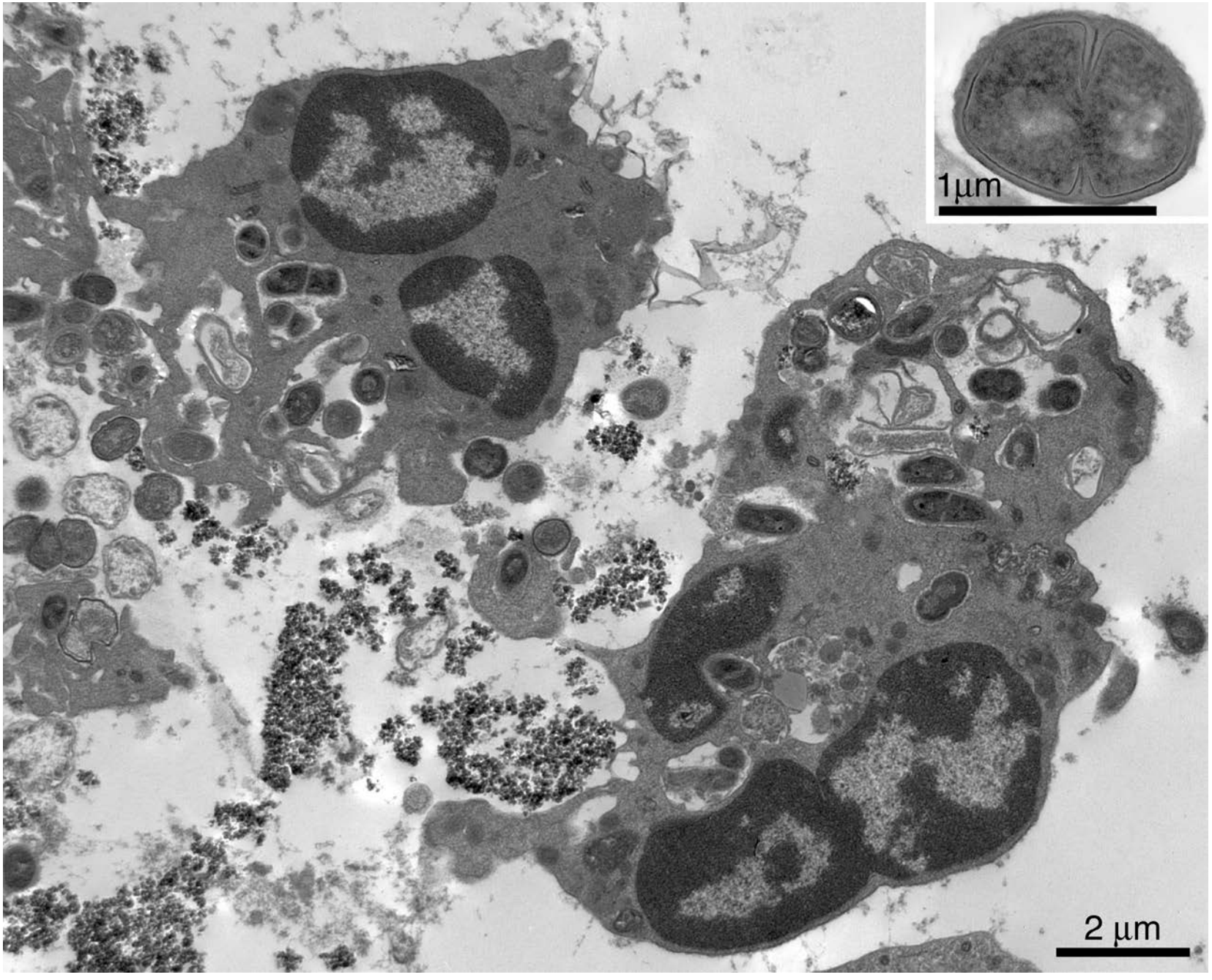
Differential Diagnosis: Herpesvirus



Herpesvirus



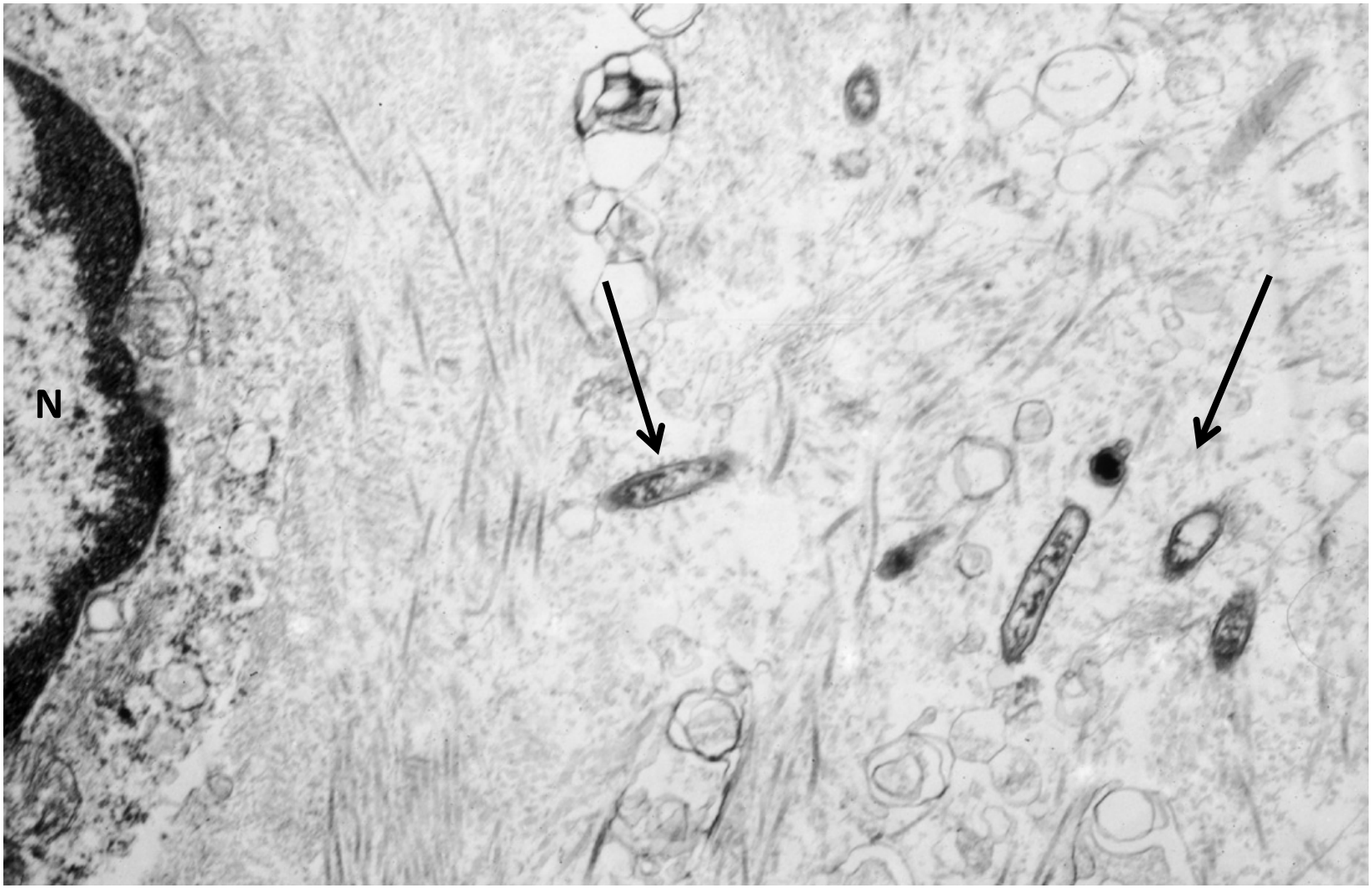
Bacterial Superinfection



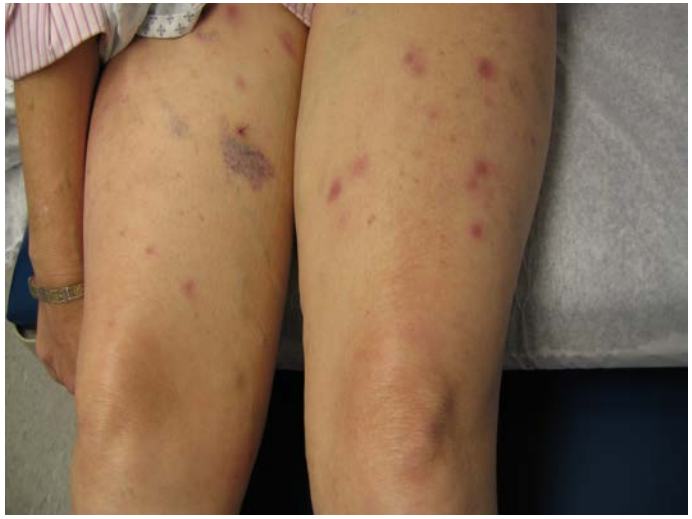
EM Frequently a Last Resort

- **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**

Whipple Bacilli



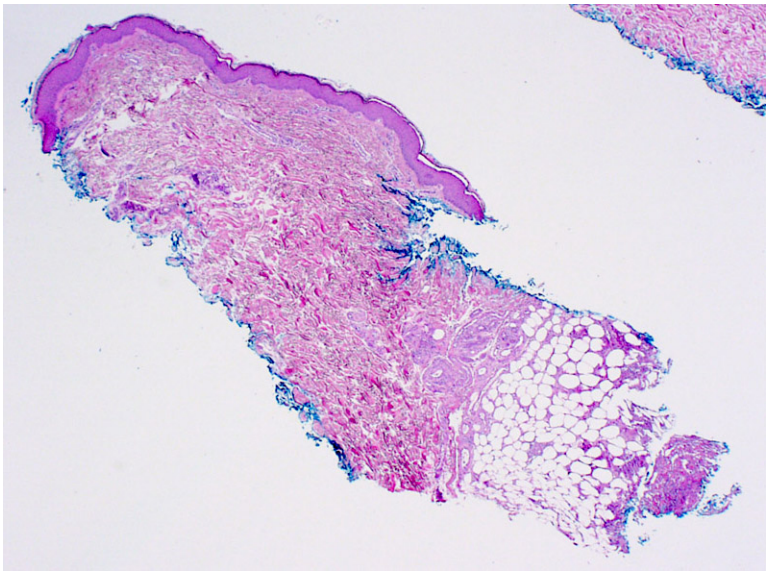
Courtesy of Dr. Dan Kenan; micrograph by Walter Fennell.

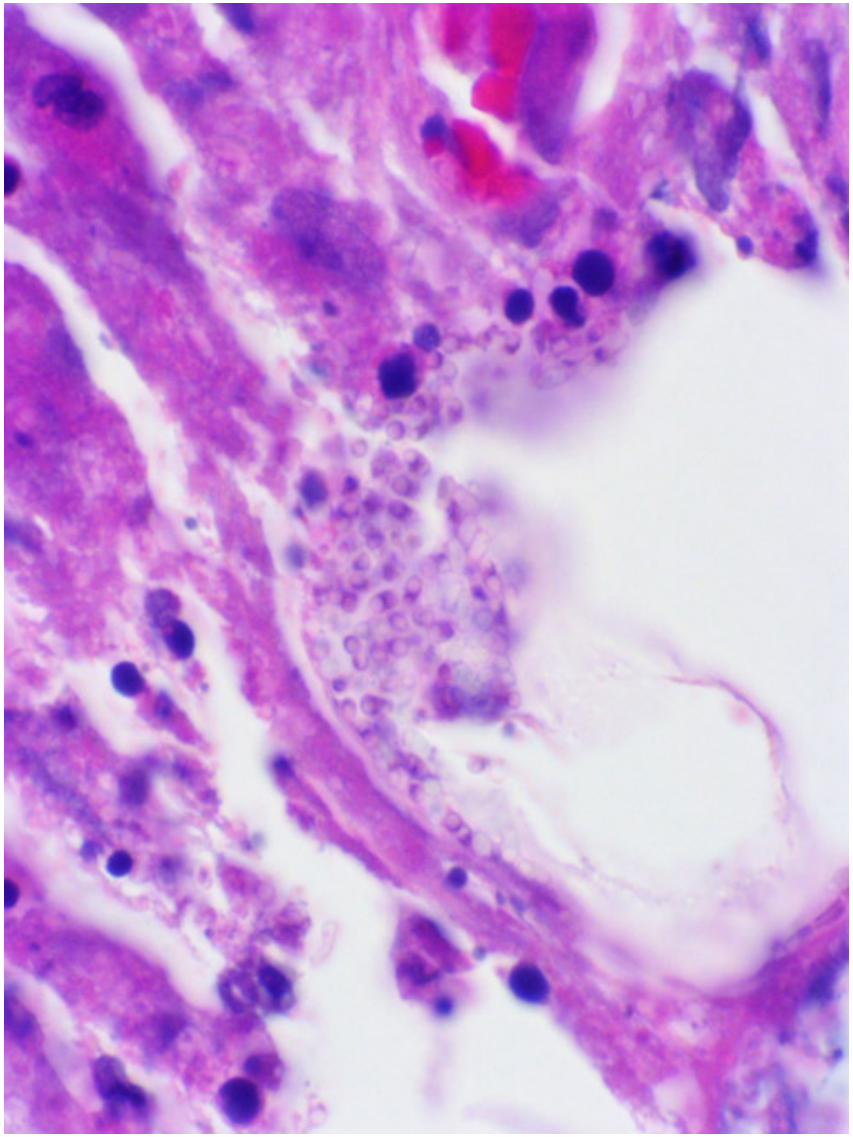


Clinical History

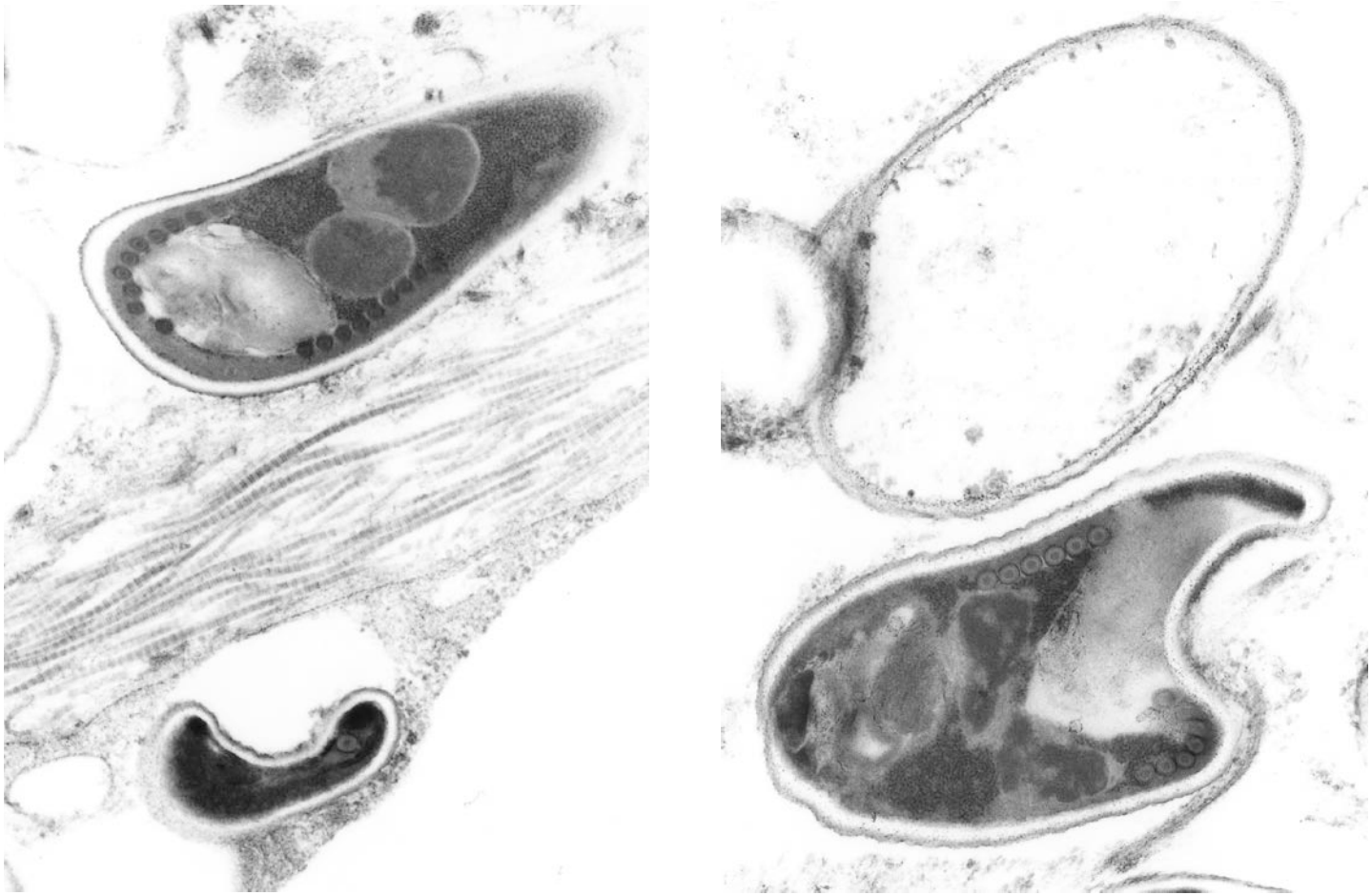
- 59-year-old woman with refractory CLL
- Multiple erythematous nodules on bilateral proximal upper and lower extremities
- No diarrhea, abdominal pain, cough, or fevers

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:

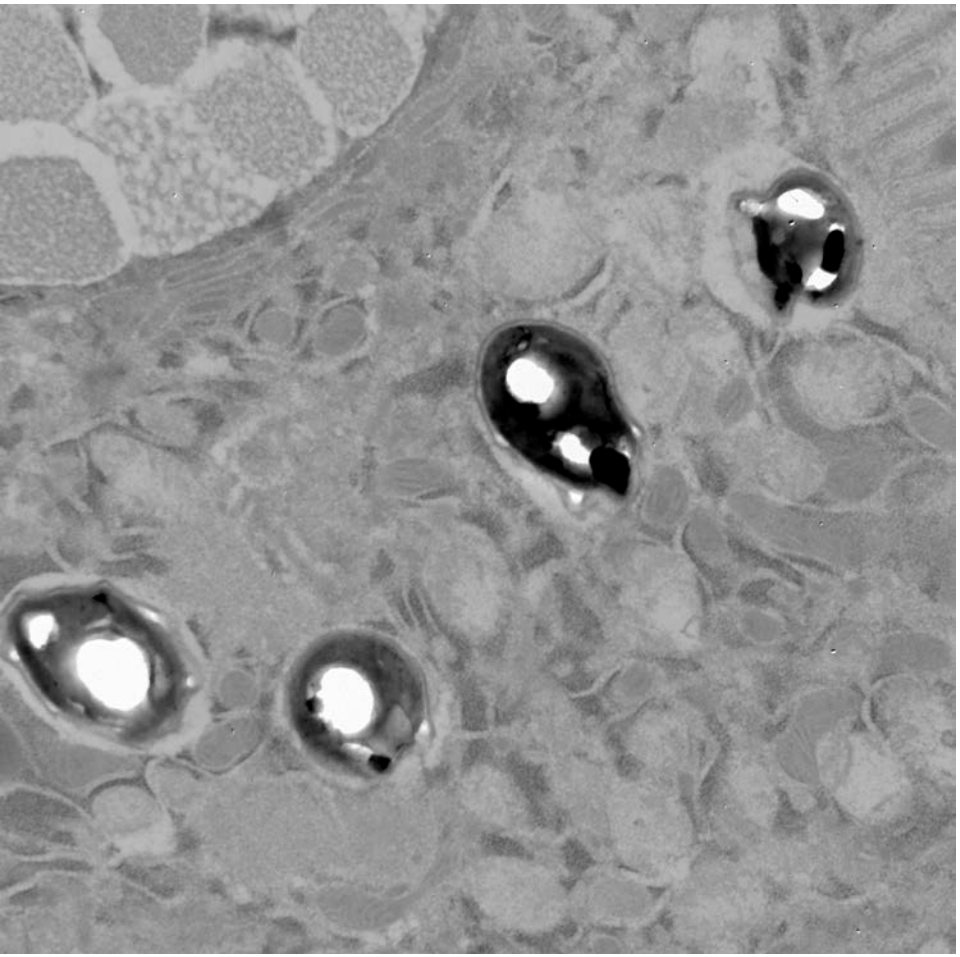
- ***Encephalitozoon***
- **2.0-2.5 x 1.0-1.5 μm, polar tube in one row of 6 turns**

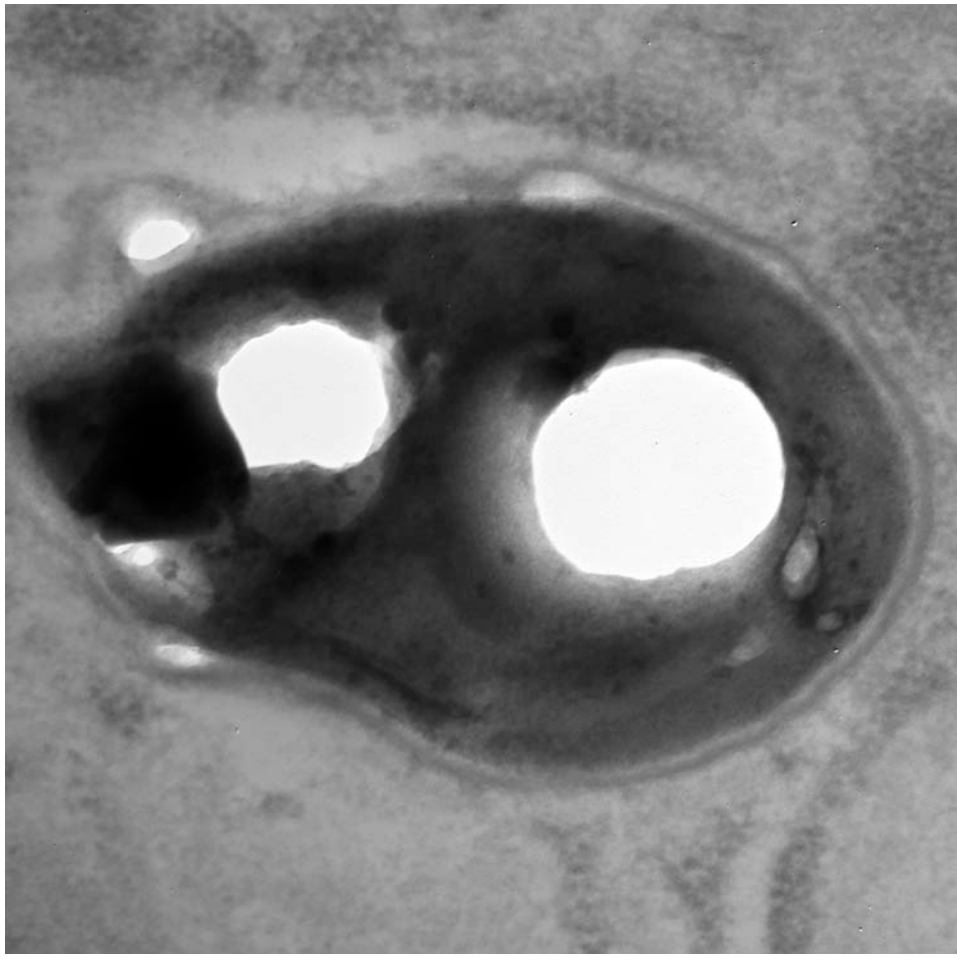
Duke EM:

- **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





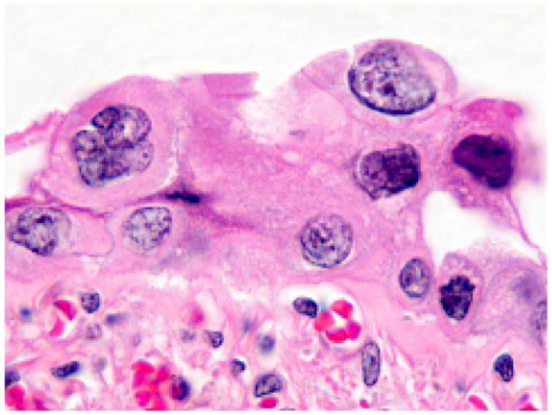
Potential Solutions for Soft Blocks

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

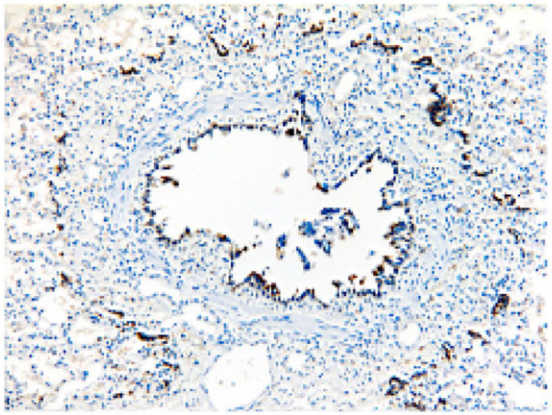
- Get more tissue
- Longer infiltration times (days)
- Use microwave processing

Immunosuppressed Child: Parainfluenza Virus Positive Culture During Life

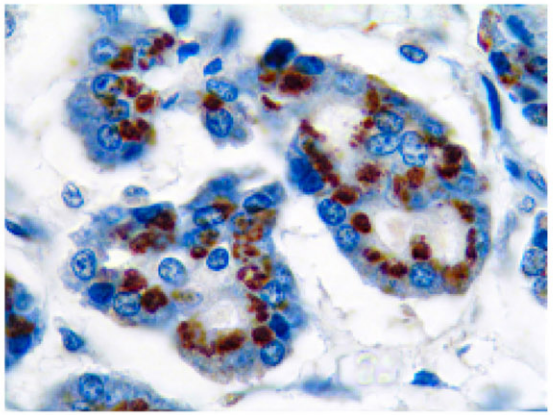
Parainfluenza Virus Immunostain of autopsy tissue



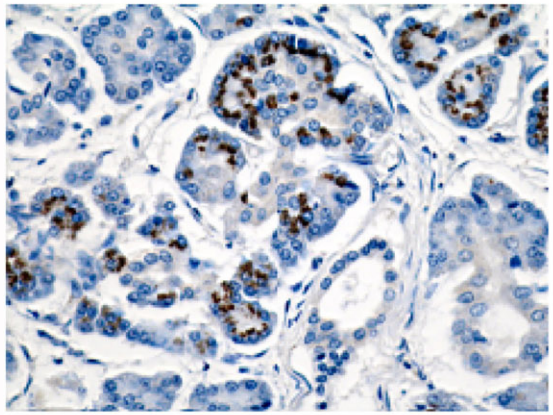
Giant cells, lung parenchyma



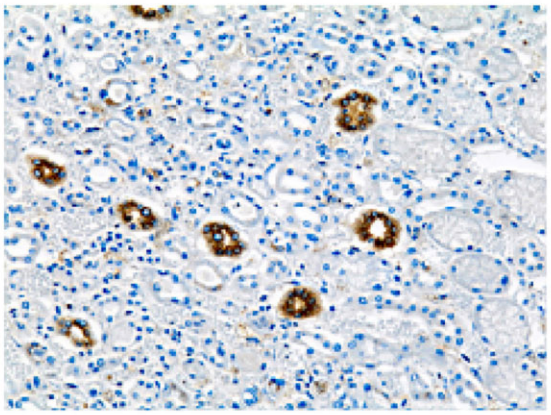
αpflu+ Bronch, Trach Epith



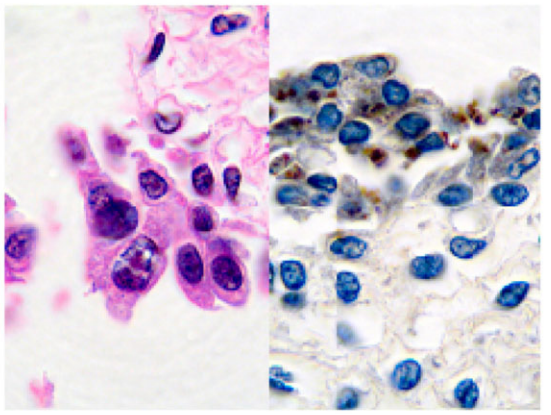
αpflu+ Bronch Mucus Glds



αpflu+ Pancr Acinar Cells



αpflu+ Kid Tub Epith Cells

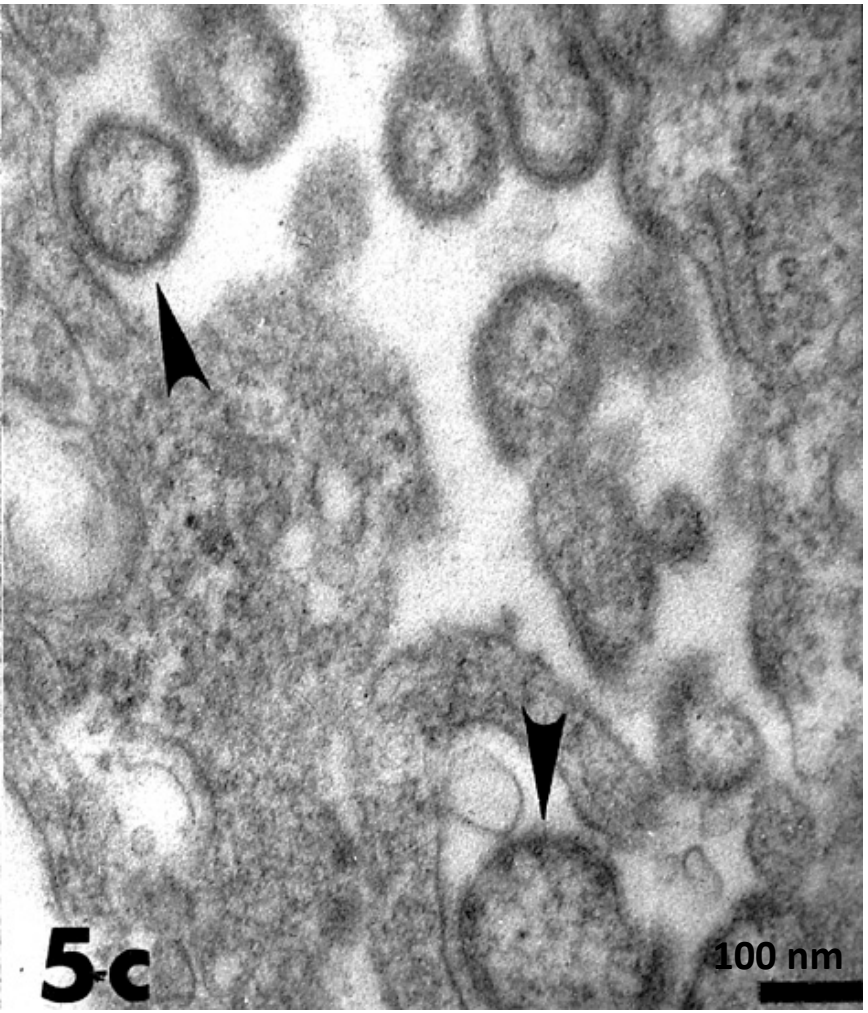


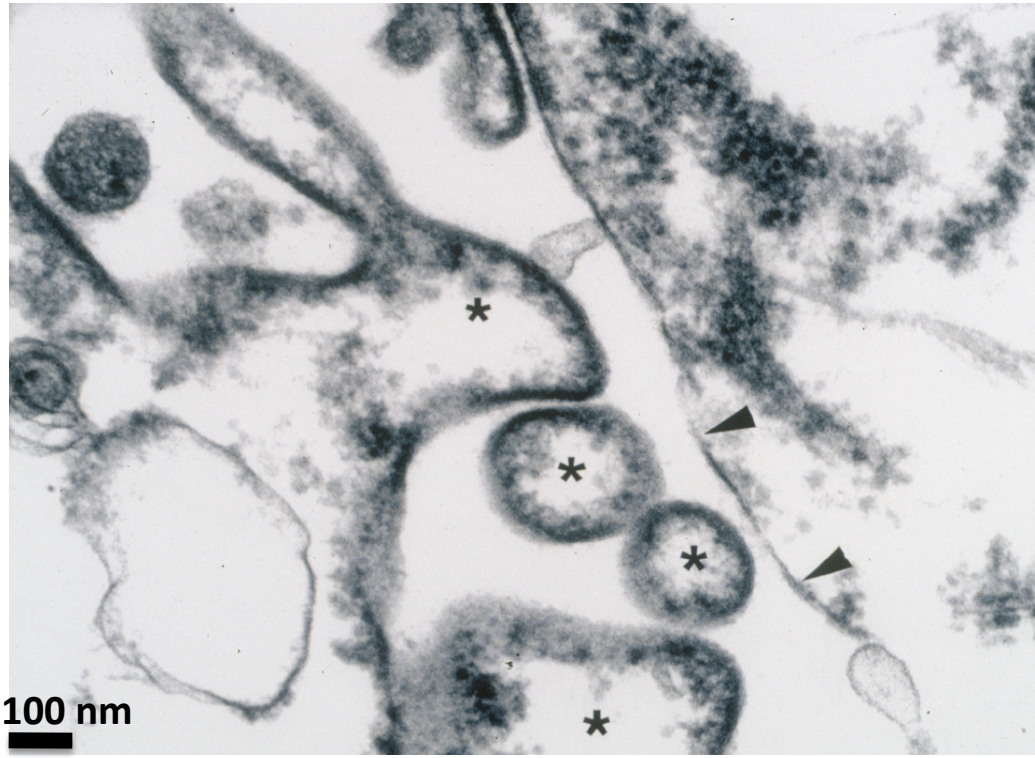
Giant Cells/αpflu+ Bladder Ep

Hum Pathol 35:594-603, 2004.

Paramyxovirus

Conventional Fixative





Tissue retrieved from a paraffin block

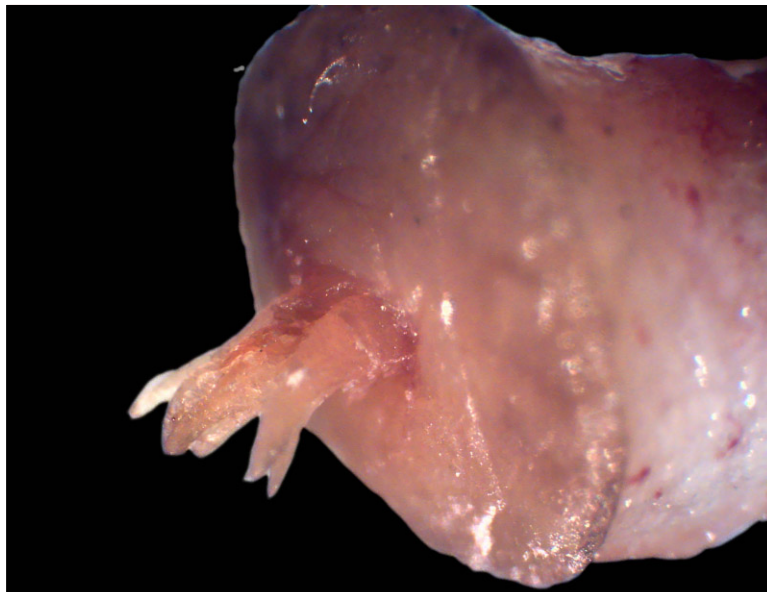
Trichodysplasia Spinulosa: Clinical History

- 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

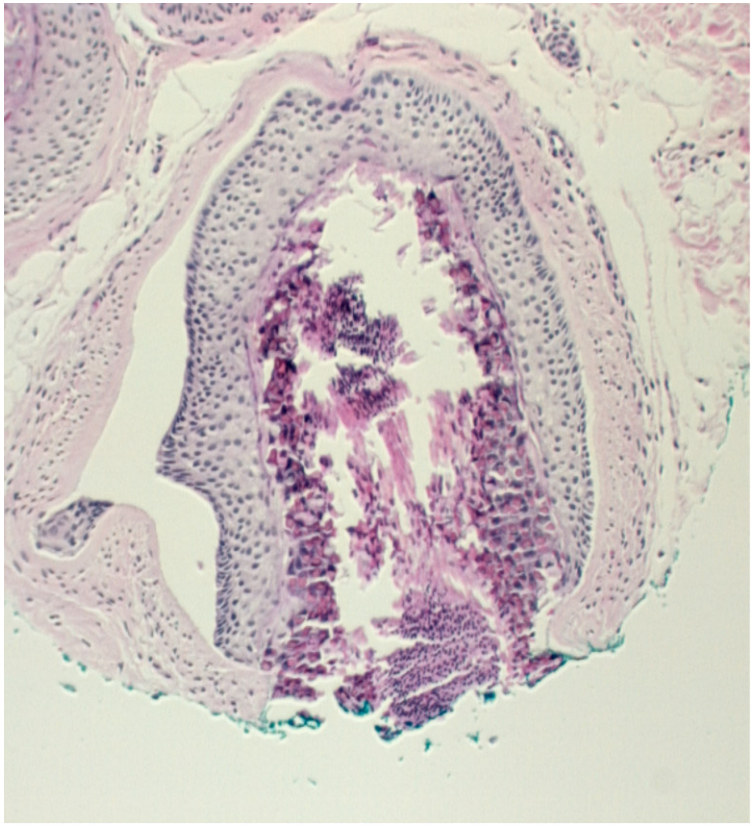
Punch biopsy



Trichodysplasia Spinulosa Studies

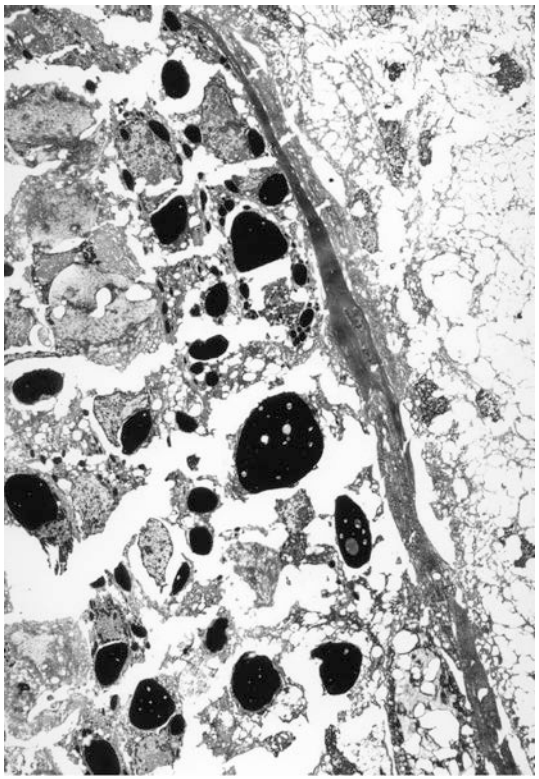
- **EM**
  - **Polyomavirus identified**
- **Polymerase chain reaction (PCR)**
  - **Negative for HPV subtypes 6/11, 16, 18, 31/33/35/39, 40/42/53/54, 51/52/55/58, 45/56**
- **Immunoperoxidase staining**
  - **Negative for HPV (broadly crossreacting and several subtype-specific mAb)**
  - **Negative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

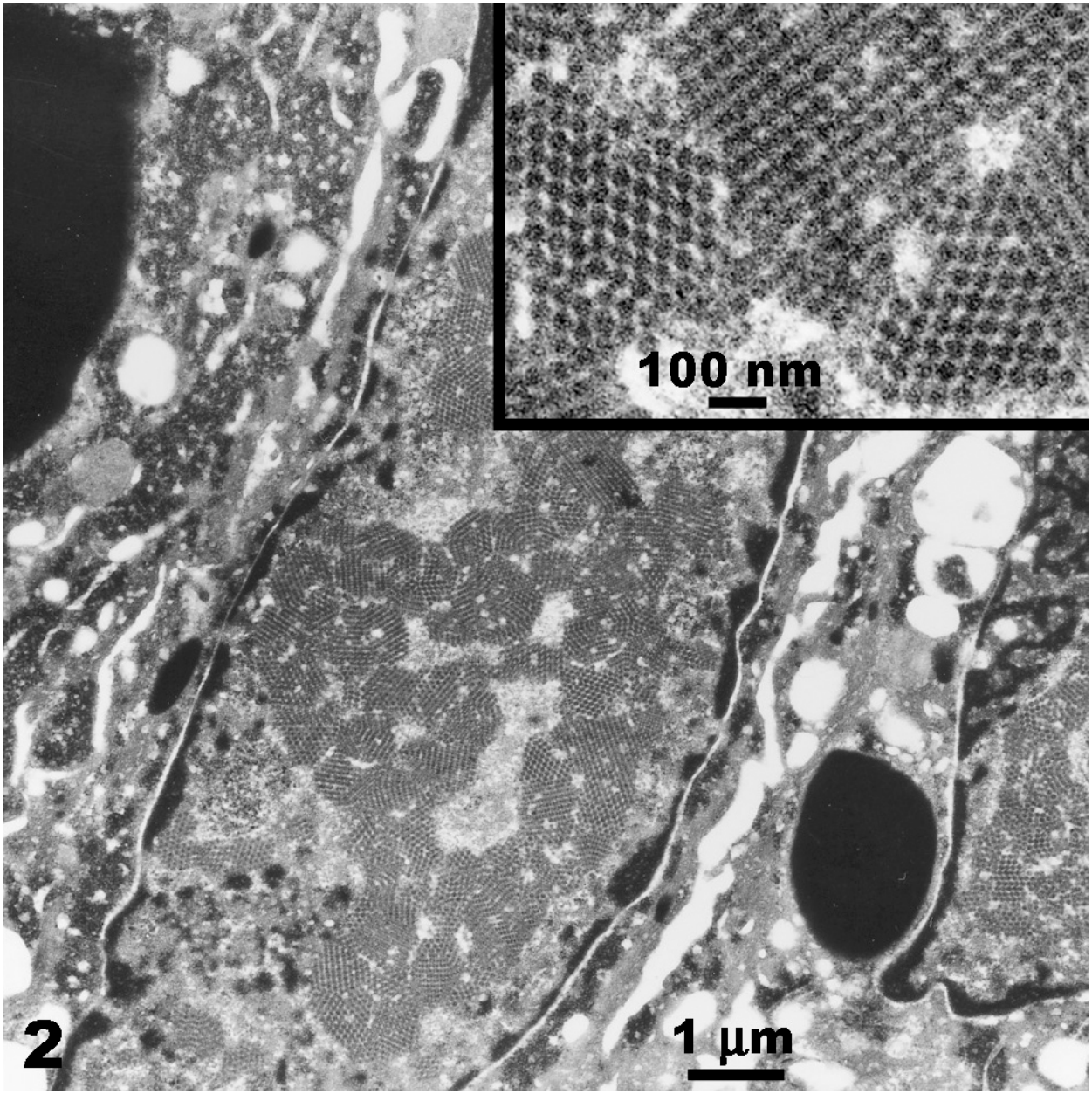
H&E of Unusual Skin



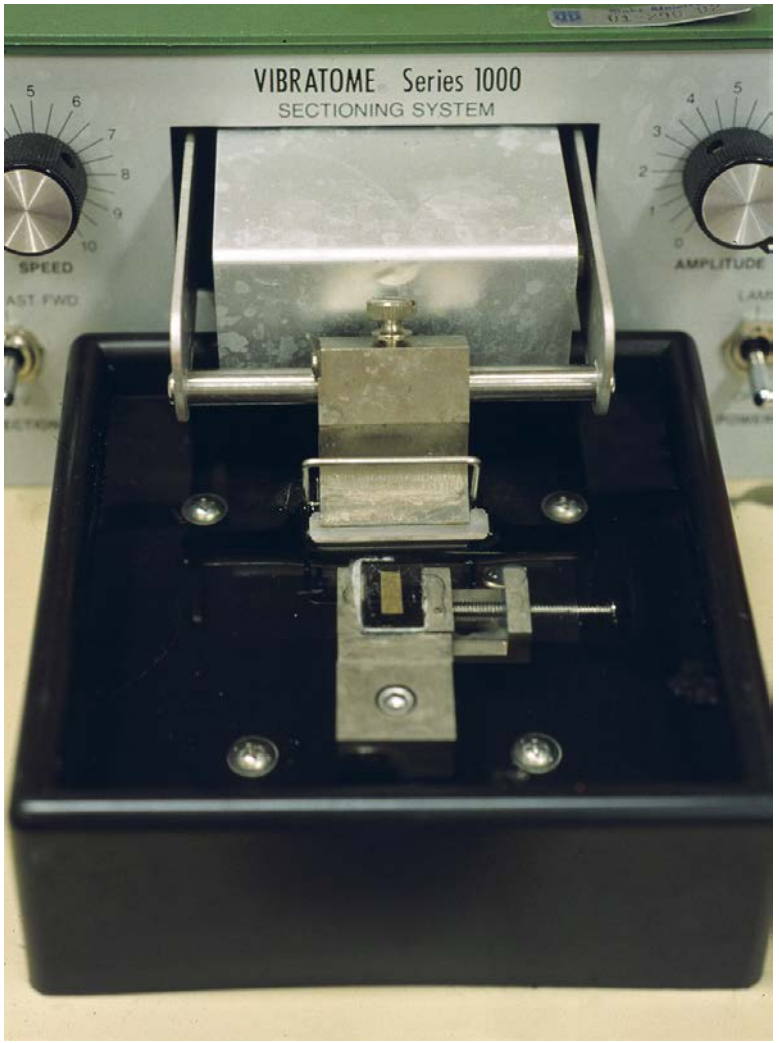
J Investig Dermatol Symp Proc 4(3):268-71, 1999.

Thin Section of Polyomavirus in Hair Follicle



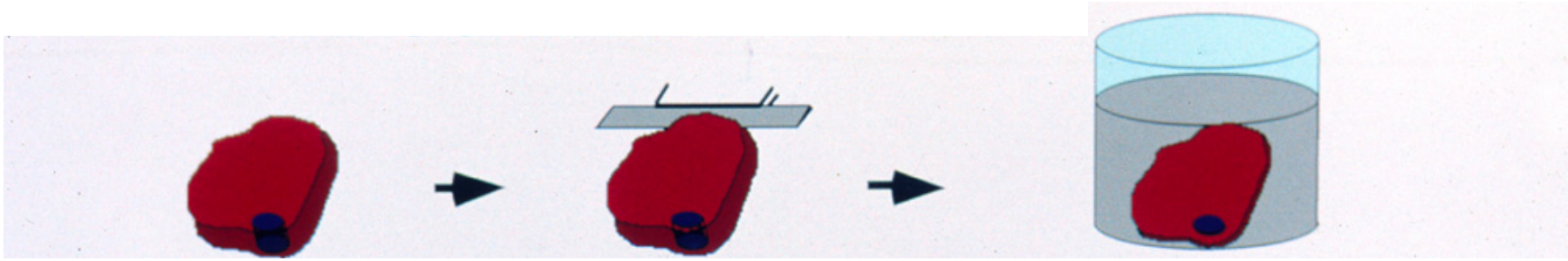


Location of Small and or Focal Pathology



Vibrating Tissue Slicer

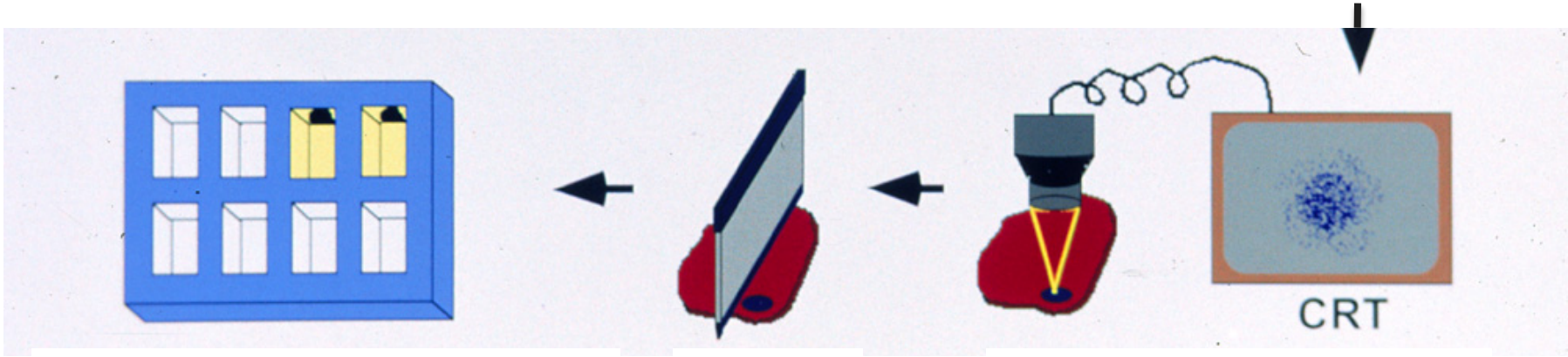
Selection of Tissue for EM Exam By Confocal Microscopy



Fix tissue slab 1x1x0.5 cm) in glutaraldehyde

Cut thick sections (100-200) on vibrating microtome

Stain thick sections with propidium iodide



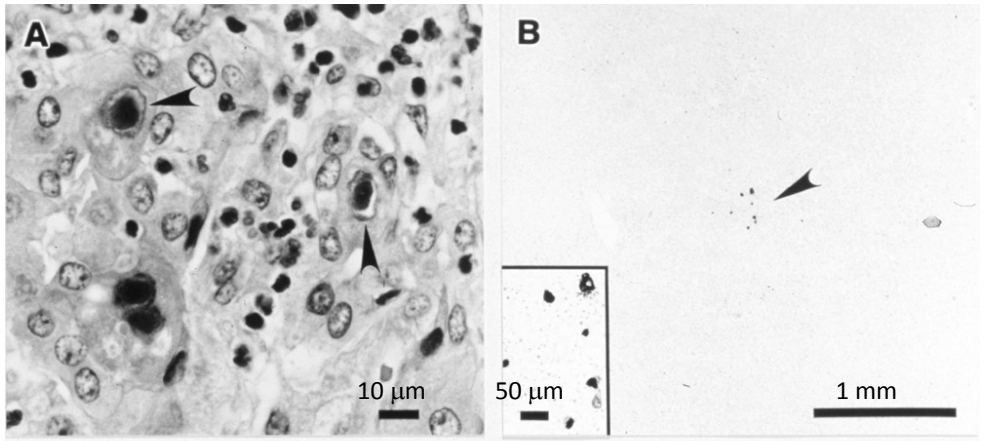
Embed tissue flat surface parallel to eventual plane of thin section

Excise area of interest

Examine thick sections with confocal microscope to identify area of interest

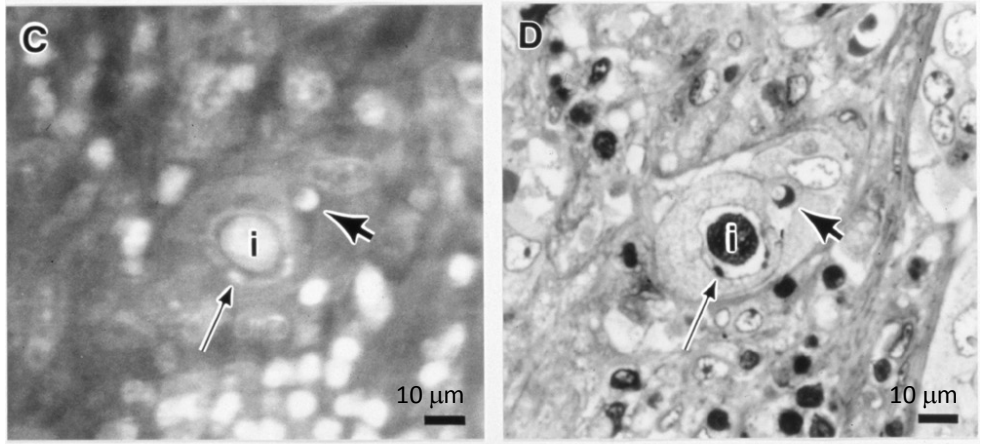
Miller SE, Levenson RM, Aldridge C, Hester S, Kenan DJ, Howell DN. Ultrastruc Pathol 21:183-93, 1997.

Focal Pathology Identified by Confocal Microscopy of Wet Tissue



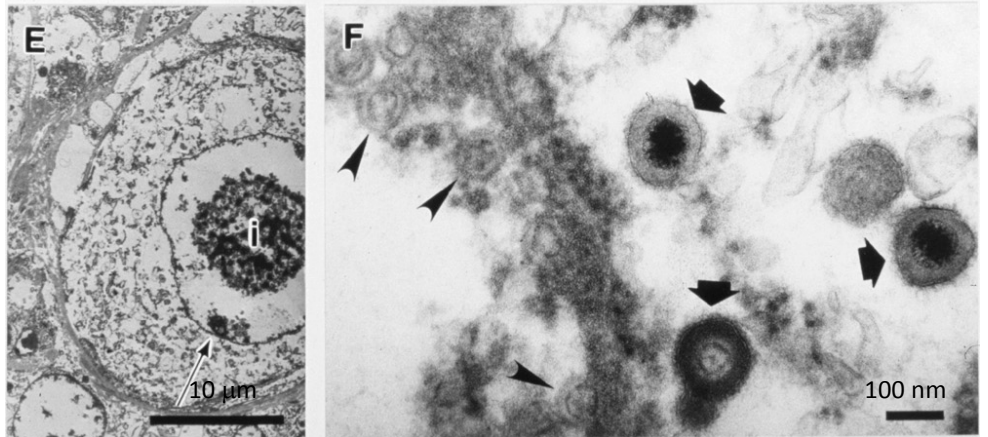
H&E

Peroxidase



Confocal

Confocal B&W Reversal

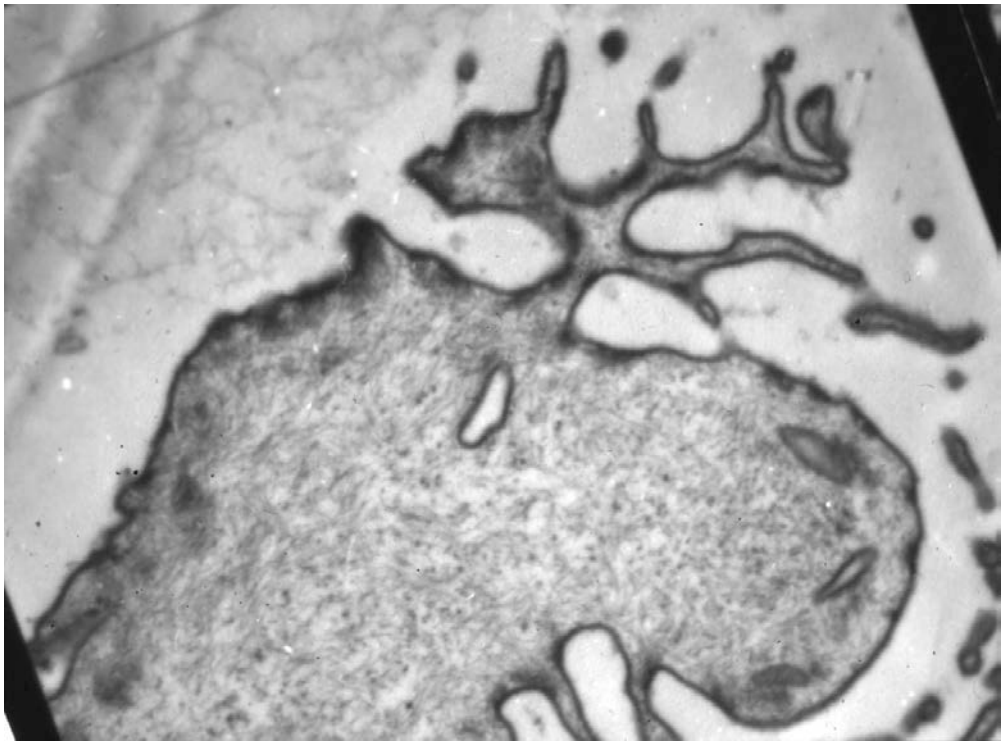


EM

EM

Miller et al.
Ultrastruc Pathol
21:183-93, 1997.

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.
http://wwwnc.cdc.gov/eid/article/9/3/02-0327_article.htm

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.
Clin Microbiol Rev. 2009;Oct;22(4):552-63. doi: 10.1128/CMR.00027-09. Review

Detection and identification of viruses by electron microscopy. J Electron Microsc Tech 4:265-301;1986.