Electron Microscopy in Diagnosis of Infectious Diseases

Sara E. Miller, Ph. D.

Department of Pathology
Duke University Medical Center
Durham, NC 27710
saram@duke.edu
(919) 684-9141

A

Duke Medicine

January 29, 2013

Society for Ultrastructural Pathology

Outline Part 1

A. Virology
   1. Advantages of using EM in diagnostic virology
   2. Limitations of using EM in diagnostic virology
   3. Workarounds
   4. Virus structure
      a. In negative stains
      b. In thin sections

Outline Part 1

B. Other organisms

1. **Bacteriology**
2. **Mycology**
3. **Photology (algae)**
4. **Protozoology**

Questions/Discussion

Outline Part 2

C. Virus look-alikes

1. Examples of confusing things in fluids
2. Examples of cell organelles that resemble viruses in tissues

Outline Part 2

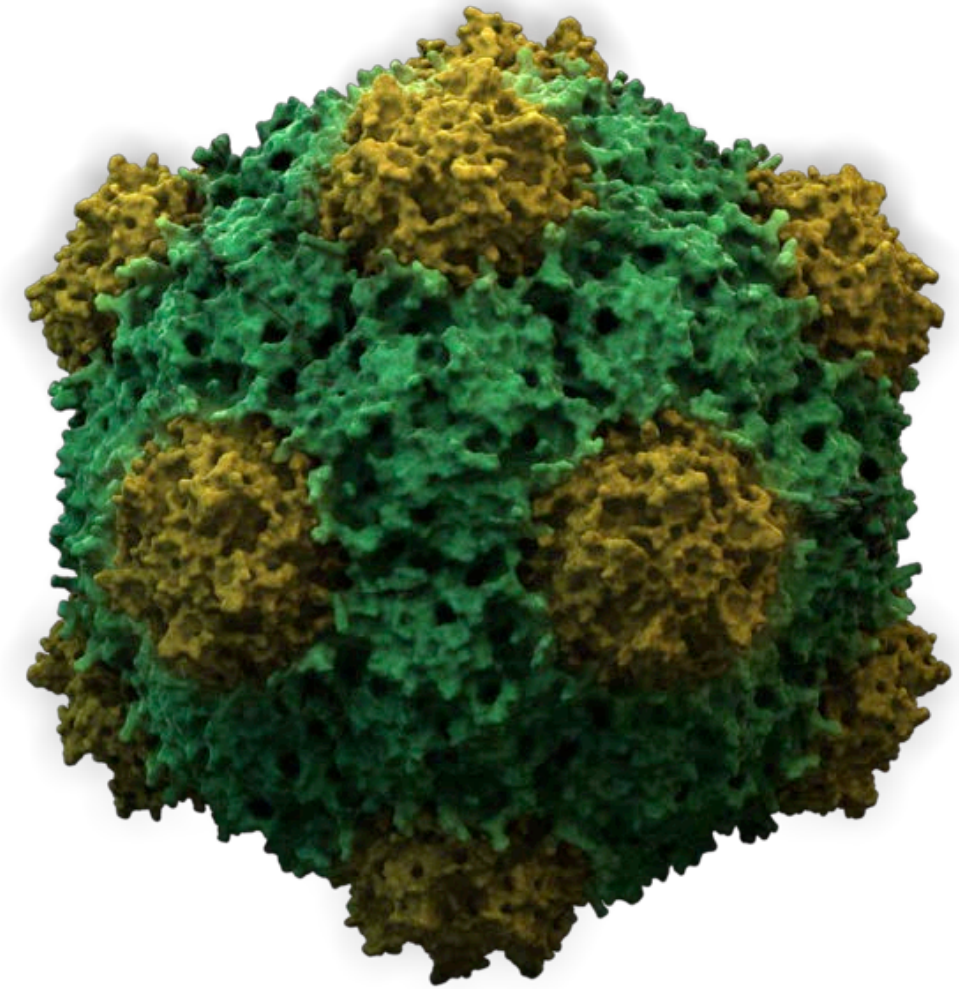
D. Real cases

1. **Examples of organisms diagnosed from patients**
2. **Quiz for fun**

Outline Part 1

A. Virology
   1. Advantages of using EM in diagnostic virology
   2. Limitations of using EM in diagnostic virology
   3. Workarounds
   4. Virus structure
      a. In negative stains
      b. In thin sections

A. Virology



Advantages of EM in Virus Diagnosis/Identification

- Fast
- Does not require living organisms
- Does not require special reagents (antibodies, nucleic acid standards, protein standards)
- Can visualize other organisms: odd agents, odd locations
- Works when other modalities are not enough (mutant genomes may not react in PCRs)
- No false positive results (cross-reactions with similar things)
- What you see is the real thing

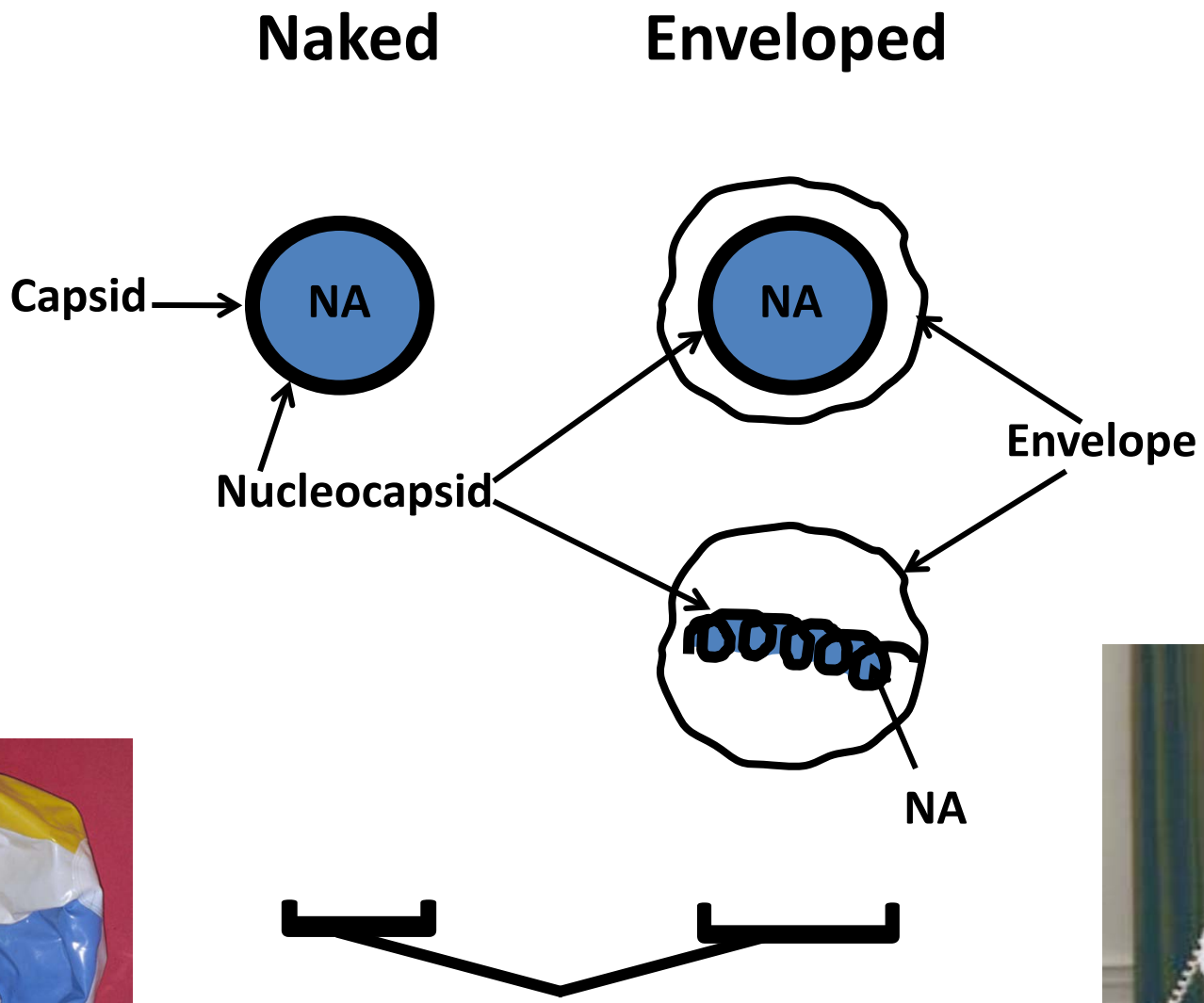
Limitations of EM in Virus Diagnosis/Identification

- **Less sensitive than some other tests**
- **Requires expensive instruments (EM)**
- **Requires virology knowledge**

Ways To Get Around Limitations

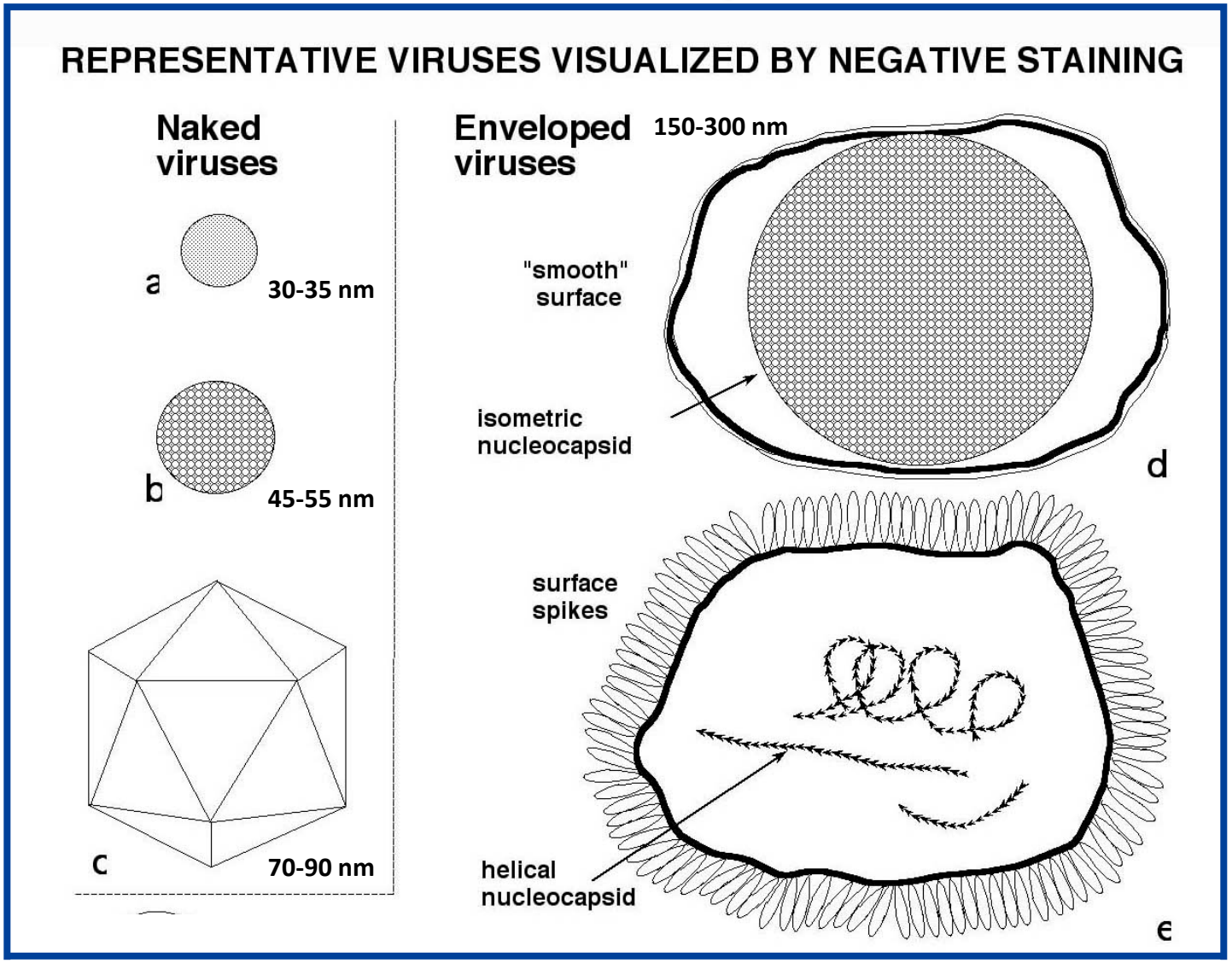
- Ultracentrifugation
- Antibody concentration
- Confocal microscopy of wet tissue slabs
- Multiple tissue locations
- Semi-thin sections of epoxy-embedded tissues
- Where to look in tissues: inflammation, nucleated cells, necrosis edge, unusual ultrastructure for the tissue type), syncytia

Virus Terminology



Identification of Viruses in Fluids

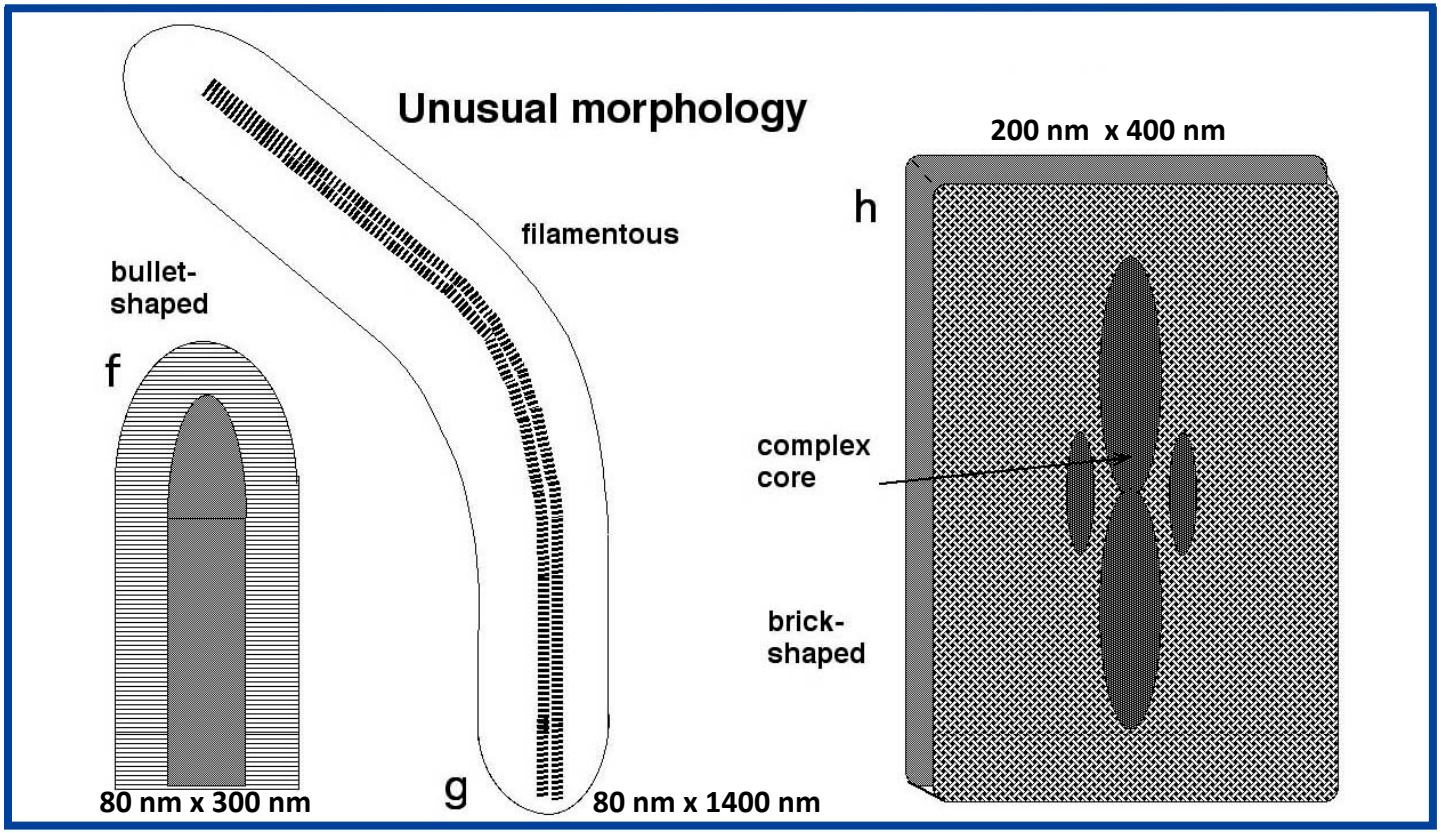
Naked: Icosahedral **Enveloped:** Pleomorphic



Miller SE. 1991. *In* de la Maza LM, Peterson EM (eds), Medical Virology, Vol. 10. Irvine, CA. pp 21-54.

Identification of Viruses in Fluids, Con't.

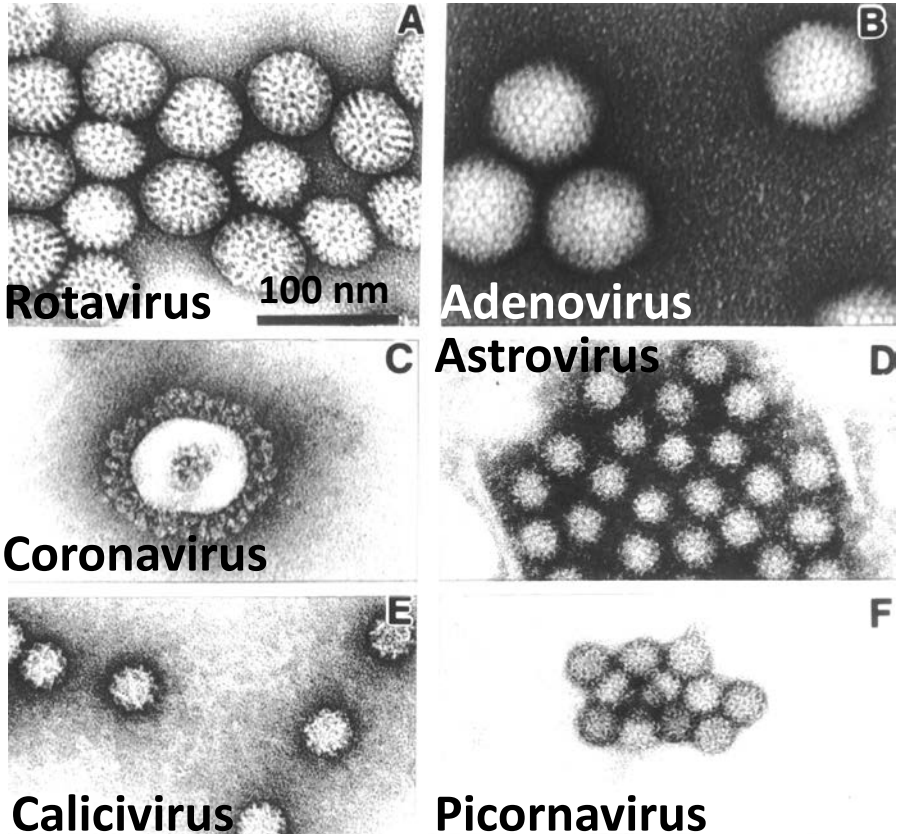
Enveloped: Not Pleomorphic



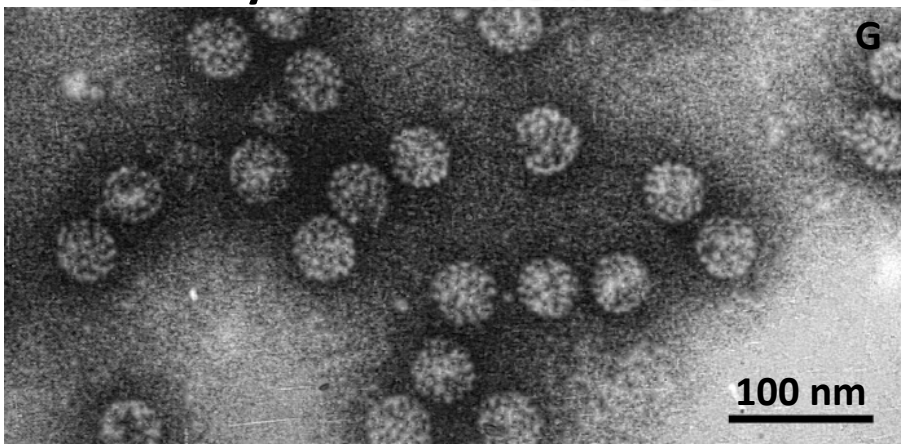
Miller SE. 1991. *In* de la Maza LM, Peterson EM (eds), Medical Virology, Vol. 10. Irvine, CA. pp 21-54.

Negative Staining of Naked and Enveloped Viruses

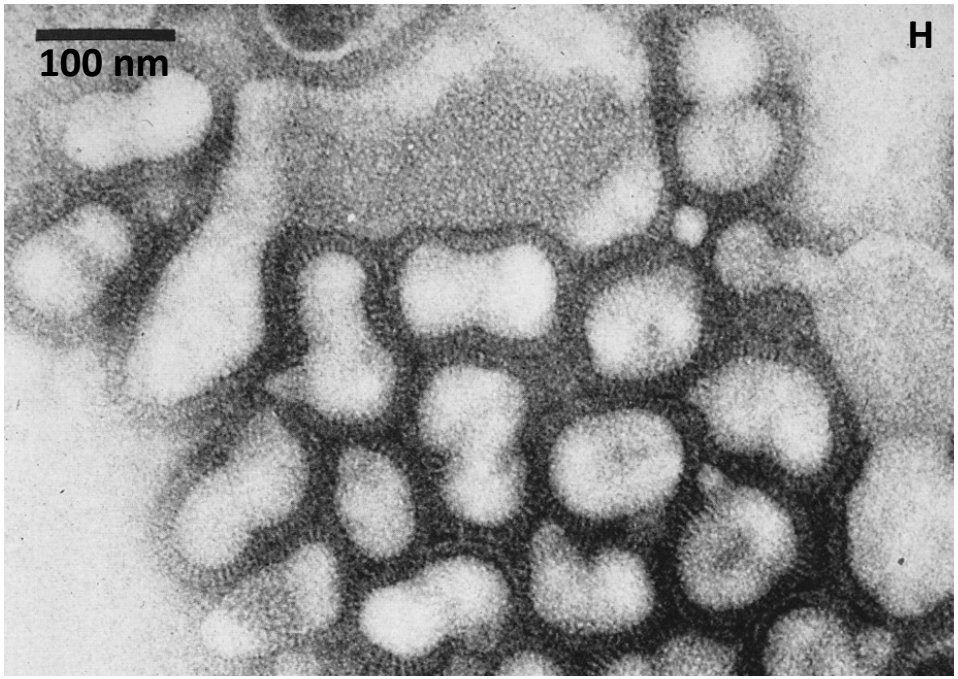
Enteric Viruses



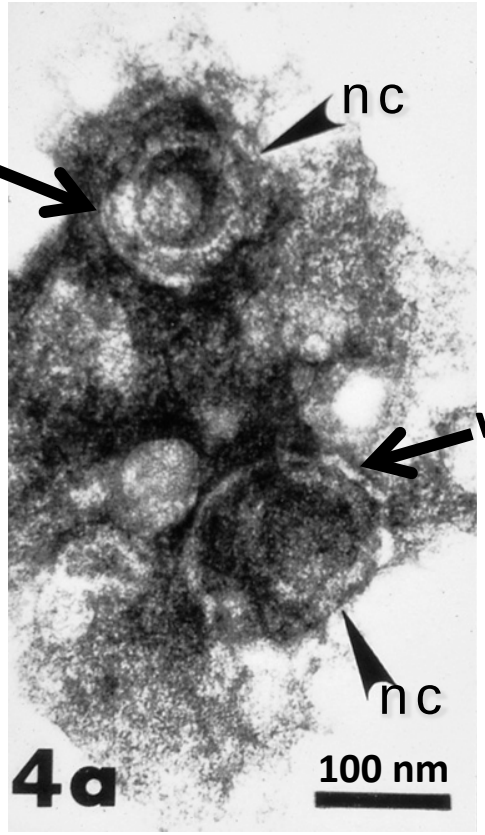
Polyomavirus from Urine



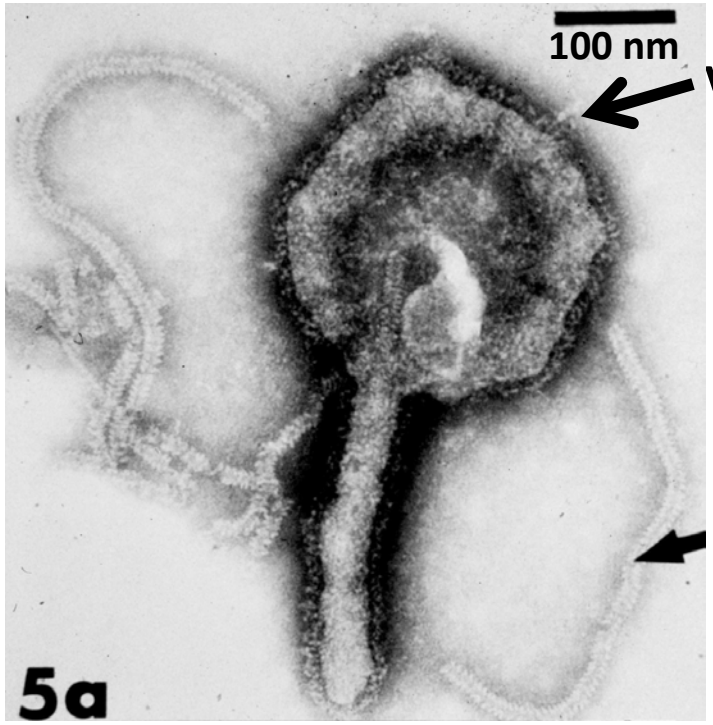
Influenzavirus



Negative Staining of Spherical and Helical Nucleocapsids



Herpesvirus



Measlesvirus

Identification of Viruses in Tissues

Naked (Icosahedral)

- 3 size ranges
- Paracrystalline arrays

Location in cells

- DNA--nucleus
- RNA--cytoplasm

Enveloped (Pleomorphic)

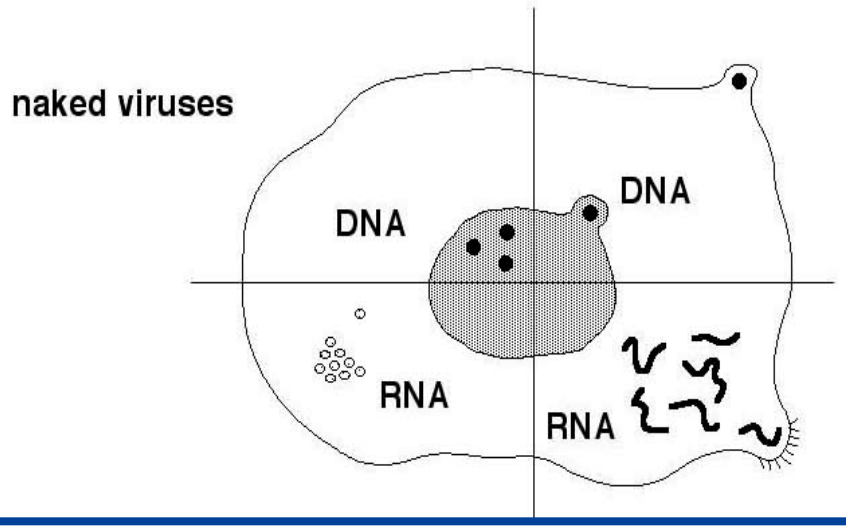
Membrane-associated

- "Smooth"
- Fringed

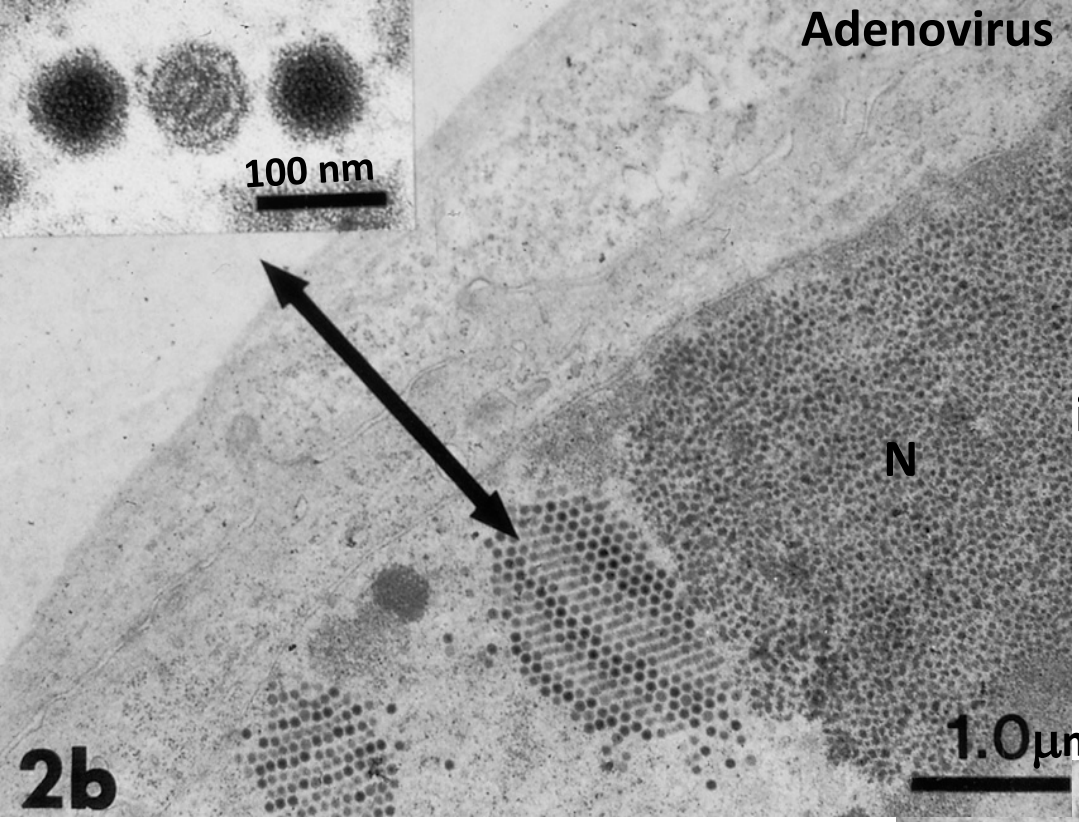
Nucleocapsids

- Spherical
- Helical

REPRESENTATIVE VIRUSES VISUALIZED BY THIN SECTIONING

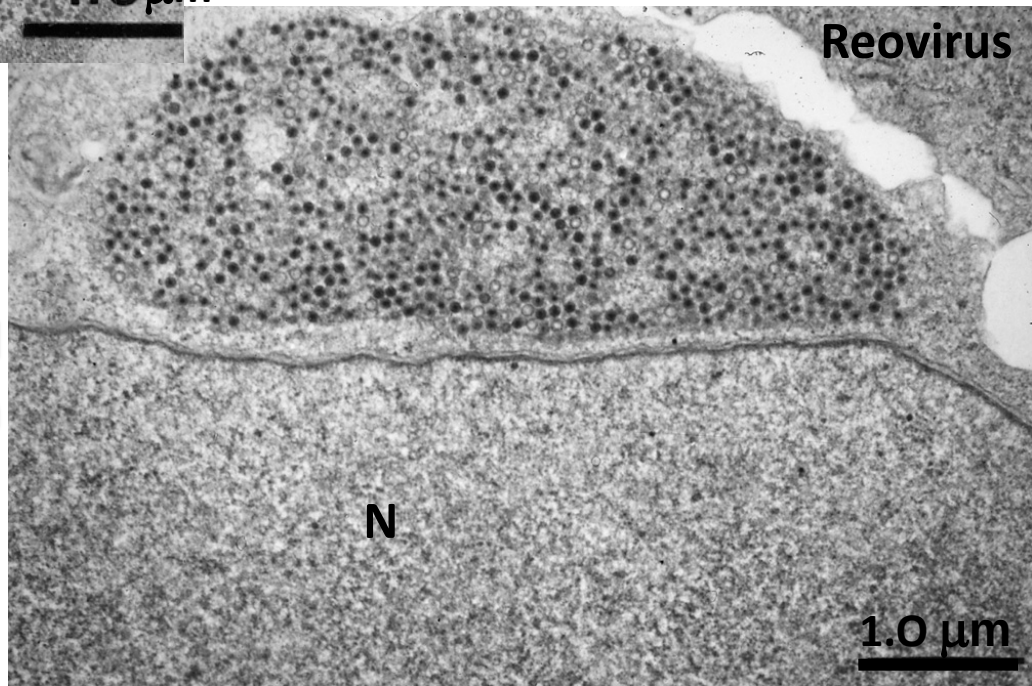


From: Miller, SE. 1991. Evaluation of electron microscopic information available from clinical samples. In LM de la Maza and EM Peterson (eds), Medical Virology 10. Plenum Press, New York. pp. 21-54.

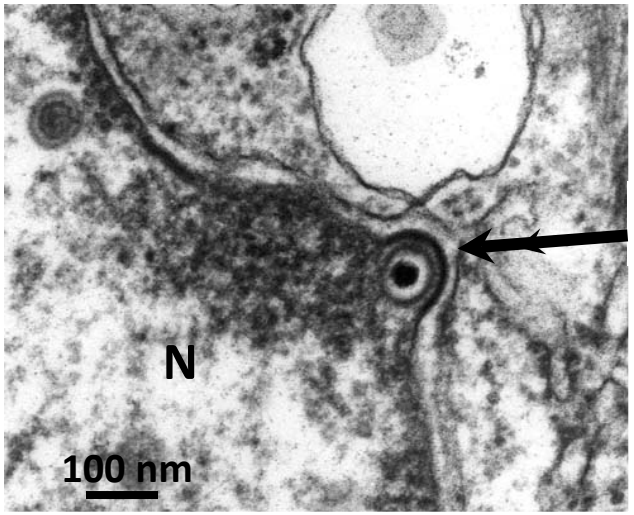


DNA Viruses: Usually produced in the nucleus

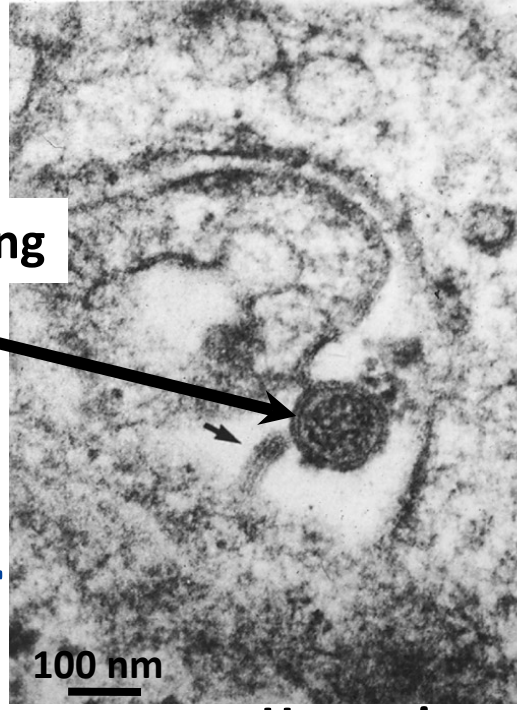
RNA Viruses: Usually produced in the cytoplasm



Nuclear Membrane Budding

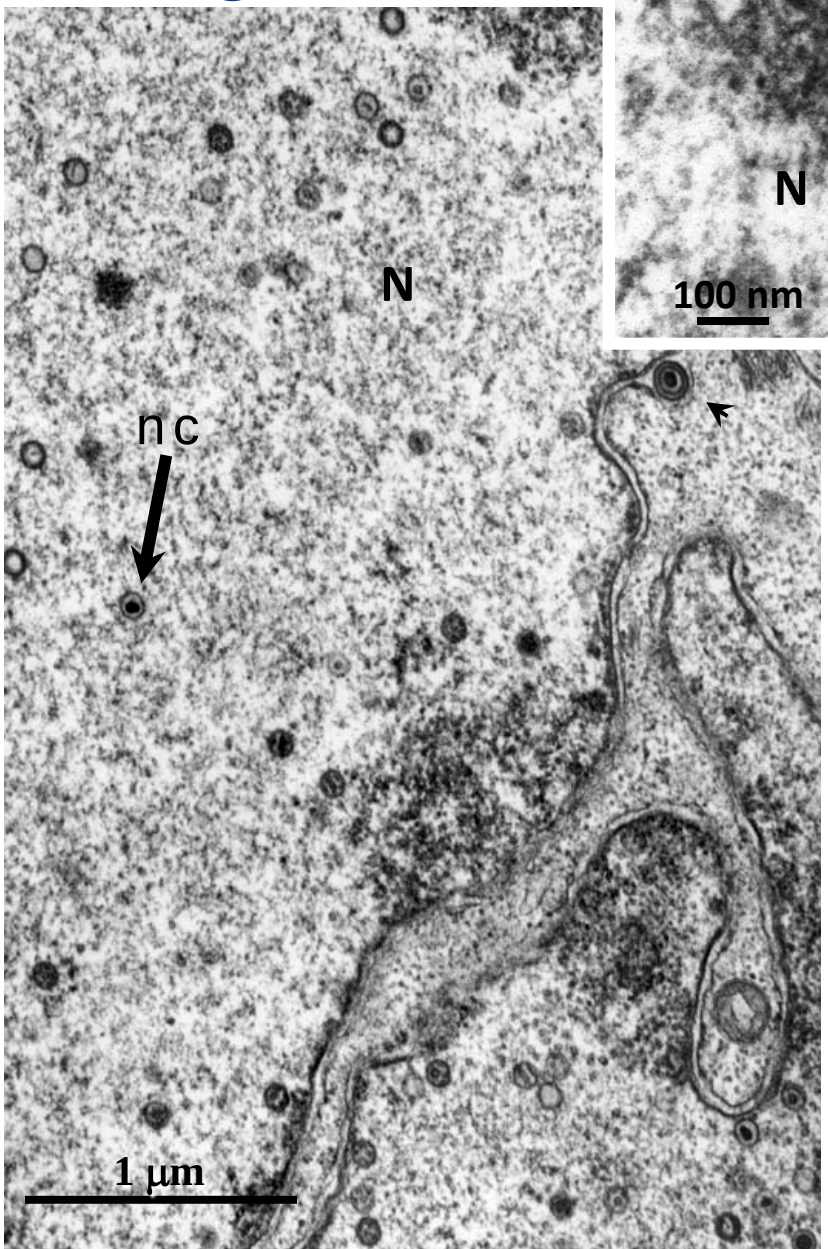


Budding virus



Hantavirus

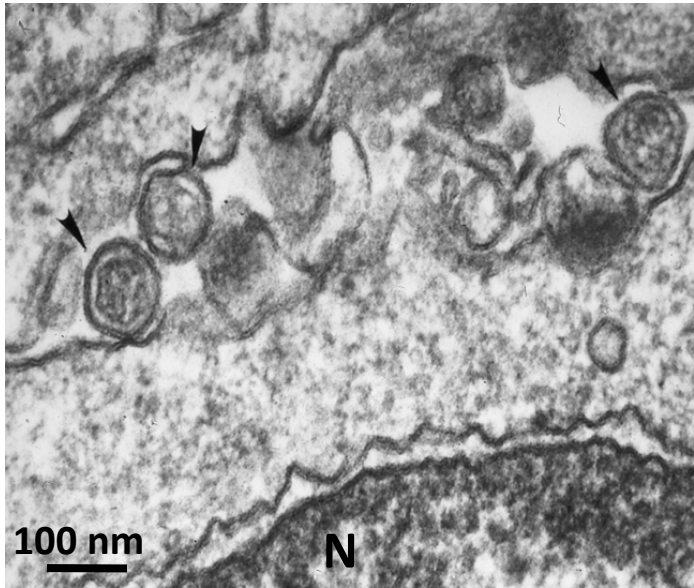
Vesicular Budding



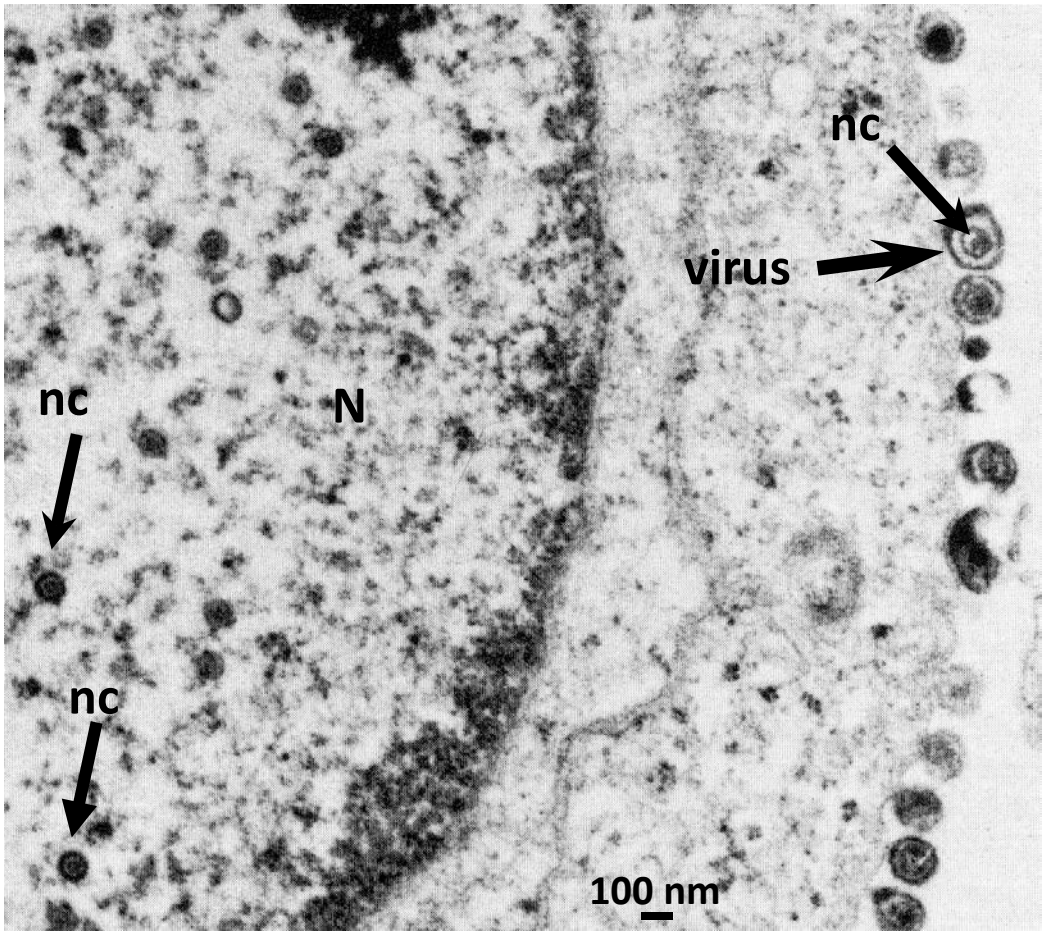
Herpesvirus

Plasma Membrane Budding

HTLV

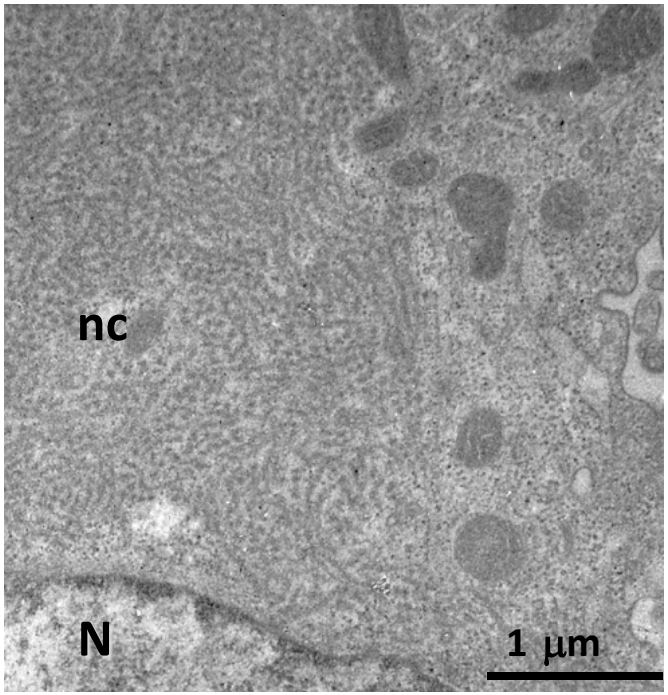


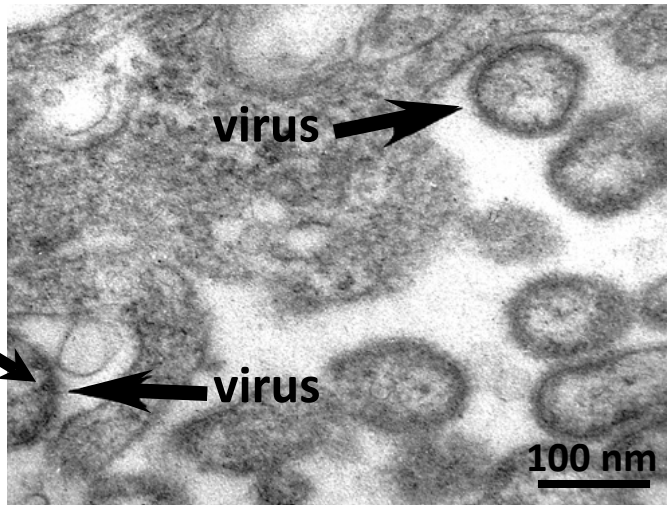
Spherical Nucleocapsids



Herpesvirus

Helical Nucleocapsids





Measlesvirus

EM in Surveillance of Bioterrorism and Emerging Diseases

Bioterrorism: the intentional release or dissemination of biological agents such as bacteria, viruses, or toxins to create disease and fear.

Laboratory Response Network (LRN)

- **Collaboration: FBI & Association of Public Health Laboratories**
- **Started: August, 1999**
- **Includes: State & local public health labs, veterinary, agriculture, military, environmental, & water- & food-testing labs**
- **Function: rapid testing, timely notification, & secure results reporting from public health emergencies (biological/chemical terrorism & emerging diseases)**

LRNs and Electron Microscopy

- **About half the LRNs are paired with electron microscopy laboratories.**
- **The Duke Electron Microscopy Service (DEMS) is the EM partner of the NC State Public Health Laboratory in the NC LRN.**
- **If asked to process biohazard sample, read Dos and Don’ts article.**

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.

Class A Viral Agents of Bioterrorism

Smallpox virus

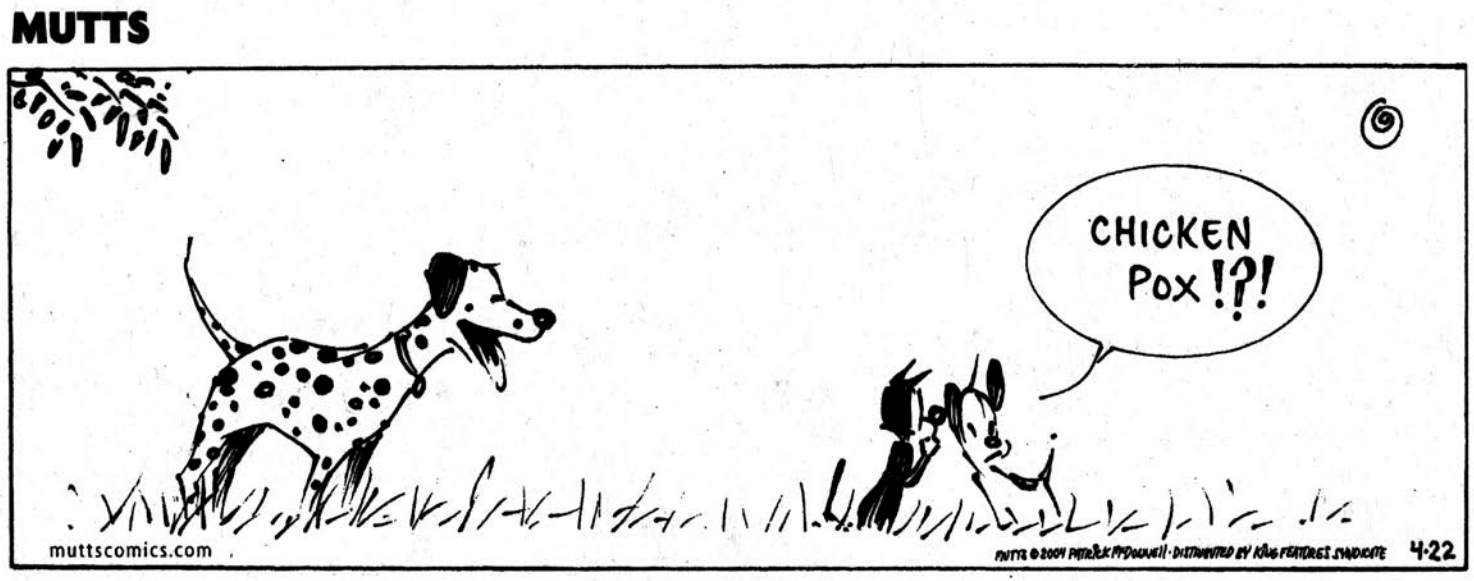
- **Easily disseminated**
- **Easily weaponized**
- **High mortality rate, survivors badly scarred**
- **Previously mass-produced**
- **Believed to have been distributed after USSR collapse**

Hemorrhagic fever viruses

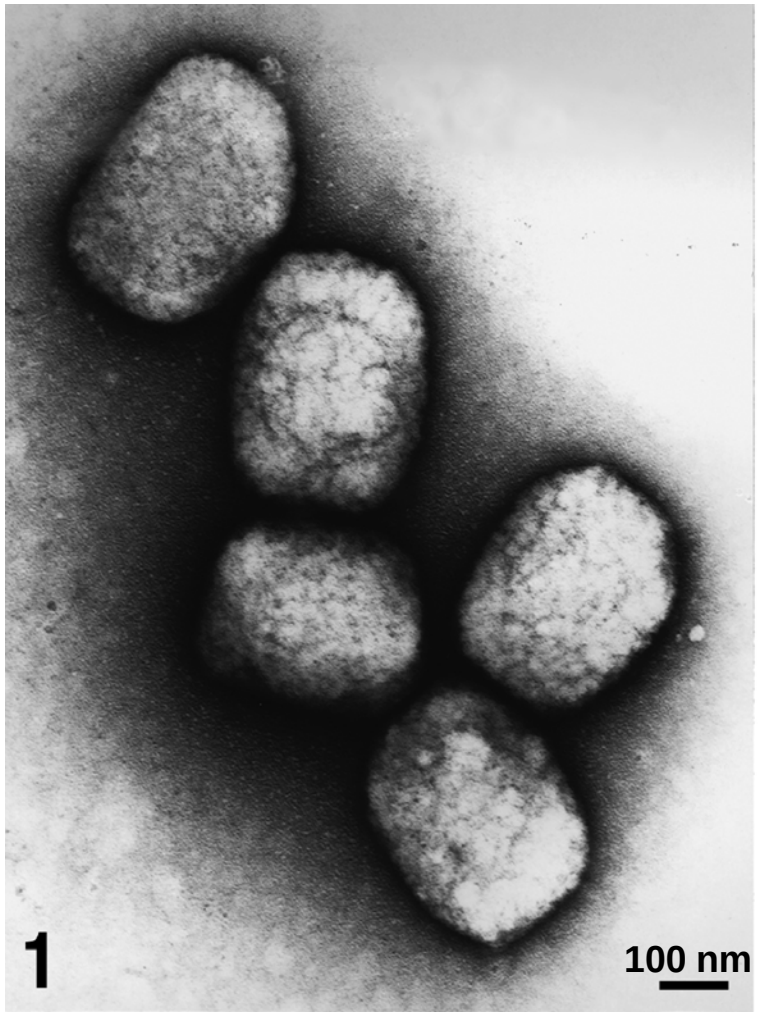
- **Filoviruses (Marburg, Ebola)**
- **Arenaviruses (Lassa, Machupo)**

Because of ID speed, EM is on the front line in bioterrorism surveillance (e.g., smallpox).

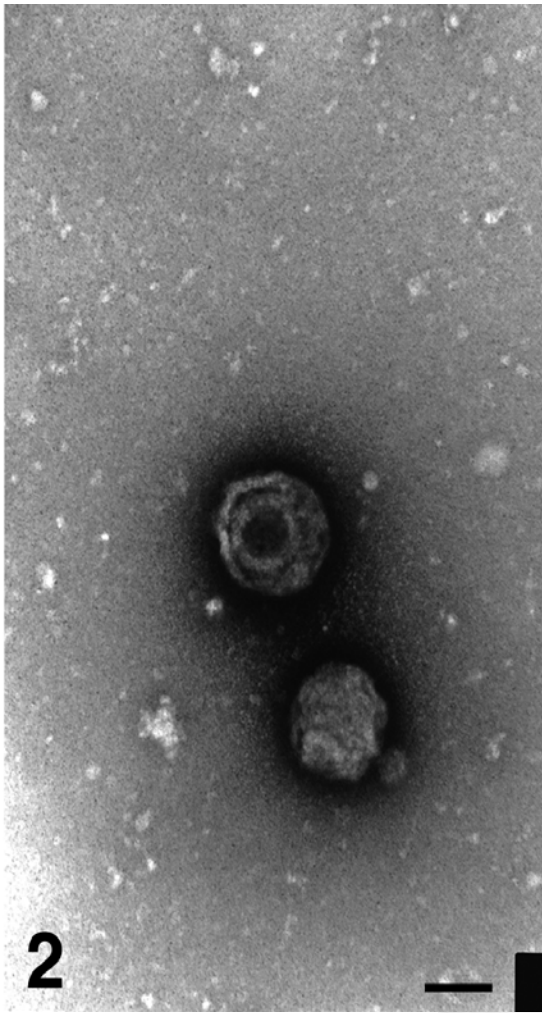
Poxvirus lesions are most likely to be confused with varicella-zoster virus (VZV) (a herpesvirus) lesions.



EM in Surveillance of Bioterrorism



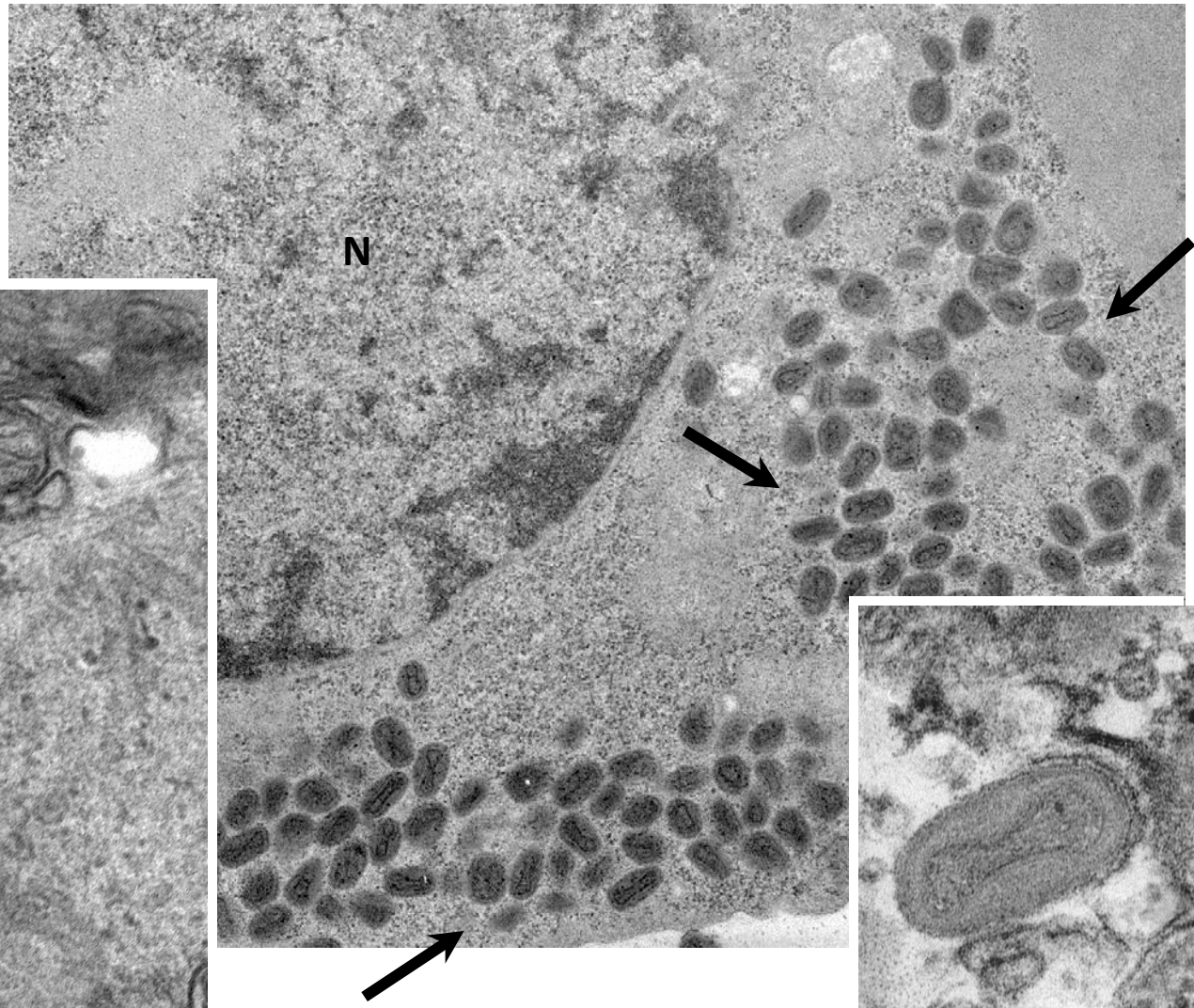
Orthopoxvirus

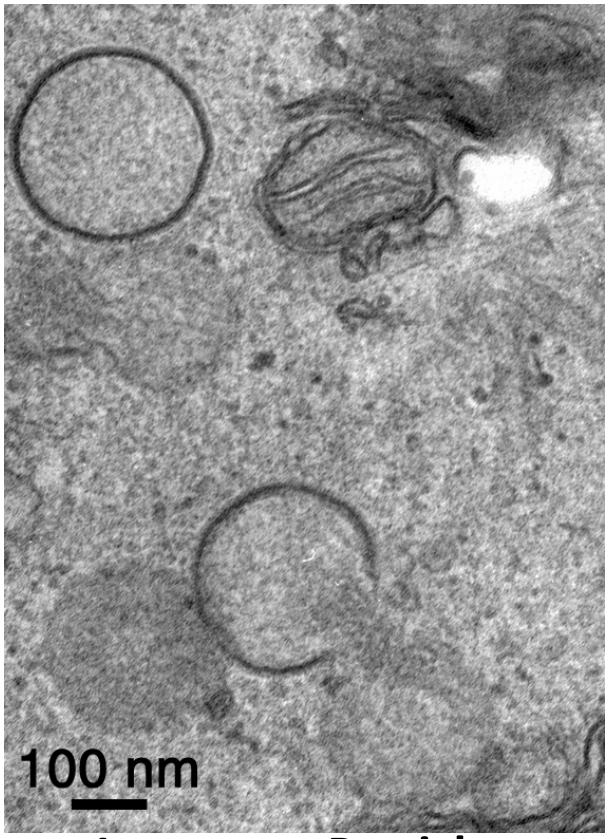


Herpesvirus

Parapoxvirus

Poxvirus

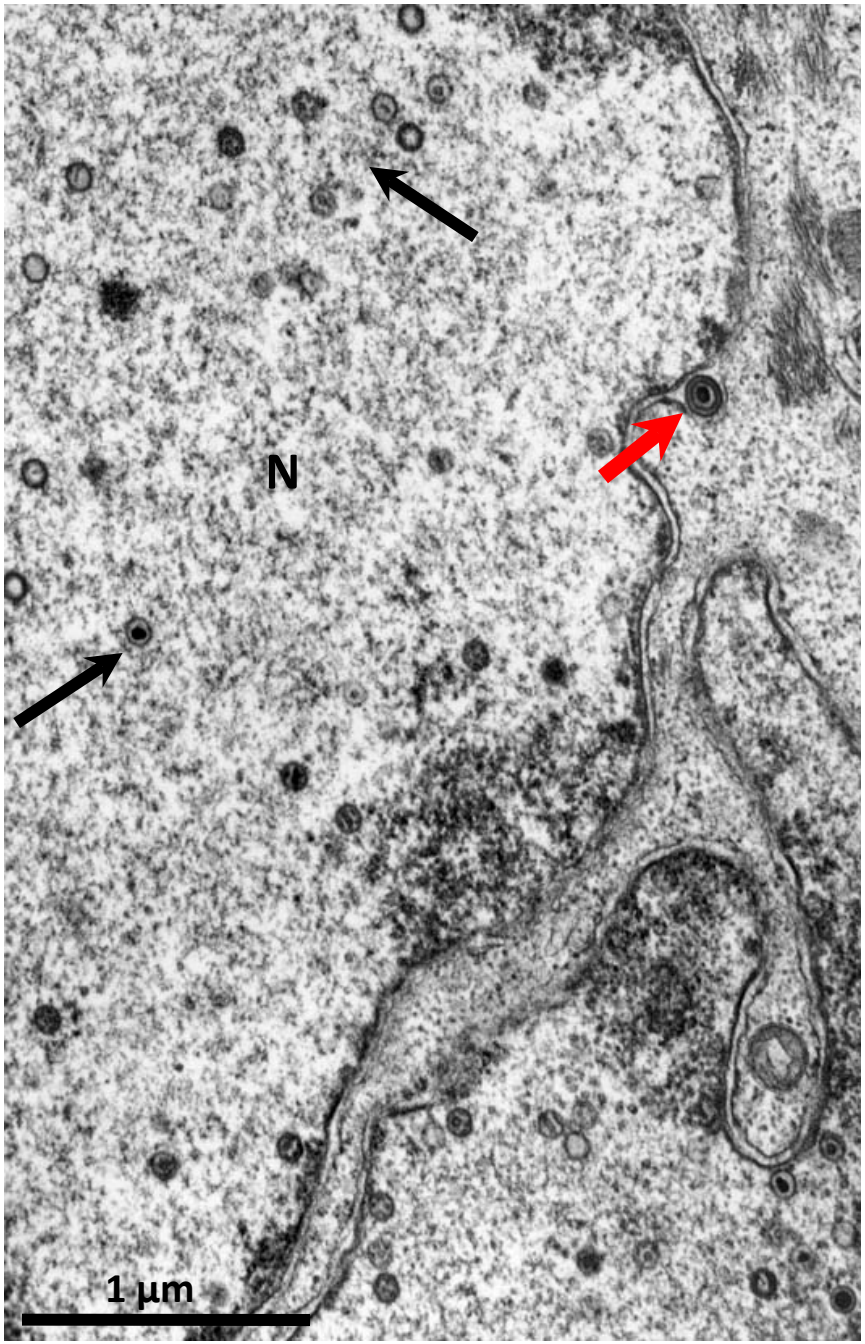


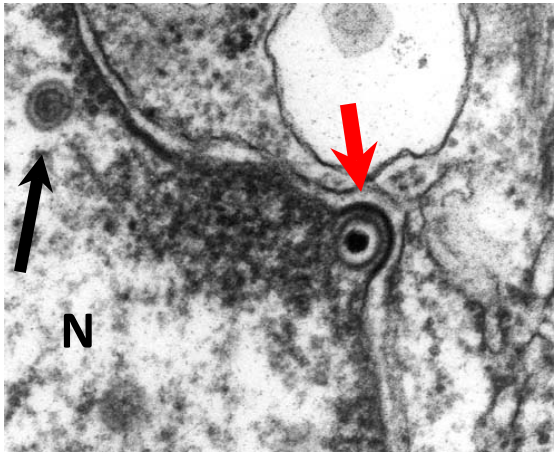


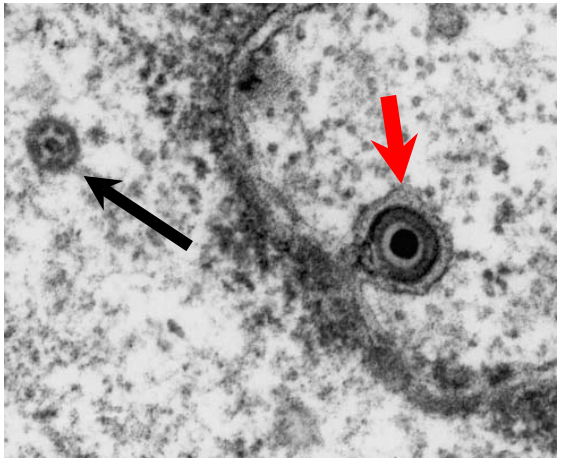
Immature Particles

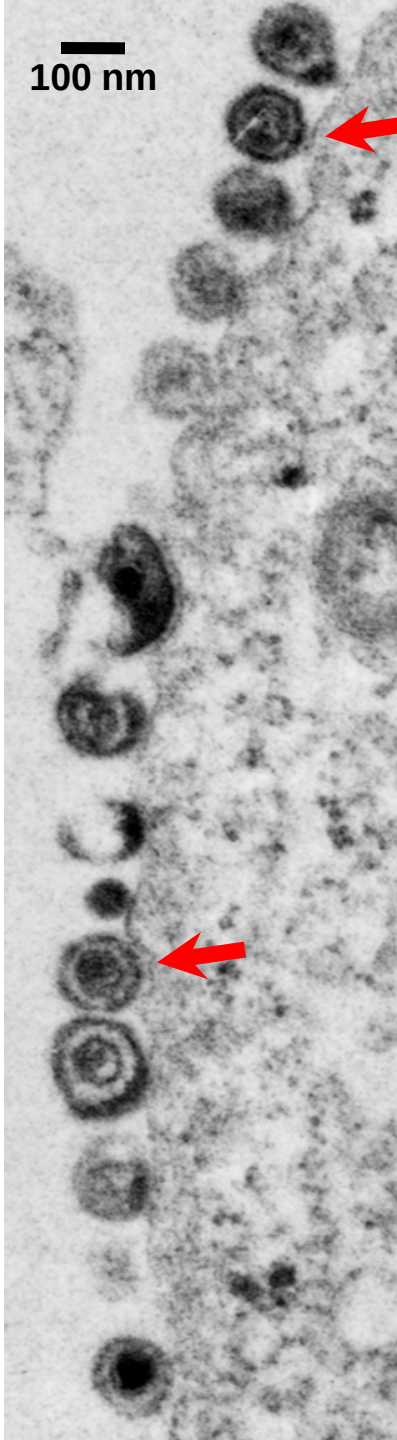
Mature Particles

Herpesvirus









EM in Surveillance of Emerging Diseases

Viral emerging diseases identified first by EM

- **Parvovirus B-19**
- **Monkeypox**
- **SARS coronavirus**
- **Metapneumovirus**
- **Morbilivirus**
- **Nipah virus**

EM was crucial in identifying the coronavirus in the SARS outbreak.

Costs (11/02 – 7/03):

774 Deaths, 9.6% fatality

(China, Hong Kong, Taiwan, Canada)

$11 billion (Asia alone)

Staff members wearing double layers of surgical masks talk at a hospital in Guangzhou in southern China Thursday. China's disclosure of a sharply higher death toll from a new flu-like disease has raised fears of a wider outbreak.

Screening urged for flu-like illness

Health agency issues 1st warning that disease might be spread on planes

BY EMMA ROSS
Associated Press

Airlines flying out of a handful of cities hit hard by a mystery disease should question passengers at check-in desks for signs of sickness, a global health agency said Thursday.

In its first warning that suggests the disease can be spread on airplanes, the World Health Organization said passengers with flu-like symptoms or who may have been exposed to severe acute respiratory syndrome, SARS, should not be allowed to fly.

"If the passengers are sick, health workers will be recommending to the airline that they not board the plane," said Dr. David Heymann, WHO's infectious diseases chief.

The WHO advice — it can only make recommendations to governments — is directed at flights leaving cities where the disease is spreading locally: Toronto; Singapore; Hanoi, Vietnam; Hong Kong; Taiwan; Beijing; Shanghai and the Chinese

A woman wears a protective mask as she rides a streetcar near St. Michael's Hospital in Toronto, Canada. Doctors ordered Toronto hospitals closed to almost all visitors.

fallen ill with SARS and 53 people have died; that doesn't include a death Thursday that Hong Kong officials were reporting.

The United States has 51 suspected cases, said Dr. Julie Gerberding, head of the U.S. Centers for Disease Control and Prevention.

The death rate has remained around 4 percent since the outbreak began, experts said. There have been three deaths in Canada and none in the United States.

areas," Heymann said.

However, the CDC's Gerberding encouraged Americans to defer vacations to Asia if they were able to do so.

"This is now a global epidemic and potentially a global pandemic," if it's not quickly brought under control, she said.

The WHO has teams of infectious disease experts in the affected countries offering support. But some places continue to have problems containing the disease, which apparently got its start last winter in Guang-

end concerts by the Rolling Stones were postponed.

In Ontario, Canada's most populous province, health authorities declared a state of emergency and called for a 10-day quarantine of people who had visited a hospital where the outbreak spread.

The number of people under quarantine could be "in the thousands," said Dr. Sheela Basrur, Toronto's medical officer of health.

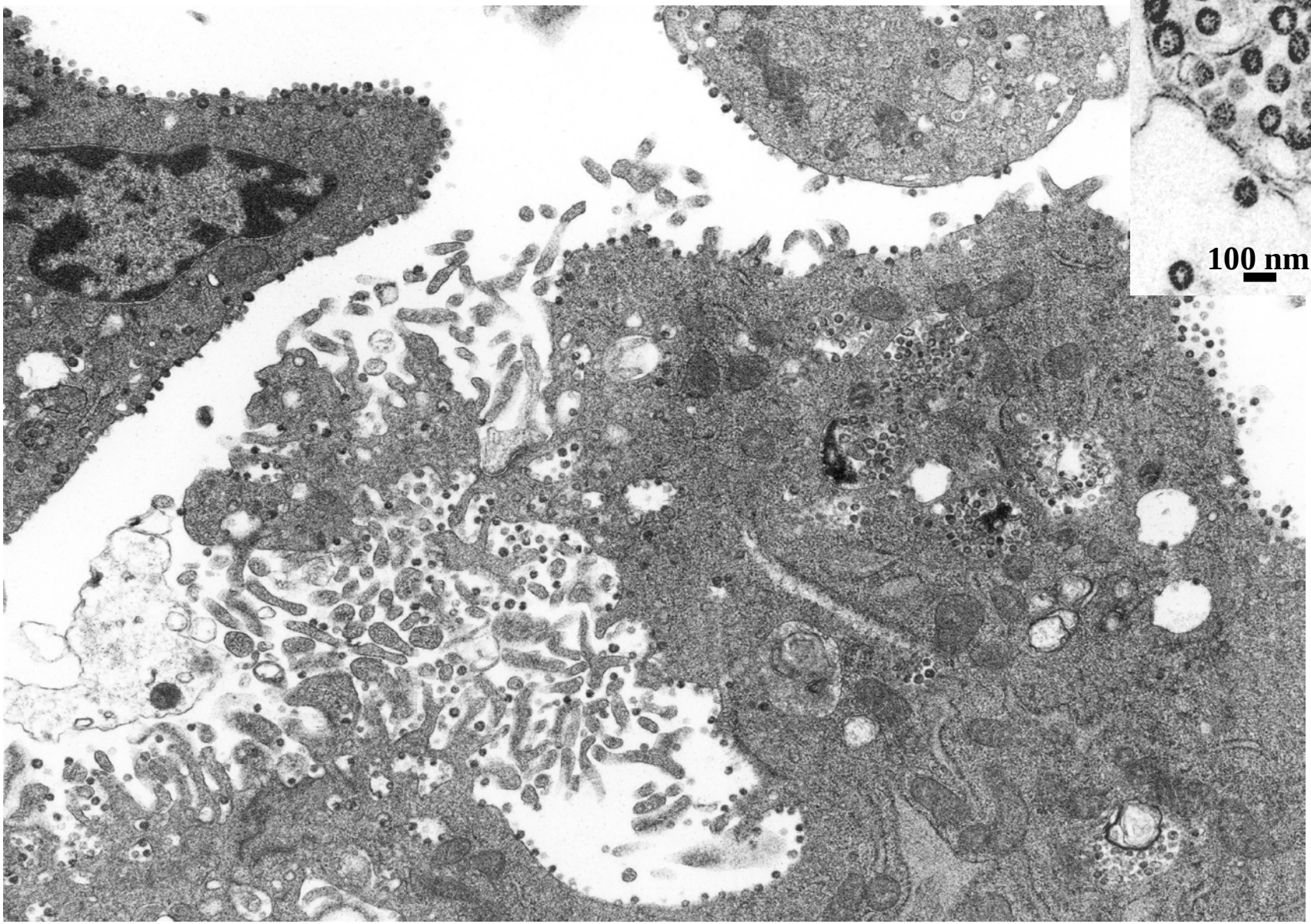
Ontario health officials said Thursday they had bought all the high-grade surgical masks available in Canada to protect medical workers.

"One hundred thousand were delivered yesterday, 200,000 will be delivered today and the last 40,000 were purchased early this morning," said Dr. Colin D'Cunha, Ontarios Commissioner of Public Health.

Health officials already knew the disease had spread beyond Asia by international air travel on March 15, when the Canadian cases turned up. But at the time it was not clear whether those people were sick on the plane or whether they got sick after coming home.

"Now we know that there are people who are traveling when they are sick," Heymann said. "There have been more and

Thin Section of SARS Coronavirus



Courtesy of Cynthia Goldsmith, CDC, Atlanta.

EM was crucial in identifying the monkeypox virus outbreak

ASSOCIATED PRESS | DOUGLAS C. PIZAC

A giant Gambian rat (like the one shown above) likely has infected prairie dogs with monkeypox disease. The illness has infected 33 people in Midwestern states.

Number of likely monkeypox cases rises to 33 in Midwest

BY NICOLE ZIEGLER DIZON
Associated Press

CHICAGO — Federal health officials investigating an outbreak of monkeypox that apparently spread from pet prairie dogs to people in three Midwestern states said Monday the number of possible cases has risen to at least 33.

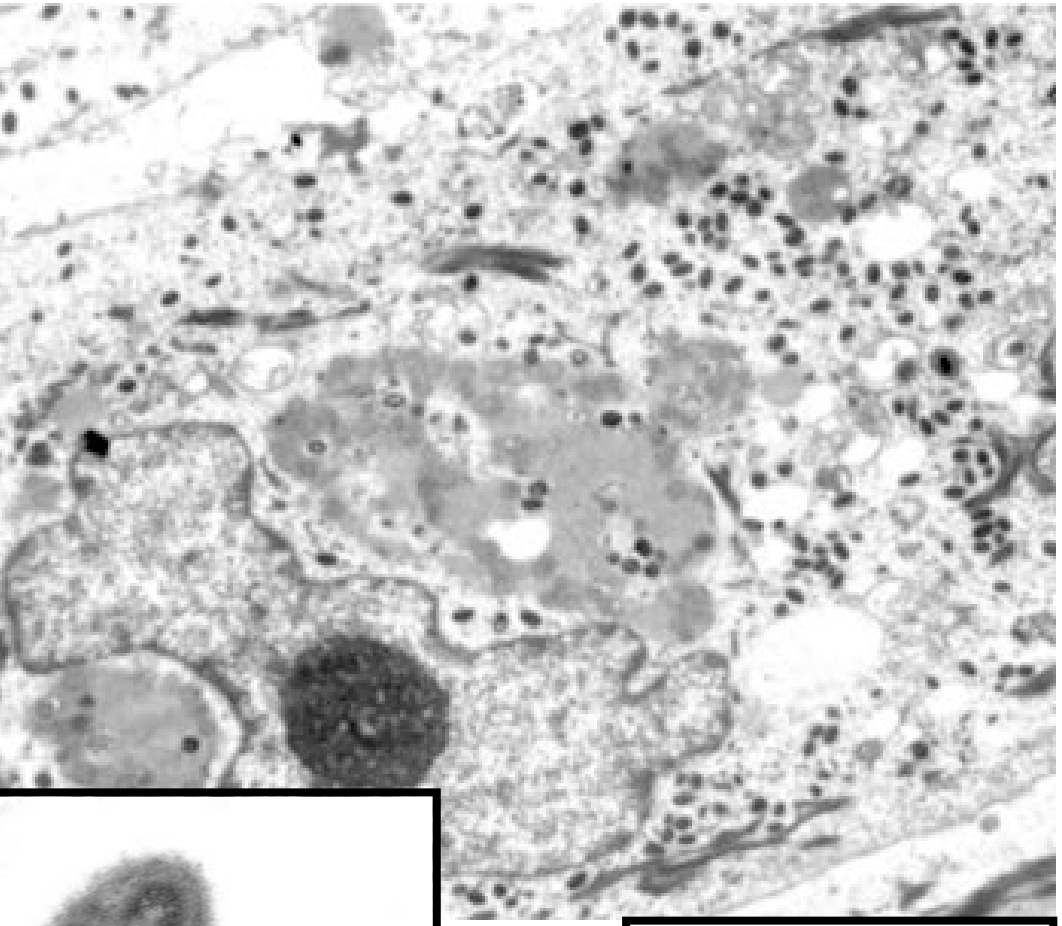
The Centers for Disease Con-

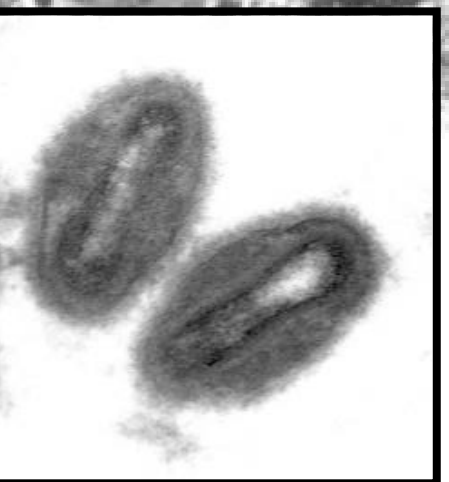
"For the average citizen, I would not necessarily be concerned at this point of being exposed to monkeypox."

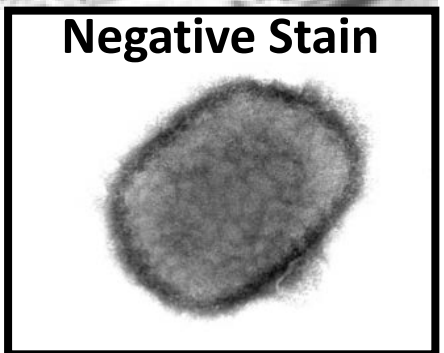
gowns and masks. Other suspected victims were treated and released.

The human mortality rate from monkeypox in Africa has ranged from 1 percent to 10 percent, but the virus may be less lethal in the United States because people are typically better nourished and medical technology is far more

Thin Section of Monkeypox Virus Infected Cell







Summary: Identification of Viruses

- **In fluids (negative stains)**
  - **Naked icosahedral: size, surface structure**
  - **Enveloped: size, surface structure of core/envelope**
- **In tissues (thin sections)**
  - **Naked**
  - **Enveloped (usually budding)**
  - **Location: nucleus/cytoplasm**