Aerosolized filoviruses in three species of nonhuman primates

Differences in disease course and pathology

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Filoviruses: a potential biological weapon?

- Aerosol dissemination is the most efficient way to disperse biological weapons for large scale attacks
- Epidemiology studies have suggested that aerosol transmission is not a concern during ***natural*** outbreaks of filoviruses

….***but***….

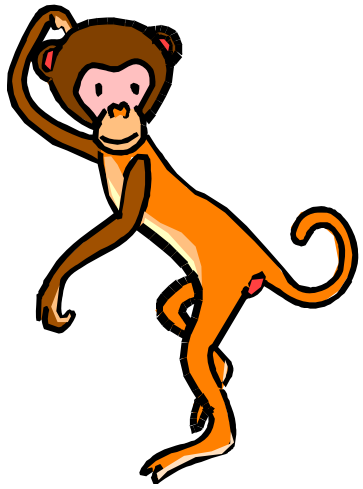
- Ebola Reston – possible aerosol transmission between NHPs
- Documented transmission of filoviruses from infected to naive NHP housed in the same room
- Experimental evidence for aerosol infection of NHP and guinea pigs
- The former Soviet Union studied Marburg and Ebola viruses as potential offensive biological weapons
- Aum Shinrikyo was reportedly interested in obtaining samples of Ebola virus for use as a biological weapon

Animal models for filoviruses

- parenteral inoculation

- Rodent
  - Requires adaptation
  - Guinea pig
  - Mouse

- NHP
  - Cynomolgus macaque
  - Rhesus macaque
  - African green monkey
  - Baboon (Russian studies)



Experimental evidence for aerosol infection with filoviruses

- Russian scientists published several studies in the 1990s and early 2000s describing aerosol infection with the Popp strain of Marburg or Ebola Zaire in guinea pigs and nonhuman primates
  - Doses used (guinea pig LD50) do not translate easily to conventional pfu
  - Guinea pig adapted viruses rather than wild type
- USAMRIID scientists demonstrated that aerosol exposure to Ebola Zaire caused lethal disease in rhesus macaques
  - Limited number of animals; single study

Animal Models of Aerosol Infection with Filoviruses – Goal

To develop animal models suitable for use in efficacy studies to support licensure of medical countermeasures under the Animal Rule for protection against exposure to aerosolized filoviruses.

Animal Models of Aerosol Infection with Filoviruses – Objectives

- Develop guinea pig and NHP models
- Determine appropriate challenge dose
  - Historical 1000 pfu for parenteral challenge
  - LD50/99 not empirically established but presumed to be very low
- Compare disease course & pathology in multiple rodent & NHP models
  - “Most commonly used” might not necessarily be the “most relevant” model of the human disease
  - For Ebola Zaire and Marburg Ci67, pathogenesis studies in cynomolgus macaques available for comparison
- Evaluate use of telemetry to monitor & record physiological changes in exposed animals
  - Not previously done for filovirus-infected animals

Aerosols with Ebola Zaire ‘95

Why?

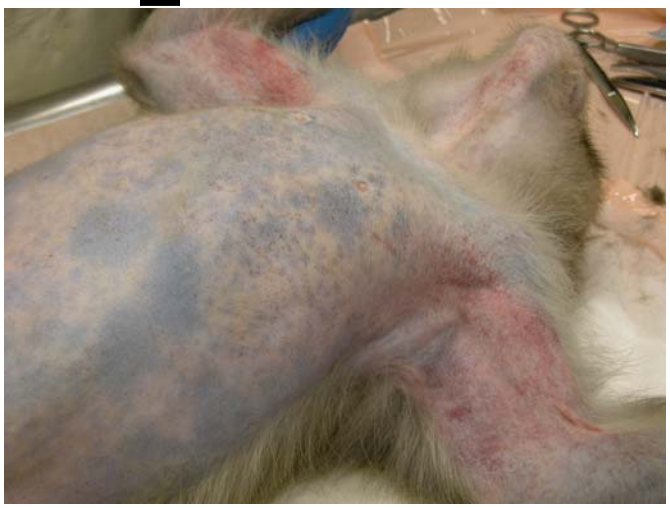
- ~70-80% mortality rate in human outbreaks Prior to Marburg outbreak in Angola, most virulent of known filoviruses
- Highly virulent in NHP
- Prior pathogenesis study for parenteral inoculation of cynos
- Prior vaccine studies with parenteral challenge

Clinical observations & pathology

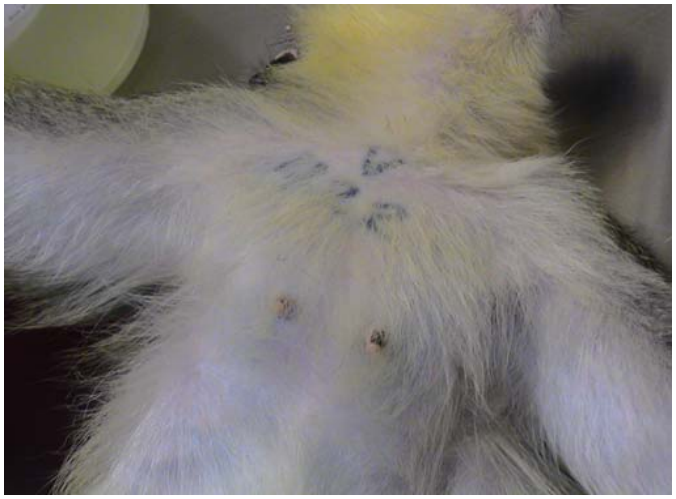
- Clinical Observations
  - Both species of macaques develop a notable petechial rash similar to parenteral inoculation
  - AGM do not develop a significant rash
  - Observations of gastrointestinal discomfort & anorexia are common with both macaque species but not AGM
- Gross necropsy findings
  - Lungs of cynomolgus macaques and AGM have mild pulmonary congestion and edema
  - Rhesus macaques looked more like parenteral inoculation

- Microscopic analysis of tissues is in progress

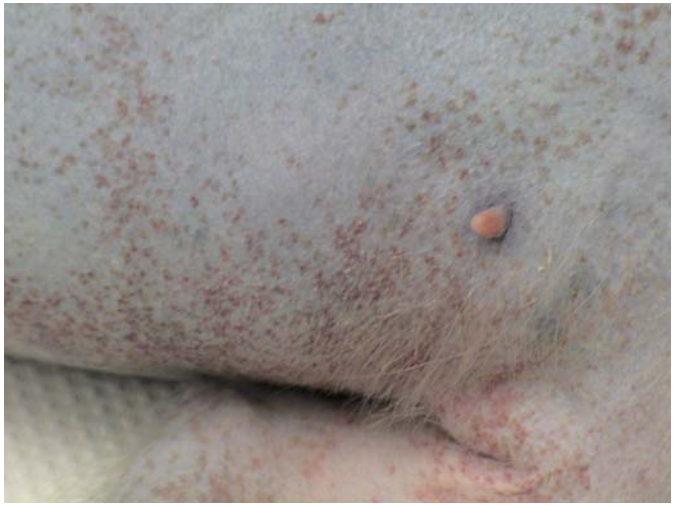
Development of rash postexposure



Rhesus macaque

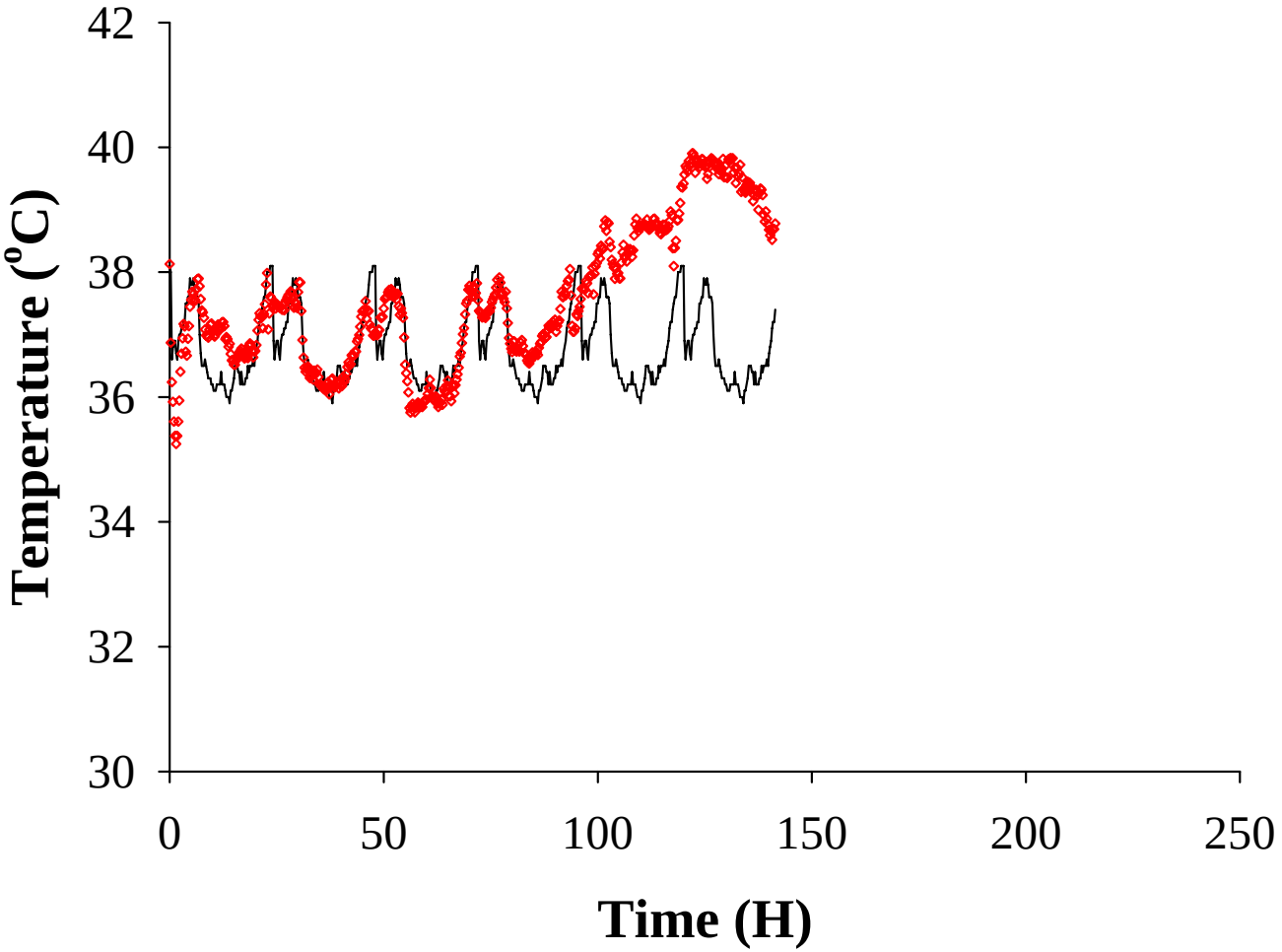


African green monkey

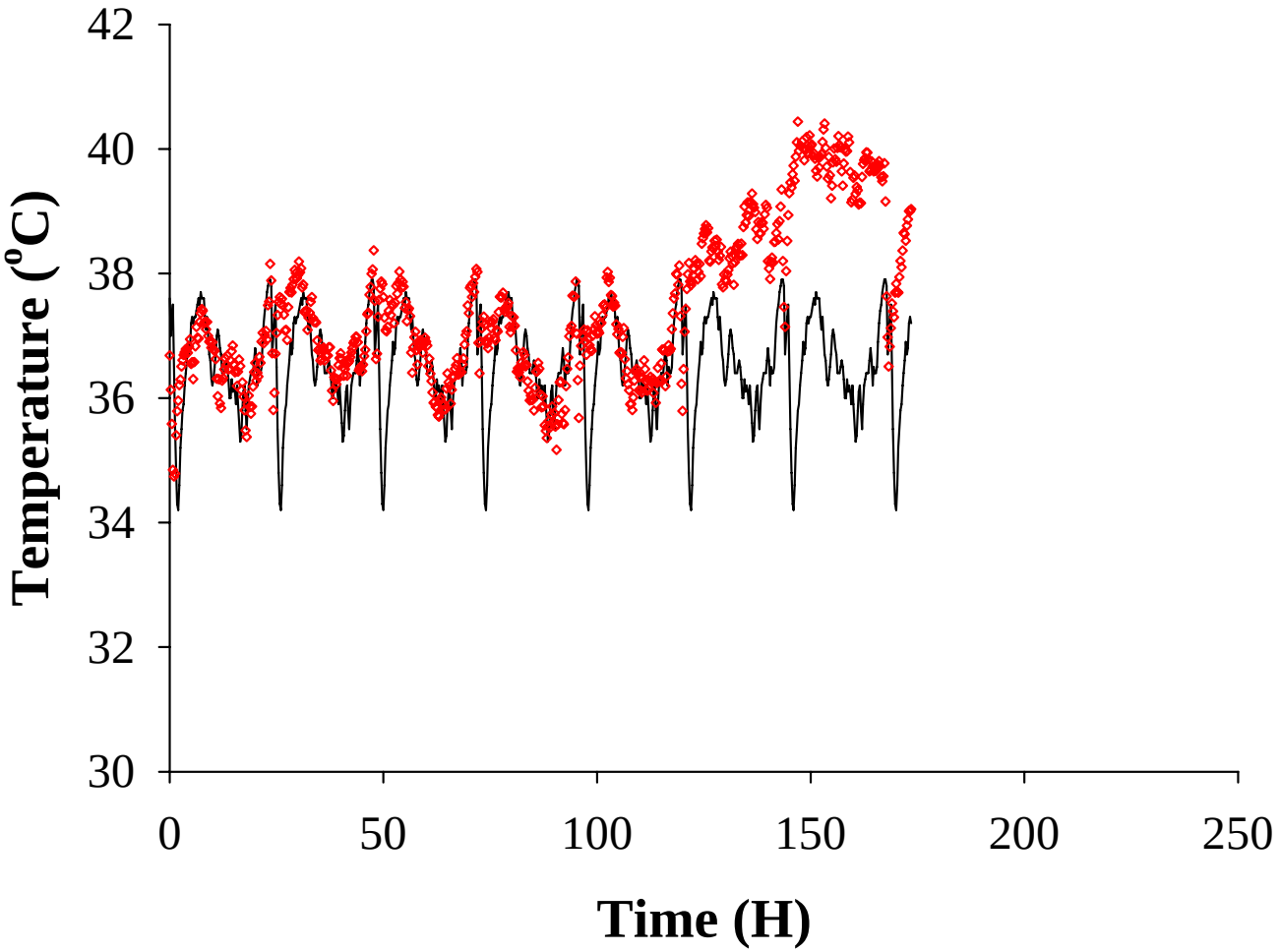


Cynomolgus macaque

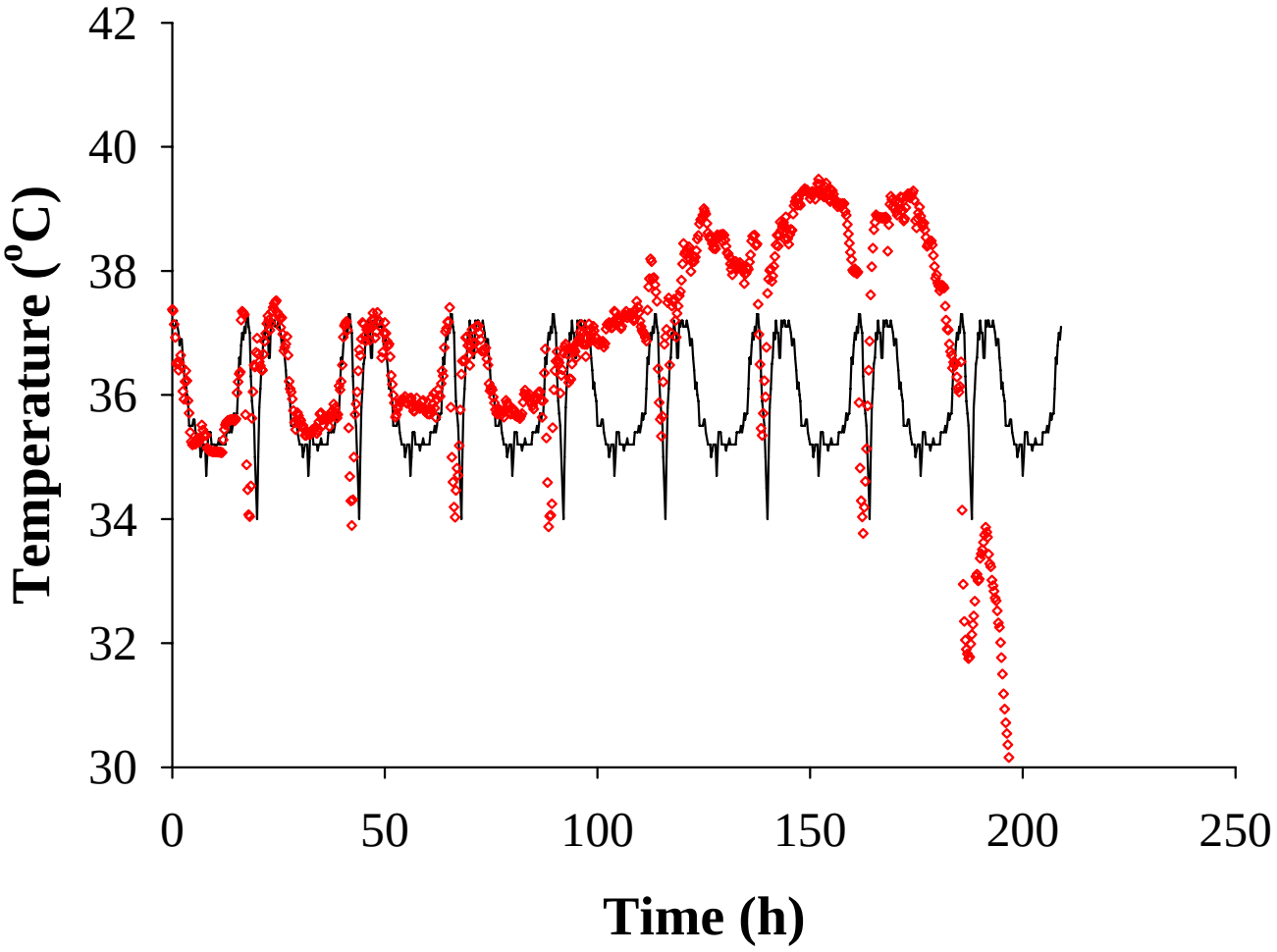
Fever response in cynos after aerosol exposure to Ebola Zaire



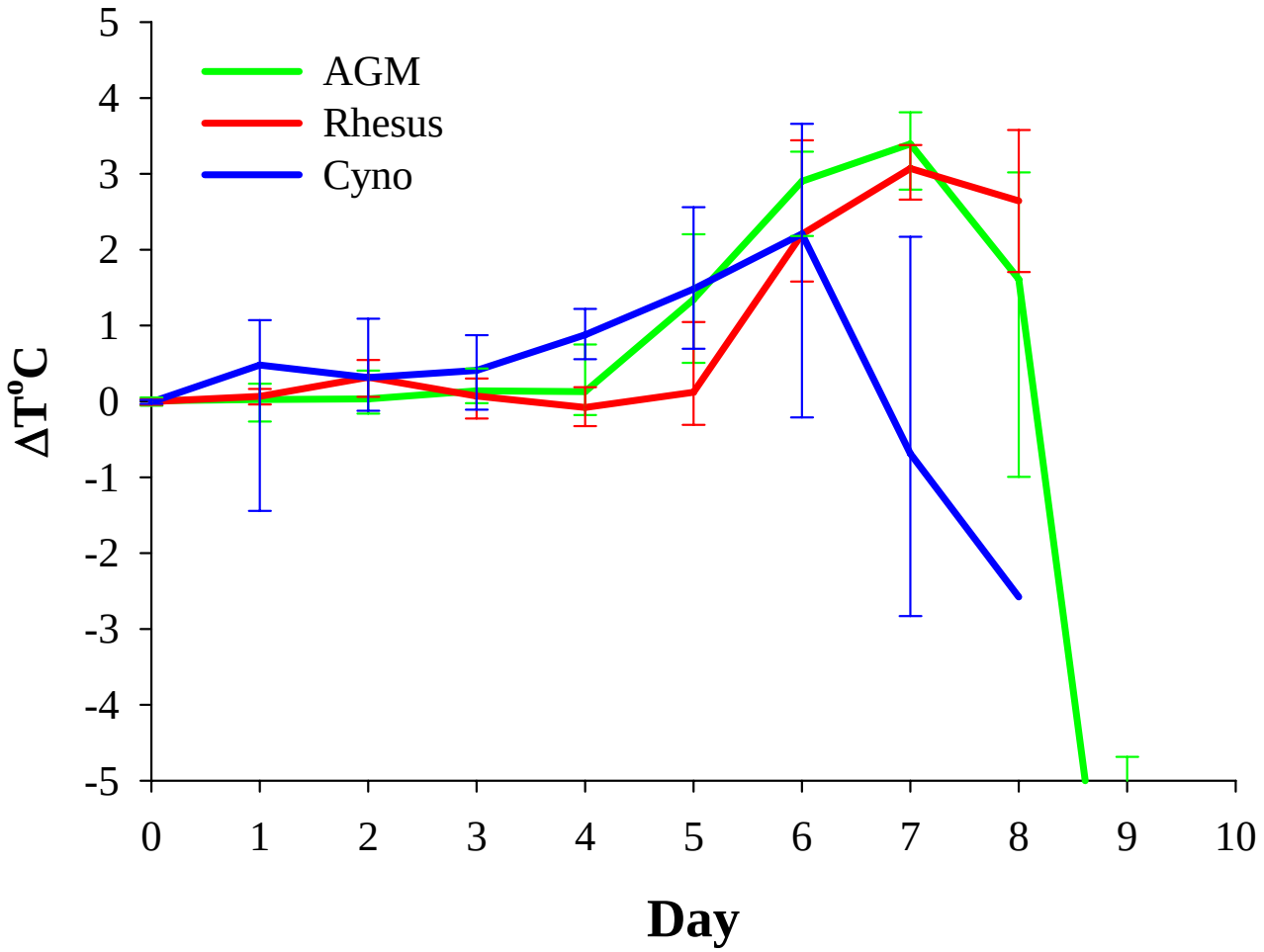
Fever response in rhesus after aerosol exposure to Ebola Zaire



Fever response in African green monkeys after aerosol exposure to Ebola Zaire



Fever response in NHP after aerosol exposure to Ebola Zaire – comparison



Aerosol exposure to Ebola Zaire – cynomolgus macaques

Challenge Dose	Date Moribund
128.3	8
0.8	
4.2	
11.0	7
2.0	10

Challenge		Fever						
Dose	Date Moribund	Onset	Tmax	RU Max	Duration (h)	Duration (d)	Fever-hrs	Ave Elev.
351	8	5	40.1	4.2	99.8	4	156.0	1.6
154	6	4	39.9	2.8	71.3	3	118.2	1.7
156	7	3	37.8	2.0	77.5	3	77.9	1.0
272	6	4	39.9	3.4	45.8	2	77.8	1.7
251	6	2	41.0	5.1	101.5	5	190.8	1.9
223	7	2	38.6	1.5	100.5	5	101.5	1.0

MTD: 6.5 days
Ave. Fever Duration (h): 82.7
Ave. Fever-hours: 120.3

Aerosol exposure to Ebola Zaire – rhesus macaques

Challenge		Fever						
Dose	Date Moribund	Onset	Tmax	RU Max	Duration (h)	Duration (d)	Fever-hrs	Ave Elev.
8			38.9	1.3	10.3	0	7.2	0.7
14	12	10	39.7	3.5	52.3	3	116.5	2.2
56	10	8	39.7	4.2	73.5	3	155.6	2.1
389	11	7	39.8	3.2	96.8	4	213.8	2.2
1237	7	5	40.1	3.2	66.0	3	130.4	2.0
1136	9	7	39.5	3.2	38.3	2	70.9	1.9

Challenge		Fever						
Dose	Date Moribund	Onset	Tmax	RU Max	Duration (h)	Duration (d)	Fever-hrs	Ave Elev.
598	7	6	40.4	3.5	63.0	2	152.9	2.4
1048	7	6	40.6	3.9	54.8	2	113.8	2.1
1330	7	6	40.3	2.7	76.8	2	150.9	2.0
1089	8	5		1.8	84.8	4	192.8	2.3
1091	7	6	40.0	1.6	39.3	2	75.9	1.9
913	8	6	39.8	1.6	57.5	3	125.9	2.2

MTD: 7.3 days
Ave. Fever Duration (h): 62.7
Ave. Fever-hours: 135.4

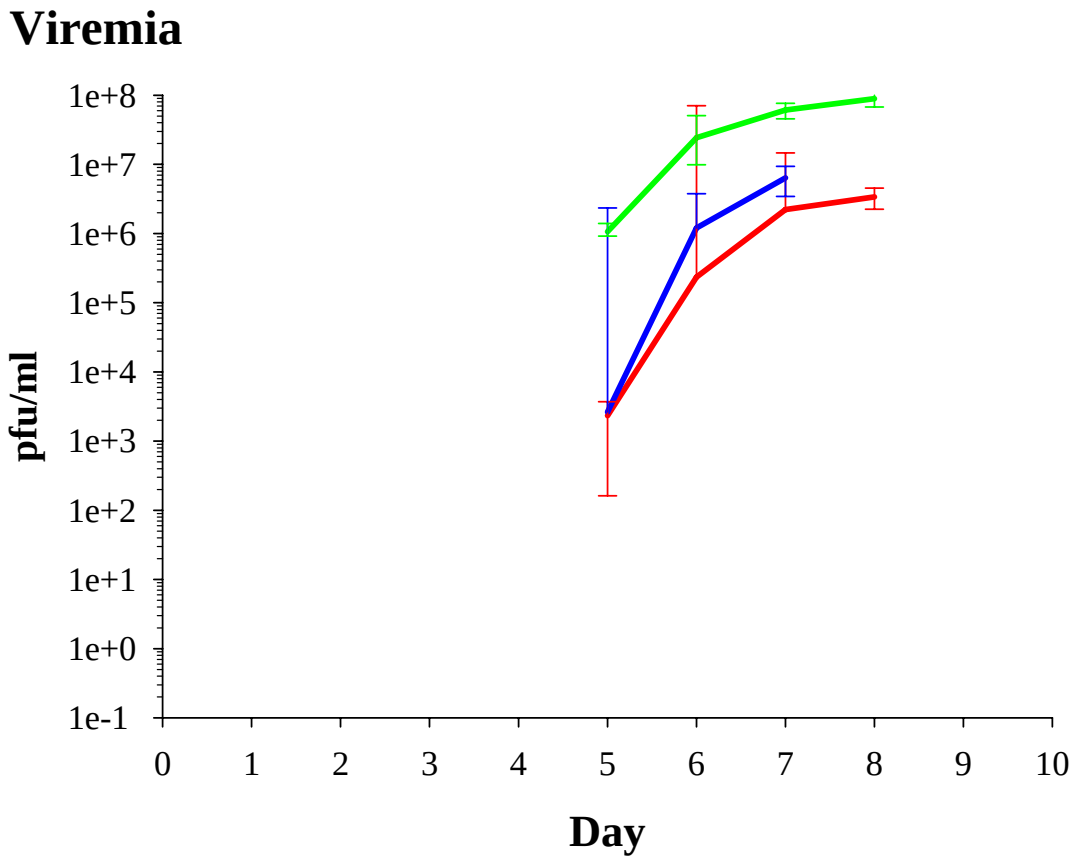
Aerosol exposure to Ebola Zaire – African green monkeys

Challenge		Fever						
Dose	Date Moribund	Onset	Tmax	RU Max	Duration (h)	Duration (d)	Fever-hrs	Ave Elev.
6	9	6	40.0	5.0	116.3	5	259.1	2.2
7	10	8	39.7	5.0	75.8	3	190.3	2.5
42	10	6	39.0	4.1	99.3	4	221.0	2.2
154	9	5	40.0	3.7	104.8	5	217.4	2.1
202	10	6	39.9	4.1	100.0	4	225.9	2.3
104	8	5	39.5	3.9	91.3	4	213.3	2.3

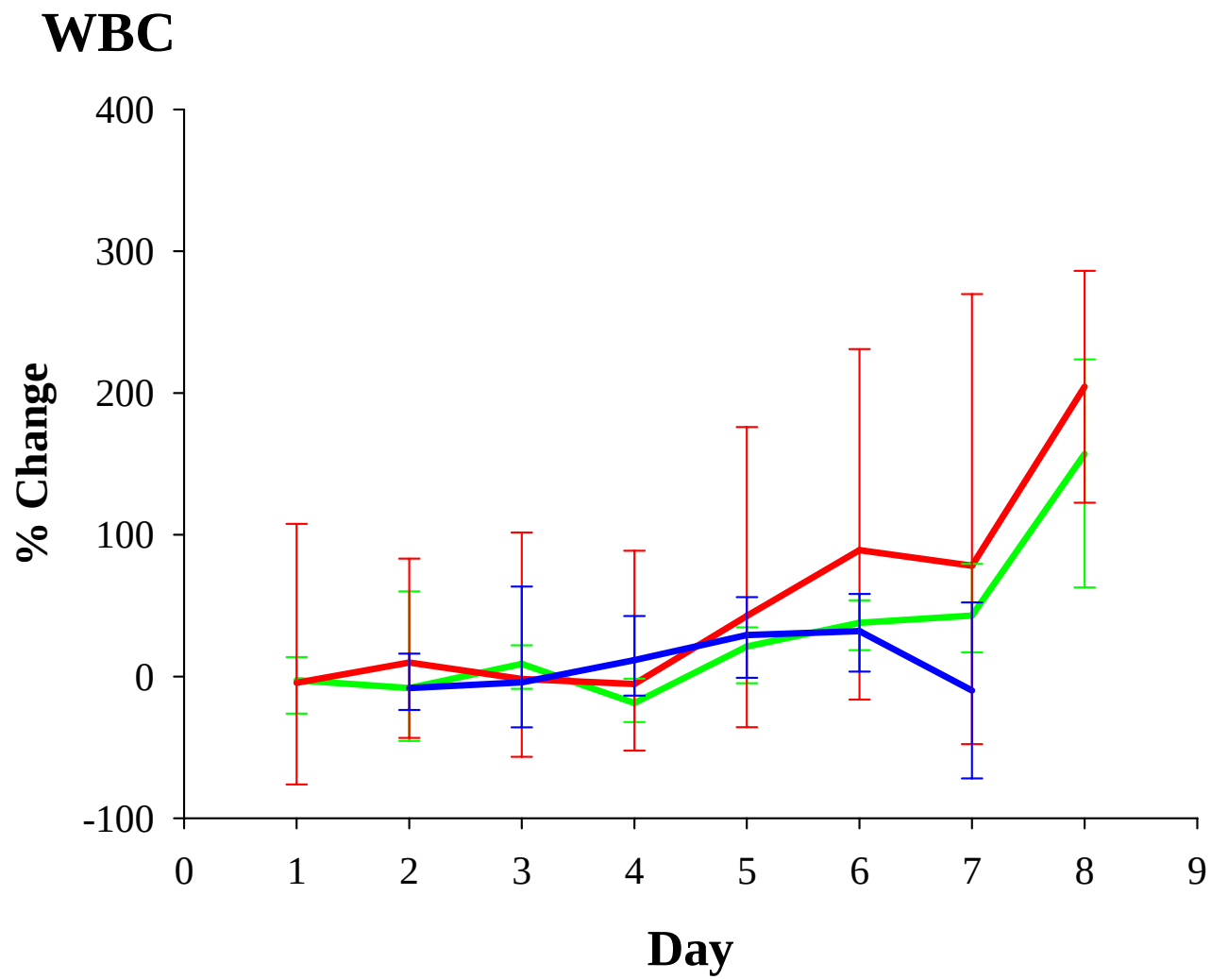
Challenge		Fever						
Dose	Date Moribund	Onset	Tmax	RU Max	Duration (h)	Duration (d)	Fever-hrs	Ave Elev.
1131	8	4	39.2	4.3	110.8	4	182.6	1.6
1396	9	5	39.5	4.8	89.5	4	203.0	2.3
383	8	6	40.0	4.7	74.0	3	155.3	2.1
562	8	5	39.7	4.0	102.3	4	224.7	2.2
318	8	5	39.7	4.6	87.8	4	231.1	2.6
487	9	6	39.2	3.9	66.3	3	141.6	2.1

MTD: 8.3 days
Ave. Fever Duration (h): 88.4
Ave. Fever-hours: 189.7

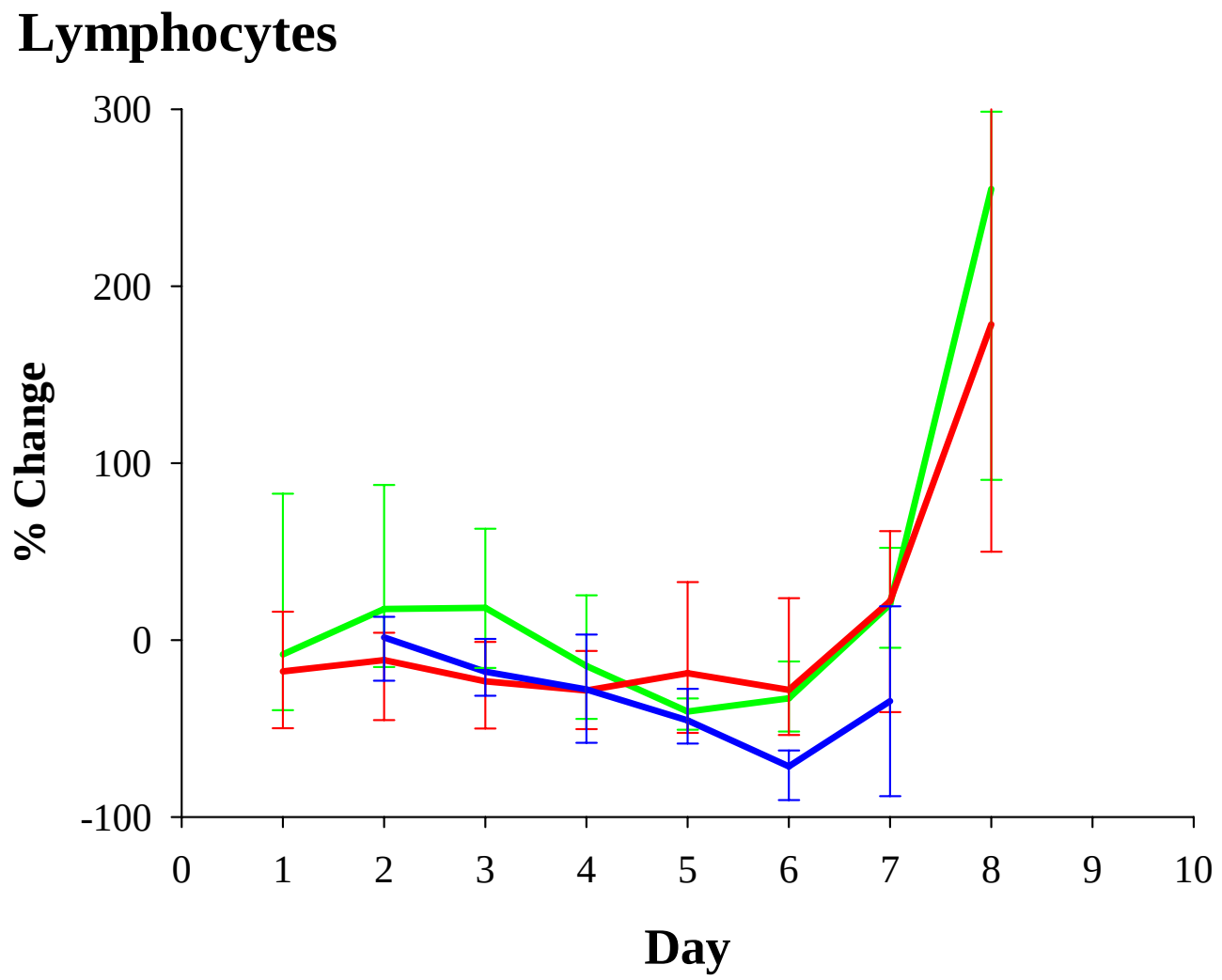
Serum viremia in NHP after aerosol exposure to Ebola Zaire



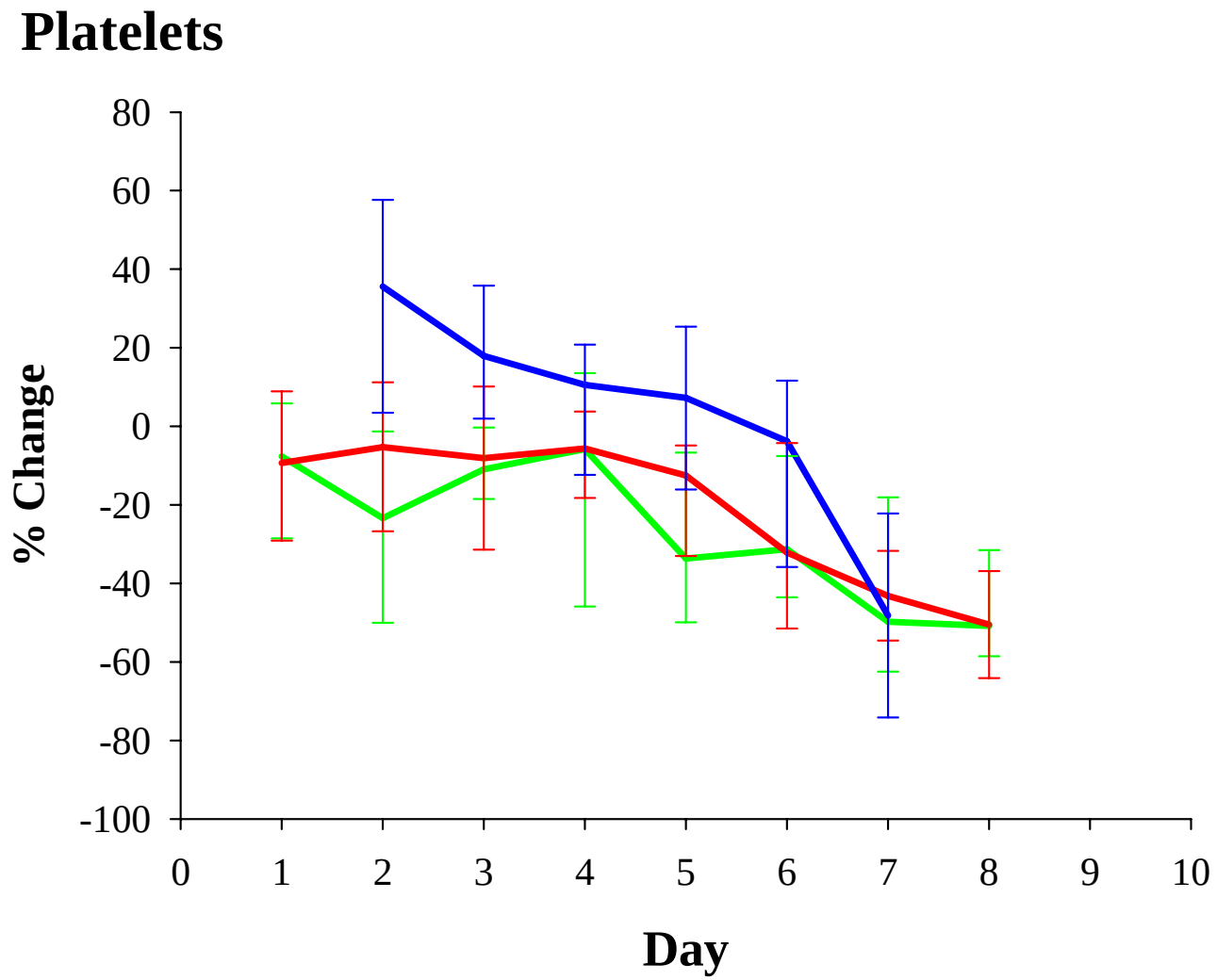
Changes in WBC count postexposure



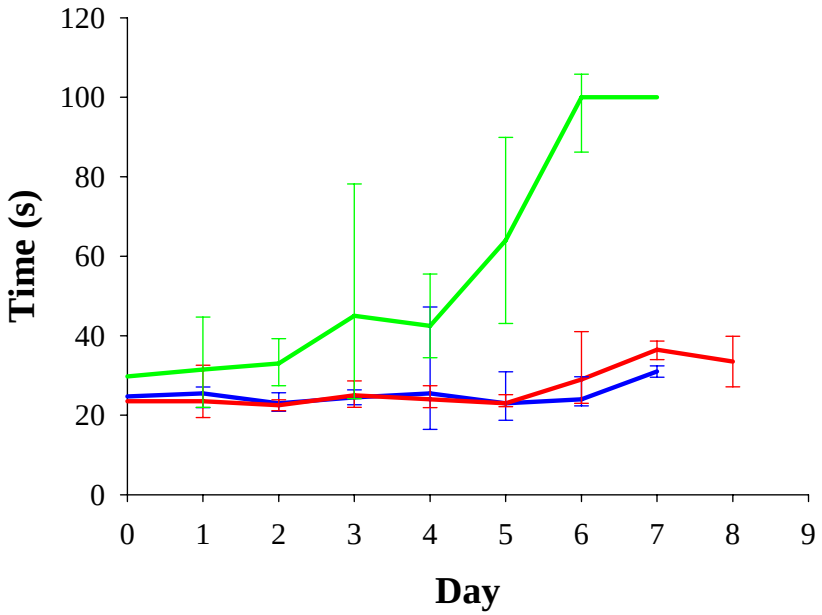
Changes in peripheral blood lymphocytes postexposure



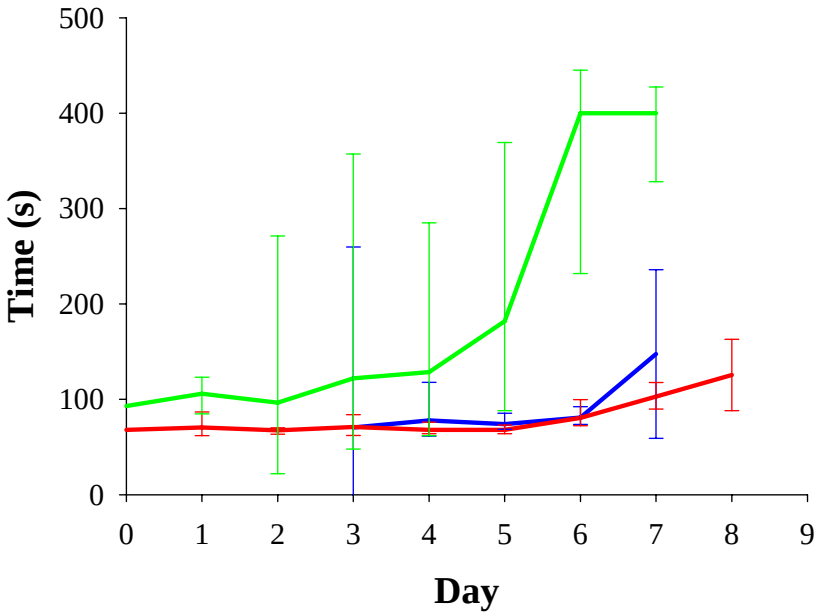
Decline in platelet counts postexposure



Disruption of coagulation pathways

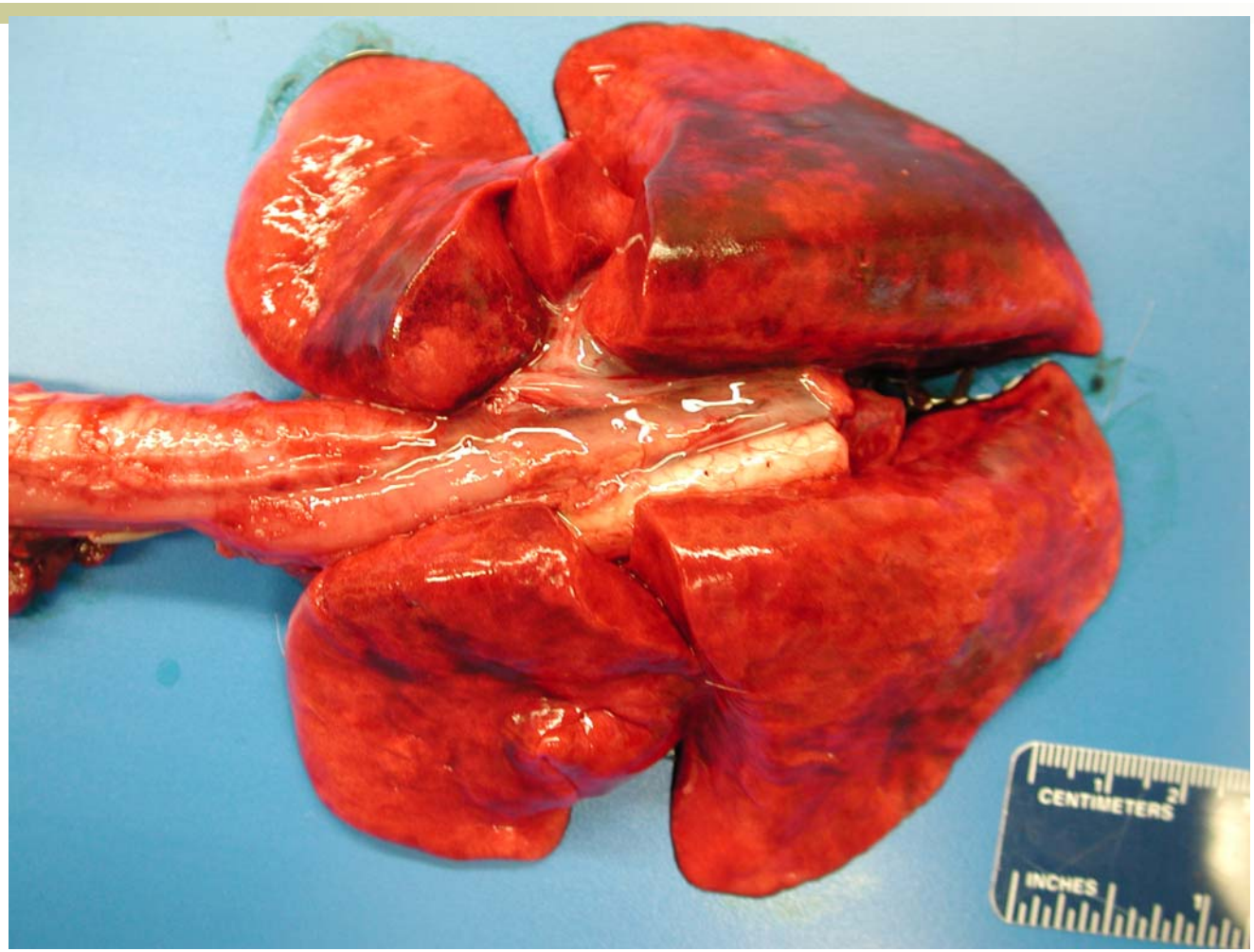


PT (extrinsic)



APTT (intrinsic)

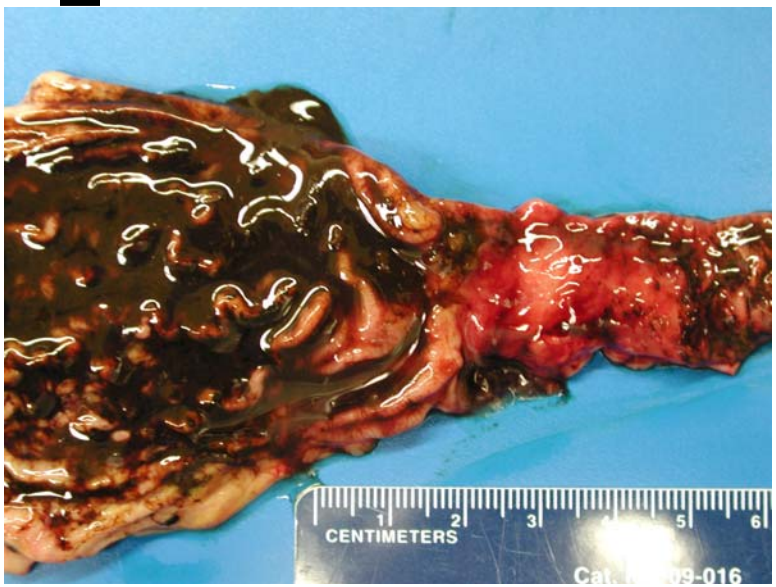
Pathogenesis of aerosolized Ebola Zaire

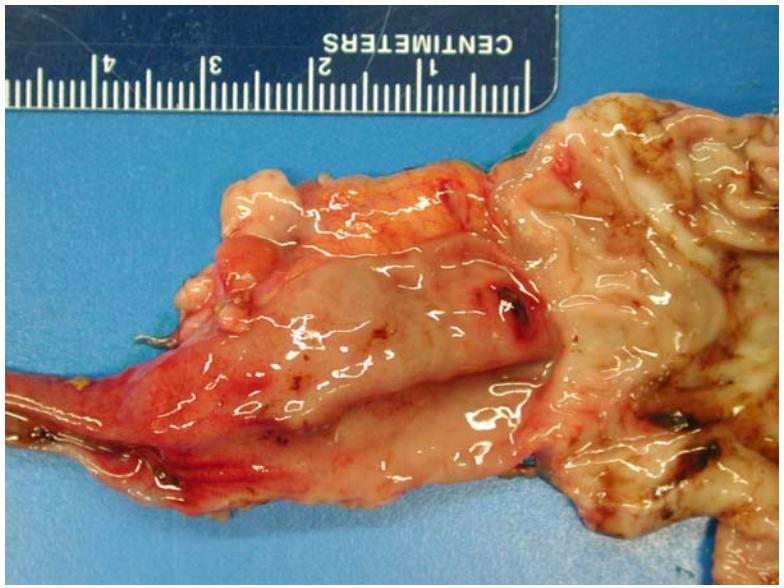


Mediastinal edema in African green monkey infected with Ebola Zaire

Photographs courtesy of Don Nichols, Pathology Division, USAMRIID

Differences in pathogenesis between NHP species





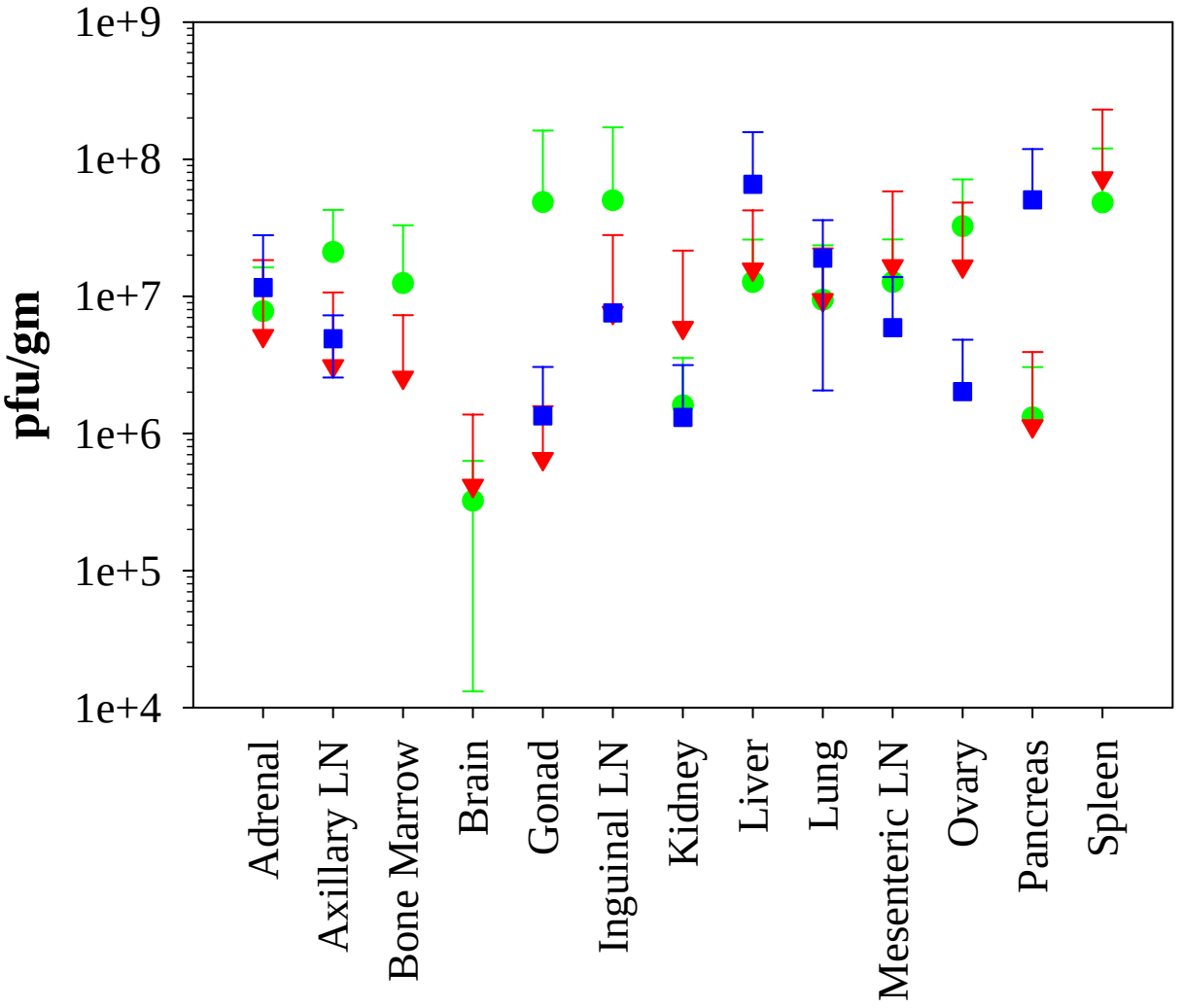
Photographs courtesy of Don Nichols, Pathology Division, USAMRIID

Congestion and/or hemorrhage in the mucosa of the duodenum

Found in macaques infected parenterally but not common (<50%)

With aerosol, seen in 2 of 6 rhesus, 1 of 6 cynos, and 5 of 6 African green monkeys exposed to ~1000 pfu

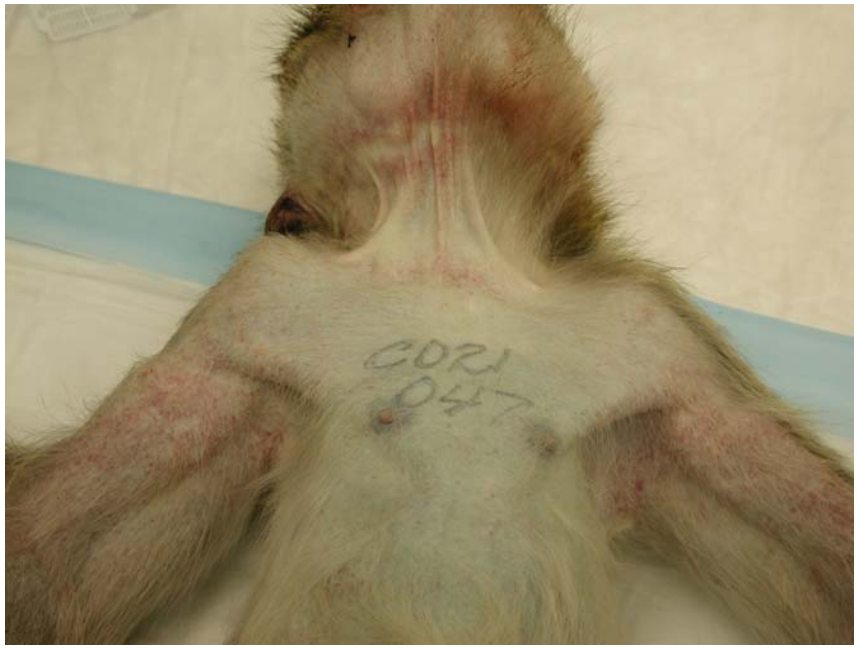
Aerosol exposure – viral isolation from tissues

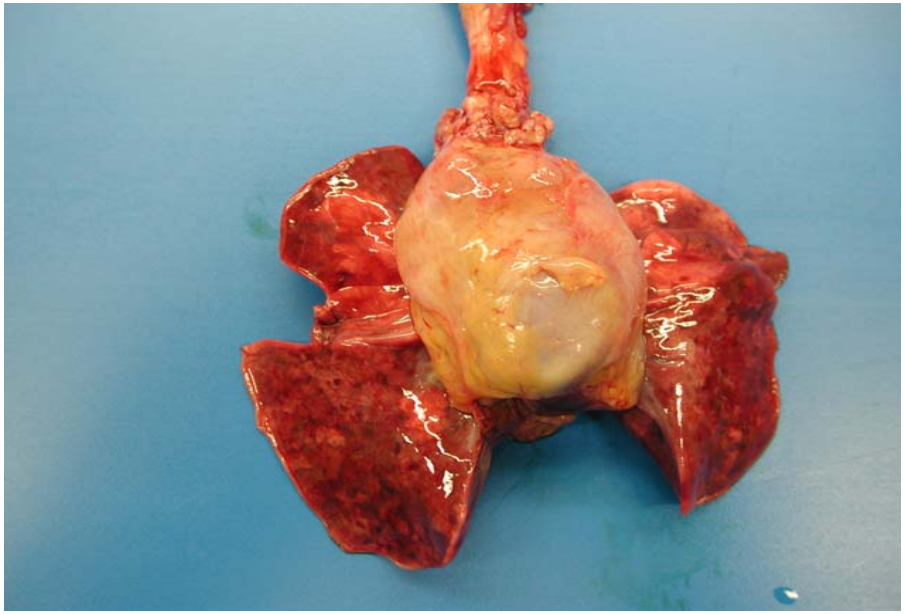


Conclusions – Ebola Zaire

- Aerosolized Ebola Zaire ’95 is highly virulent in all three NHP, even at very low doses. Rhesus may be slightly more resistant to infection.
- Time course of infection is extended at lower doses but at high doses aerosol the time course is comparable to parenteral infection.
- There are clear differences between NHP species in the disease caused by aerosol infection
  - MTD longest in African green monkeys, shortest in cynos
    Slightly accelerated in rhesus macaques (relative to parenteral)
  - Clinical observations/signs (rash, gastrointestinal discomfort)
  - Fever duration/severity worse in African green monkeys
  - Gross necropsy findings
  - Changes in extrinsic/intrinsic coagulation pathways
- Which species is the more relevant to humans?

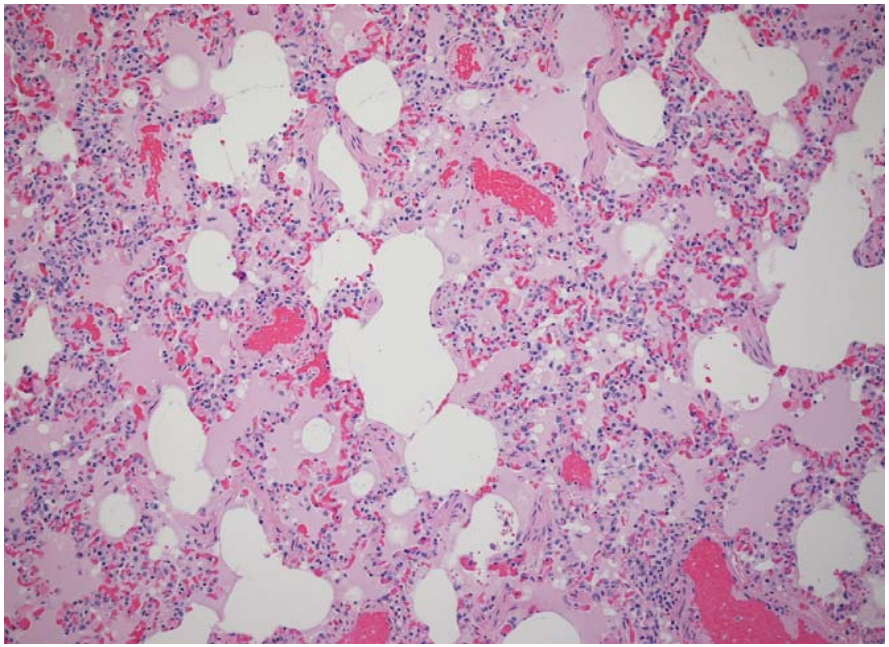
Pathology from Ci67-infected cynomolgus macaque

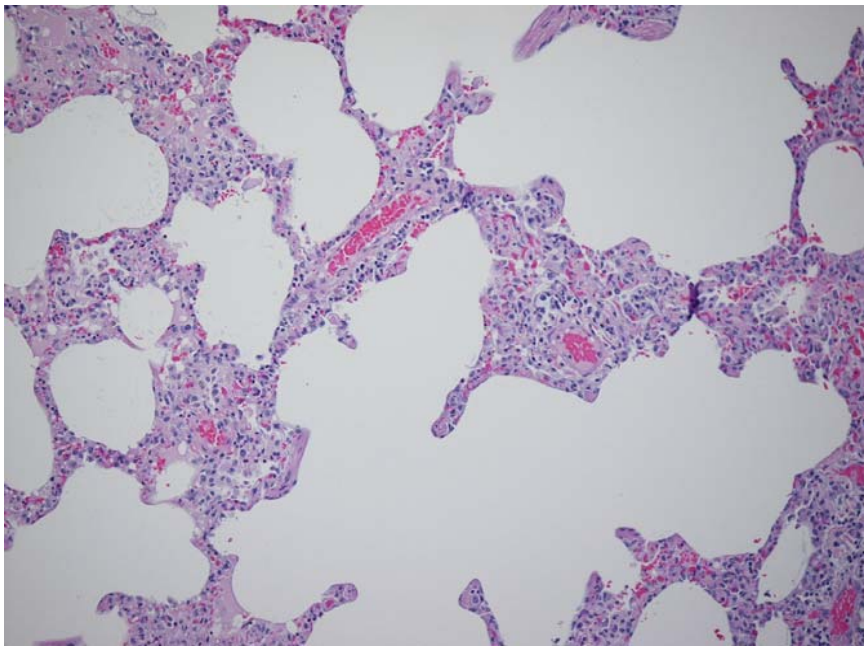




Photographs courtesy of Tony Alves, Pathology Division, USAMRIID

Pathology of Ci67 in lungs of cynomolgus macaques – Histology





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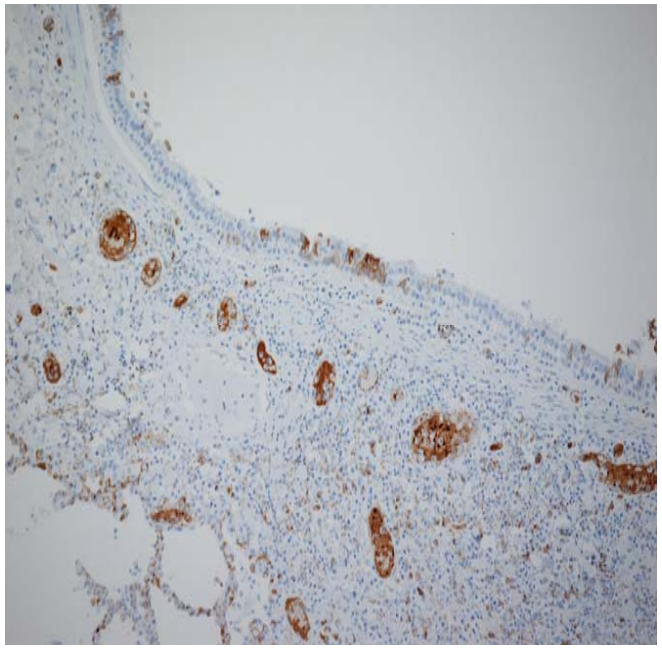
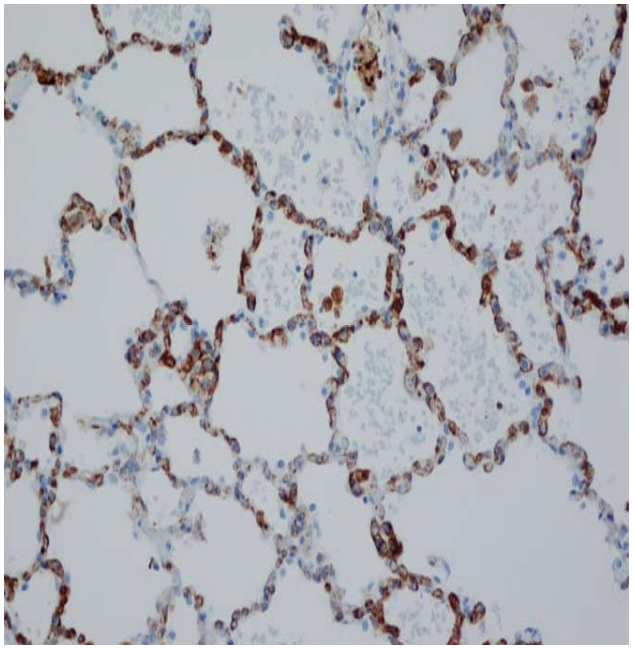
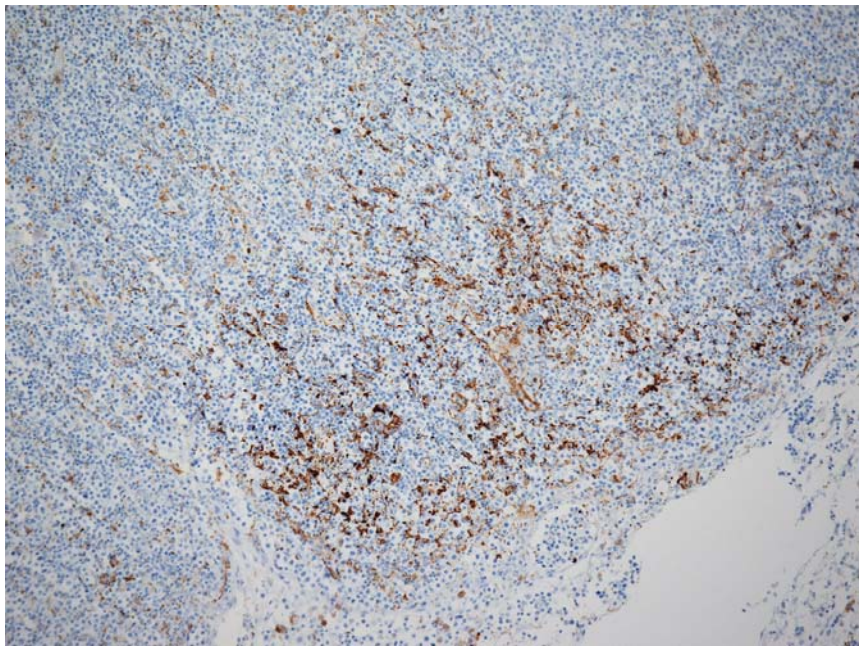
Pathology of Ci67 in lungs of cynomolgus macaques - IHC

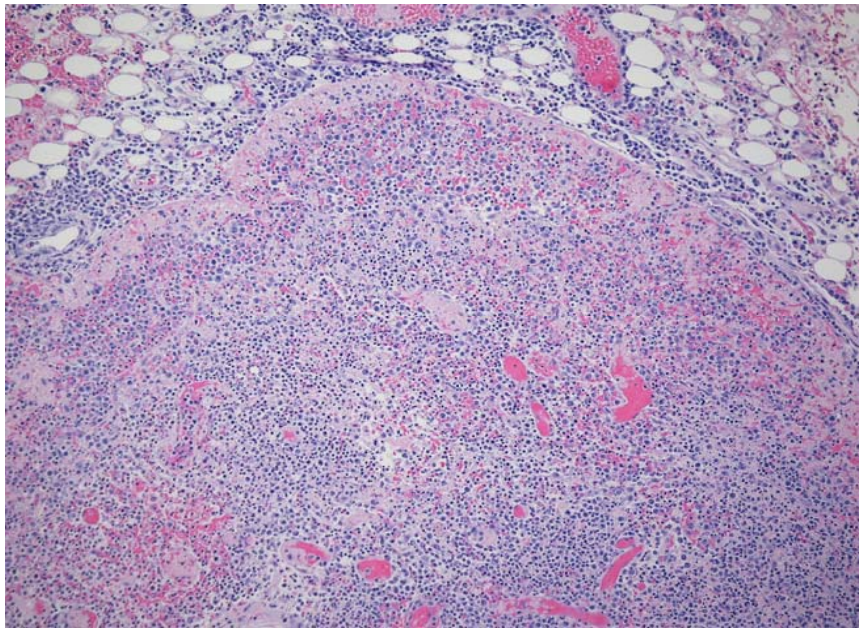




Photographs courtesy of Tony Alves, Pathology Division, USAMRIID

Pathology of Ci67 in mediastinal LN – cynomolgus macaque





Photographs courtesy of Tony Alves, Pathology Division, USAMRIID

Studies done to date

- Ebola Zaire
  11 cynos, 12 African greens, 12 rhesus, 102 Hartley guinea pigs
- Ebola Sudan
  3 cynos
- Marburg Ci67
  11 cynos, 9 African greens, 9 rhesus, 120 Strain 13 guinea pigs, 116 Hartley guinea pigs
- Marburg Musoke
  5 cynos, 120 Hartley guinea pigs, 24 strain 13 guinea pigs
- Marburg Ravn
  60 Hartley guinea pigs
- Marburg Angola
  6 cynomolgus macaques

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