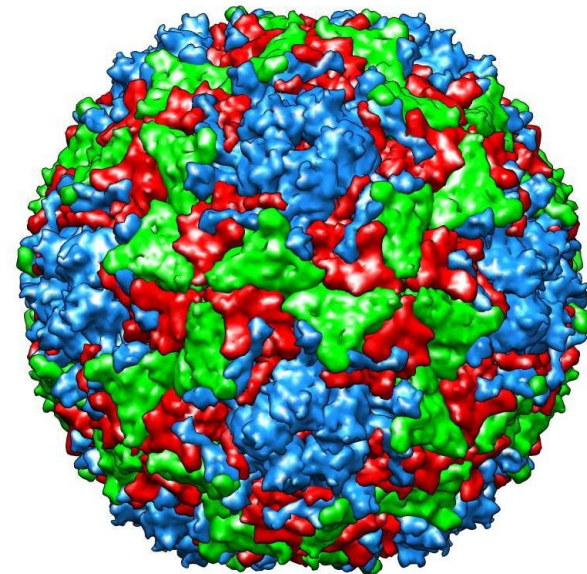


Parechovirus leukoencephal -**opathy** or -**itis** ?

Clayton A. Wiley MD/PhD
June 14, 2013



Collaborators

Pittsburgh

Stephanie Bissel

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Caitlin Winkler

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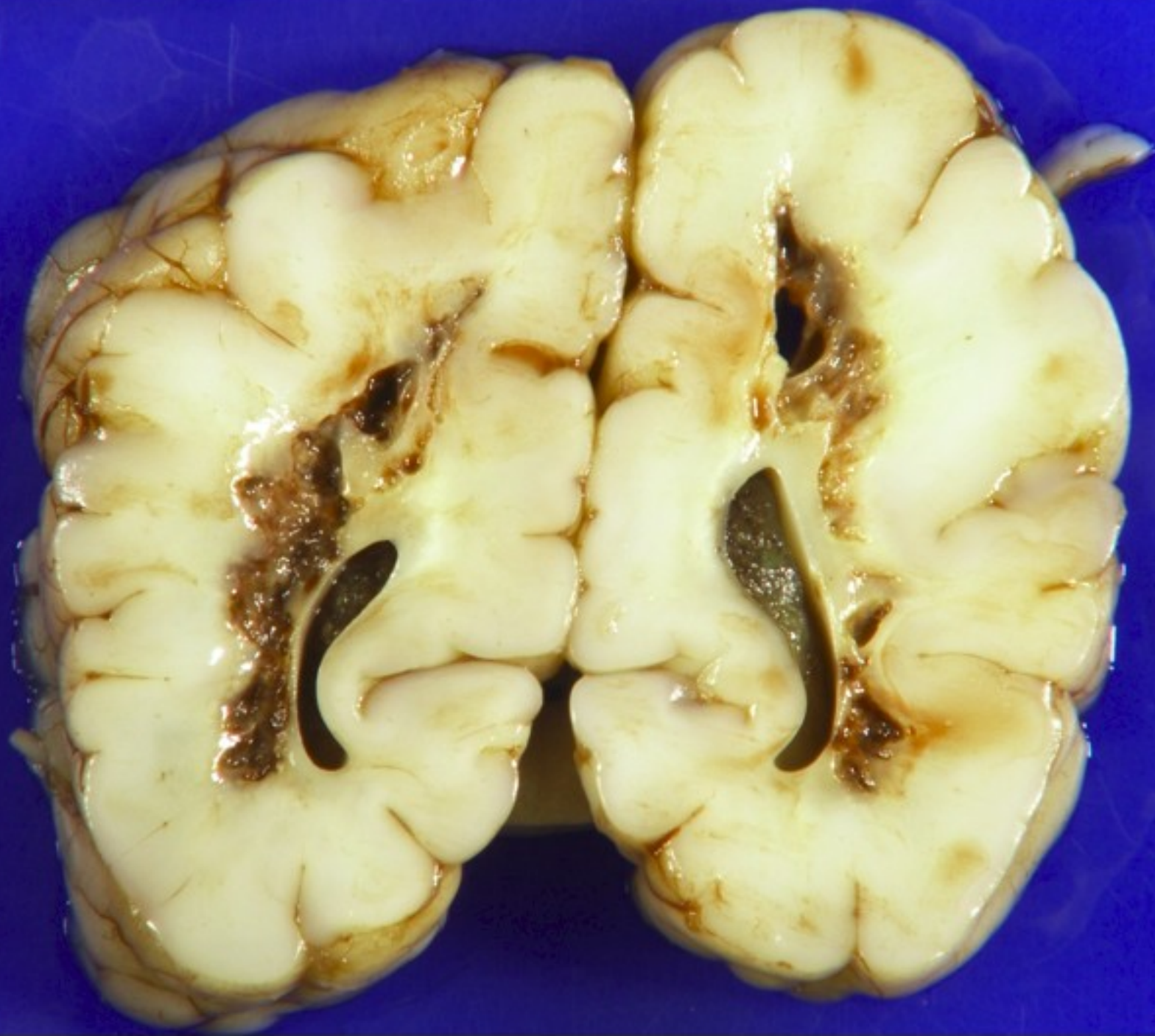
Atlanta CDC

W. Allan Nix

Disclosure

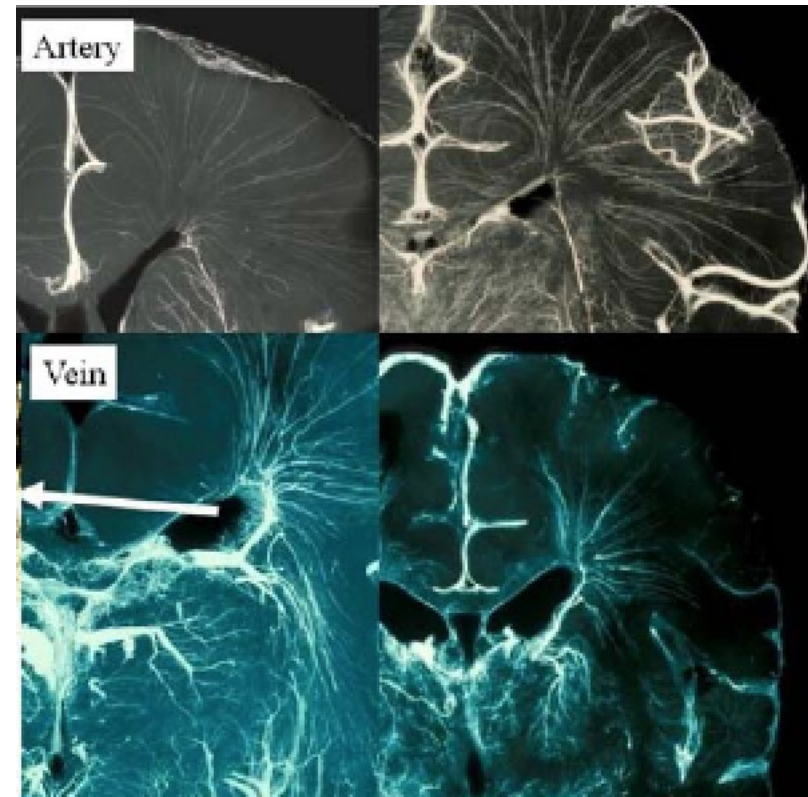
Nothing, but open to suggestions

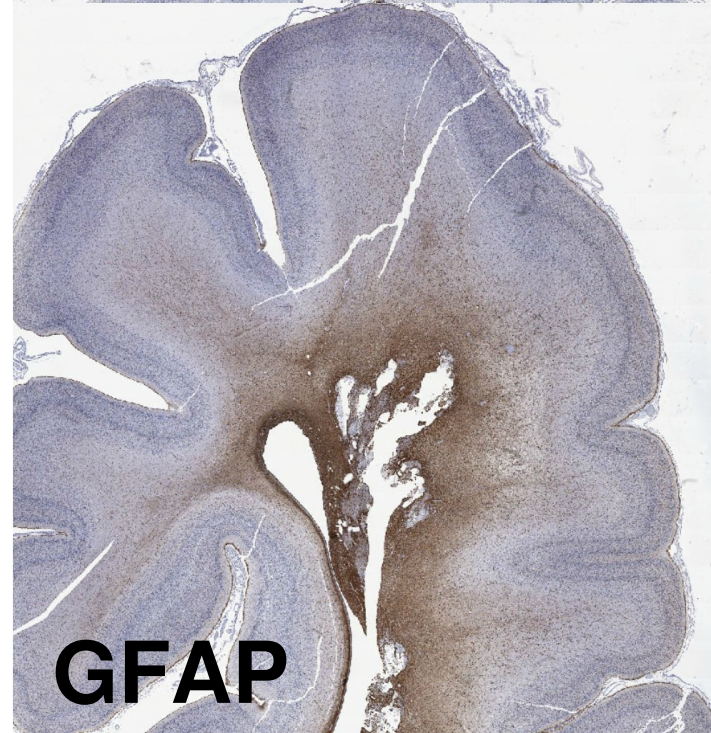
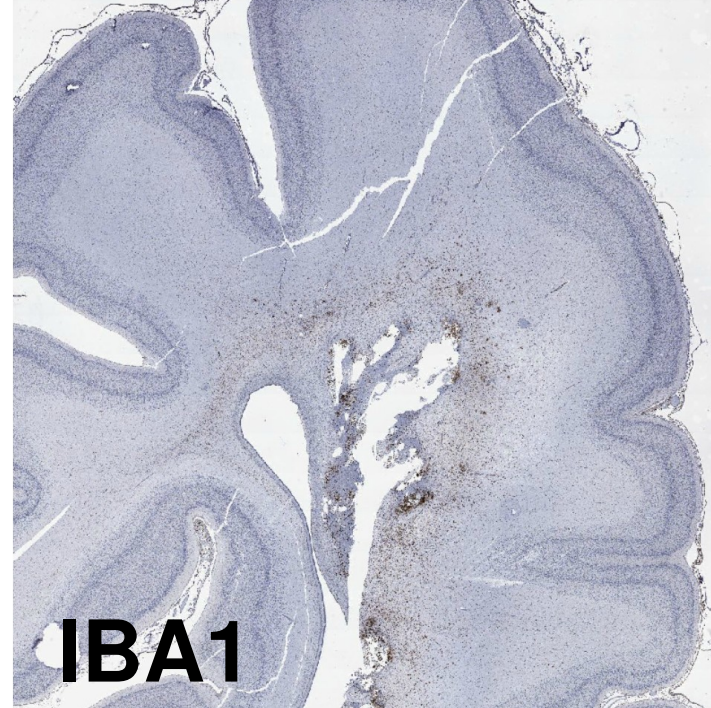
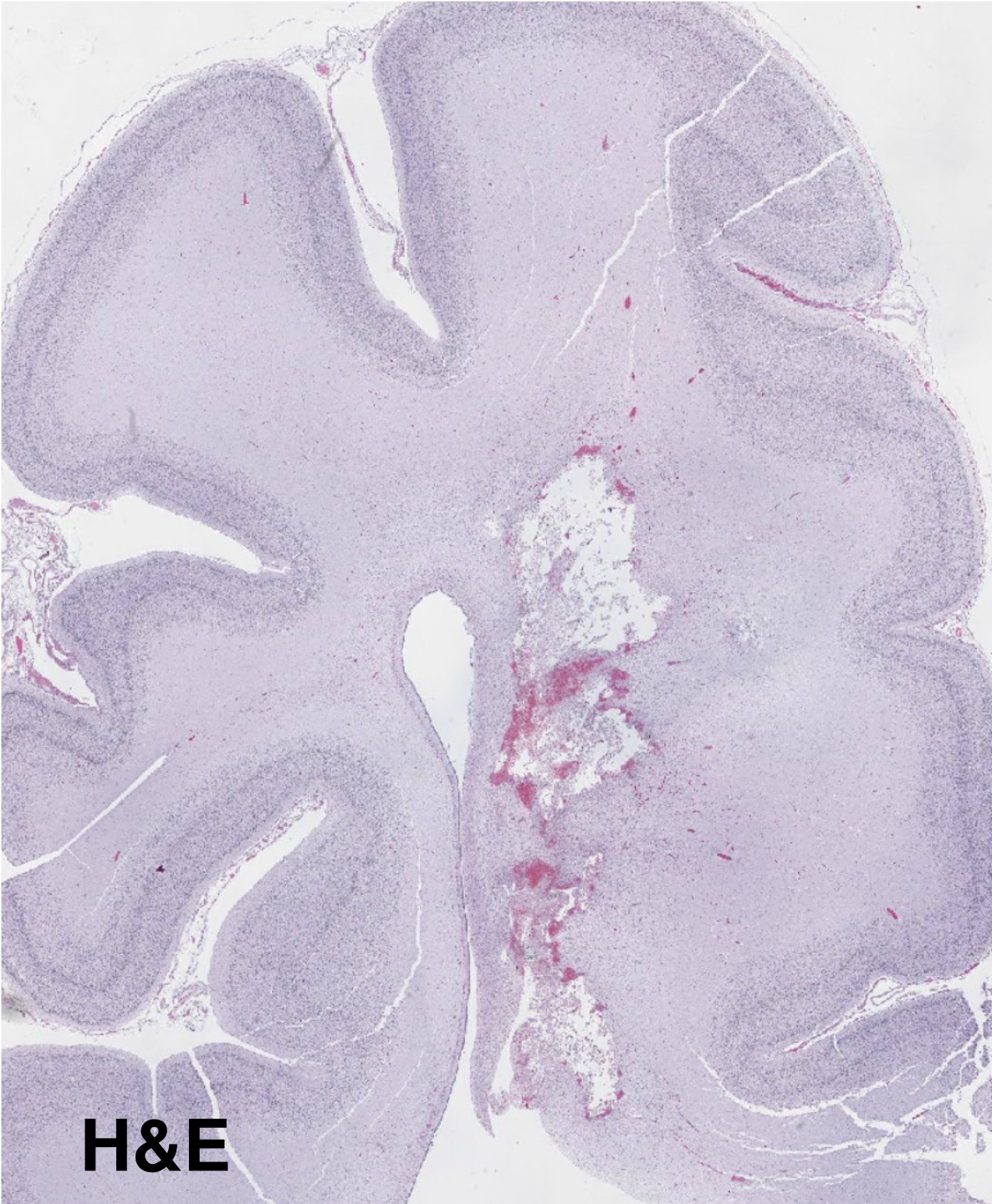


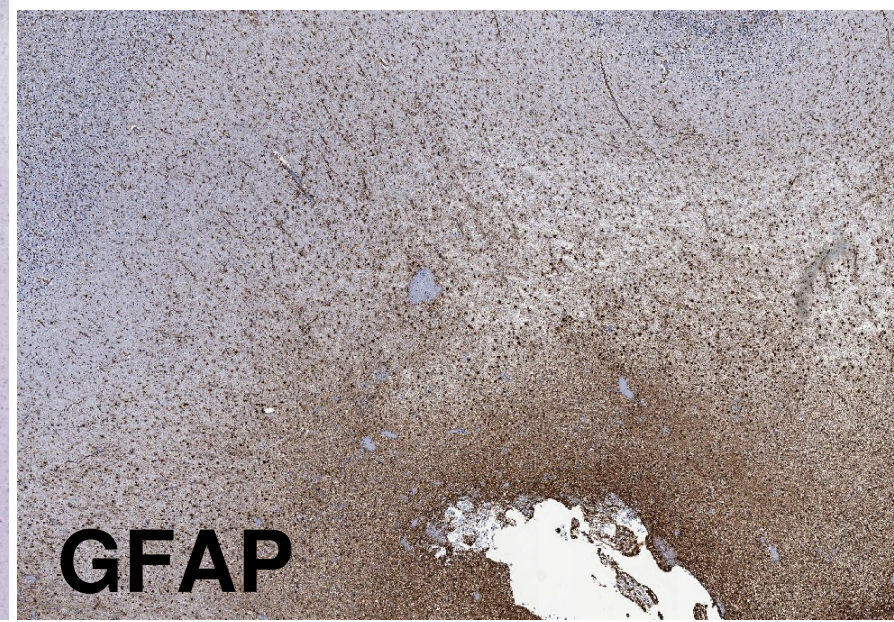
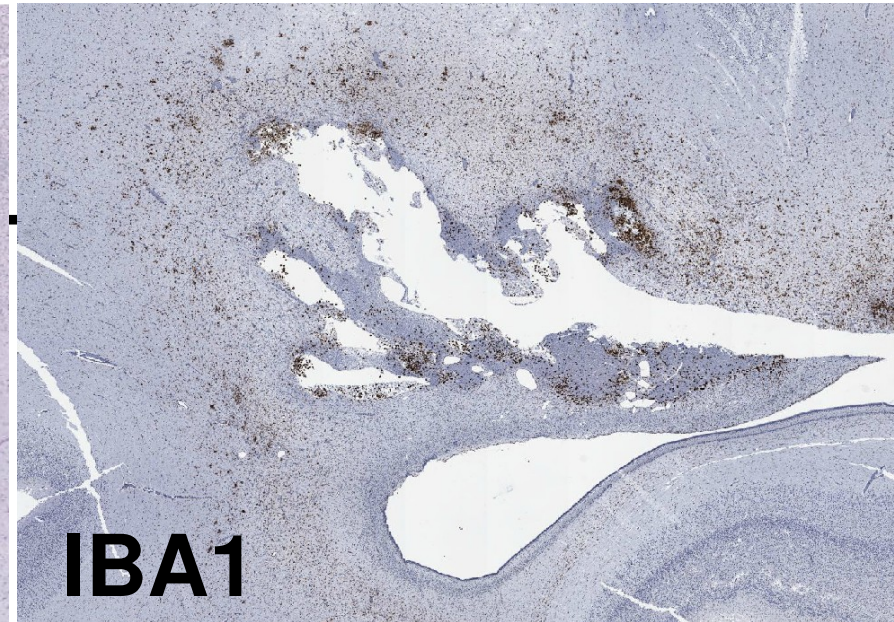
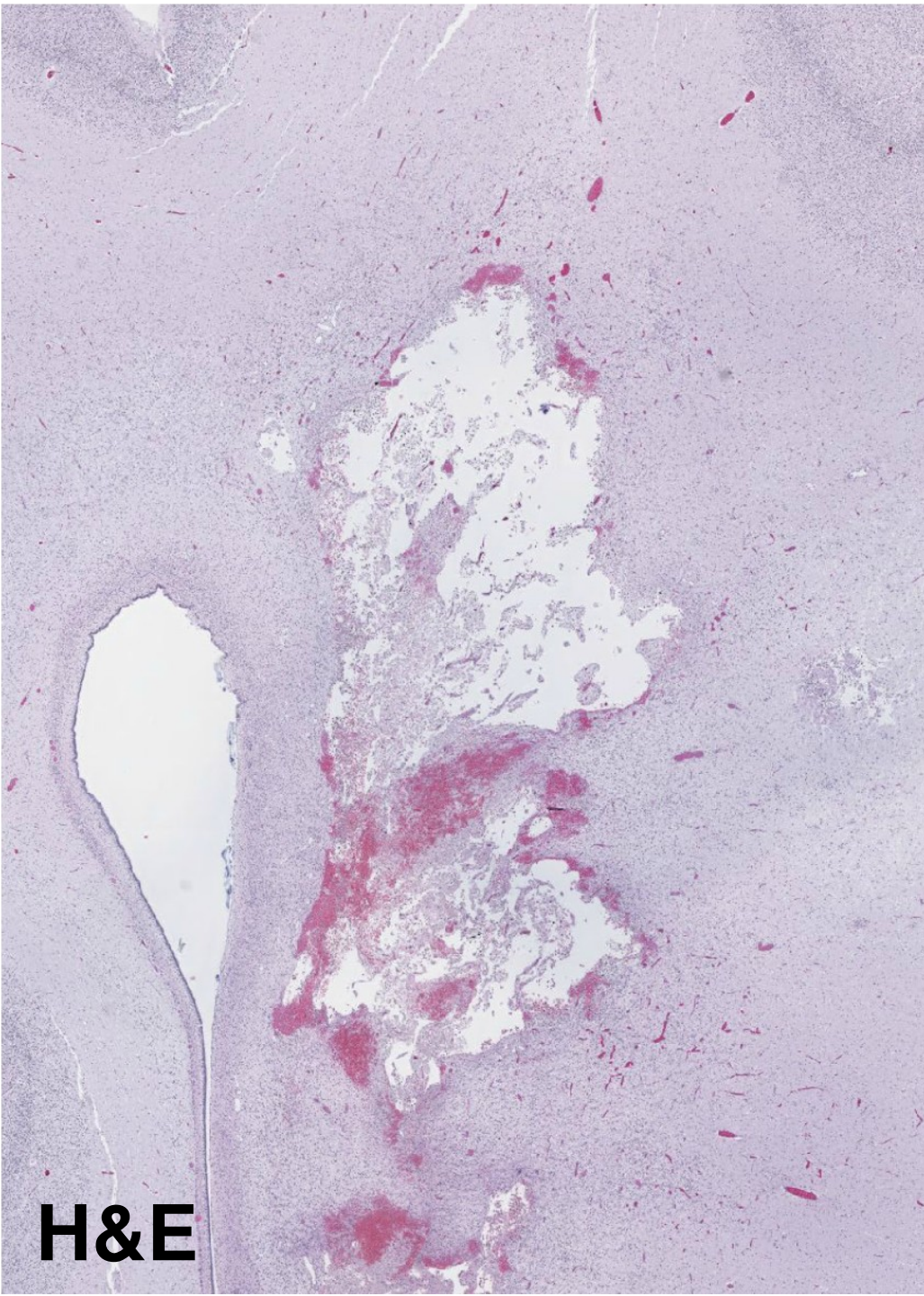


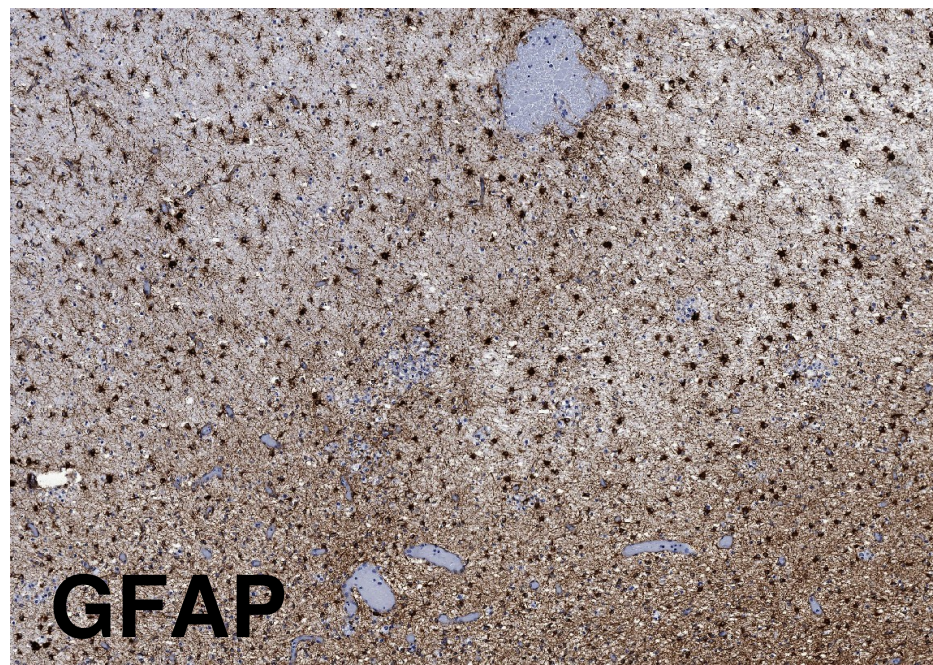
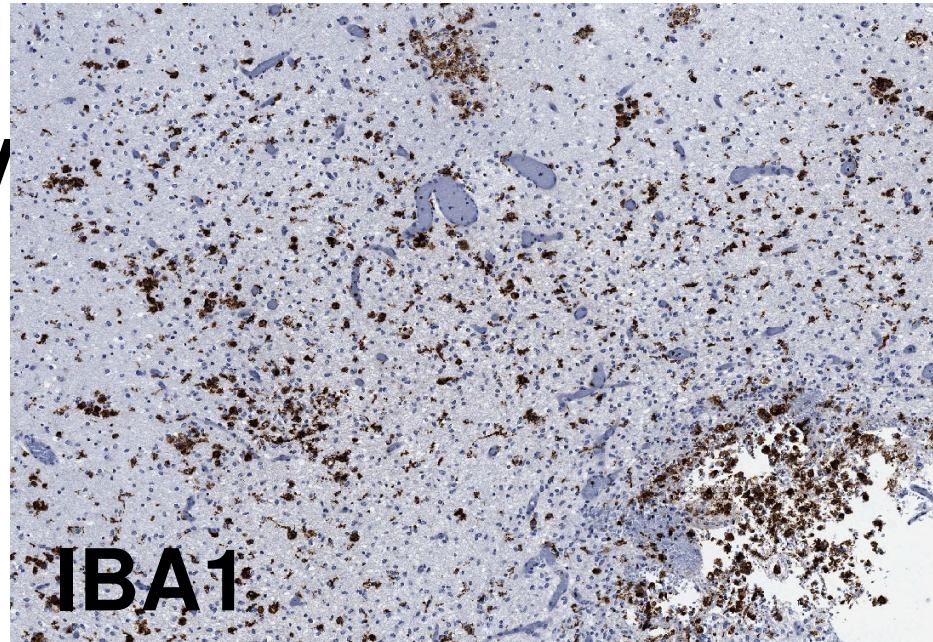
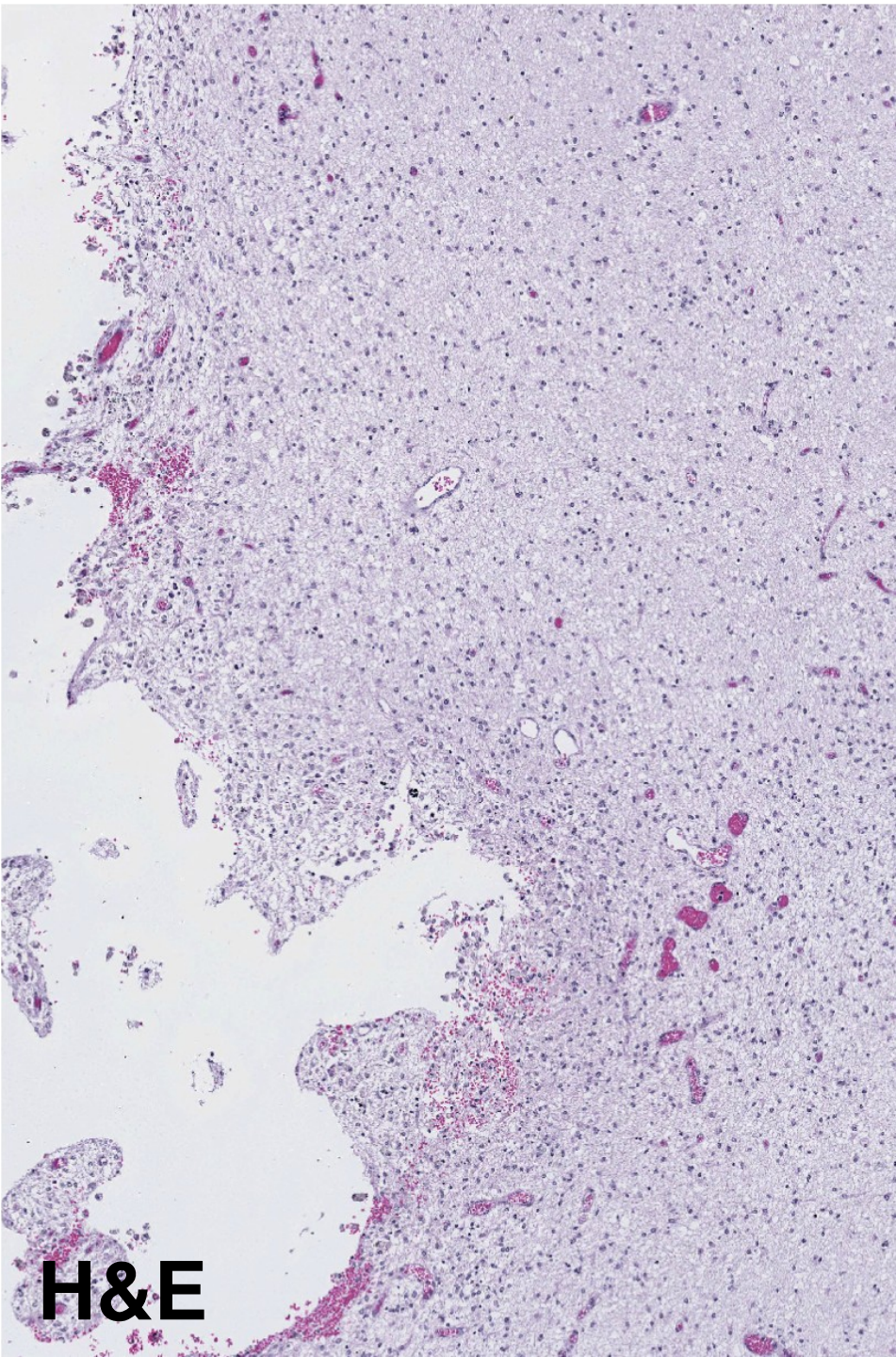
PVL

- First described by Virchow
- Cystic disease diagnosable by US
 - DWMG not diagnosable with US (? MRI)
- Compromise of distal field of vascular perfusion
 - Gestational age most important risk factor
 - Intrapartum cardiopulmonary instability
 - Seen in preterm to term neonates up to 2 months
 - 5-10% of preterm infants 24-33 weeks
 - 25% of SIDS





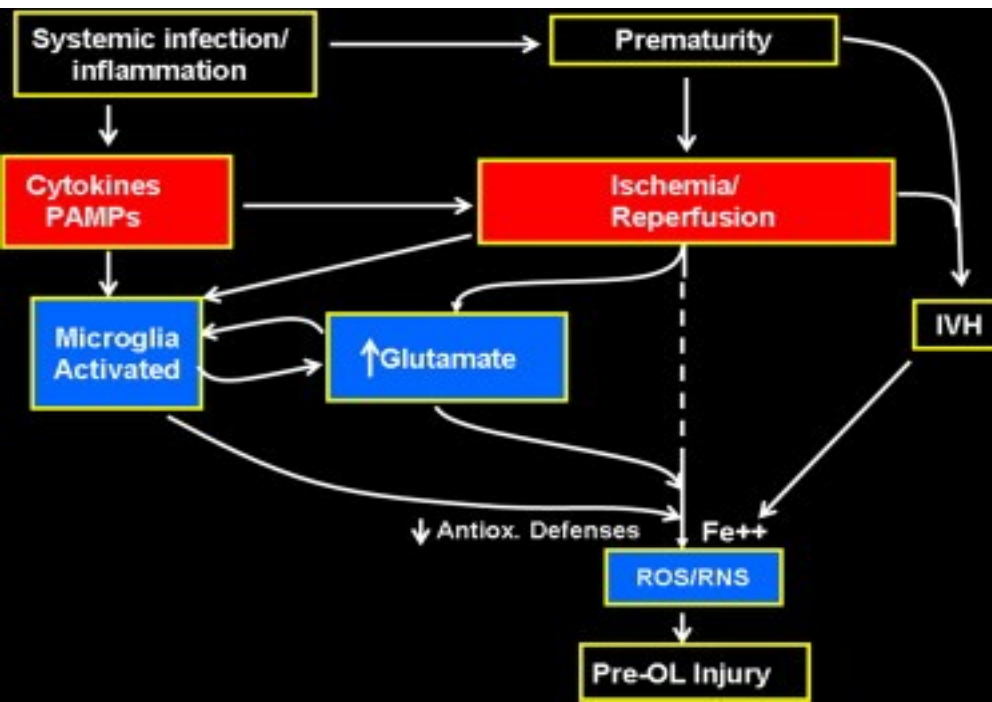




PVL: Pathogenesis

What is Periventricular Leukomalacia?

Periventricular leukomalacia (PVL) is characterized by the death of the white matter of the brain due to softening of the brain tissue. It can affect fetuses or newborns; premature babies are at the greatest risk of the disorder. PVL is caused by a lack of oxygen or blood flow to the periventricular area of the brain, which results in the death or loss of brain tissue. The periventricular area-the area around the spaces in the brain called ventricles-contains nerve fibers that carry messages from the brain to the body's muscles. Although babies with PVL generally have no outward signs or symptoms of the disorder, they are at risk for motor disorders, delayed mental development, coordination problems, and vision and hearing impairments. PVL may be accompanied by a hemorrhage or bleeding in the periventricular-intraventricular area (the area around and inside the ventricles), and can lead to cerebral palsy. The disorder is diagnosed by ultrasound of the head.



- Loss of developing oligos ?
 - Susceptible to IFN gamma

Joseph J. Volpe , Hannah C. Kinney , Frances E. Jensen , Paul A. Rosenberg

Intern Journal Develpl Neurosci 29, Issue 6 2011 565 - 582

Case 1

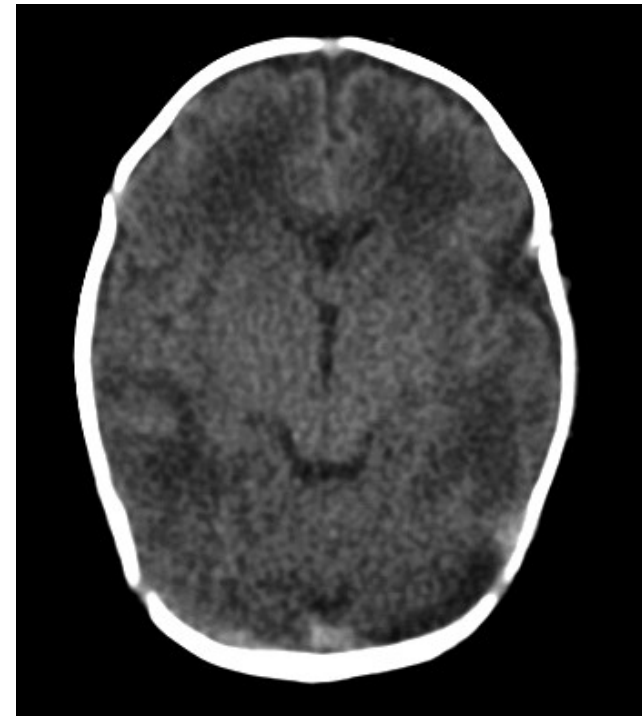
- 33 week female born to 37yo G3P2
 - Gestational diabetes, placenta previa
 - C-section
 - 1780 grams, Apgars 8/9
 - Biotinylase Deficiency
- NICU for apnea episodes
 - Intubated for surfactant 1 day
 - Screening head ultrasound @ 6 and 16 days- normal
 - Weaned from TPN
- D/C'd to home at 20 days

Case 1 cont'd

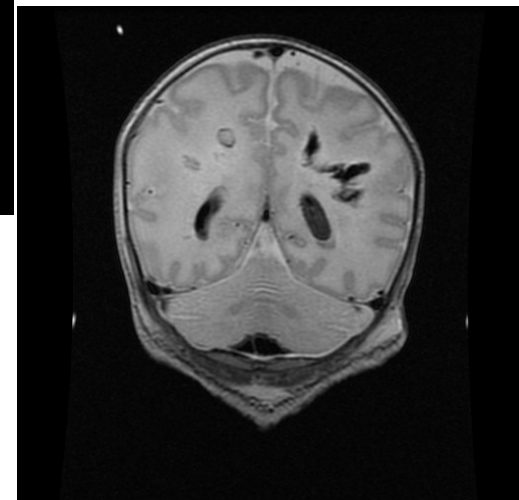
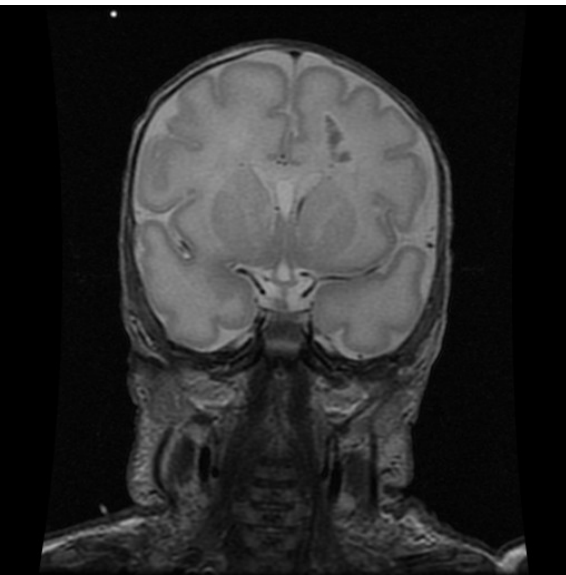
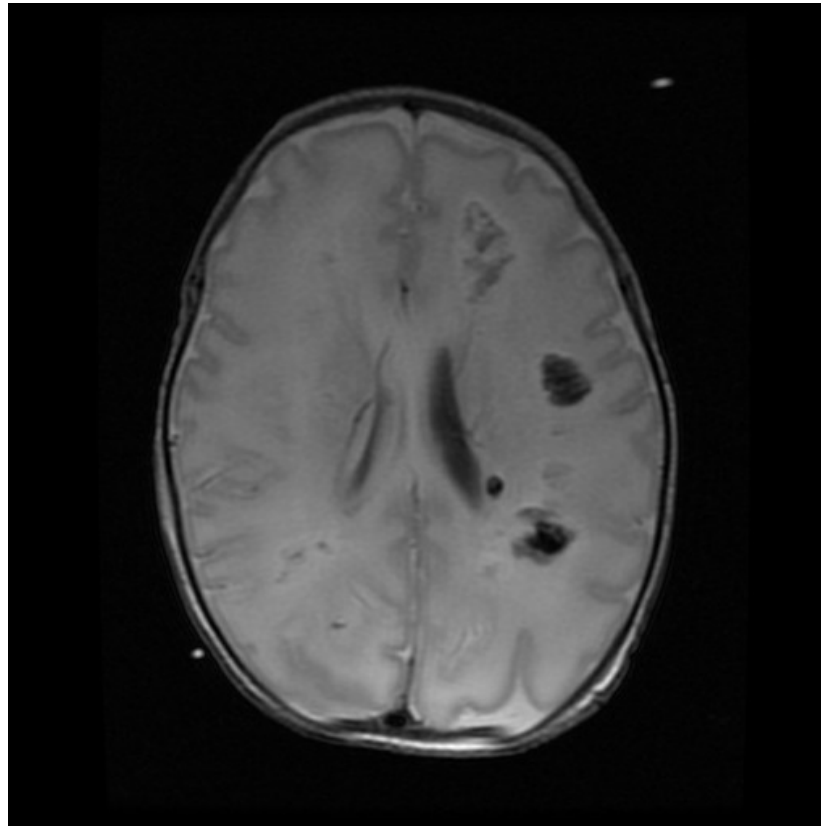
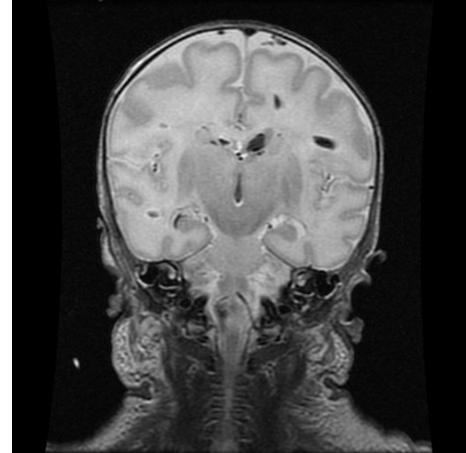
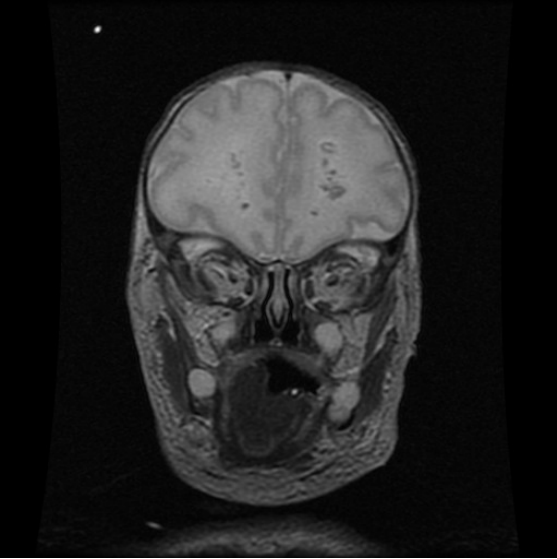
- 30 days returned to ER because of vomiting
 - Admitted to NICU for “Neonatal sepsis”
 - Somnolent with occasional episode of apnea
 - intubated



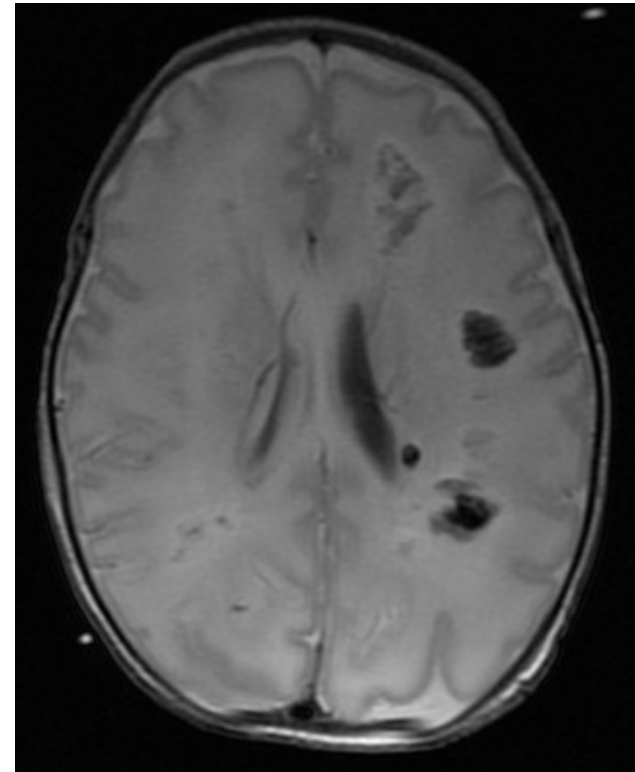
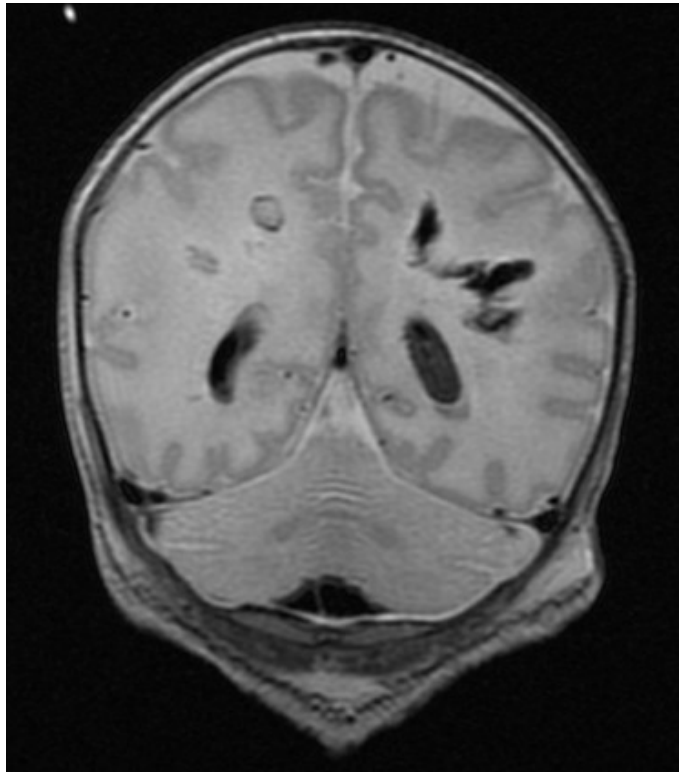
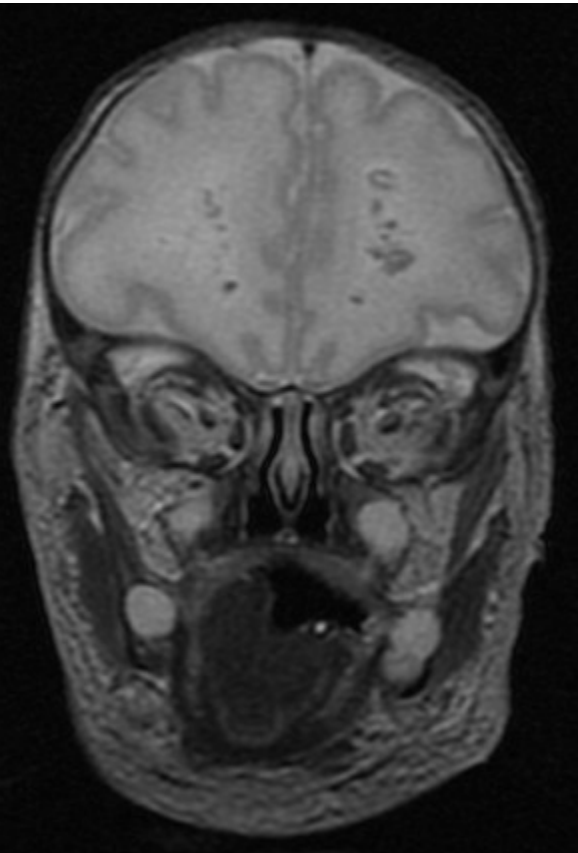
head CT



Seizures at 32 days
MRI at 33 days

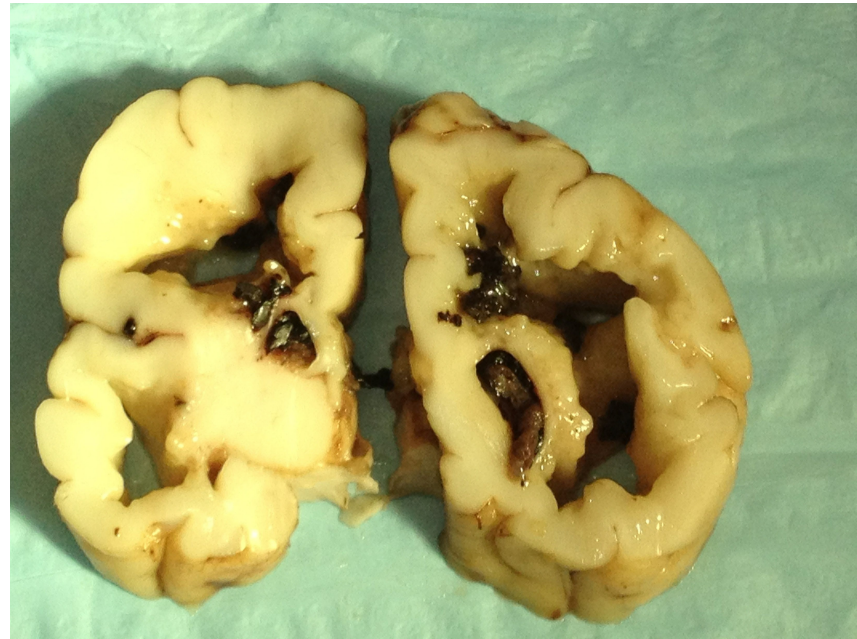


MRI at 33 days

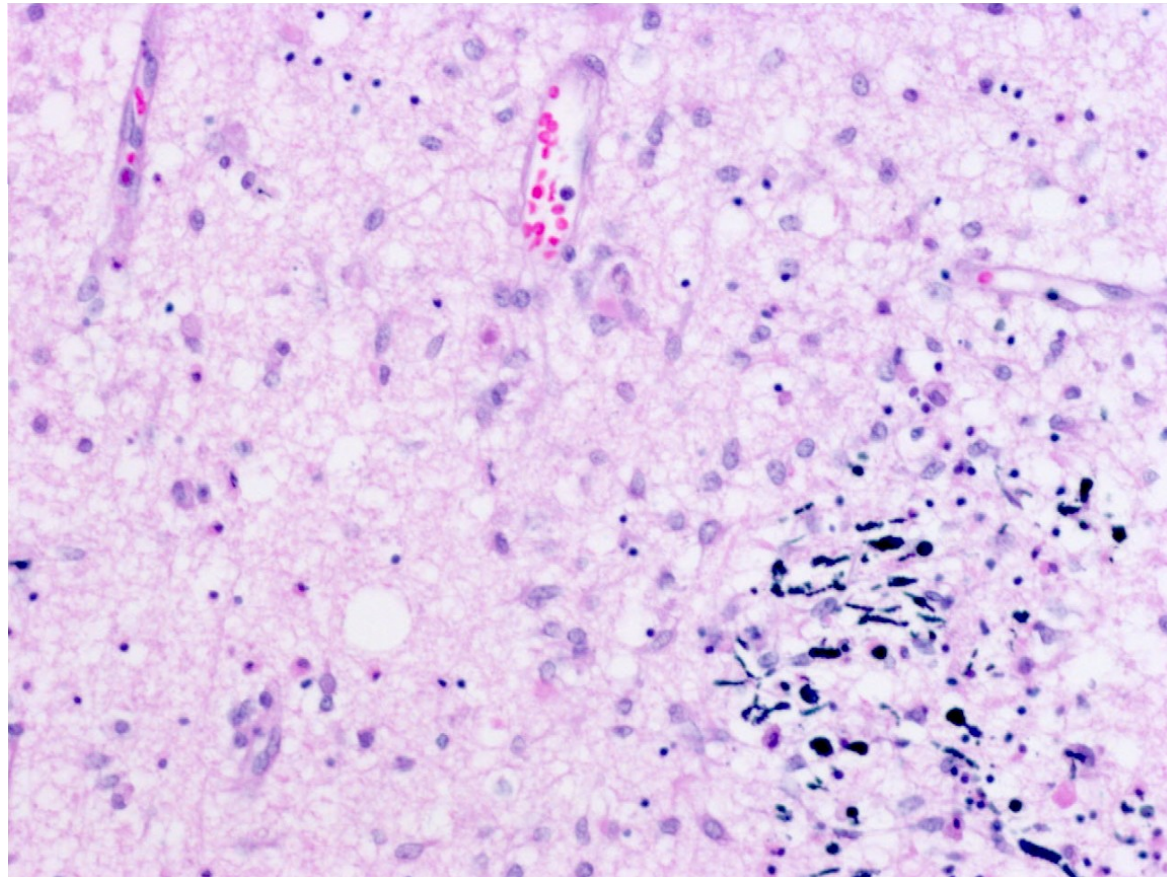
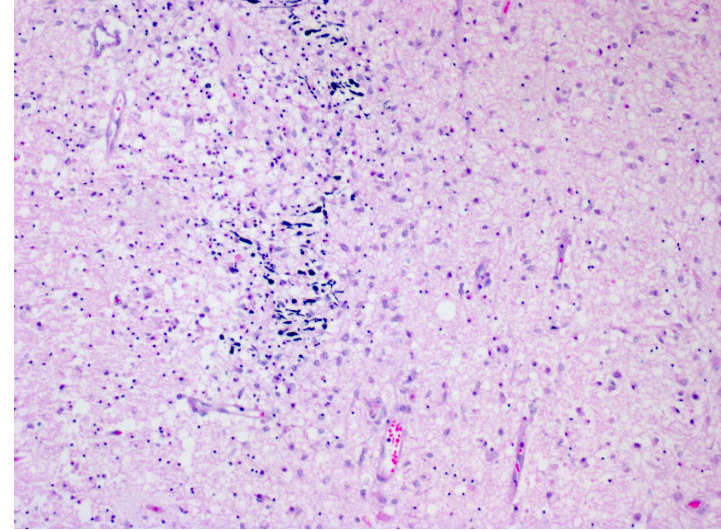
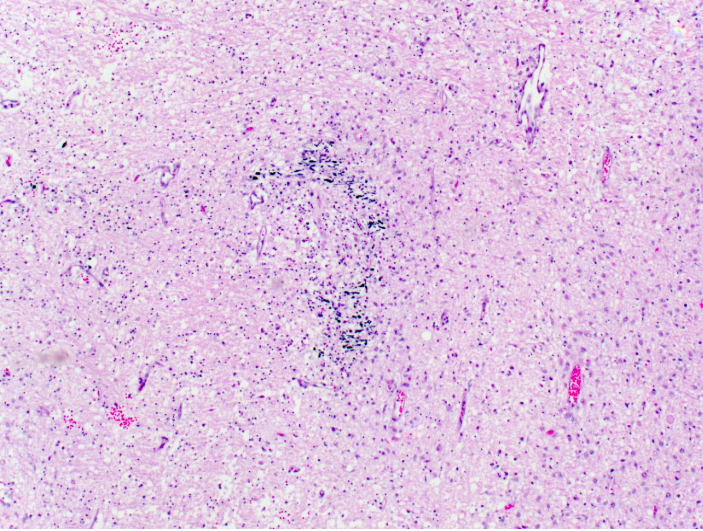


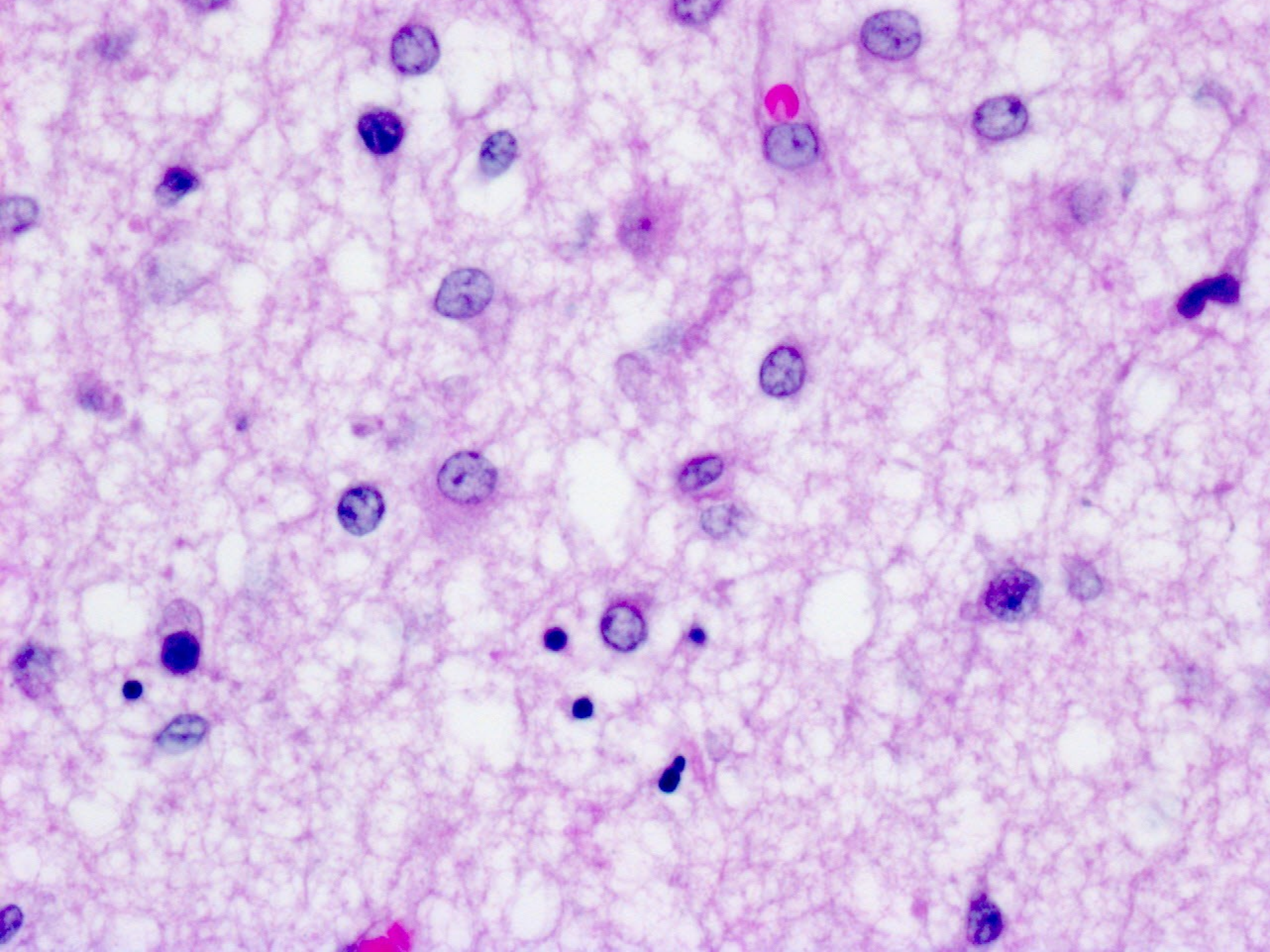
Died @ 35 days of age

- General Autopsy
 - Hepatic Steatosis
- CNS autopsy
 - Cystic periventricular leukomalacia



H&E





Died @ 35 days of age

- CNS autopsy
 - Cystic periventricular leukomalacia
- CSF RT PCR (drawn at time of MRI 33 days)
 - Human Parechovirus RNA detected

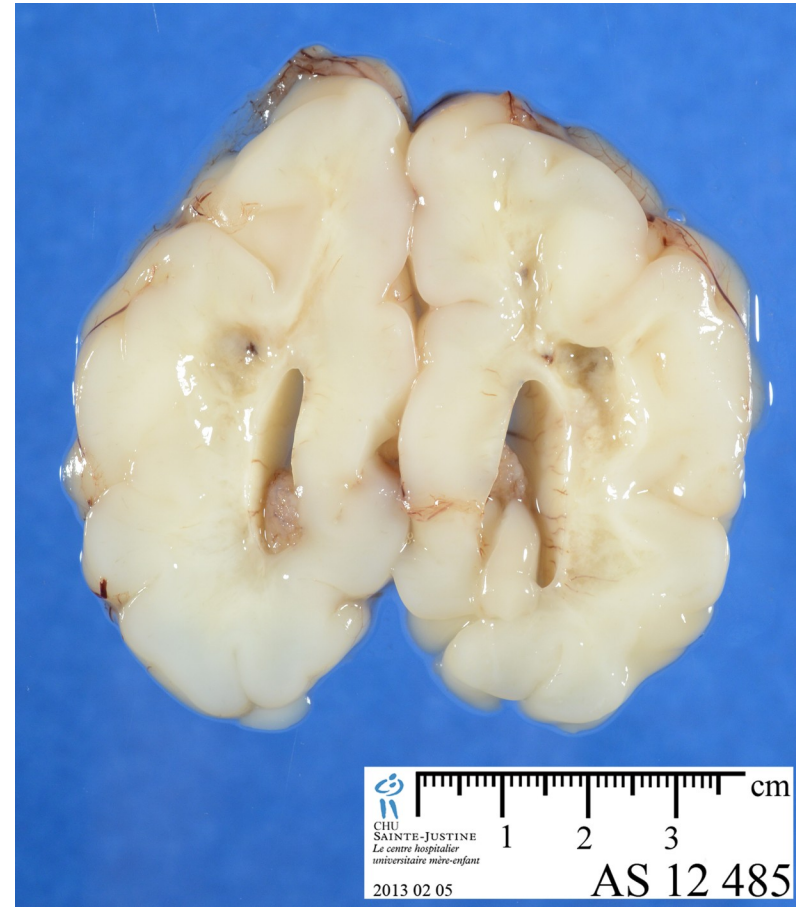
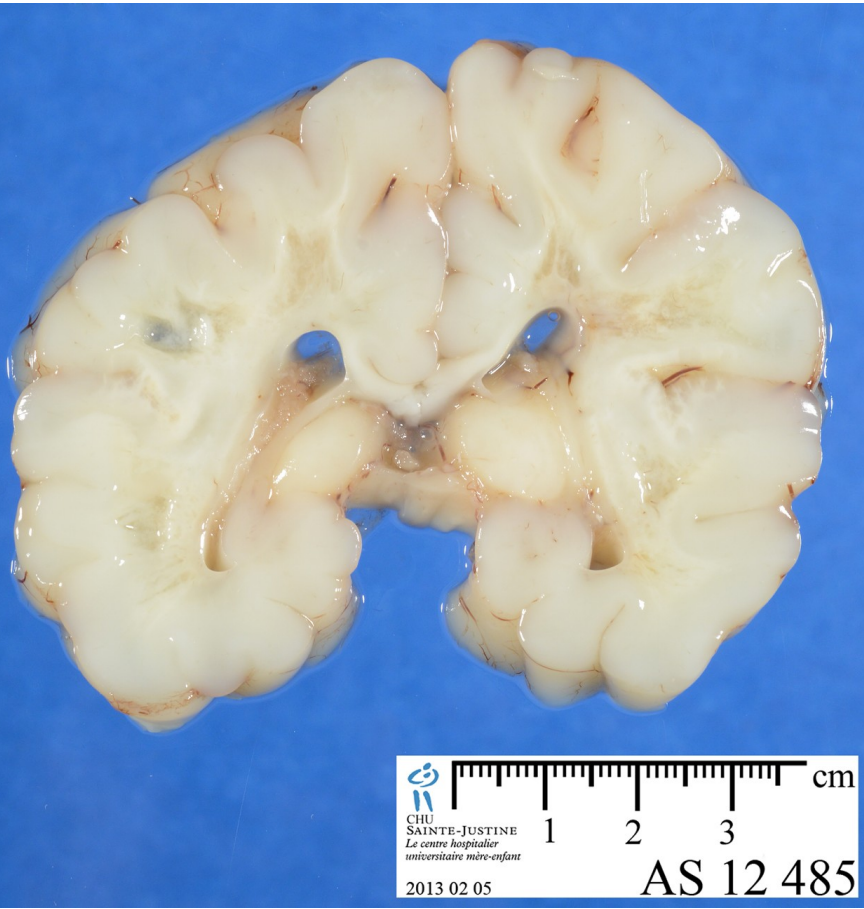
Case #2

Dr. Roland Auer and Dr. Maxime Richer

- Male twins born at 33 weeks to 34yo G2P1
 - Twin B admitted to different hospital for respiratory distress syndrome
 - Recovers sent home well
 - Twin A- 1985 gr, Apgars 9/10
 - 15 days- 2320 gr excellent health
 - Mom develops gastrointestinal infection
 - 16 days- neonatal sepsis, pancorporeal maculopapular rash
 - 18 days seizures-
 - MRI periventricular lesions with hemorrhage
 - CSF PCR “Enterovirus” NOS
 - 27 days- dies

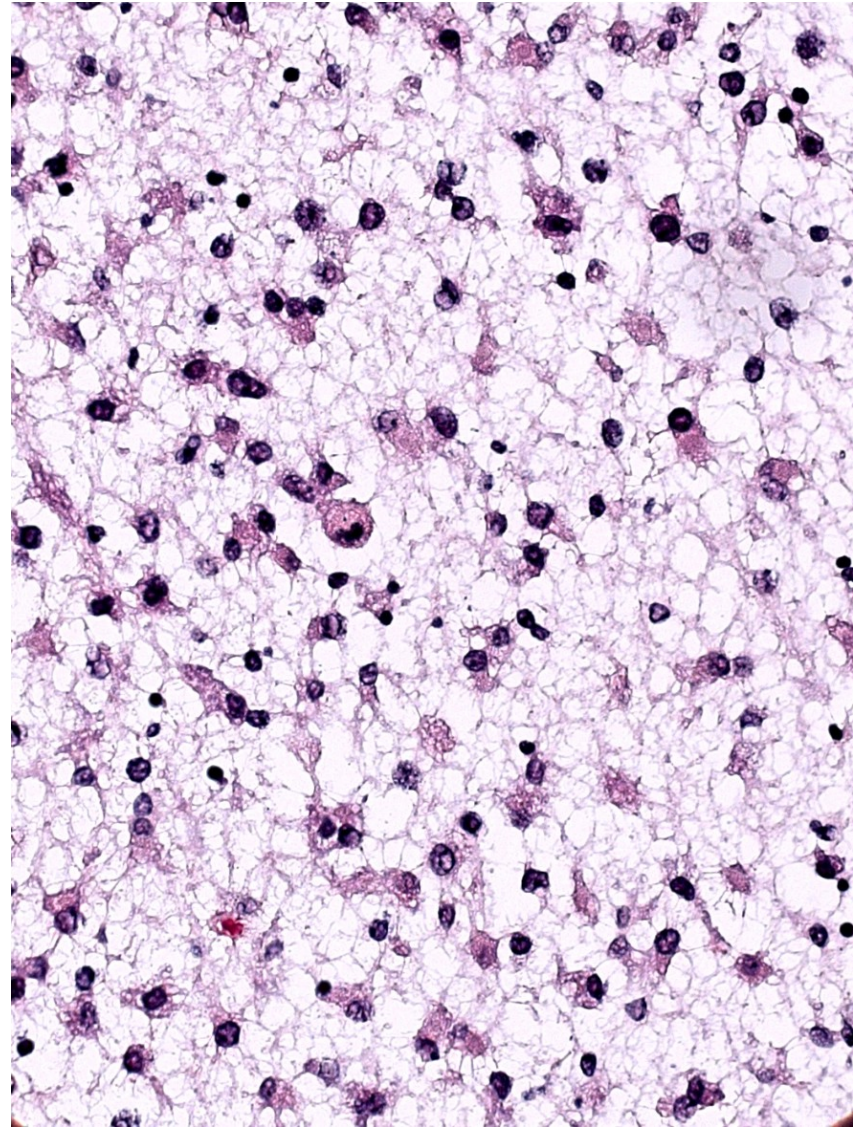
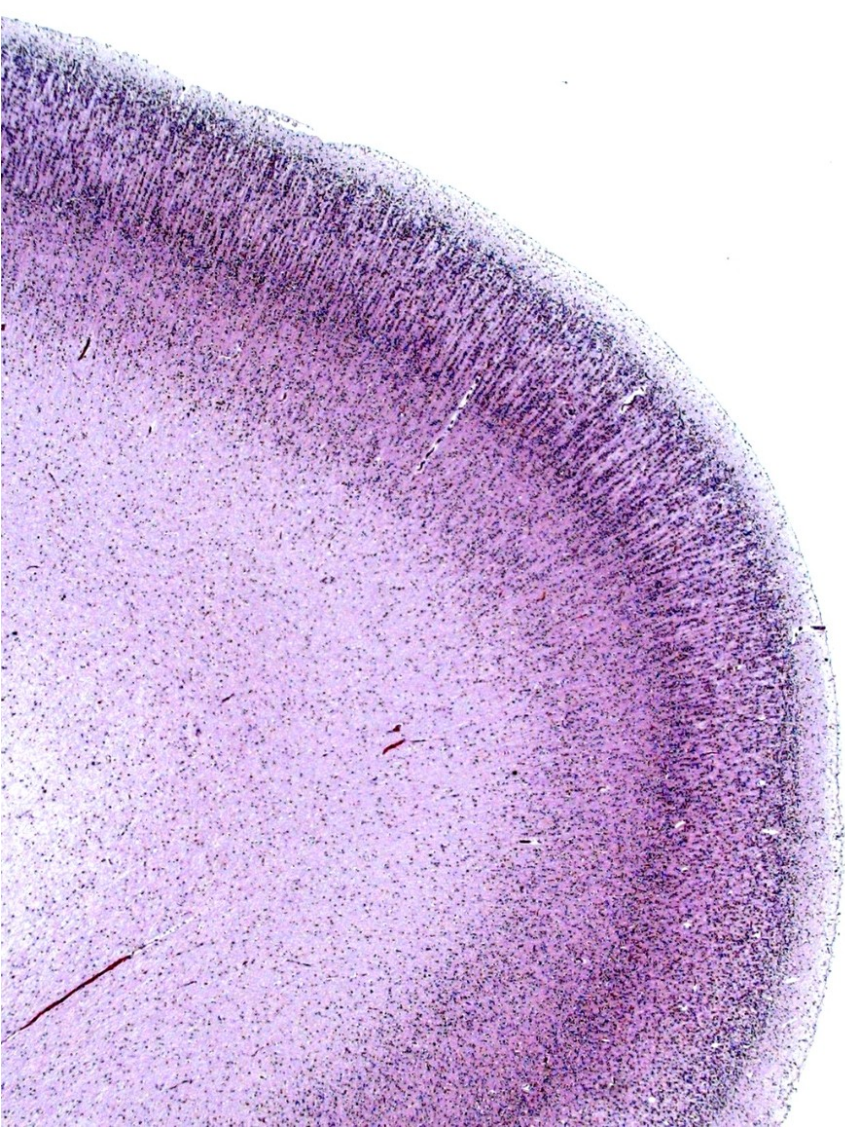
Case #2

Dr. Roland Auer and Dr. Maxime Richer



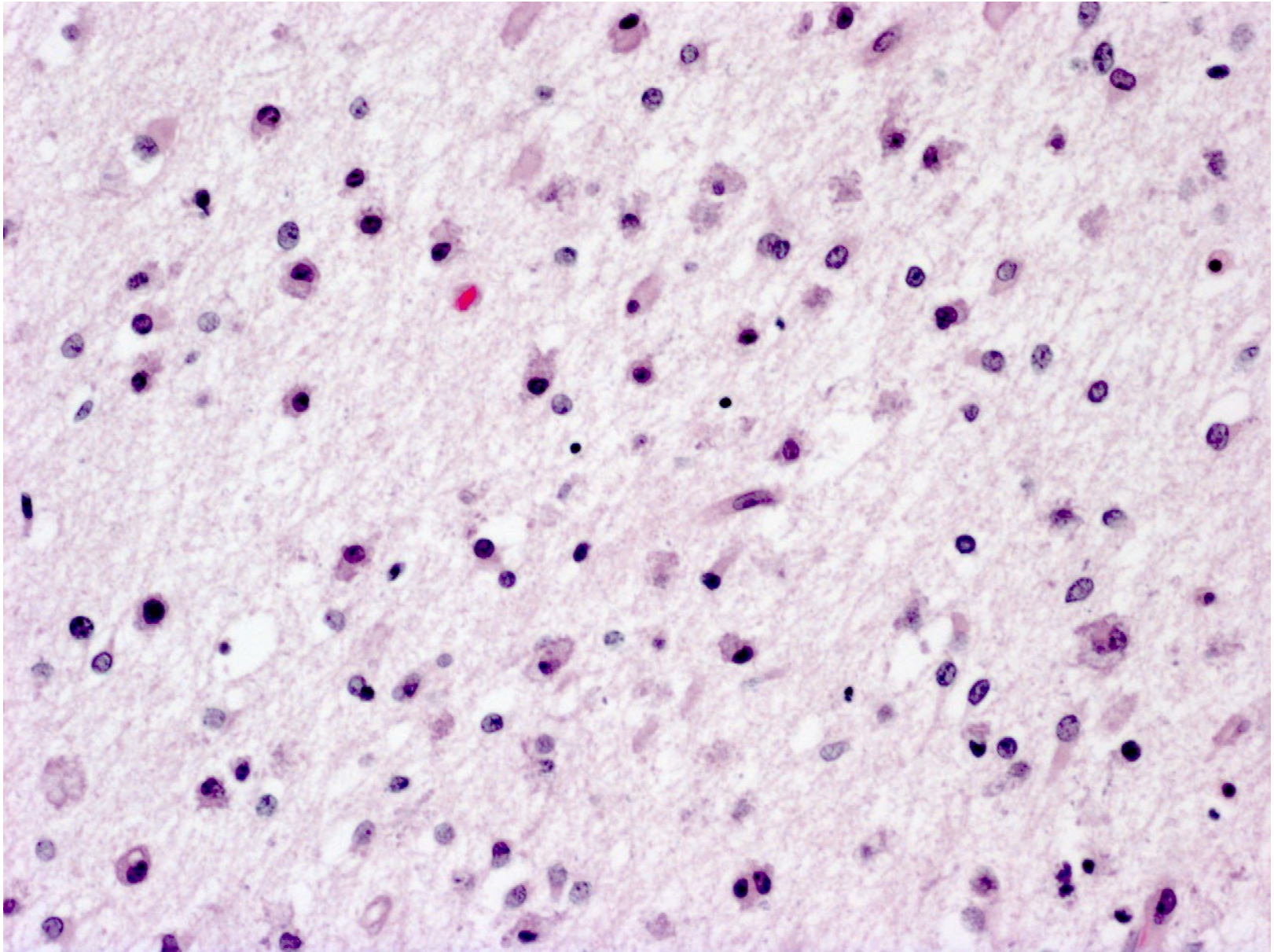
Case #2

Dr. Roland Auer and Dr. Maxime Richer



Case #2

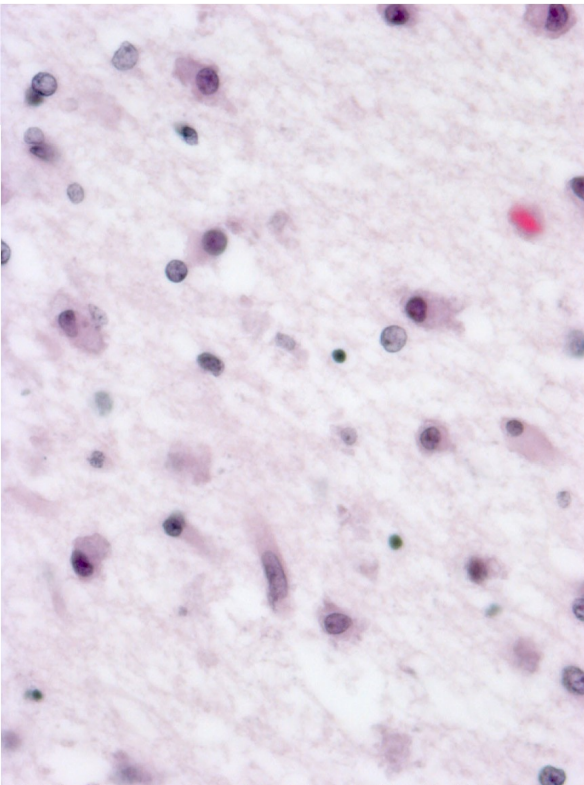
H&E CTX WM



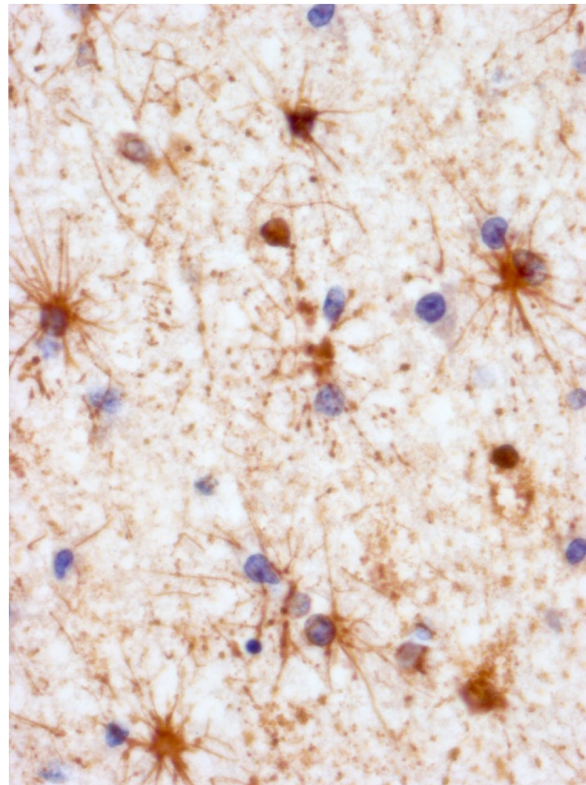
Case #2

CTX WM

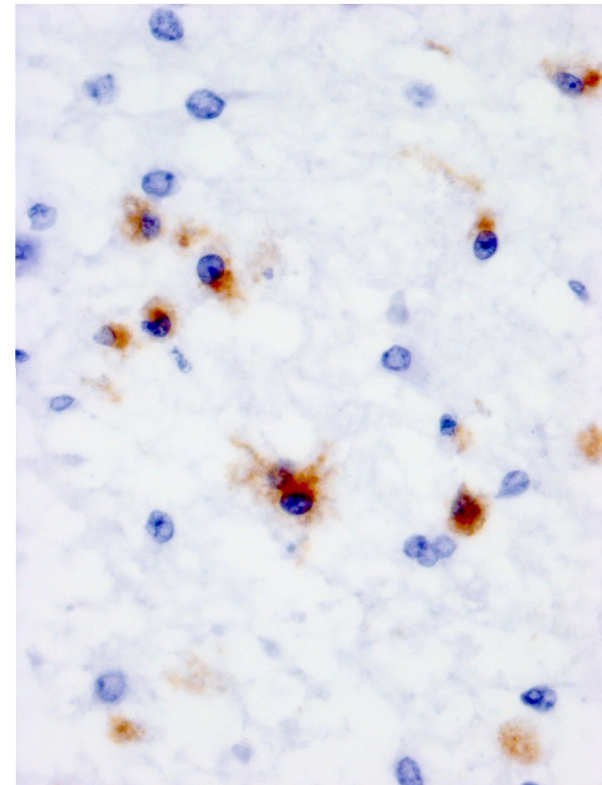
H&E



GFAP

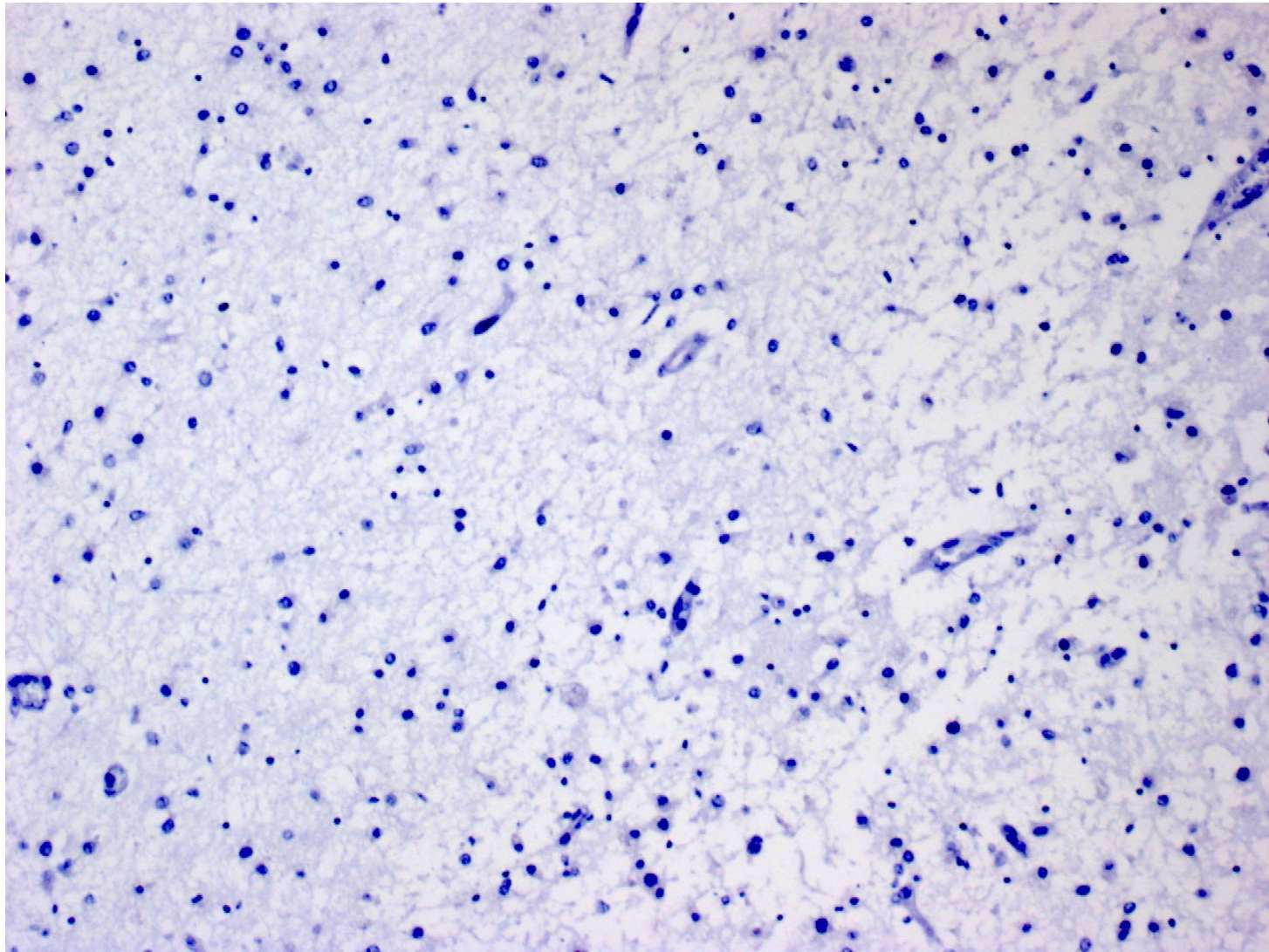


CD68



Case #2

CD3 (CD20) CTX WM



Summary of 2 cases

- Infants born slightly prematurely
- Healthy at birth
- Contact with ill adult
- Rapidly develop neonatal sepsis
- Sign of increased CNS pressure with nl CSF
 - CSF cultures negative, but
- Died within 1-2 weeks
- Histopathology- PVL without inflammation
- So what is this Parechovirus stuff?

ORIGINAL ARTICLE

Human Parechovirus Causes Encephalitis with White Matter Injury in Neonates

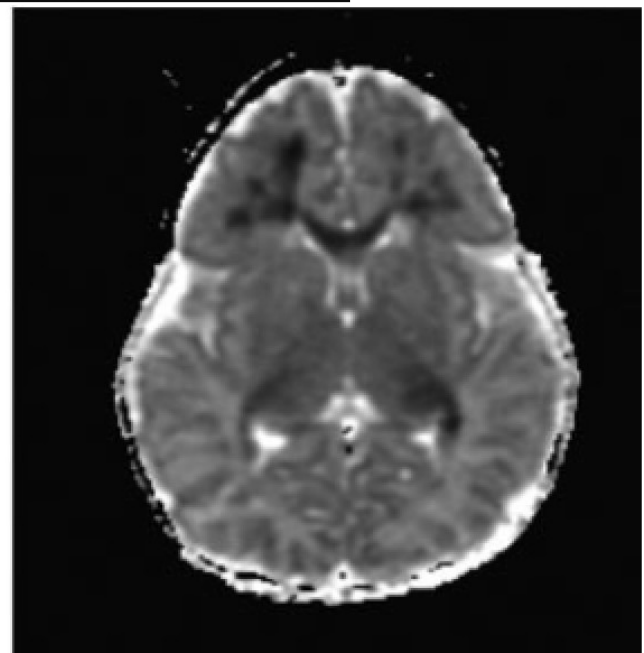
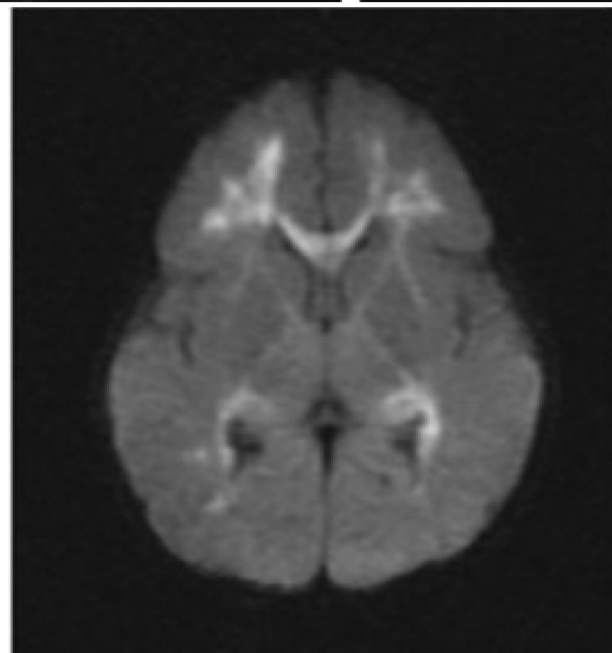
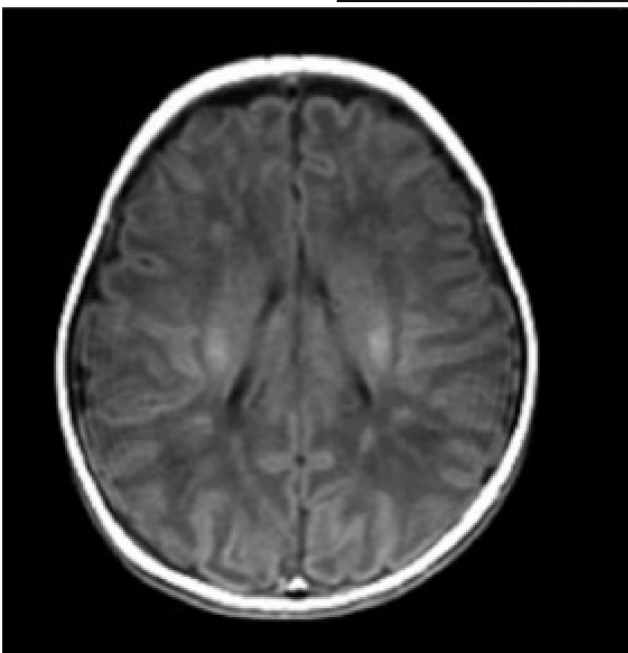
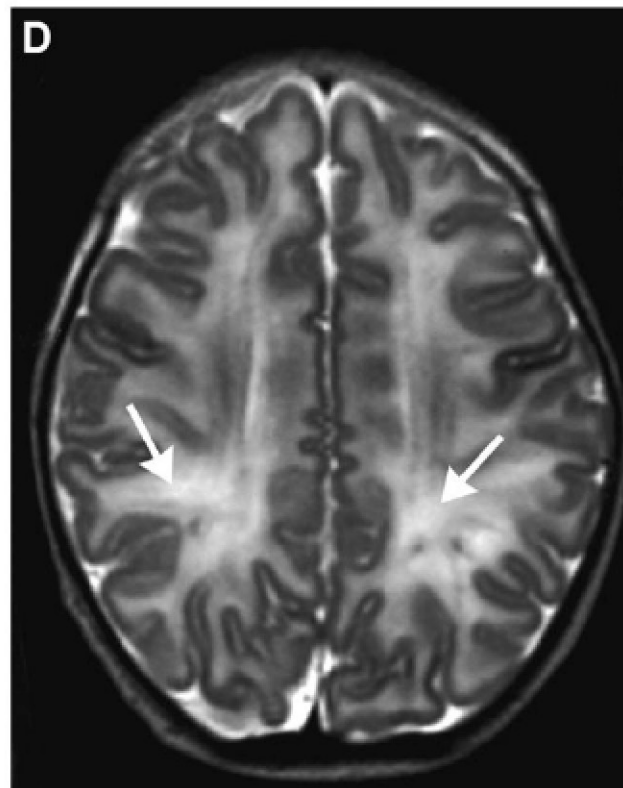
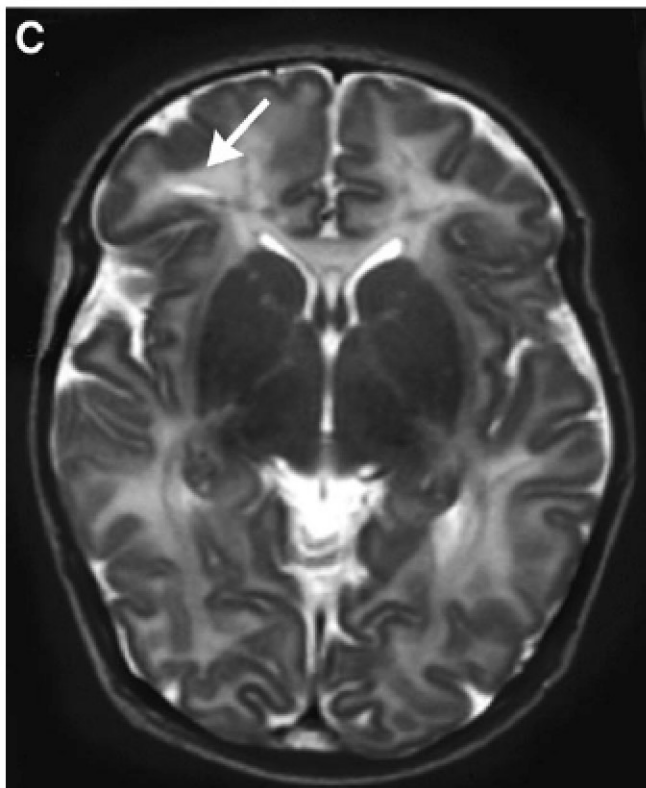
Malgorzata A. Verboon-Maciolek, MD, PhD,¹ Floris Groenendaal, MD, PhD,¹ Cecil D. Hahn, MD, MPH,²
Jonathan Hellmann, MBBCh,³ Anton M. van Loon, PhD,⁴ Guy Boivin, MD, PhD,⁵
and Linda S. de Vries, MD, PhD¹

Ann Neurol 2008;64:266–273

Human Parechovirus Causes Encephalitis with White Matter Injury in Neonates

Malgorzata A. Verboon-Macielek, MD, PhD,¹ Floris Groenendaal, MD, PhD,¹ Cecil D. Hahn, MD, MPH,²
Jonathan Hellmann, MBBCh,³ Anton M. van Loon, PhD,⁴ Guy Boivin, MD, PhD,⁵
and Linda S. de Vries, MD, PhD¹ Ann Neurol 2008;64:266–273

- 10 infants (29-41 wks) neonatal sepsis & seizures
 - 3 preterm / 7 term
 - Term infants presented within 2 wks.
 - Preterm presented several wks. after birth
- CSF- mostly acellular, nl protein / glucose
 - HPeV3 RNA: CSF 8, blood 1, feces 1
- MRI- 1-14 days after clinical onset
- **None died**
 - Cerebral palsy (1), seizures, “normal” 50%

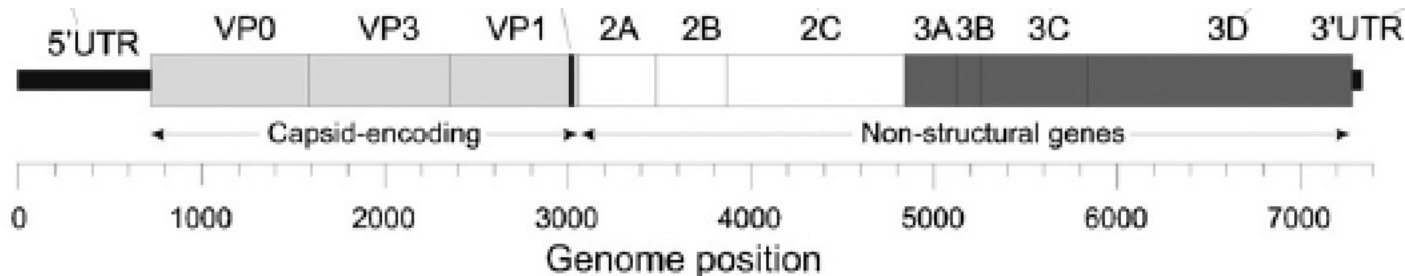


Picornaviridae

- Classification- originally done serologically
now done molecularly
- Family- Picornaviridae
 - 15 genera
 - 5 cause human infection
 - Enterovirus
 - Rhinovirus
 - Hepatitis virus
 - Poliovirus
 - Kobovirus

Picornaviridae

- 28nm, nonenveloped, single (positive) stranded RNA (~7 kb in length)



Codes for single polyprotein of ~2180 AA

Multiple host receptors (CD55, CD155, integrins)

CAR expressed in developing CNS (not adult)

Picornavirus: Enterovirus

- Mostly asymptomatic infections
 - 10 million **symptomatic** infections/yr. US
 - Most common cause of acute meningitis
 - 1870 Charcot described poliomyelitis
 - Relationship to type1 diabetes
- Lifelong immunity to individual strains
 - Not sterilizing

Enterovirus encephalitis

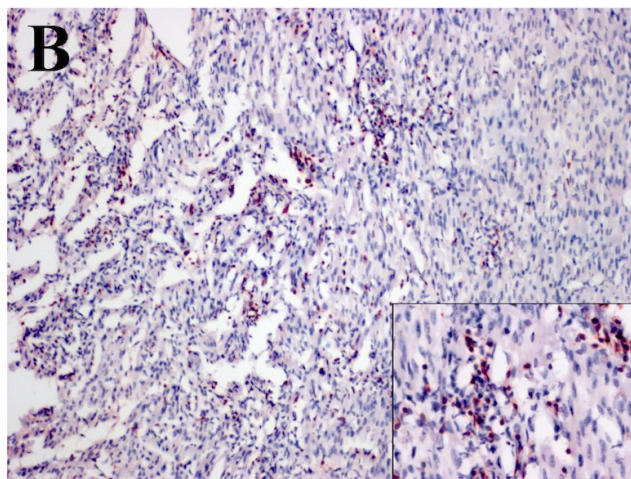
- Twins delivered by cesarean section to G4P2 22yo.
 - Perinatal care scant
 - Apgars 8/9
- d/c to home @ 4days
- Died between 1 and 2 weeks of life
- Severe myocarditis and encephalitis
- Coxsackie B Virus identified

Heart and Brain

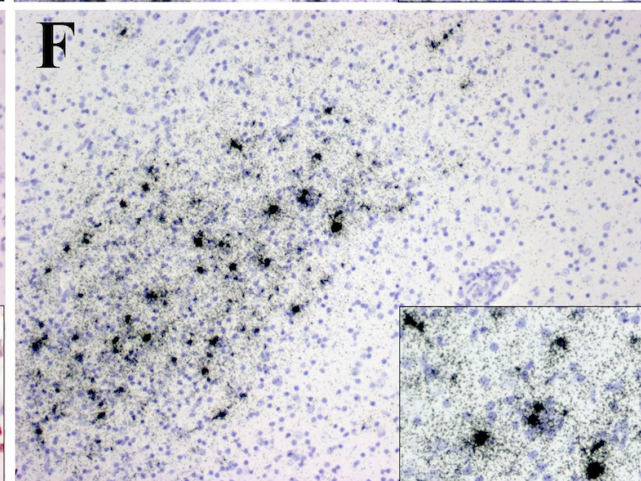
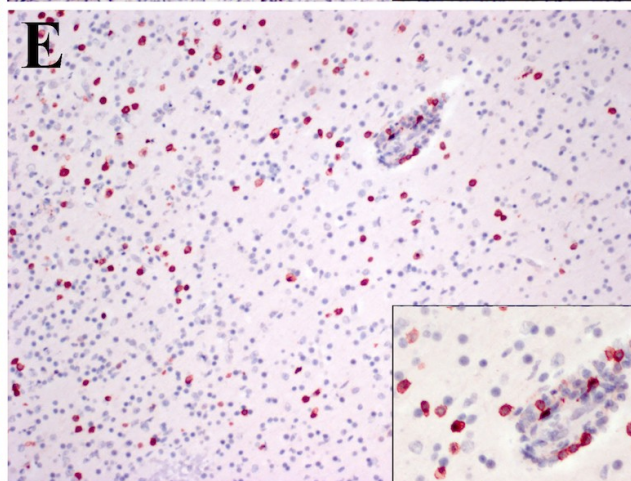
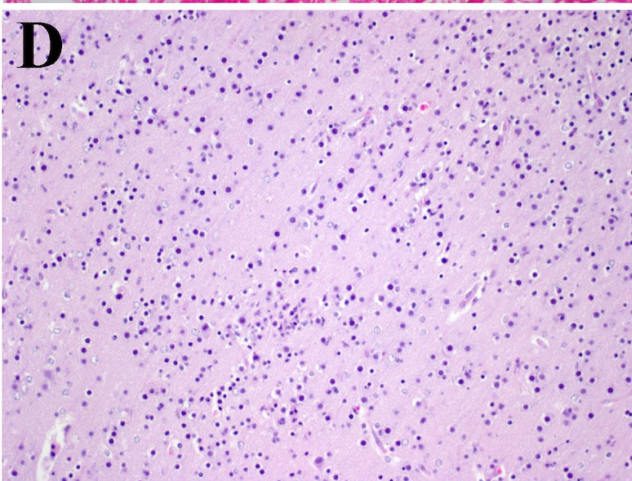
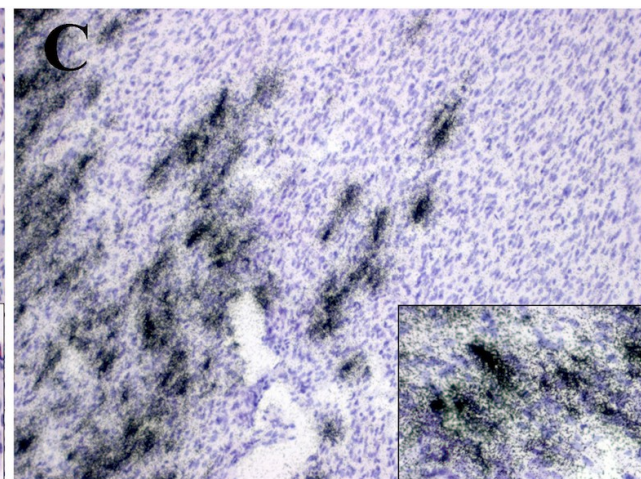
H&E
ISH



Anti-CD3



CB4

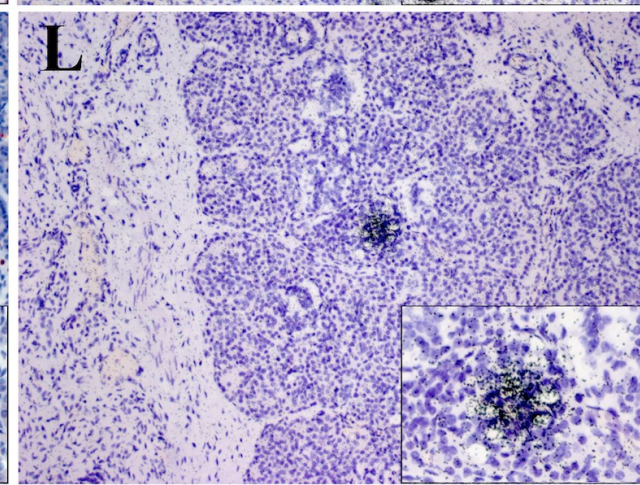
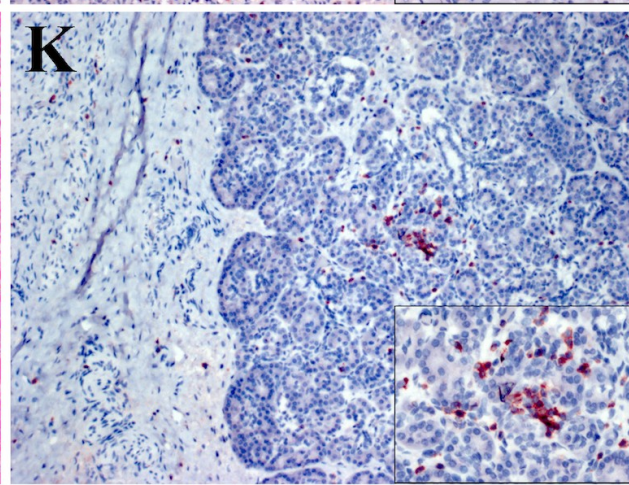
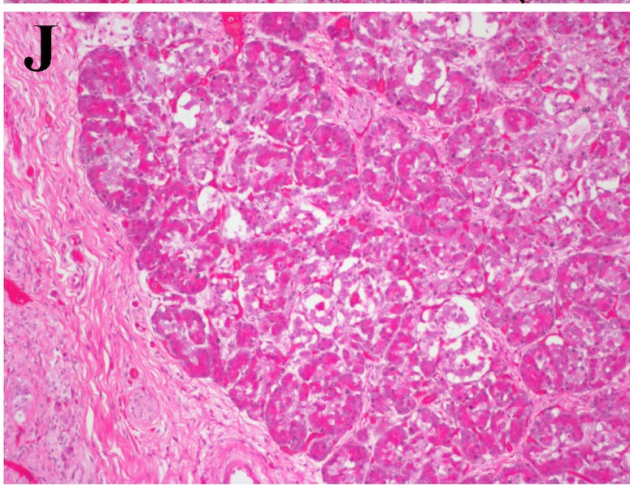
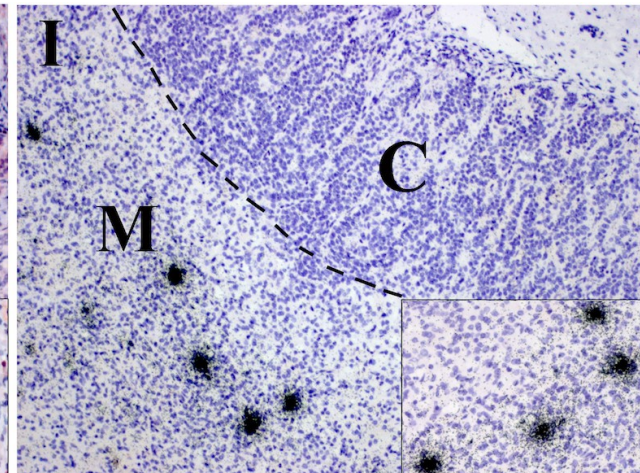
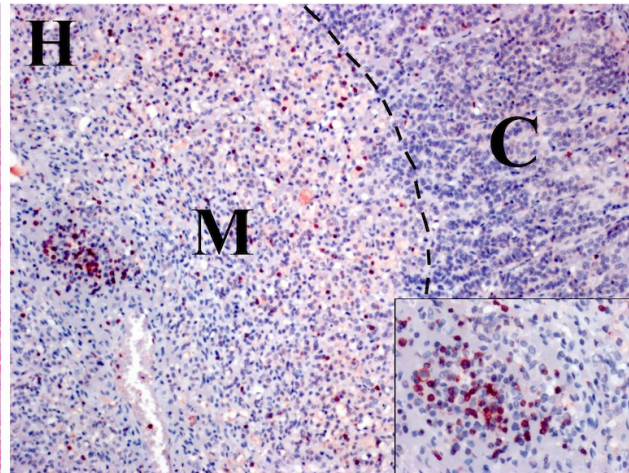
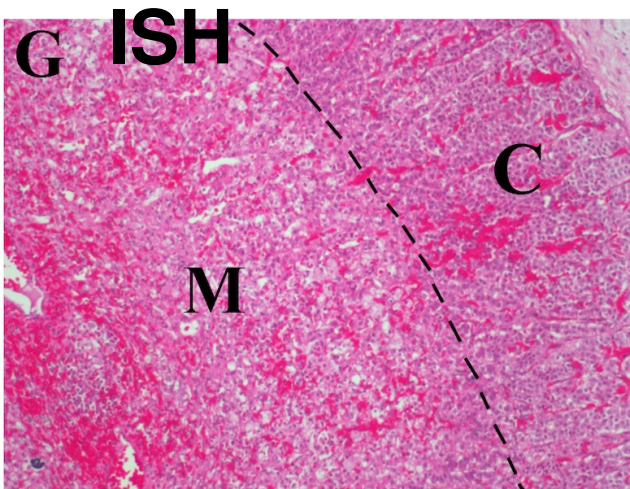


Adrenal and Pancreas

H&E

Anti-CD3

CB4

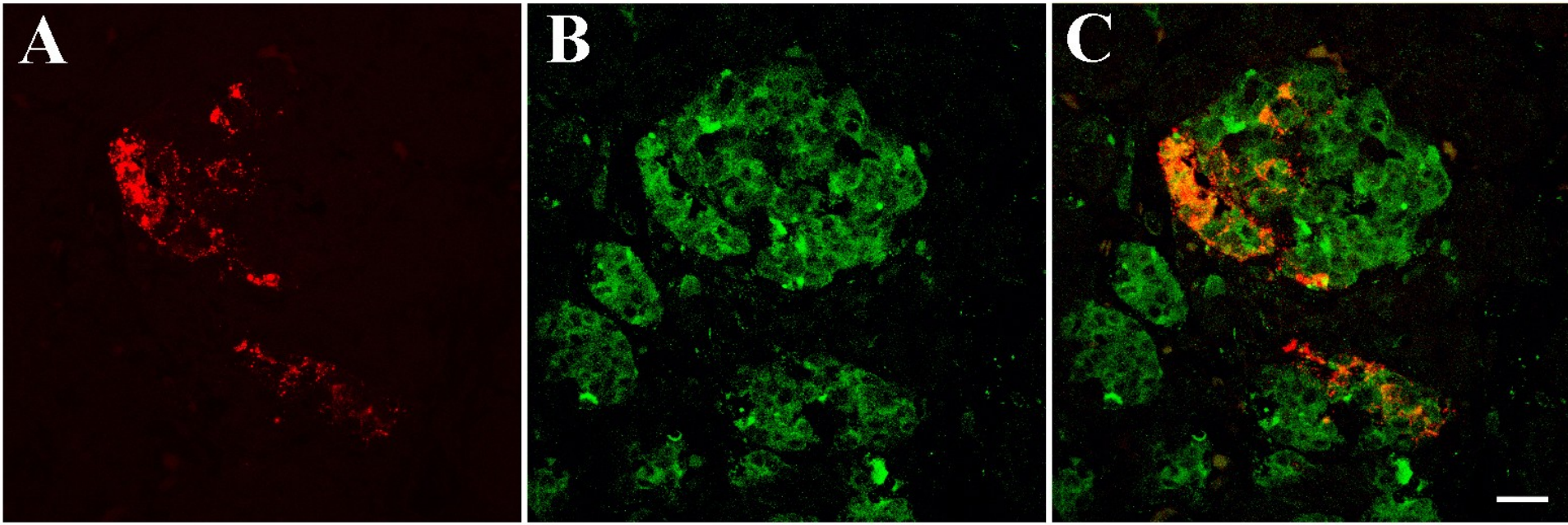


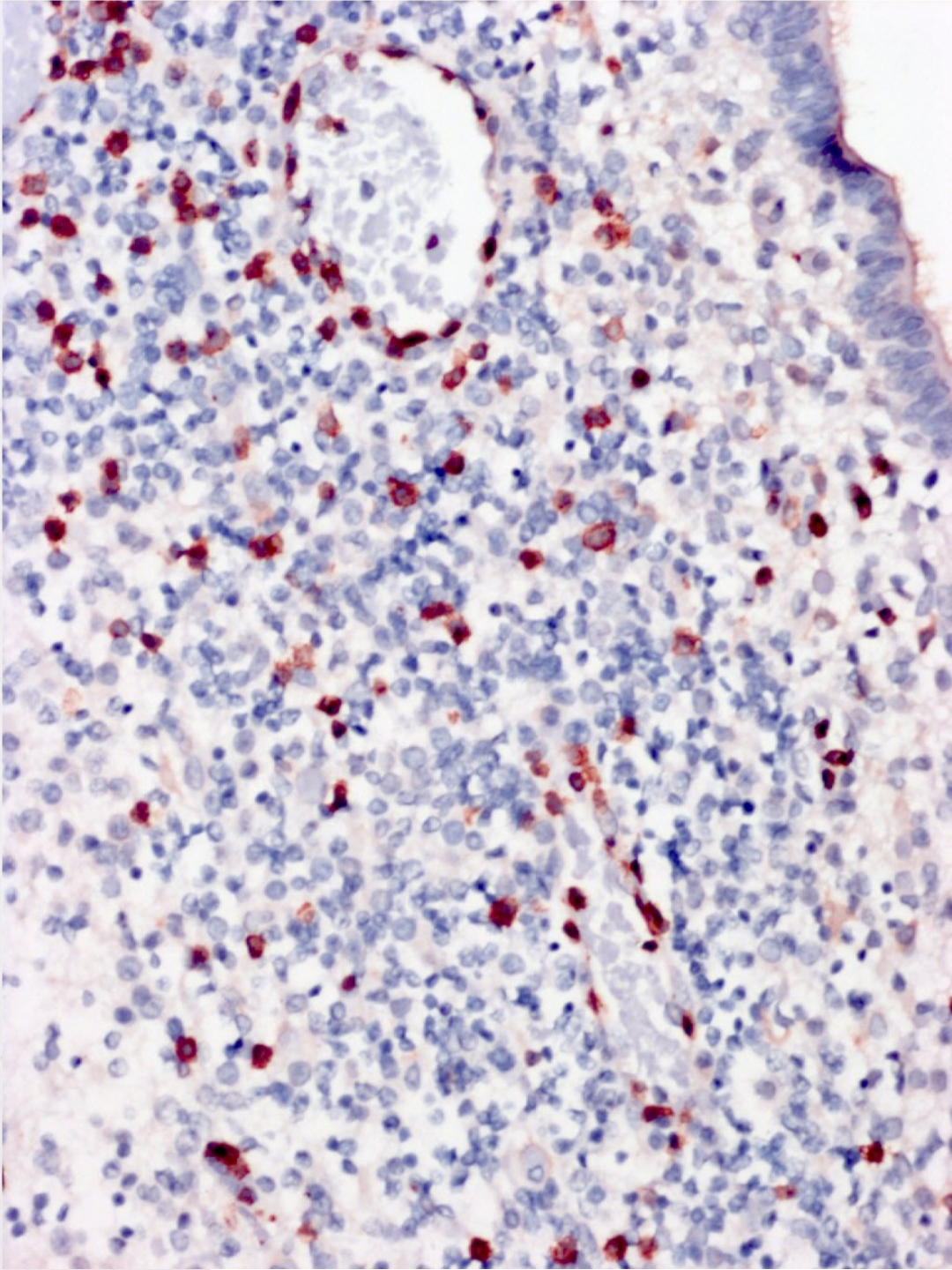
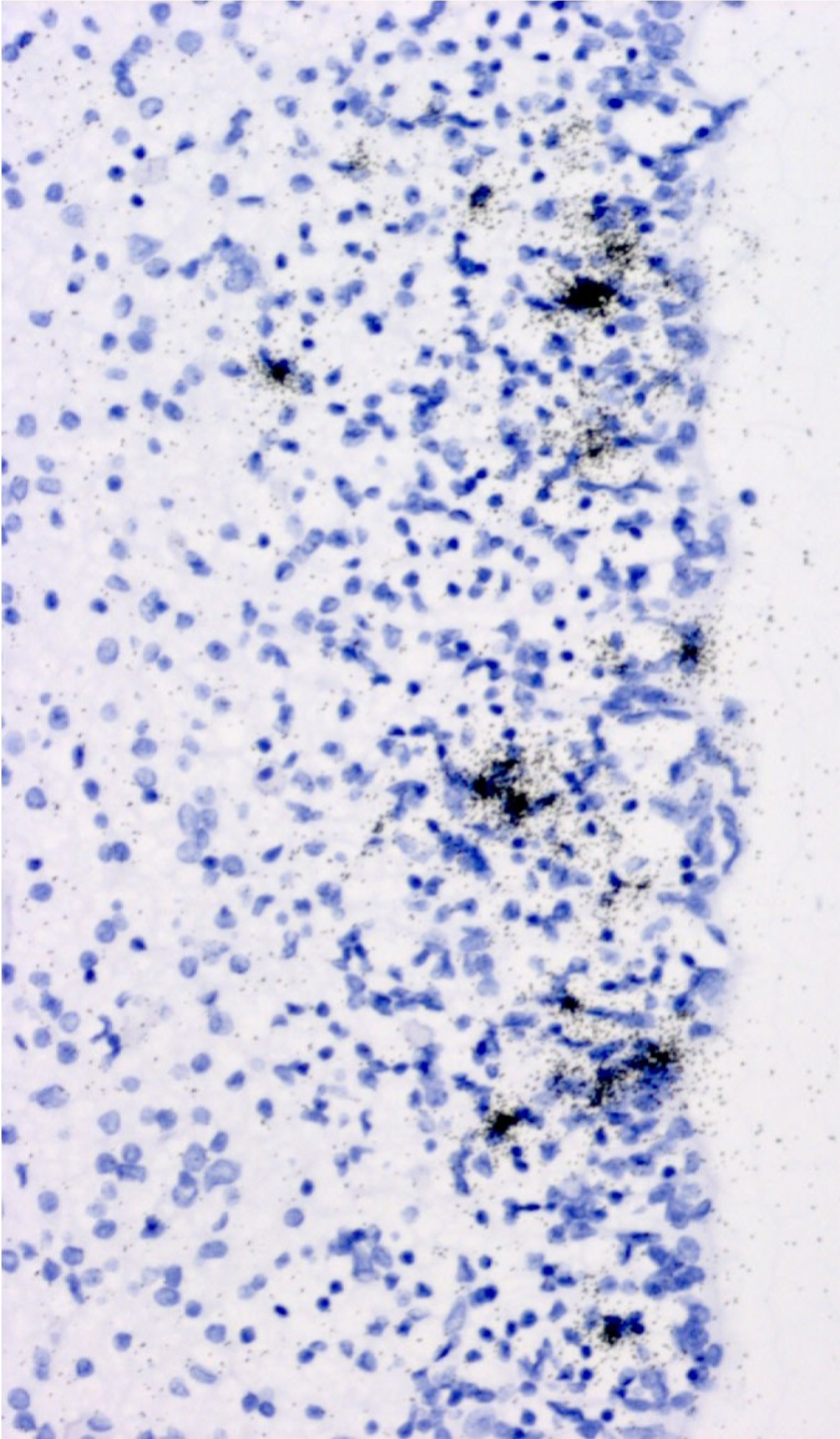
Pancreatic Islets

@EV

@Synaptophysin

Merge



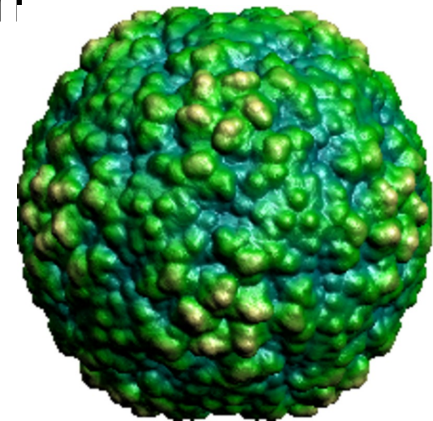


Summary: Enteroviral encephalitis

- “Classic meningoencephalitis”
- Tropism for myocardiocytes, neurons and neuronal stem elements, neural crest derivatives (adrenal medulla, pancreatic islets)
 - Coxsackie adenovirus receptor (CAR)
- T-cell immune response
 - Death vs viral clearance

Picornaviridae: Parechovirus

- HPeV 1 & 2 originally Echovirus 22 & 23
 - Isolated 50 years ago during summer diarrhea outbreak
 - Non-pathogenic to mice and monkeys
 - Enteric **C**ytopathic **H**uman **O**rphan (**ech**oviruses)
 - Currently 16 strains
 - Strains with <73% Nucleic Acid or <81% AA identity in VP1 are considered new strain
 - VP1 contains RGD motif
 - EXCEPT for HPeV3



HPeV 3

- Reported in 2004 from 1999 isolate
 - Ito et al 2004 J. Gen. Virol
- Restricted diversity suggests it emerged ~20 years ago!
 - More limited adult seroprevalence
 - Newborn risk factor absence of maternal antibody
- No RGD motif in VP1

Epidemiology

- Biennial pattern
 - Europe even years; North America odd years
 - Summer and fall are peak months for infection
- Seroepidemiology HPeV infections
 - 40% positive 6 months
 - 70% positive 1 year
 - 100% by 5 years
 - Mean age of HPeV3 infection 0.7 months
 - Mean age of HPeV1 & 2 = 14.6 and 6.3 months

HPeV: Clinical disease

- Most infections are subclinical
 - Fever & irritability (95%), Rash 17-60%, GI 29%, Respiratory 26%
- Transmission- fecal-oral and respiratory
 - High viral titers in stool with shedding to 5 months
 - necrotizing enterocolitis (non-epidemic)
 - Less common respiratory disease
 - Respiratory shedding 1-3 weeks
- Different subtypes associated with clinical disease
 - Lymphadenitis (HPEV4), Myositis (HPeV3), myocarditis and enterocolitis (HPeV1)
 - Hepatitis, steatosis, hemorrhage (HPeV5 & 6)
- Sudden unexplained infant death (1263 cases)
 - 34% viral related (5.6% HPeV)

HPeV3: Neurological disease

- Neonatal sepsis with clinical “Meningitis”
 - Without increased ICP
 - CSF analysis mostly normal (rare and mild pleocytosis)
 - Of pediatric CSFs negative for enterovirus-4.6% contained HPeV nucleic acids
 - 97% <2 years of age
 - 46% < 1 month
 - Reports of acute flaccid paralysis with HPeV3
 - No reported deaths

HPeV3 Neurological disease cont'd

- Clinical **encephalitis**
 - Exclusively neonates and young infants
 - Full term with onset in first 2 weeks
 - Premies- onset 2-3 months
 - Seizures predominant symptom in 84%
 - Rash 66% and apnea in 55%
 - CSF mostly normal
 - MRI 90% WM lesions
 - US- Periventricular echogenicity in all
 - Mostly HPeV 3 (some 1)
 - No deaths- 64% no clinical residua
 - Rest- seizures, cerebral palsy, visual impairment, learning disability

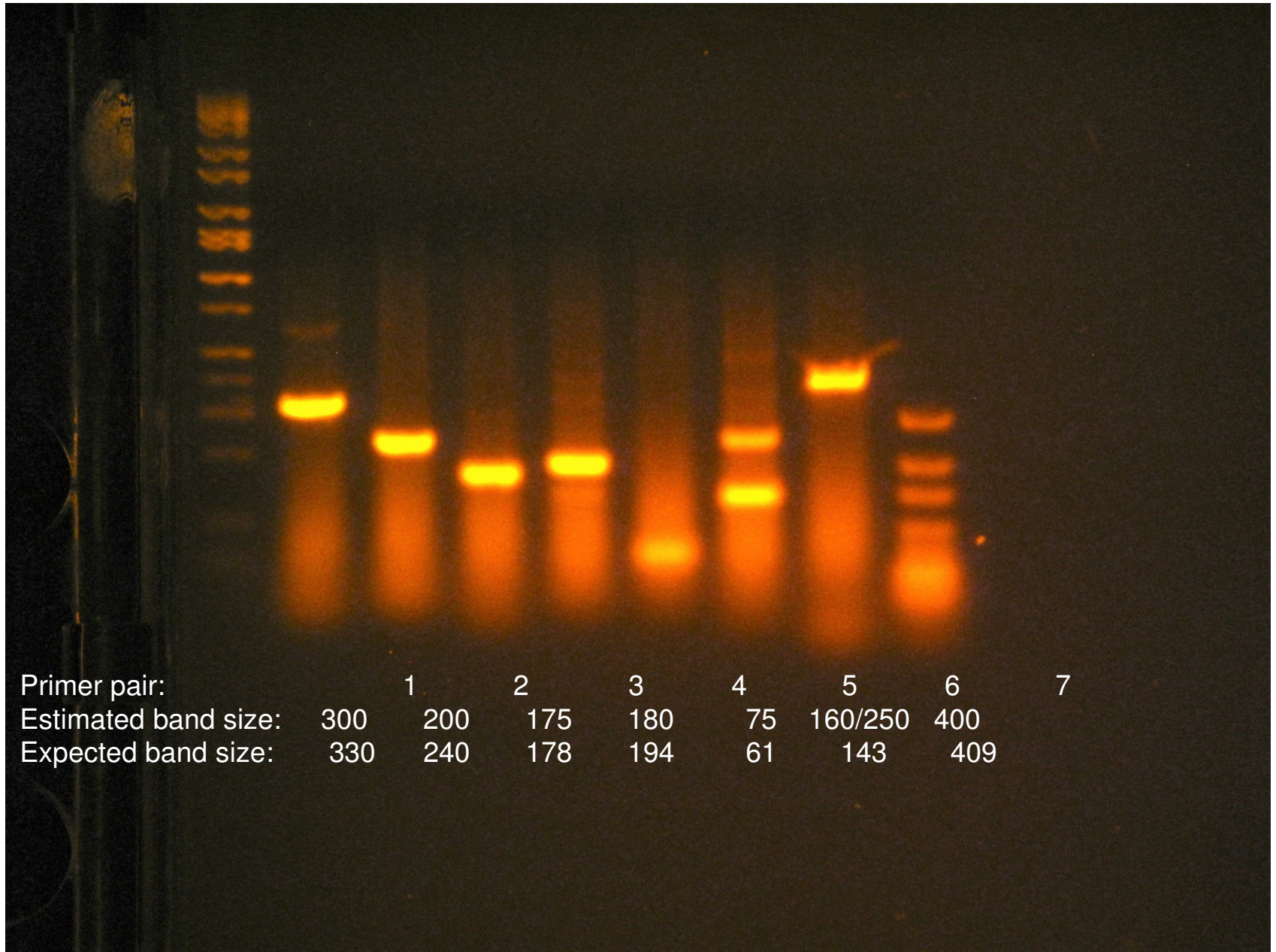
Immunity to HPeV

- Limited clinical data on HPeV
- Neutralizing antibody as with other HEV?
- Unique VPO N-terminus of dominant antigenic site

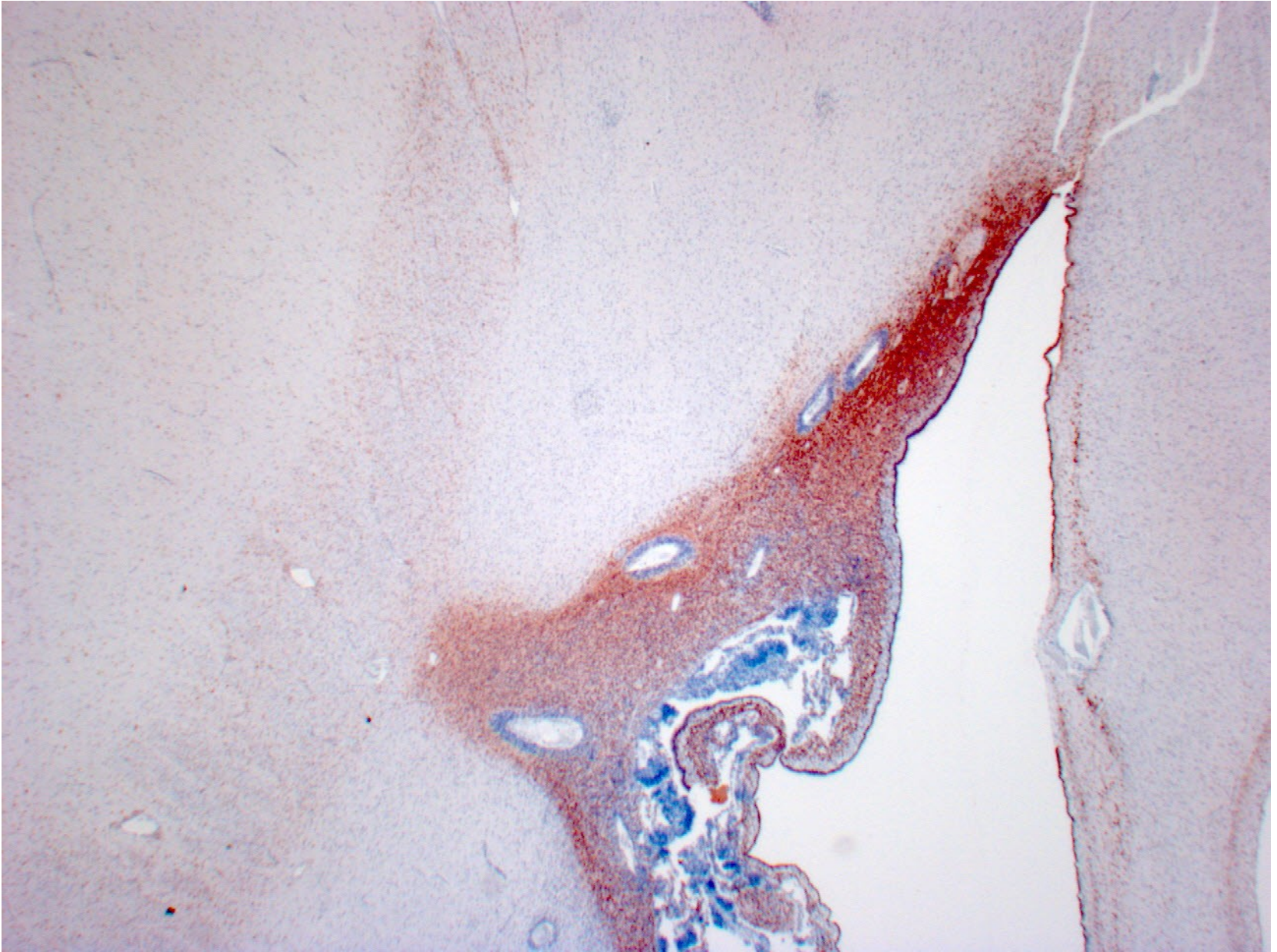
HPeV Detection

- Not detected by general enterovirus PCR
 - HPeV 3 only grows on Vero and A549 cell lines
- Specific rRT-PCR 100 to 1000 fold greater sensitivity than culture
- VP1 used for typing
 - VP1 of HPeV strains share 77% genotype and 87% AA identity
- Excreted from GI for prolonged times
 - Presence in blood and CSF limited to disease

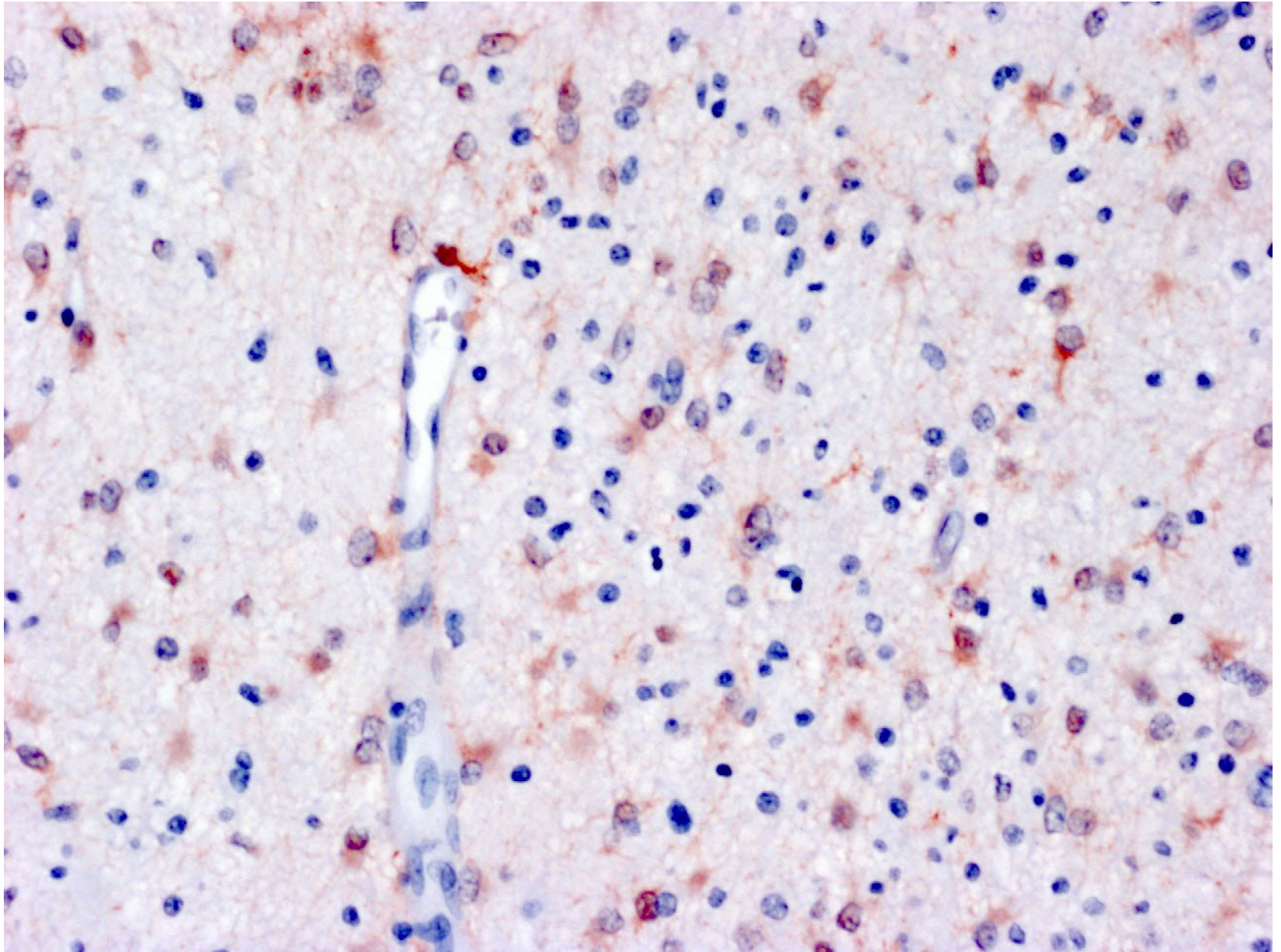
HPeV3 PCR of frozen tissue Case 1



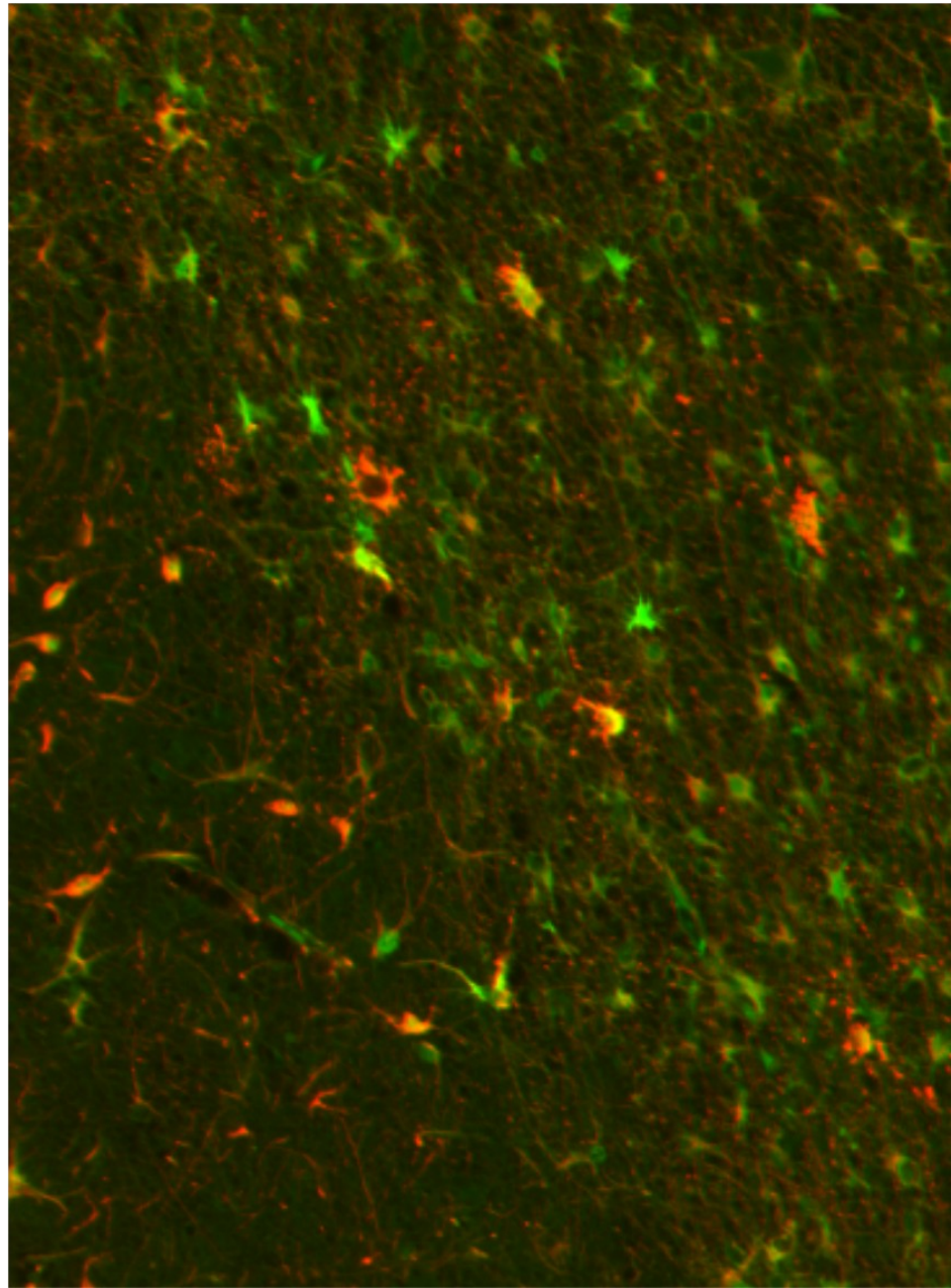
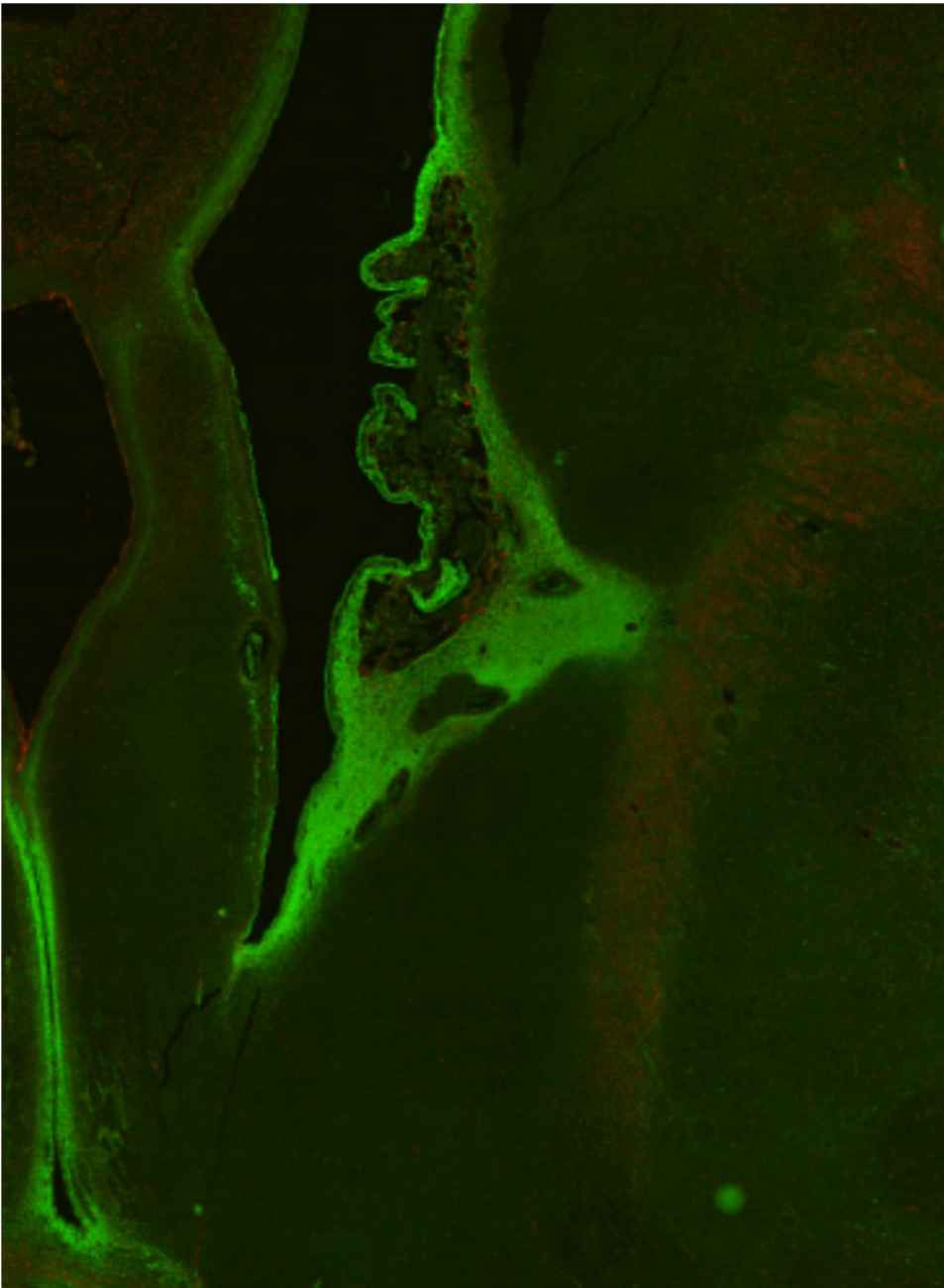
@Enterovirus



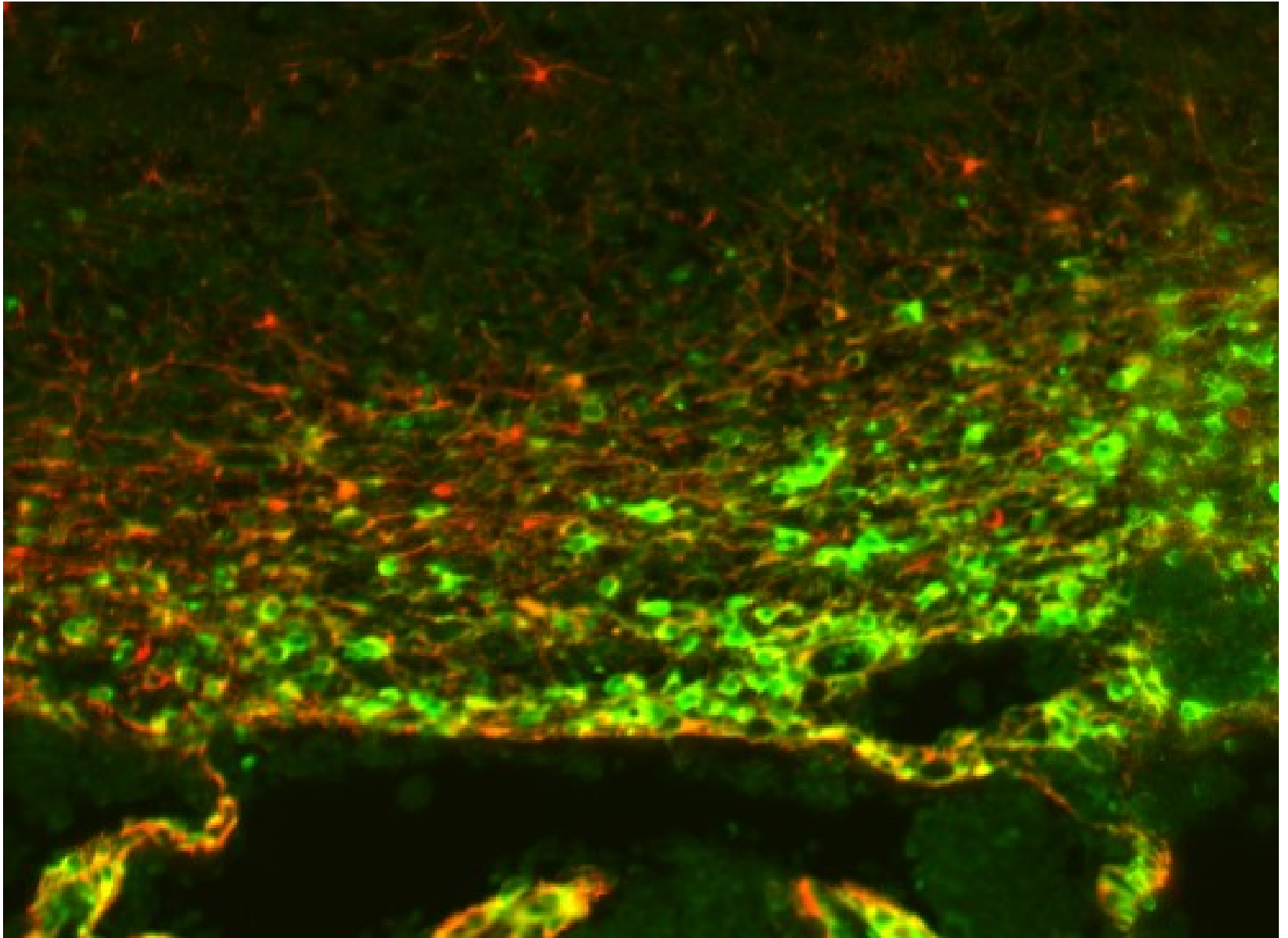
@Enterovirus



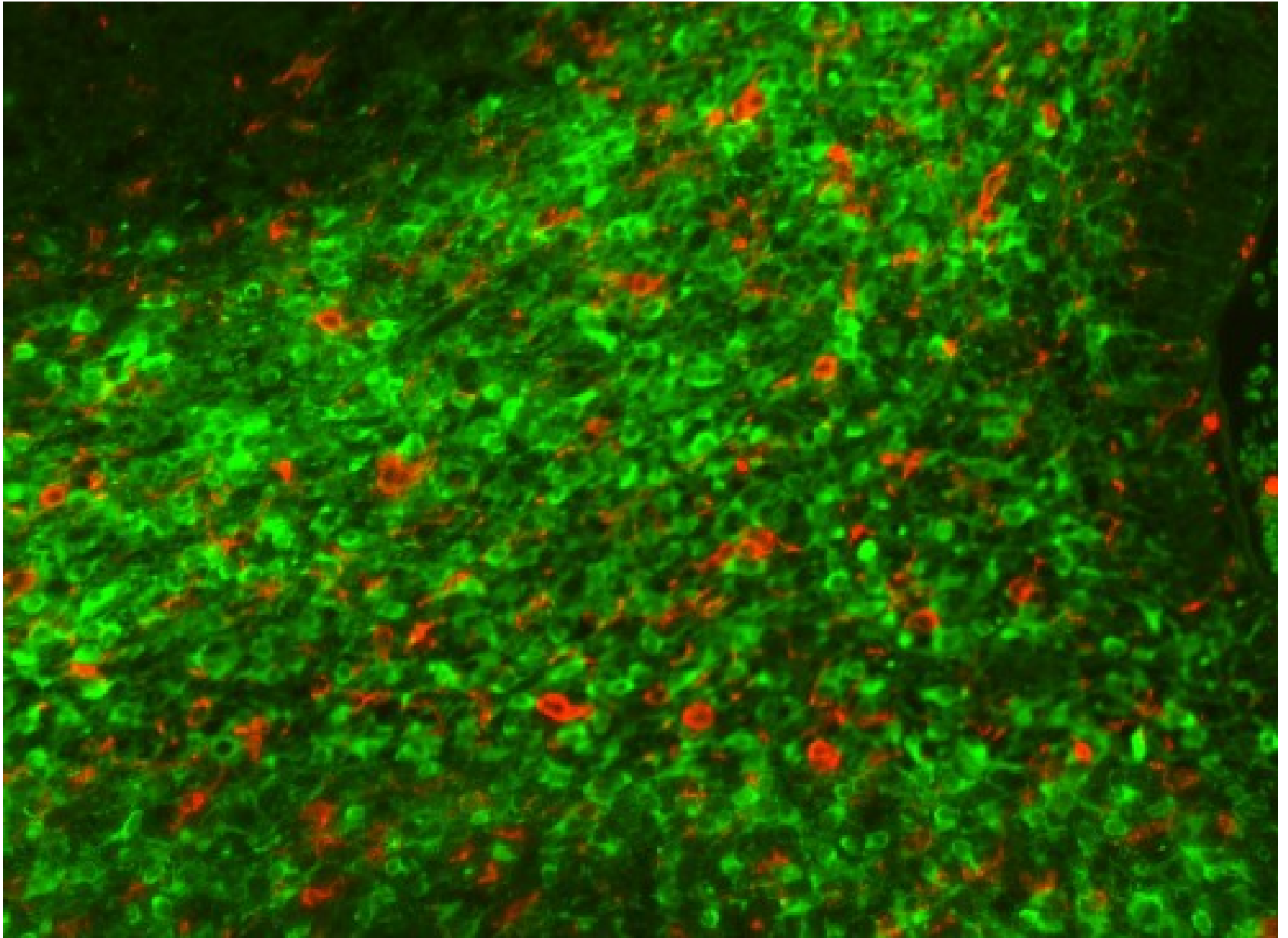
@Enterovirus / @GFAP



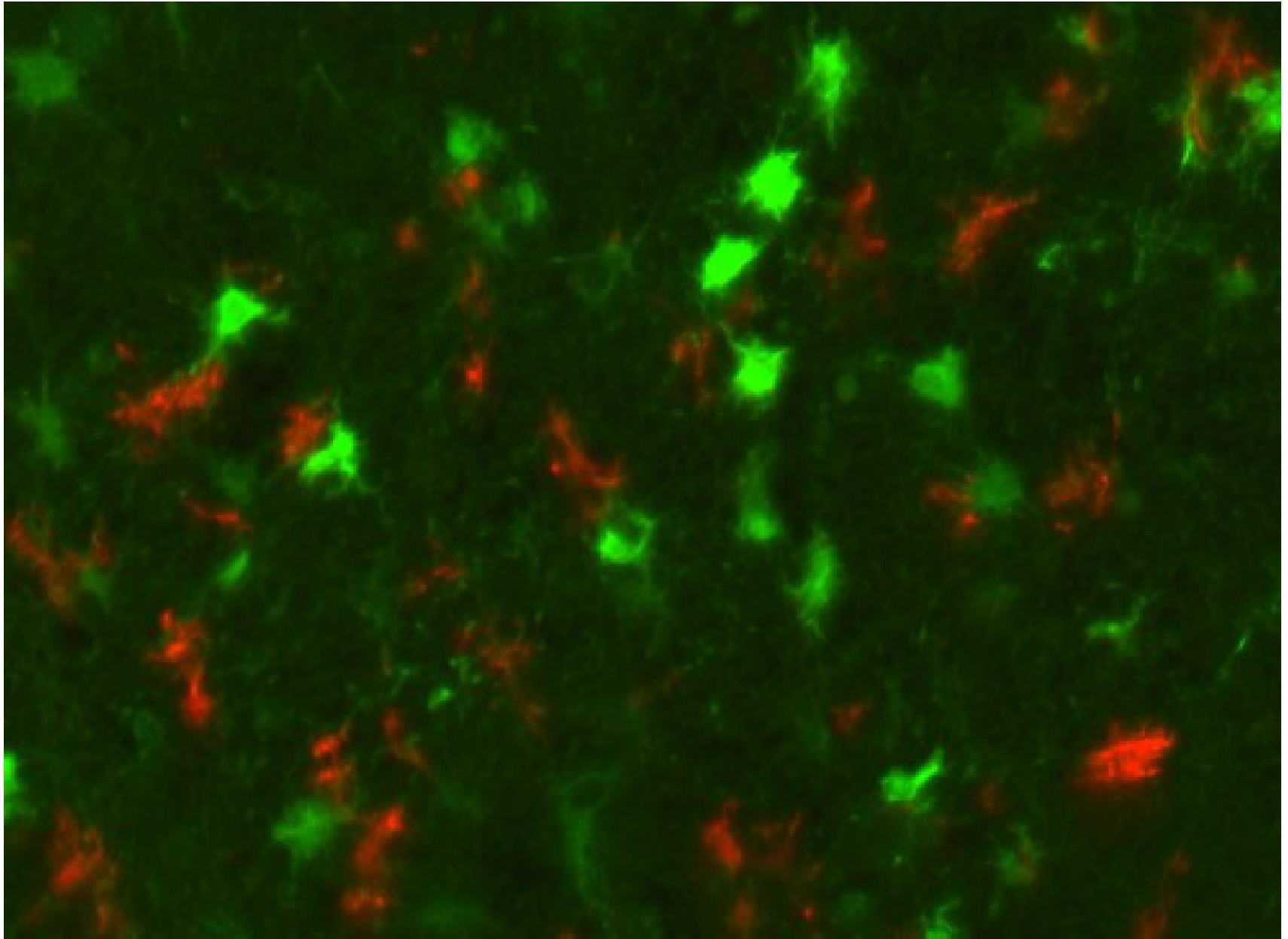
@Enterovirus / @GFAP



@Enterovirus / @IBA1



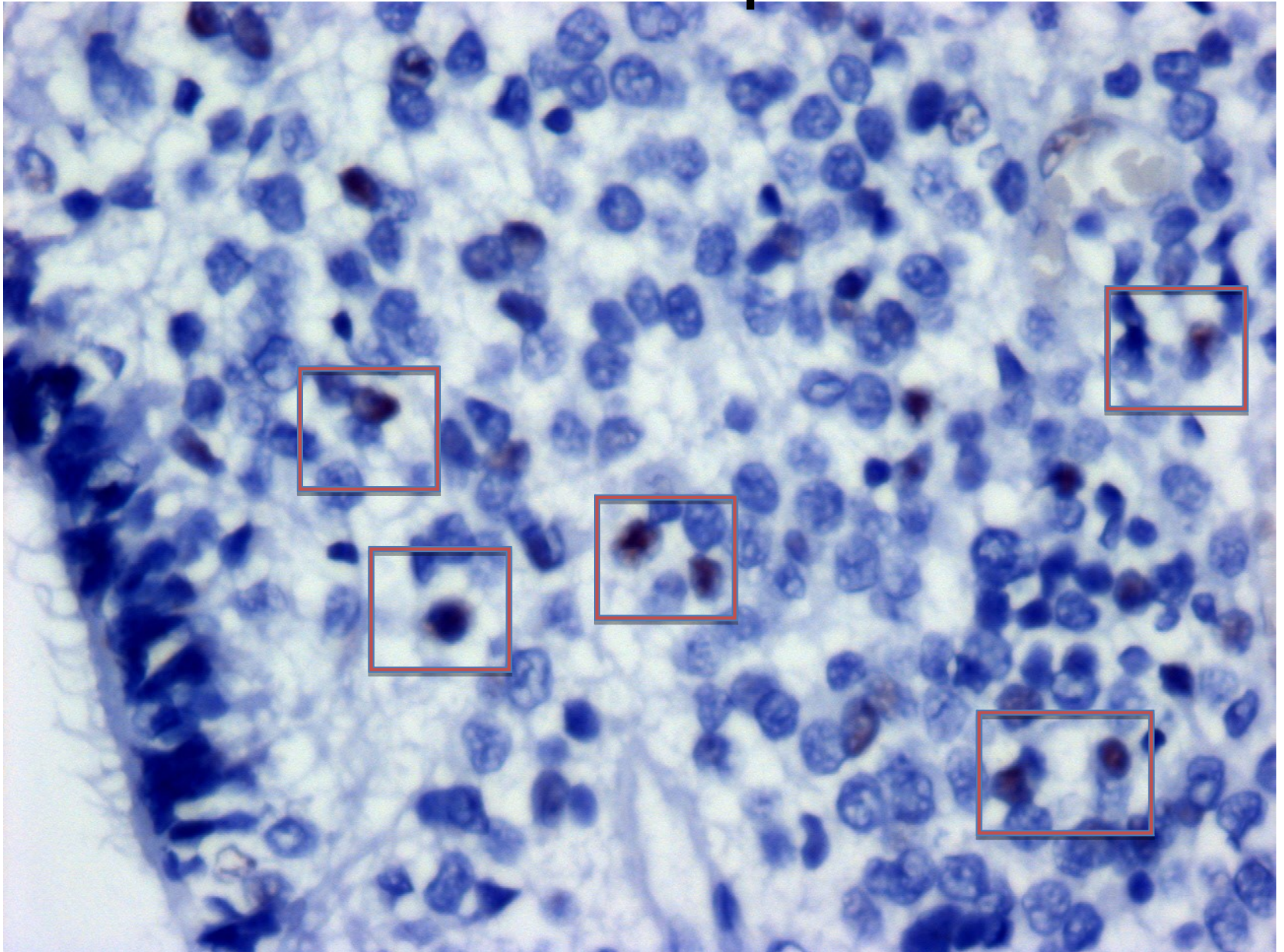
@Enterovirus / @IBA1



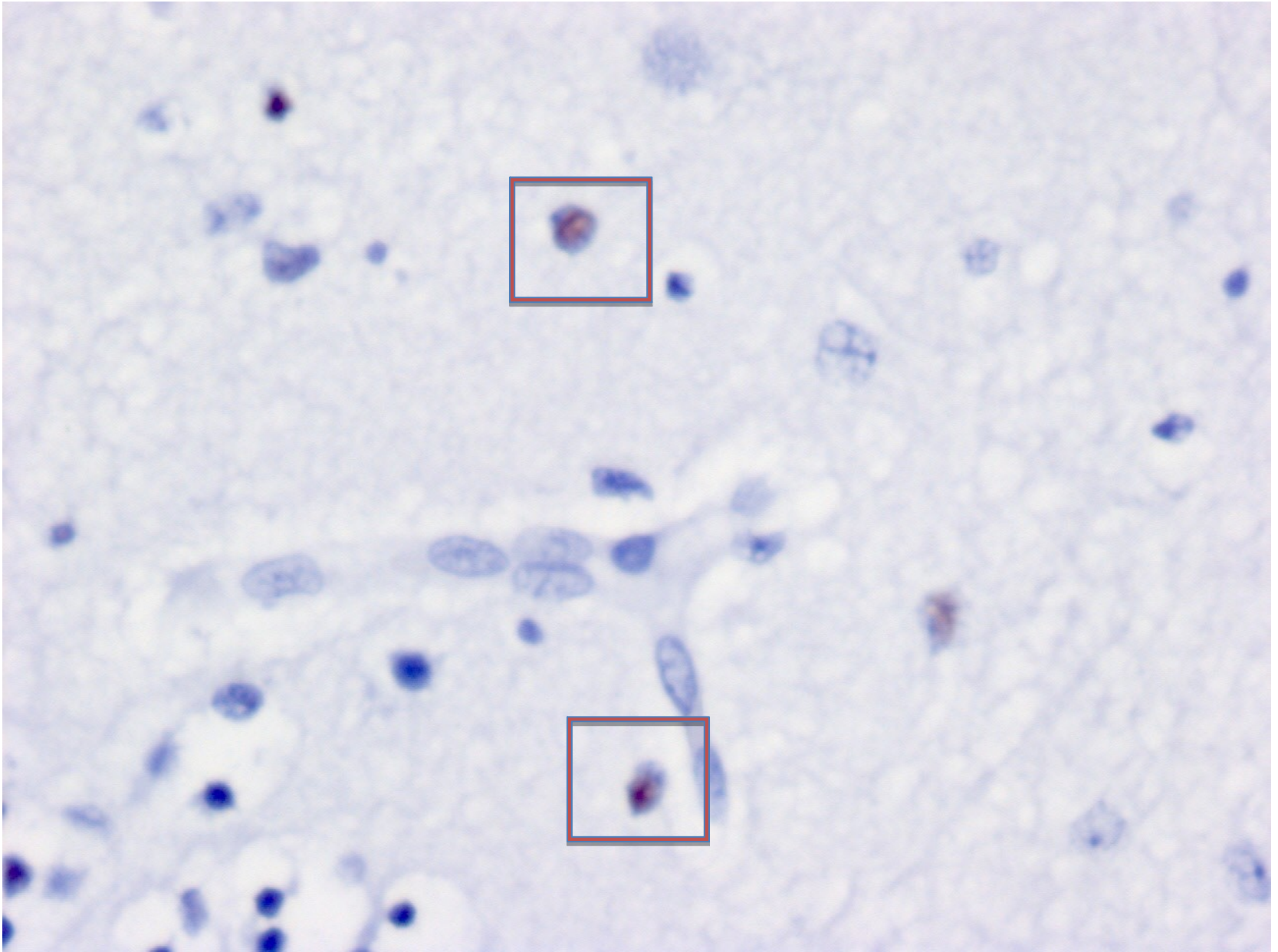
HPeV *In Situ* hybridization

³⁵S versus ACD probe

HPeV ACD probe



HPeV ACD probe



Why isn't this easy?

- Probe design problem?
- Fleeting virus?
 - Was there, now gone: only viremia?
- Viral strain?
 - Grow virus in vitro?
 - Grow mice in suckling mice?
 - Deep sequencing?
- When do you publish?

Deep Sequencing

GQ183033

Consensus

2

Coverage

0

0

100

200

300

400

500

600

700

800

900

1,000

1,100

1,200

U88

U88

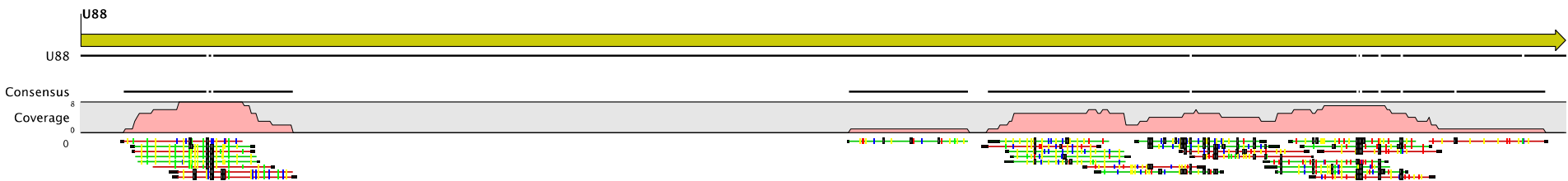
Consensus

8

Coverage

0

0



-itis

vs.

-opathy

**Analogy to other
enteroviruses**

**Timing of viral detection
in CNS and disease**

PCR detection in CNS

Strain 3 specificity

Histopathology

Histopathology

**Absence of Adaptive Immune
Response**

?IHC

?ISH

?Recovery

In vitro vs. in vivo

Animal model

Summary

HPeV3 Epidemiology

- Emergent Neuropathogen
 - 3X more common association with encephalitis than EVs
 - Probably not a zoonotic
 - But we can transmit to monkeys
 - Limited sequence diversity
 - ~1980's
 - 32% of child bearing females seronegative
 - Limited capacity to recombine with other HPeVs
 - Don't share same host receptor

Summary

Clinical features of HPeV3 encephalitis

- Preterm or term infants
 - No disease at birth
 - Infected in first 3 months
 - Role of maternal antibody
 - No available therapy
 - Future maternal immunization?
 - Rarely fatal
 - ?Neurological sequelae of stem cell infection
 - PVL-like histopathology
 - Minimal immune response (No pleocytosis, protein)

Questions and future direction

- How much neurodisease attributable to HPeV3?
- If most infants survive HPeV3 infection and it is so neurotropic (particularly neurostem elements) could it cause subtle neurological abnormalities?
- Is it an emergent viral infection, pathogenic in newborn because of absence of maternal IgG?
 - Would vaccine protect until next emergent?
- What determines neuropathogenesis of HPeV3
Cell surface receptor? Transcription factors, IIR?

