Emerging Viral Infections

Isaac H. Solomon, MD, PhD
Associate Pathologist, Brigham and Women's Hospital
Associate Professor, Harvard Medical School

Brigham and Women's Hospital
Founding Member, Mass General Brigham

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

Disclosures

- I have no relevant financial relationships to disclose

Learning Objectives

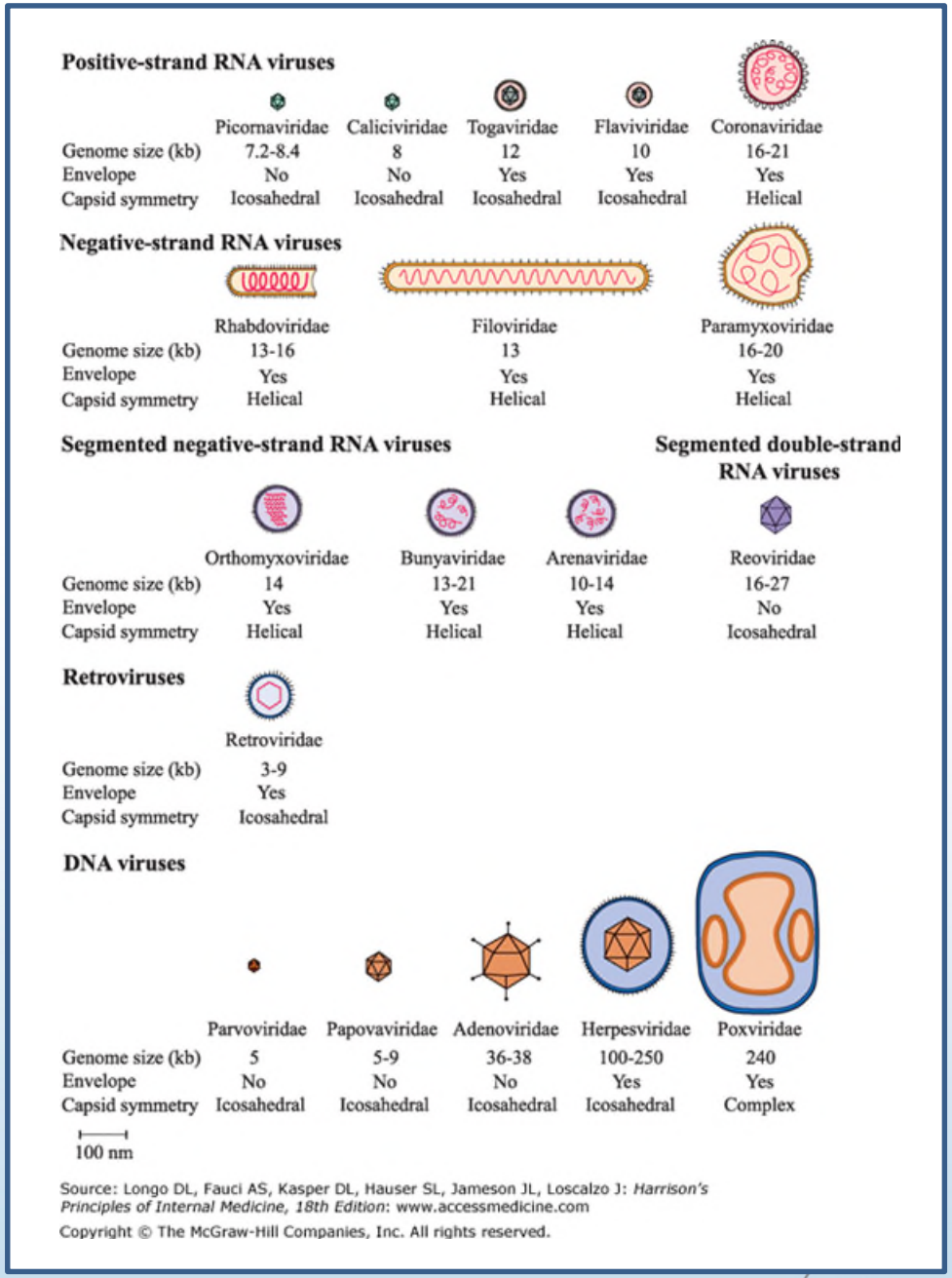
- Name at least three emerging viruses that infect the brain
- Describe the histological features associated with emerging viral infections
- Choose appropriate ancillary testing to confirm a suspected case of encephalitis due to an emerging virus

Emerging Infection

- Definition = recent or potential increase in the future
- May be:
  1) Completely new disease
  2) Disease new to or reappearing in an area
  3) Pathogens that have become resistant to antibiotics

Viruses and Human Disease

- Hundreds of viruses are known to cause disease in humans
  - Primary local infection
  - Dissemination
  - Reactivation
  - Oncogenesis
- Classified by genome structure (DNA vs RNA), capsid symmetry, and presence/absence of envelope



Viruses Associated with CNS Disease

Virus family	Viruses
DNA viruses	
Herpesviruses	Herpes simplex type 1, herpes simplex type 2, varicella-zoster, cytomegalovirus, Epstein-Barr virus
Adenoviruses	Adenovirus types 6, 7, 11, 12
RNA viruses	
Retroviruses	Human immunodeficiency virus type 1
Enteroviruses	Polioviruses, coxsackieviruses group A, coxsackieviruses group B, echoviruses, enteroviruses 70, 71
Rubivirus	Rubella
Arenavirus	Lymphocytic choriomeningitis
Paramyxoviruses	Measles, mumps
Orthomyxoviruses	Influenza, parainfluenza
Rhabdovirus	Rabies

Type	Virus
RNA viruses	
Bunyaviruses[a]	California encephalitis, Jamestown Canyon virus, La Crosse virus, Rift Valley virus, Snowshoe Hare virus (Canada), Toscana virus (Mediterranean)
Flaviviruses[a]	Dengue virus, Central European encephalitis, Japanese encephalitis (Asia), Kyasanur Forest virus (South Asia), Louping ill (British Islands), Murray Valley encephalitis virus (Australia, New Guinea), Powassan virus, St. Louis encephalitis, tick-borne encephalitis virus (three subtypes), West Nile virus
Henipaviruses[b, c]	Nipah virus[b], Hendra virus[c]
Reovirus[a]	Colorado tick fever
Togaviruses[a]	Chikungunya virus, Eastern equine encephalitis, Venezuelan equine encephalitis, Western equine encephalitis

Diamond and Mosley. (2018) Encephalitis (Viral). In: Kreutzer J.S., DeLuca J., Caplan B. (eds) Encyclopedia of Clinical Neuropsychology. Springer, Cham.

Algorithmic Approach for Diagnosis of CNS Infections

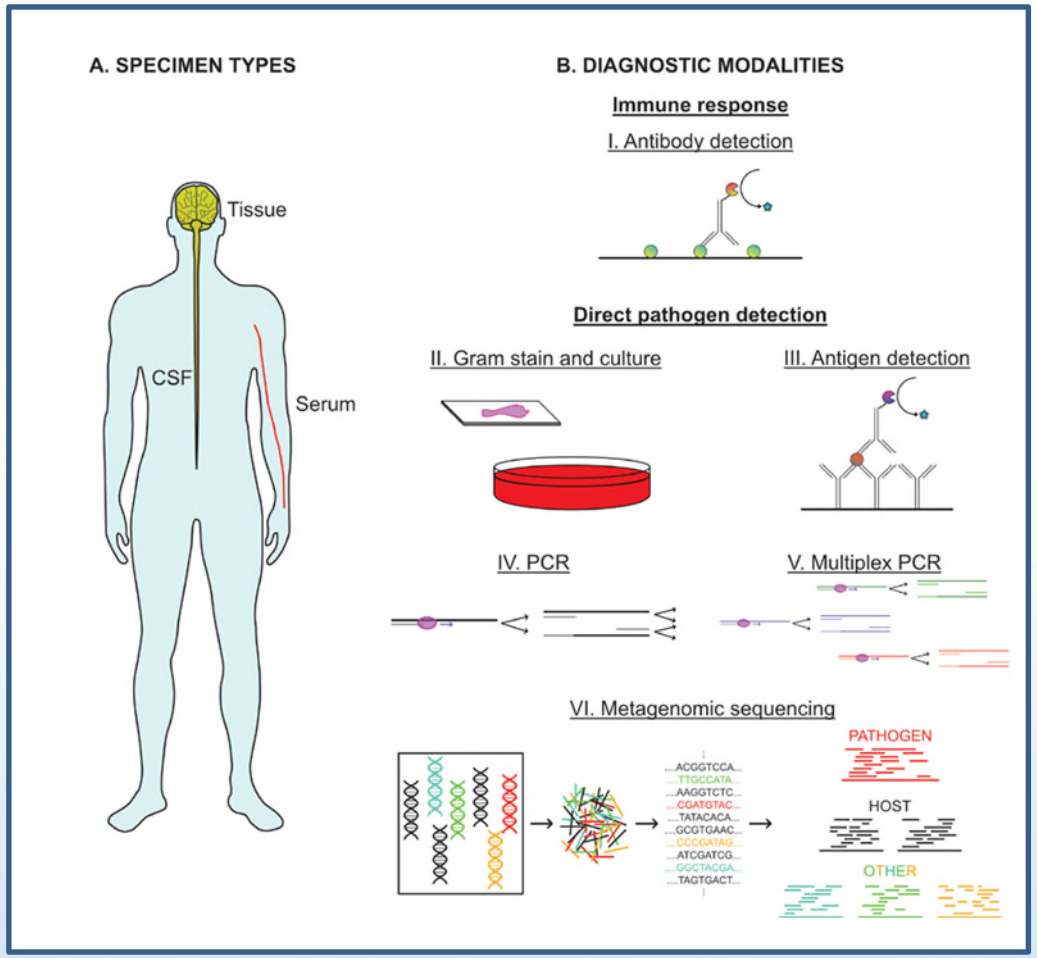
1. Review patient history, radiology, and laboratory testing results

2. Generate preliminary H&E differential diagnosis based on inflammatory pattern and observation of potential microorganisms

Ancillary testing to confirm the diagnosis:
3. Special stains
4. Immunohistochemistry/in situ hybridization
5. Electron microscopy
6. Molecular diagnostics

7. Expert consultation to help recommend or interpret histological findings and ancillary testing if needed

8. Results of ancillary testing interpreted with patient history, histopathology, and other laboratory testing to arrive at a final integrated diagnosis

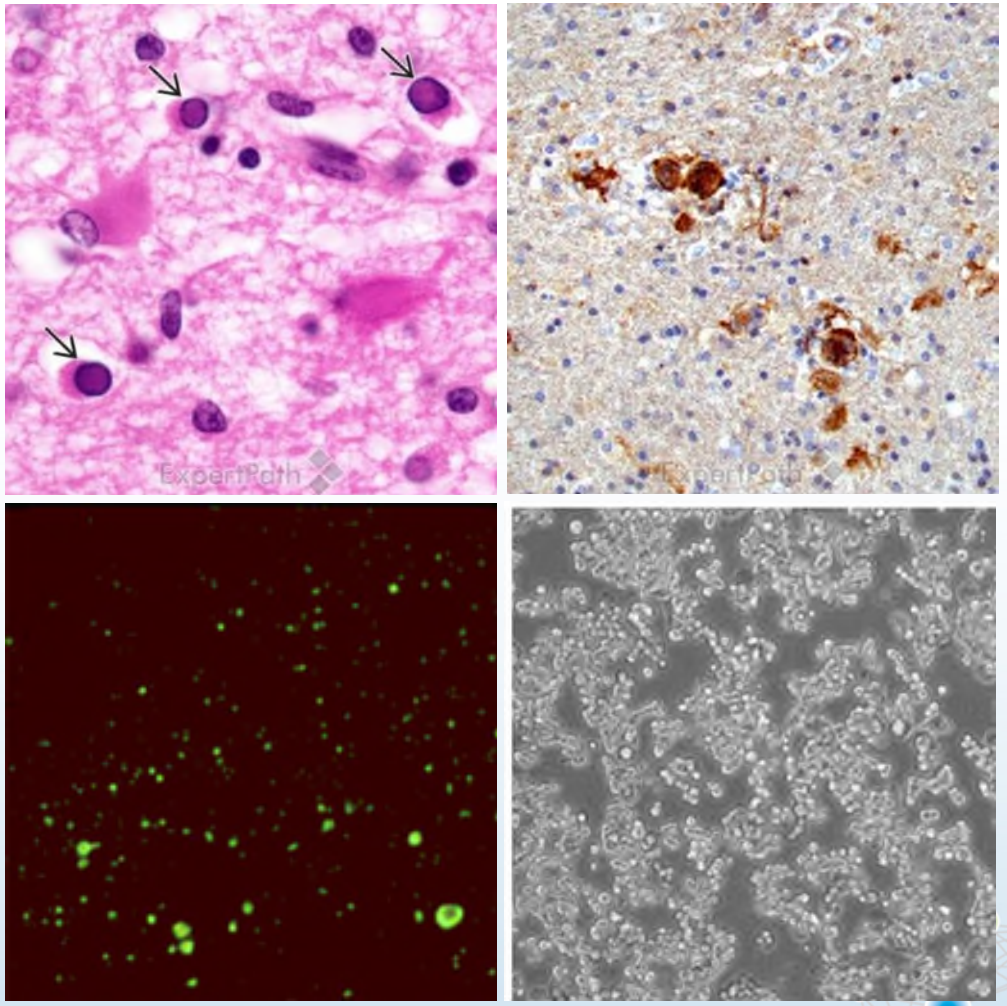


Kanjilal *et al.* (2019) *Semin Neurol.* 39(3):297-311.

Kenyon and Solomon (2025). *J Neuropathol Exp Neurol.* 84(6):447-458.

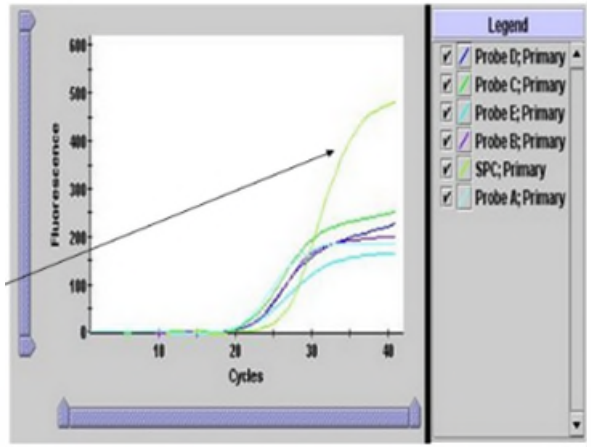
Diagnostic Tools

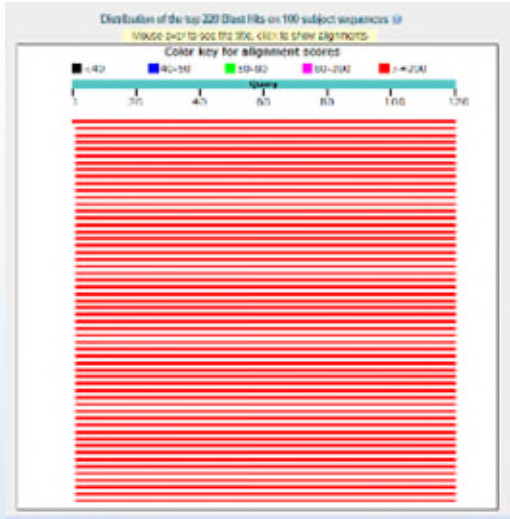
- Neuropathological Evaluation
  - Gross examination
  - Intraoperative frozen section and smear
  - Routine H&E
  - Special stains, IHC, ISH
  - Electron microscopy
- Serology (serum, CSF; IgG, IgM)
- Direct fluorescence antibody (dFA)
- Viral Culture
- Molecular testing



Molecular Testing

- Specimen type
  - Fresh/frozen tissue/fluid: performed immediately after collection; unknown distribution and prone to contamination
  - FFPE tissue: can be screened for organisms/inflammation; decreased sensitivity due to nucleic acid cross-linking
- Assay type
  - Single pathogen (e.g. HHV-6): High sensitivity; automatable with rapid turnaround; requires high degree of suspicion to select correct test
  - Targeted panel (e.g. Meningitis/Encephalitis): Conserves specimen volume; lower sensitivity/specificity; will miss unusual organisms
  - Unbiased (e.g. metagenomic next-generation sequencing): Detects any non-human nucleic acids including novel/emerging pathogens; potential false positives; most expensive





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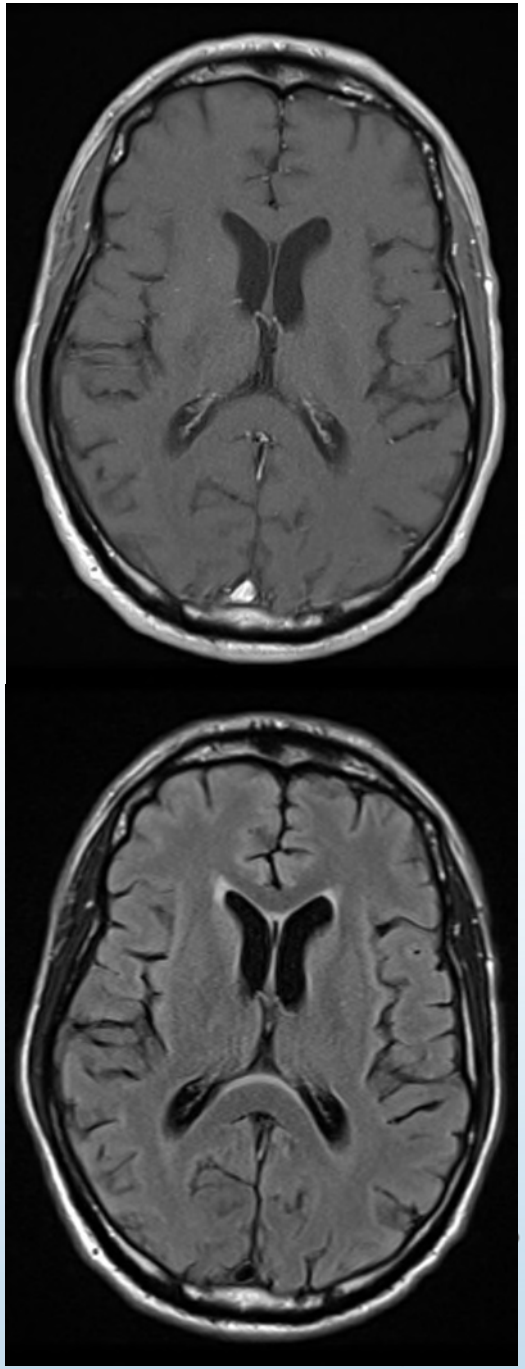
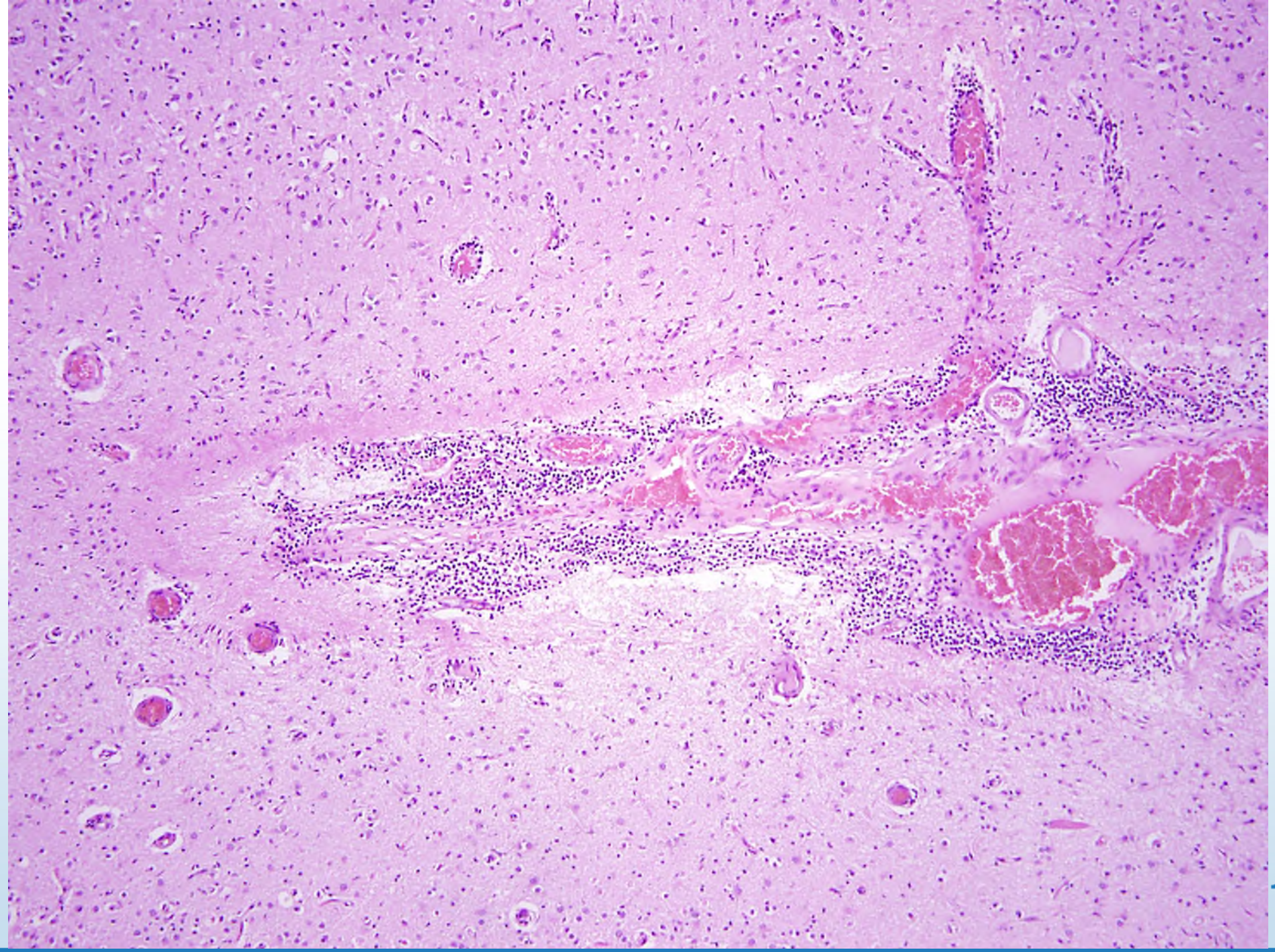
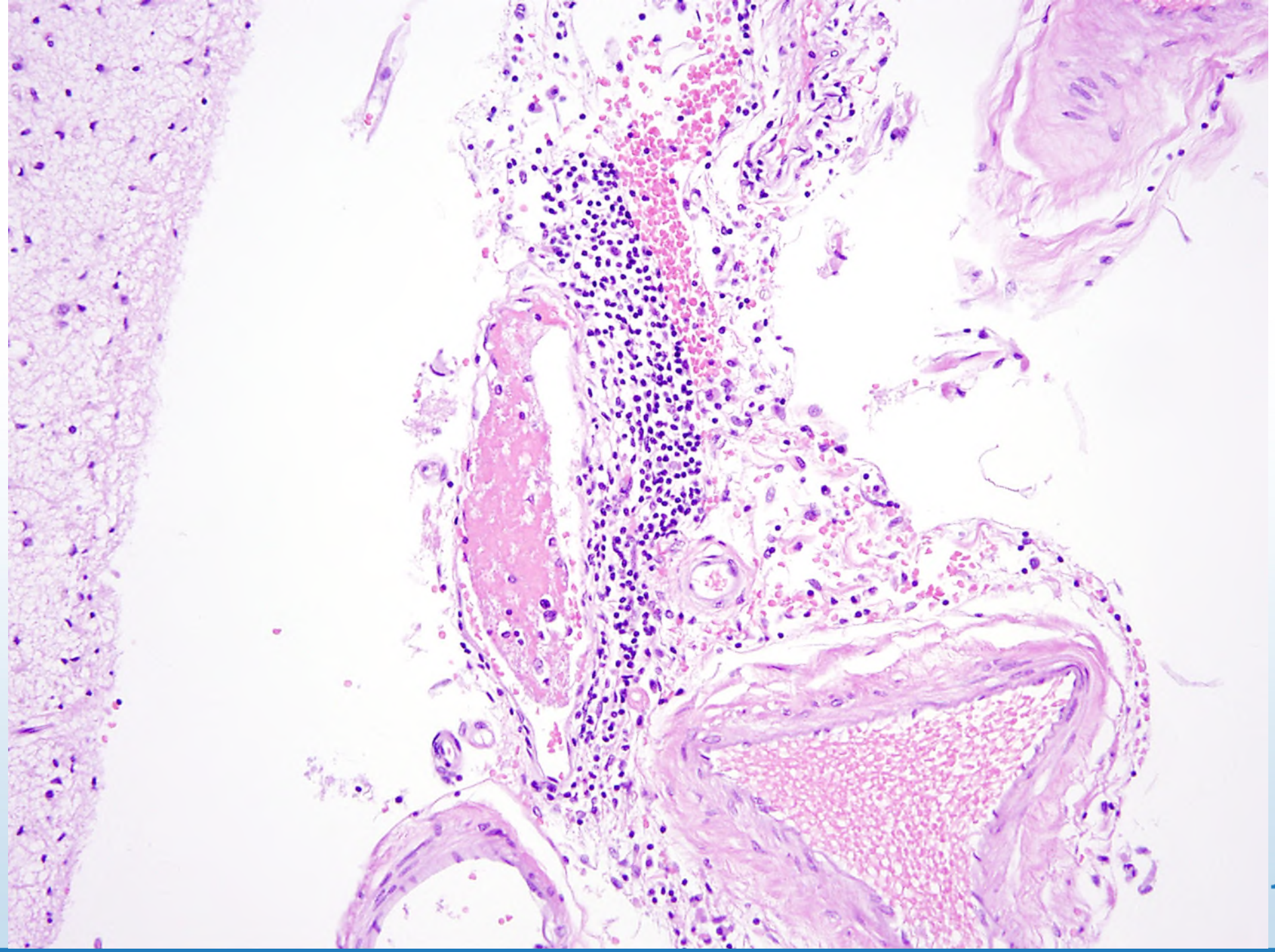
Case #1

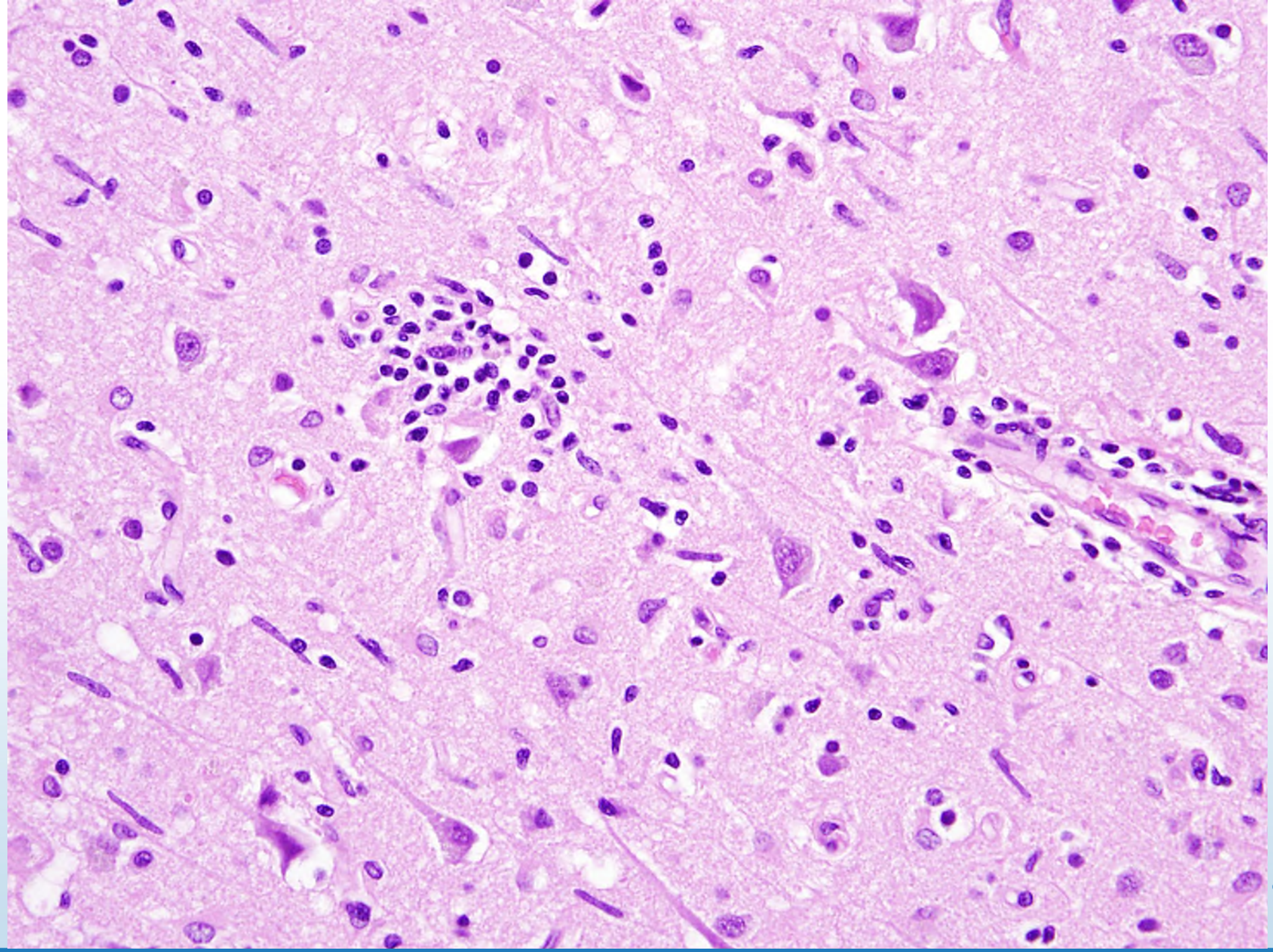
- 56-year-old man with history of mantle cell lymphoma, in remission on maintenance rituximab
- Presented with rapidly progressing dementia of unknown etiology in the setting of persistently inflammatory CSF unresponsive to high-dose steroids
- MRI showed slight ventriculomegaly attributed to atrophy, but otherwise normal
- A broad infectious and paraneoplastic workup was largely unrevealing

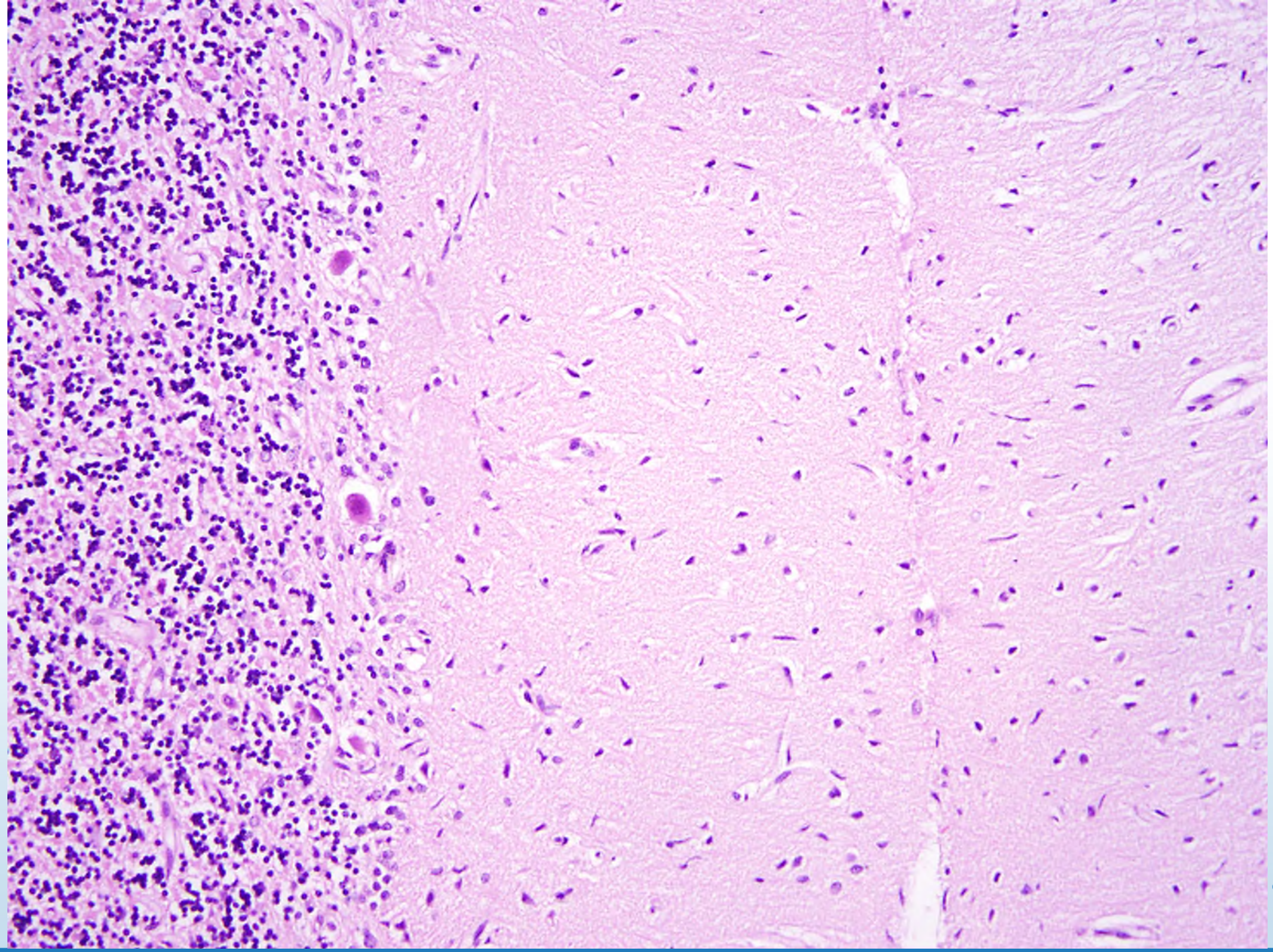


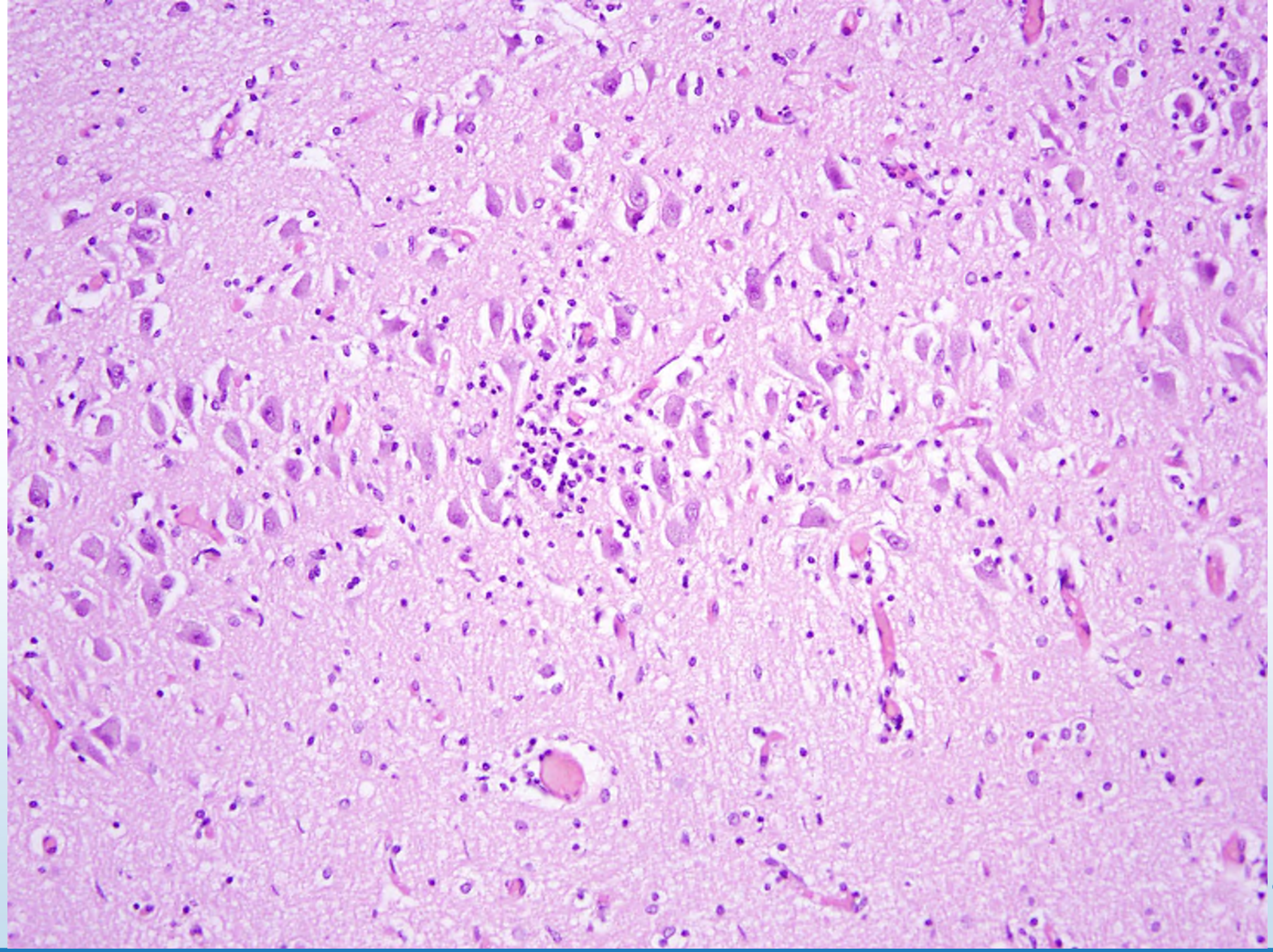
- Treated with intravenous immunoglobulins (IVIG), and two-week course of favipiravir (inhibitor of viral RNA-dependent RNA polymerase) with no significant improvement, and was transitioned to comfort measures

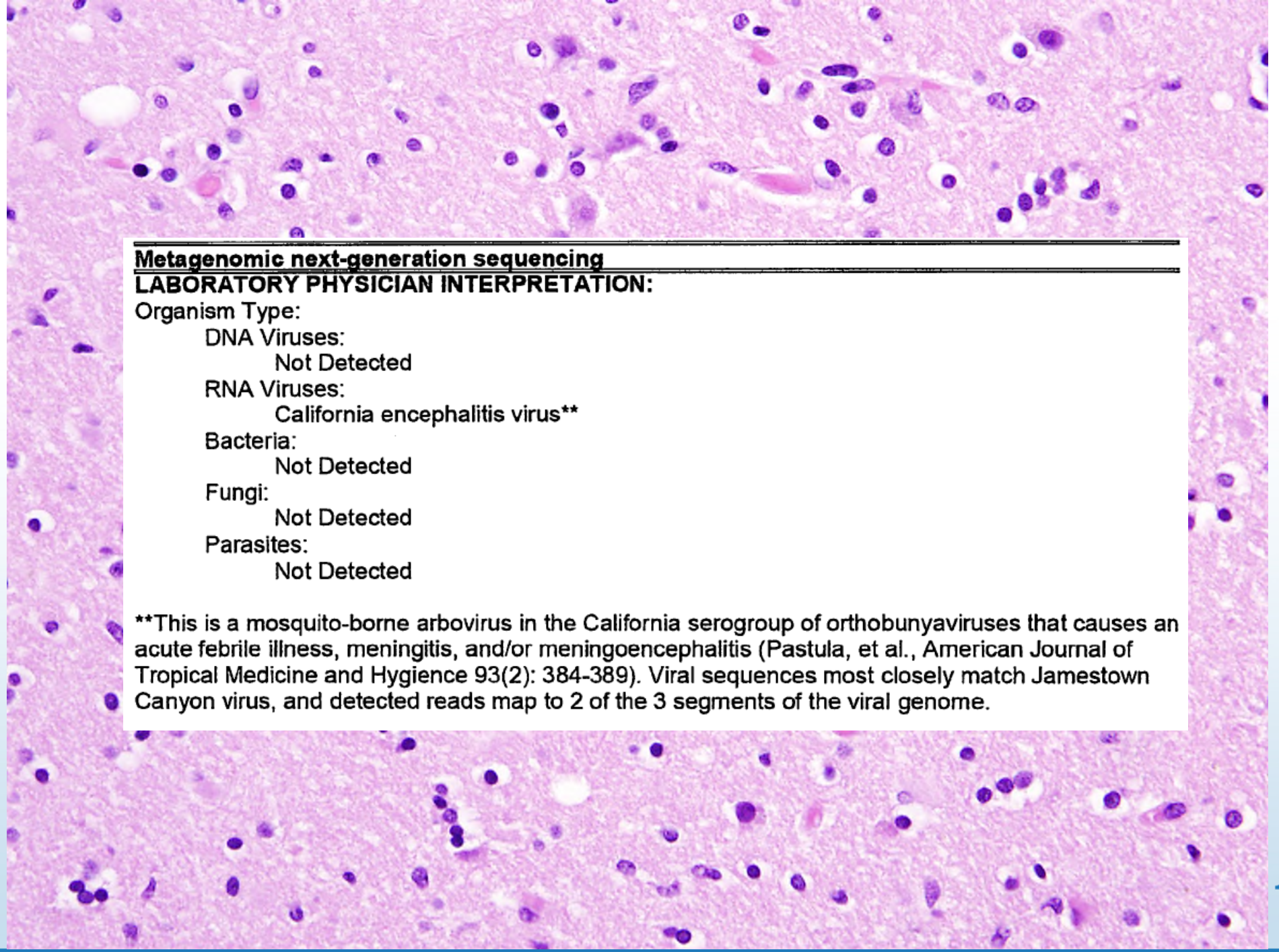












Metagenomic next-generation sequencing

LABORATORY PHYSICIAN INTERPRETATION:

Organism Type:

- DNA Viruses:
  - Not Detected
- RNA Viruses:
  - California encephalitis virus**
- Bacteria:
  - Not Detected
- Fungi:
  - Not Detected
- Parasites:
  - Not Detected

**This is a mosquito-borne arbovirus in the California serogroup of orthobunyaviruses that causes an acute febrile illness, meningitis, and/or meningoencephalitis (Pastula, et al., American Journal of Tropical Medicine and Hygience 93(2): 384-389). Viral sequences most closely match Jamestown Canyon virus, and detected reads map to 2 of the 3 segments of the viral genome.

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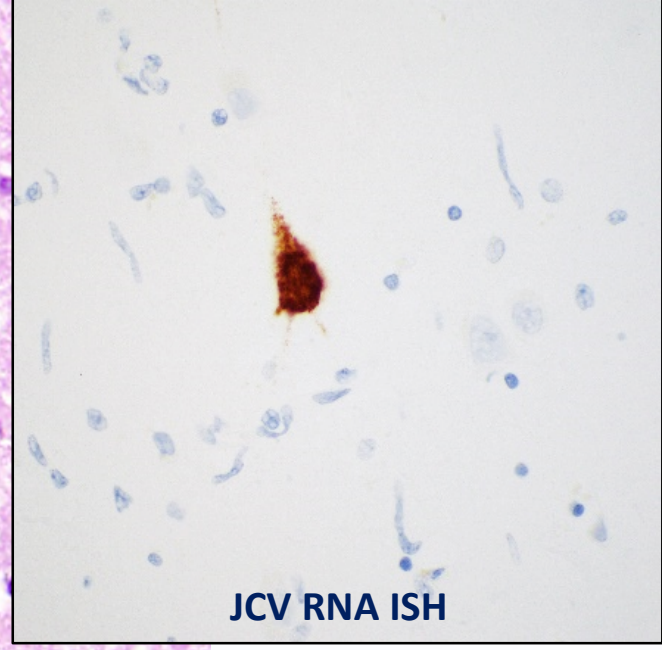

JCV RNA ISH

FINAL DIAGNOSIS:

JAMESTOWN CANYON VIRUS ENCEPHALITIS

mNGS (CSF):	Jamestown Canyon virus
IgM (blood):	negative
IgM (CSF):	negative
PCR (serum):	POSITIVE
PCR (FFPE brain):	POSITIVE
JCV ISH (FFPE brain):	POSITIVE

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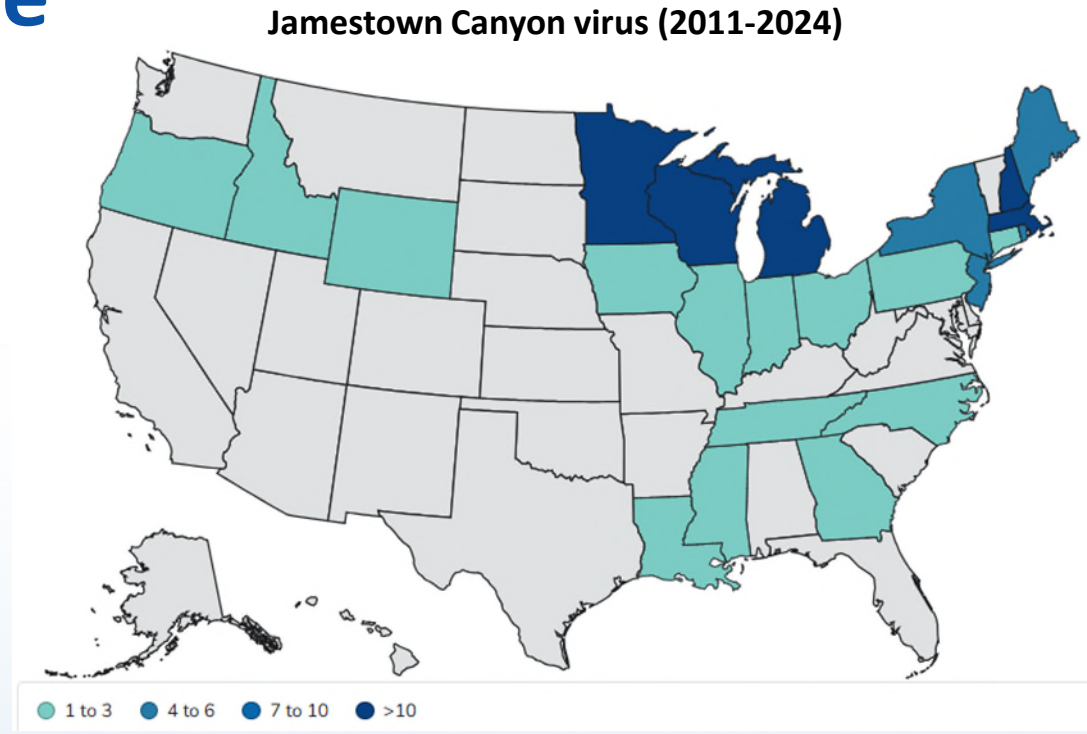
Jamestown Canyon Virus Disease

- ssRNA orthobunyavirus transmitted by mosquitoes and is rare cause of severe disease
- Histology (limited published reports):
  - Focal parenchymal inflammation, neuronal necrosis, neuronophagia, scattered glial nodules, and perivascular infiltrates, mild meningeal inflammation
  - Virus specific antibodies at CDC
  - EM for viral particles
- Laboratory: Serology, LP (lymphocytic)
- DDX:
  - Other viral causes of encephalitis: Arboviruses, HSV, VZV, EBV, CMV, Rabies, Measles, Adenovirus
  - Autoimmune encephalitis, lymphoma, low grade gliomas



CDC/ James D. Gathany

CDC

ArboNET
National Arbovirus Surveillance System

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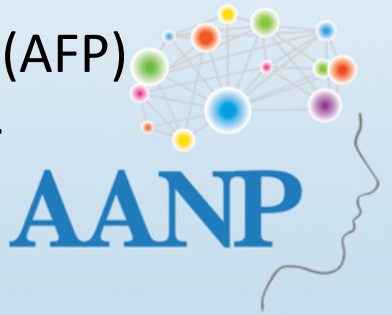
Arbovirus CNS Infections

- Arthropod-borne viruses transmitted through the bites of infected mosquitoes, ticks, sand flies, or midges (also blood transfusion, organ transplantation, perinatal transmission, breast feeding, and laboratory exposures).
- >130 arboviruses known to cause human disease
  - *Flavivirus:* Powassan virus disease, St. Louis encephalitis virus disease, West Nile virus disease
  - *Alphavirus*: Chikungunya virus disease, Eastern equine encephalitis virus disease, Western equine encephalitis virus disease
  - *Orthobunyavirus*: California serogroup virus diseases (California encephalitis, Jamestown Canyon, Keystone, La Crosse, Snowshoe hare, Trivittatus viruses)
- Most infections asymptomatic or mild febrile illness
- Neuroinvasive disease: aseptic meningitis, encephalitis, or acute flaccid paralysis (AFP)
- Laboratory Diagnosis: Isolation of virus, detection of viral antigen/nucleic acid, or detection of IgM (or increased titers of IgG) in blood, CSF, or tissue

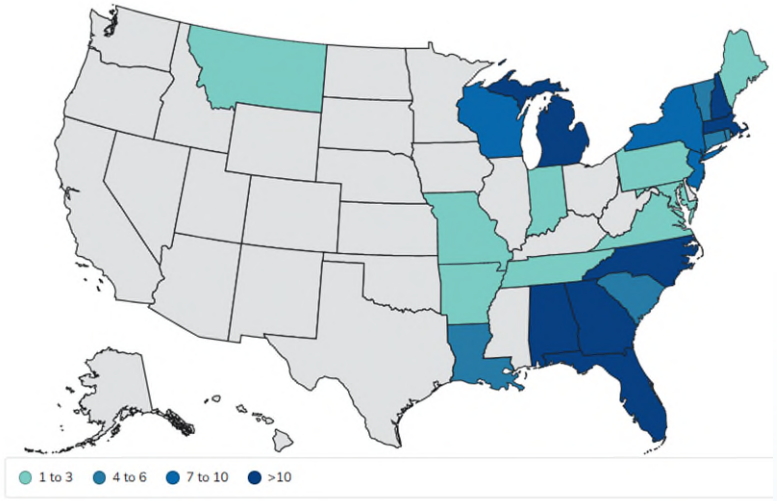


Distribution of Arboviruses in United States

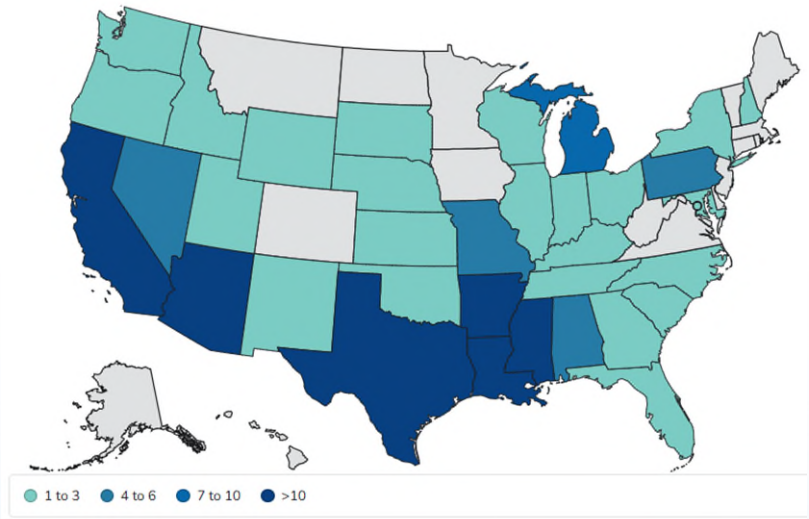
ArboNET National Arbovirus Surveillance System

CDC

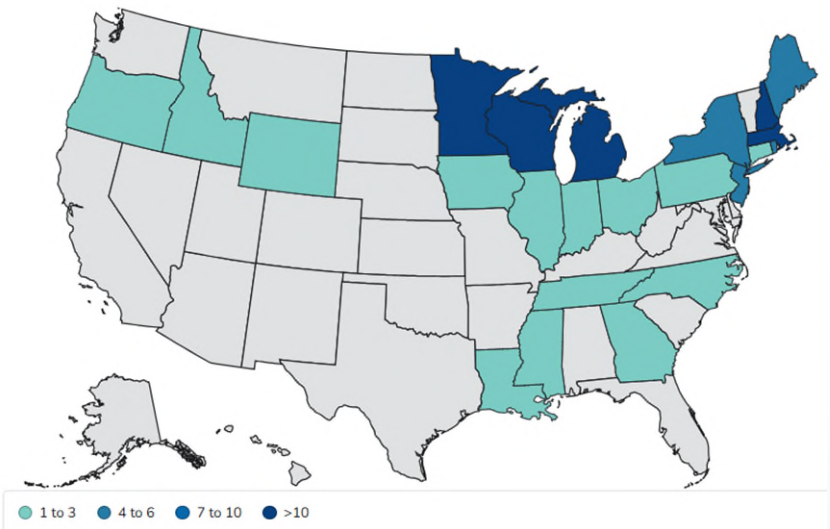
Eastern equine encephalitis virus (2003-2024)



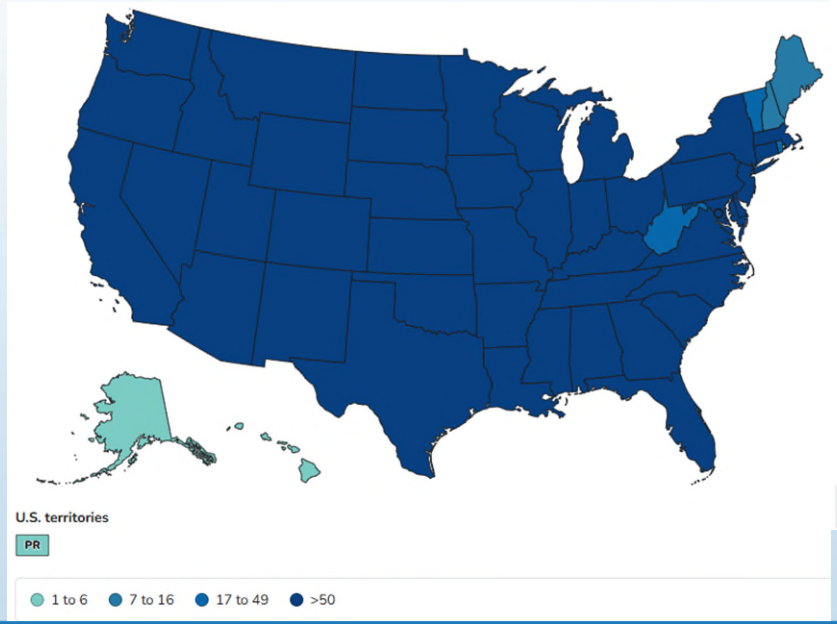
St. Louis encephalitis virus (2003-2024)



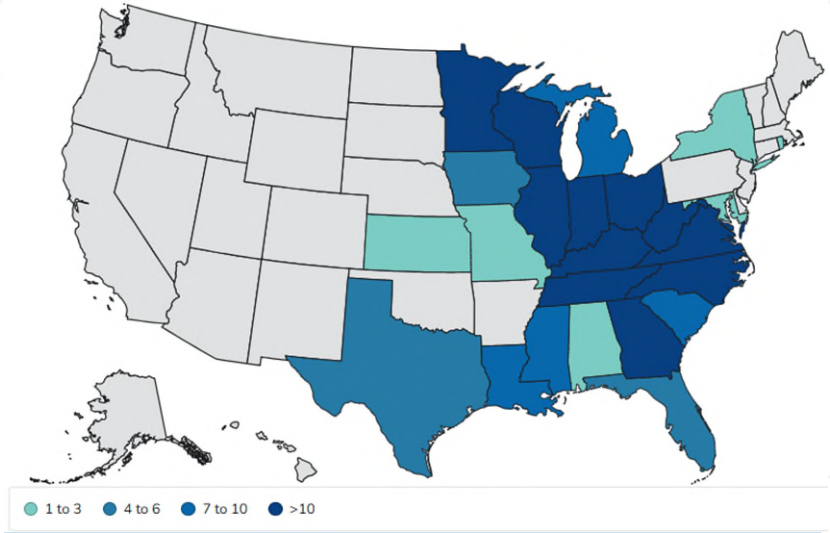
Jamestown Canyon virus (2011-2024)



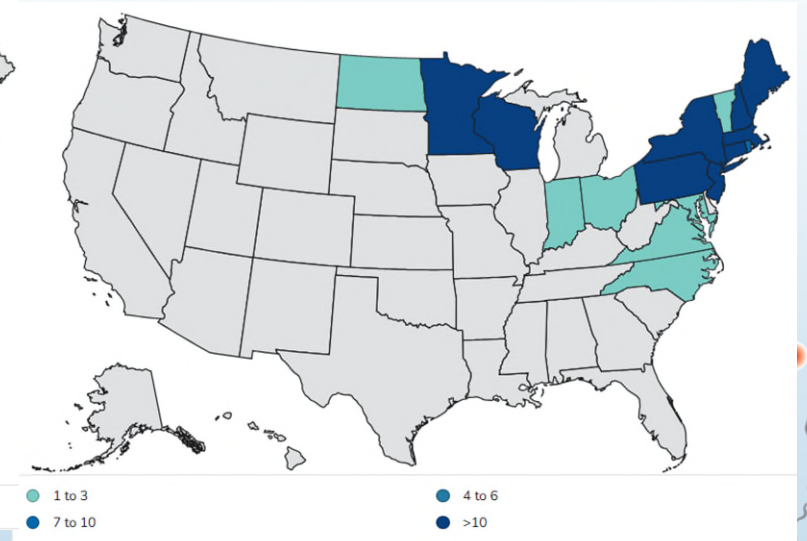
West Nile virus (2003-2024)



La Crosse virus (2003-2024)



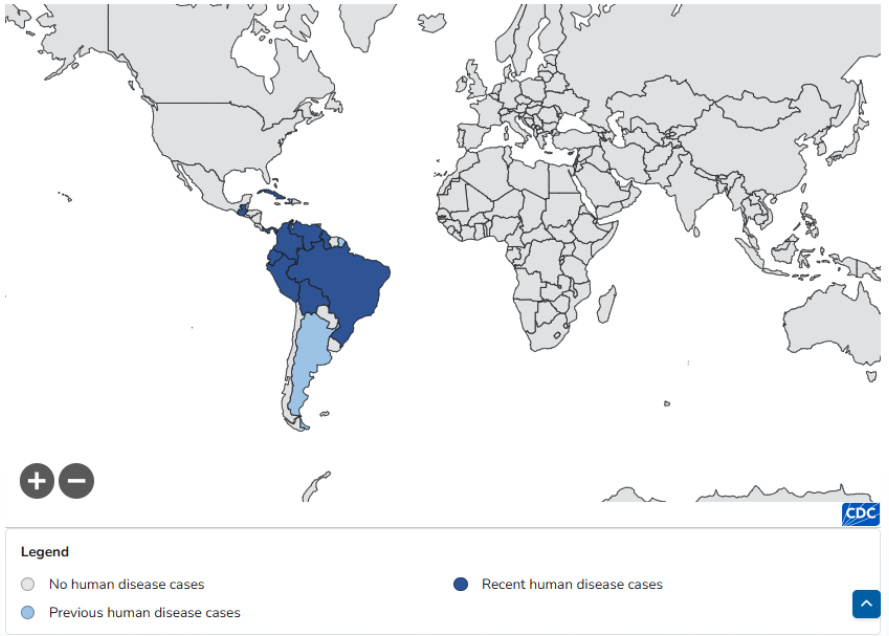
Powassan virus (2004-2024)

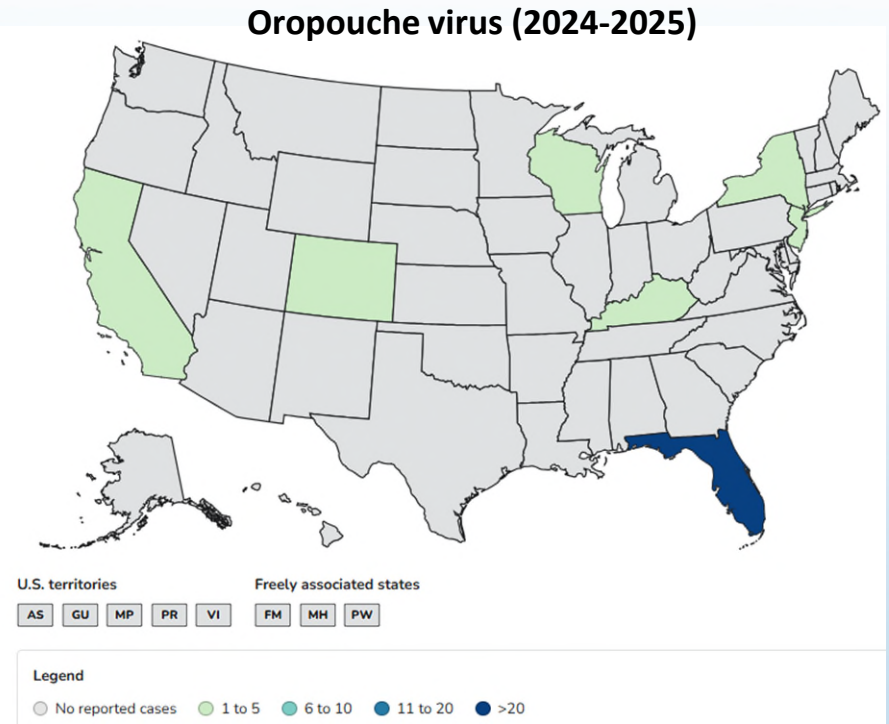


Oropouche virus (OROV)

- *Orthobunyavirus* endemic to South America, Central America, and the Caribbean with travel-imported cases to US and Europe
- Symptoms similar to chikungunya, dengue, and Zika virus infections (but can exhibit biphasic course)
- Vertical transmission likely causes adverse effects on fetal development including miscarriages
- Human cases reported of meningitis/encephalitis (5%) and peri-infectious Guillain-Barré syndrome
- Neurologic manifestations include severe headaches, frequently retro orbital, with associated photophobia
- Persistent fatigue may last weeks to months, but complete recovery is typical, with rare fatalities

Biting midge (left) and mosquito (right)
Photo courtesy: Dunpharlain

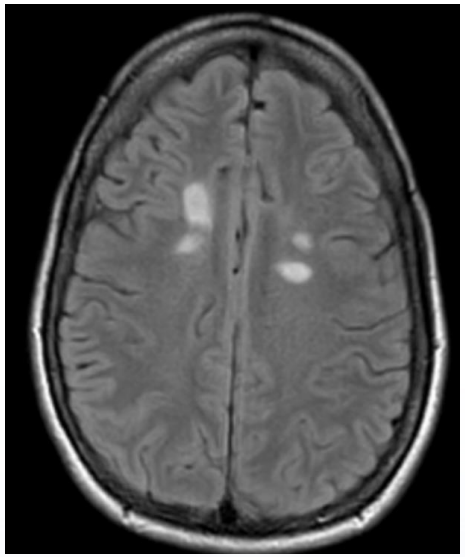




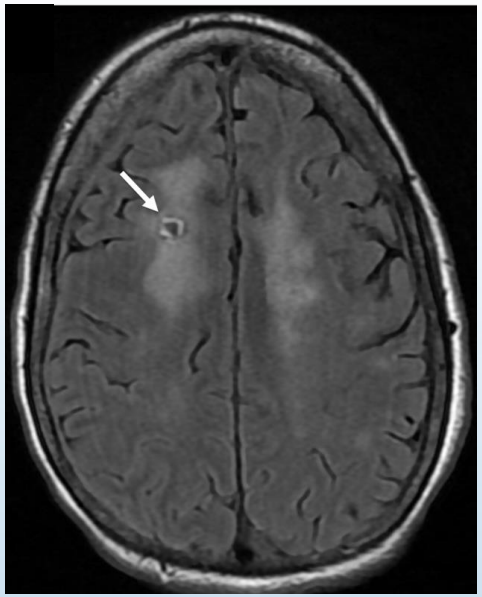
Pastula et al. (2024) *Ann Neurol*. doi: 10.1002/ana.27139.

Case #2

- 50-year-old woman with 9-year history of MS on ocrelizumab for ~3 years
- Presented with subacute decline in mental status and cognition
- MRI showed predominant bilateral frontal periventricular white matter lesions
- LP revealed elevated protein, normal glucose,1-2 nucleated cells, no oligoclonal bands
- Negative infectious workup including HSV, JC virus, CMV, toxoplasma, and bacterial cultures



10 months prior



Post-biopsy

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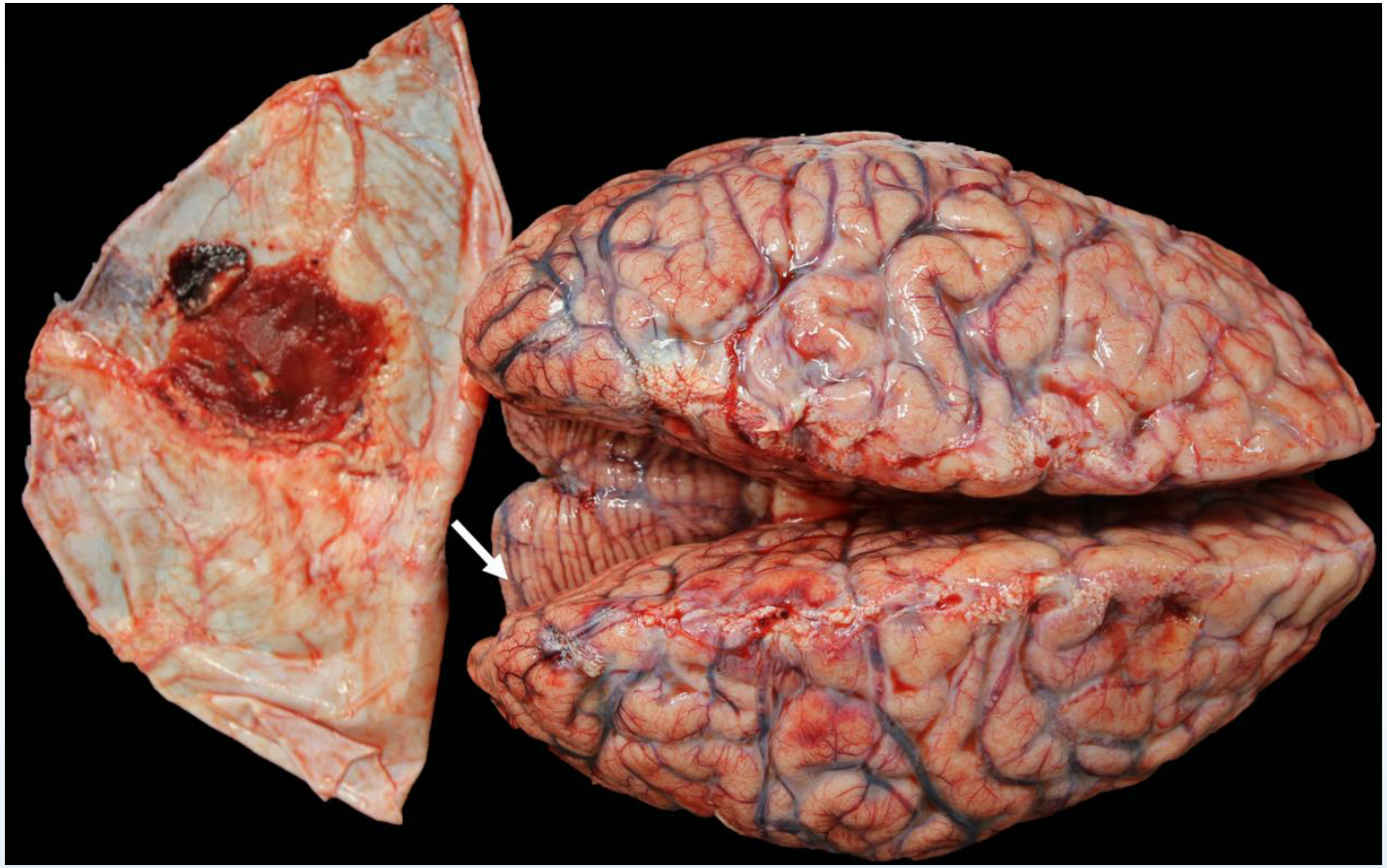
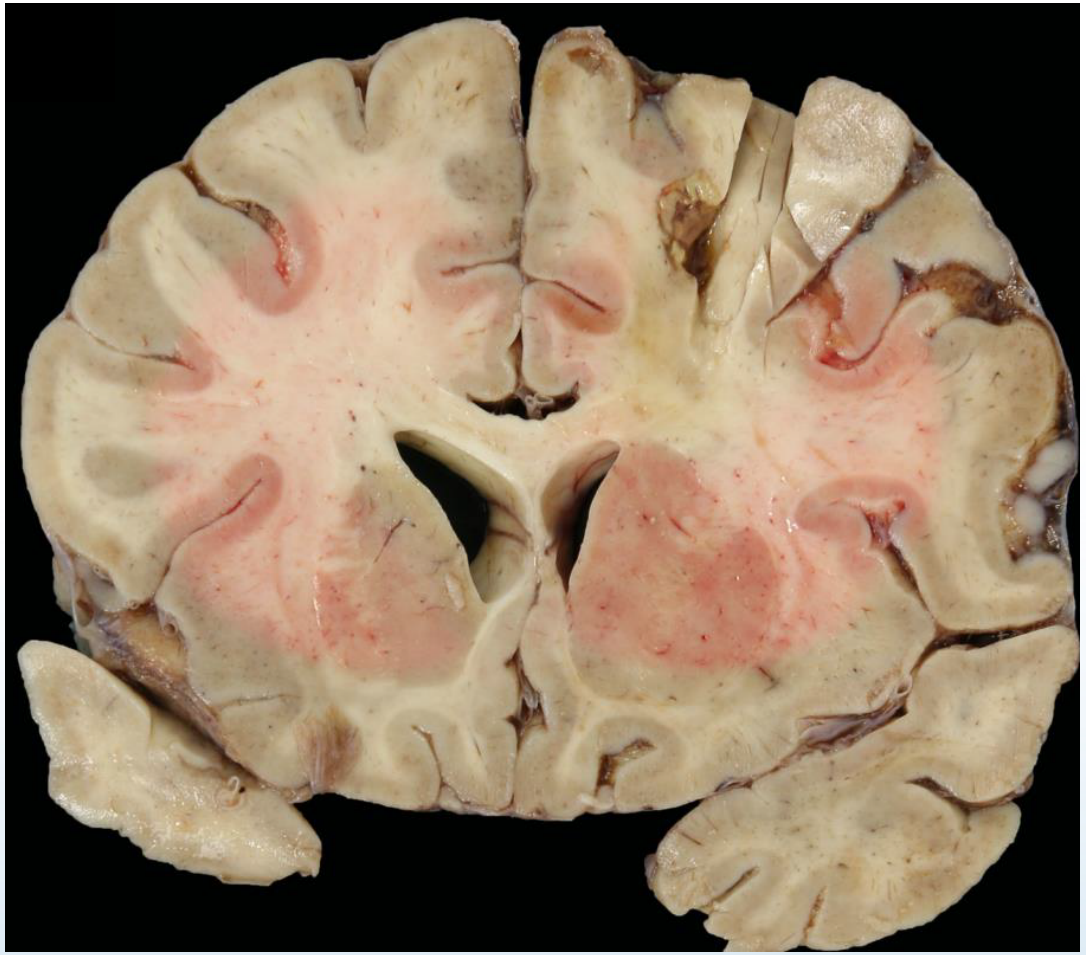
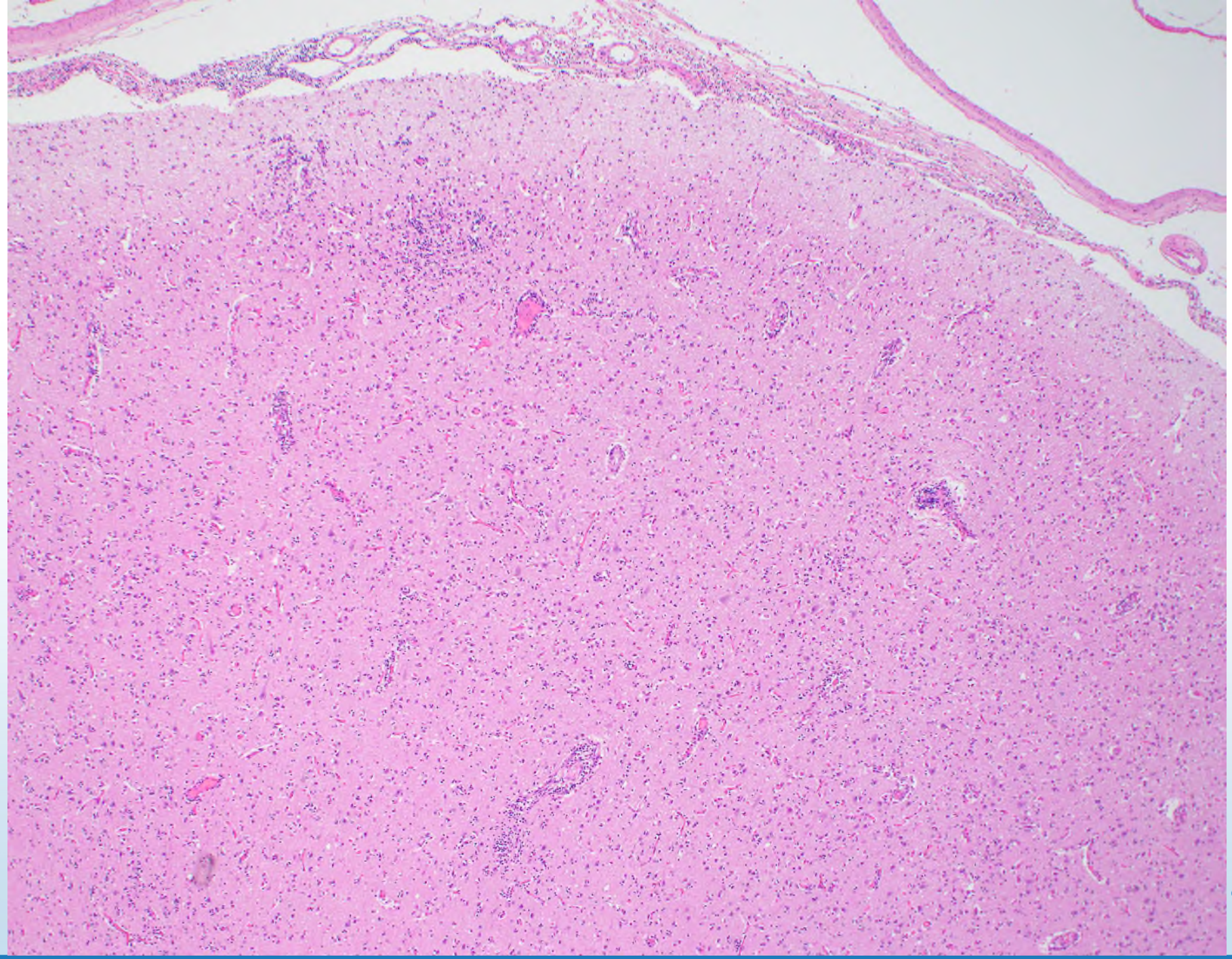
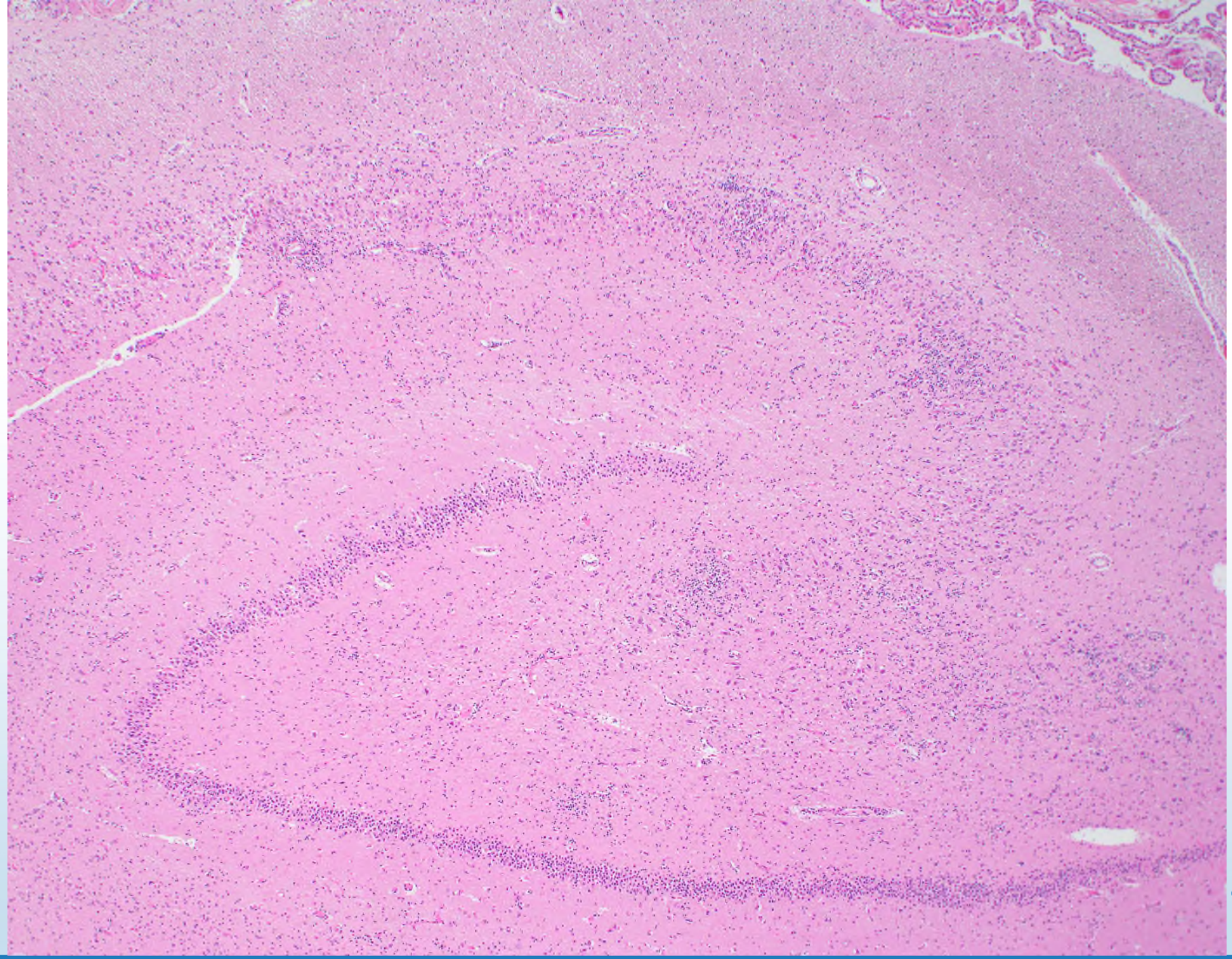
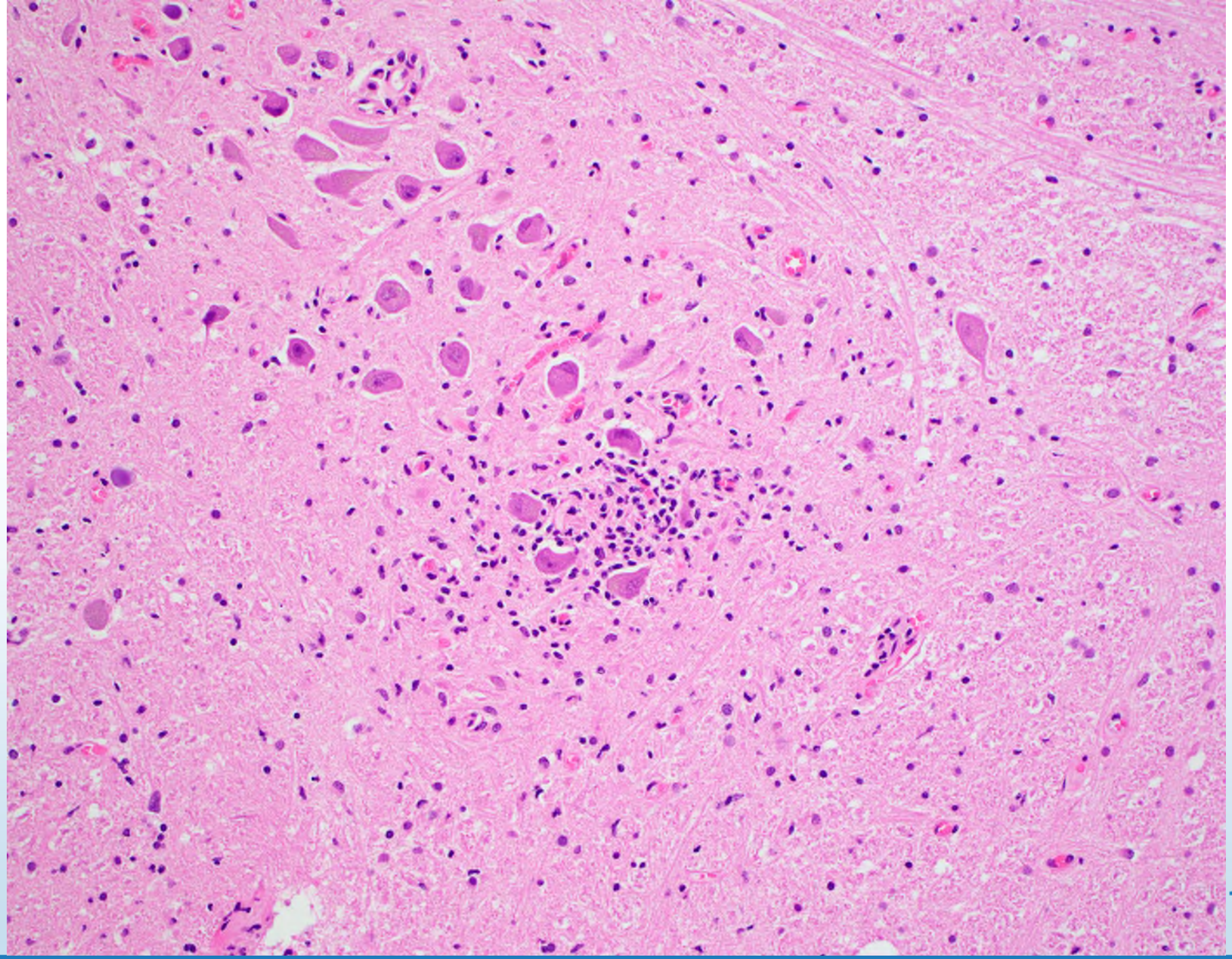


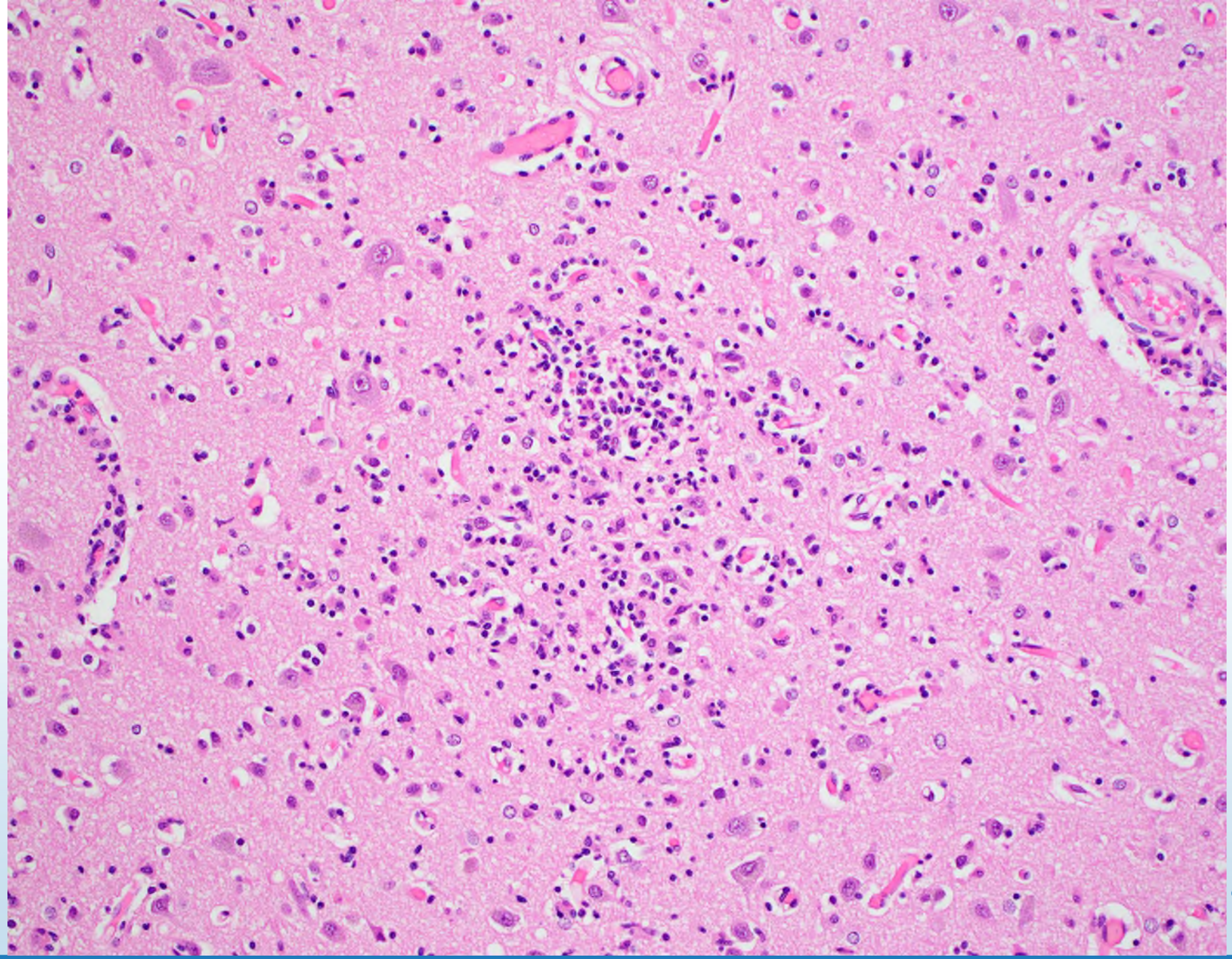


- A brain biopsy showed a generalized lymphocytic inflammatory process without clear evidence of demyelination
- After minimal improvement over 3.5 weeks of IV steroids, the patient expired and underwent a full autopsy









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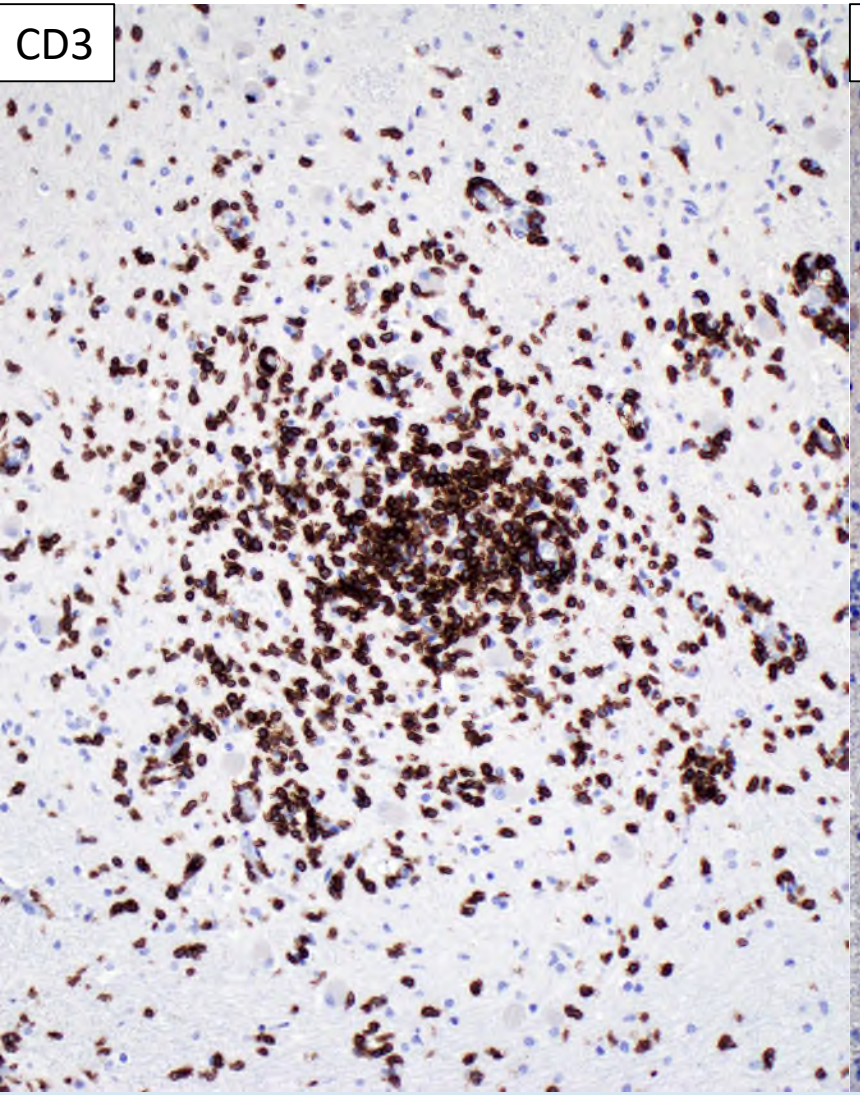
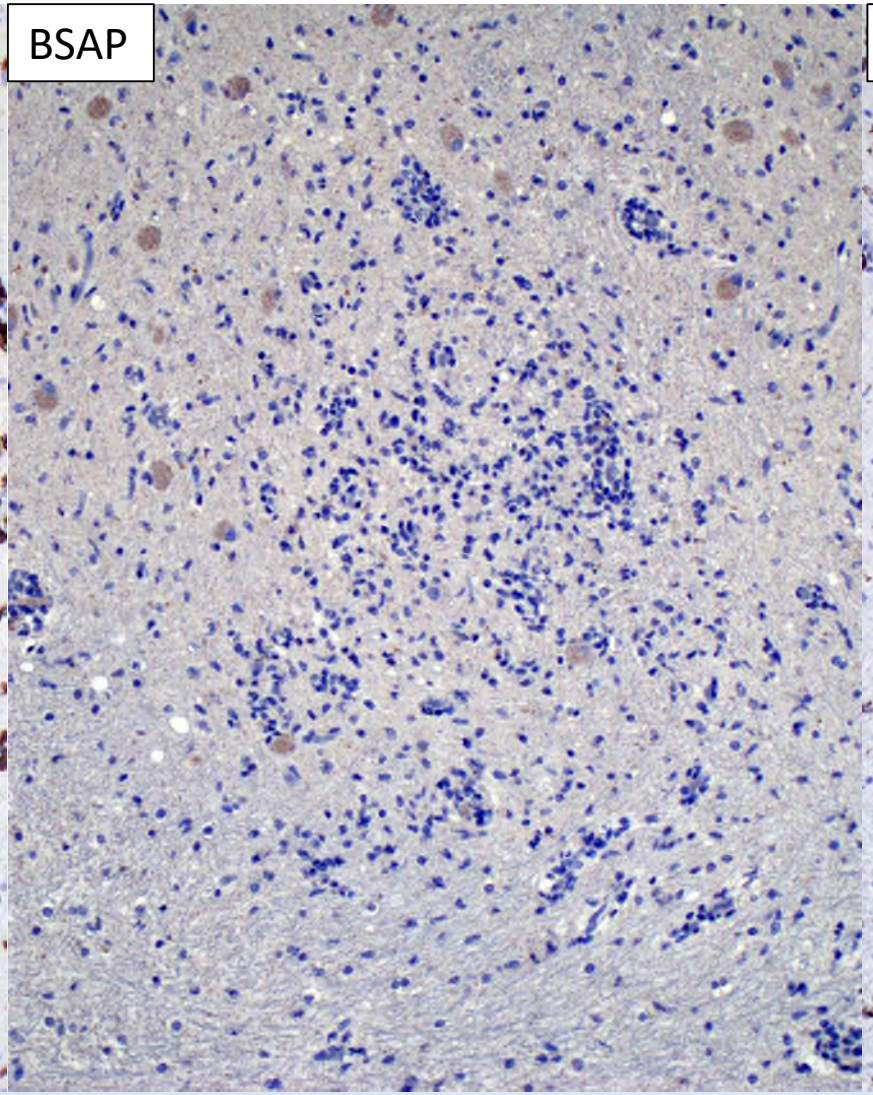
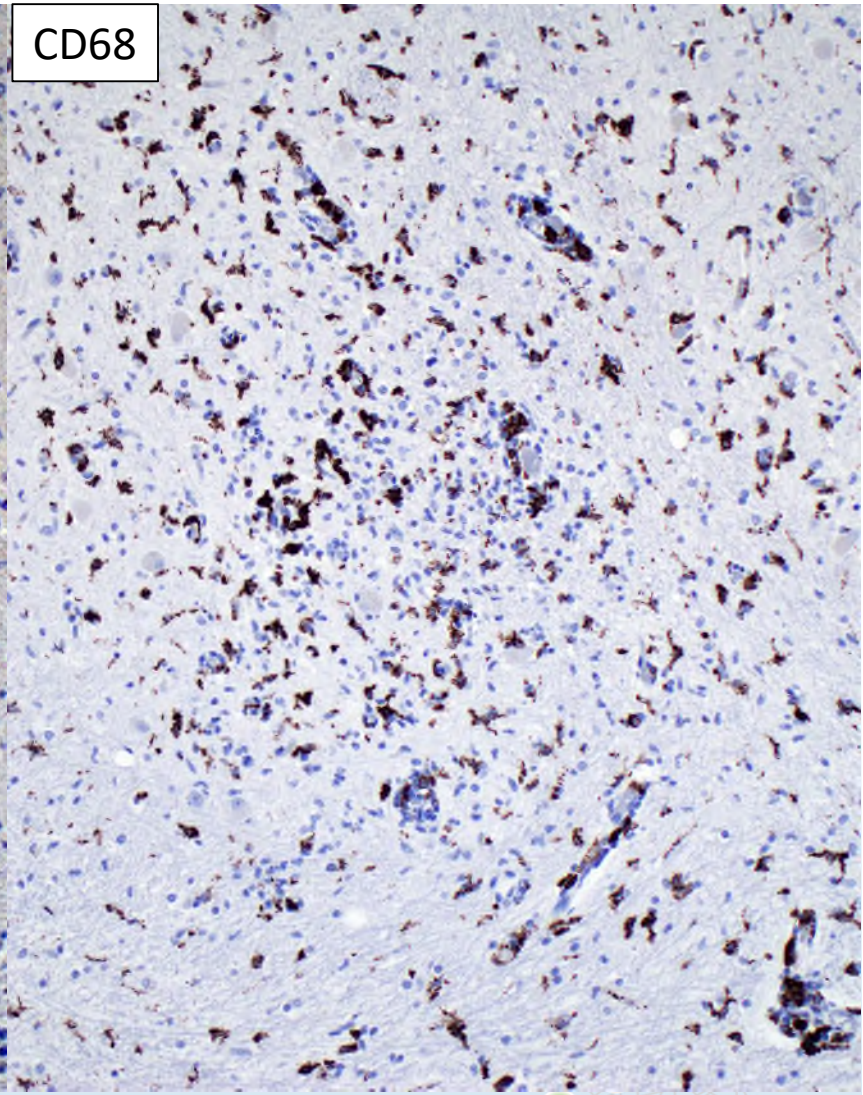
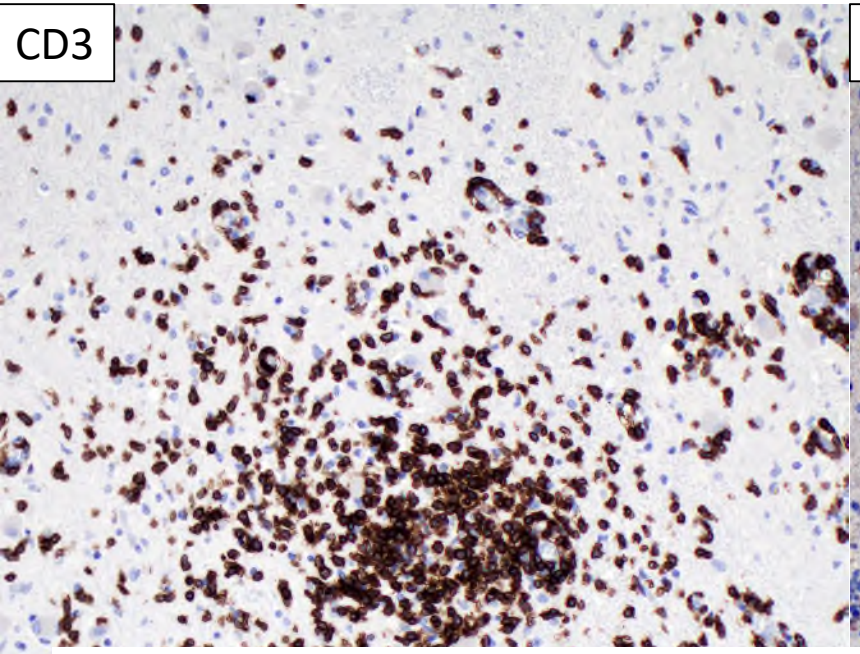
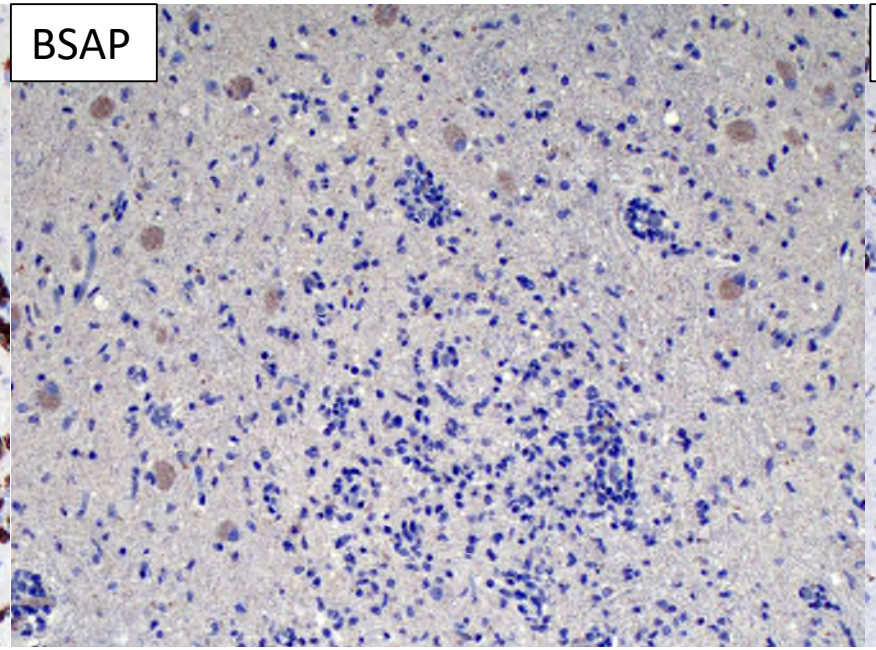
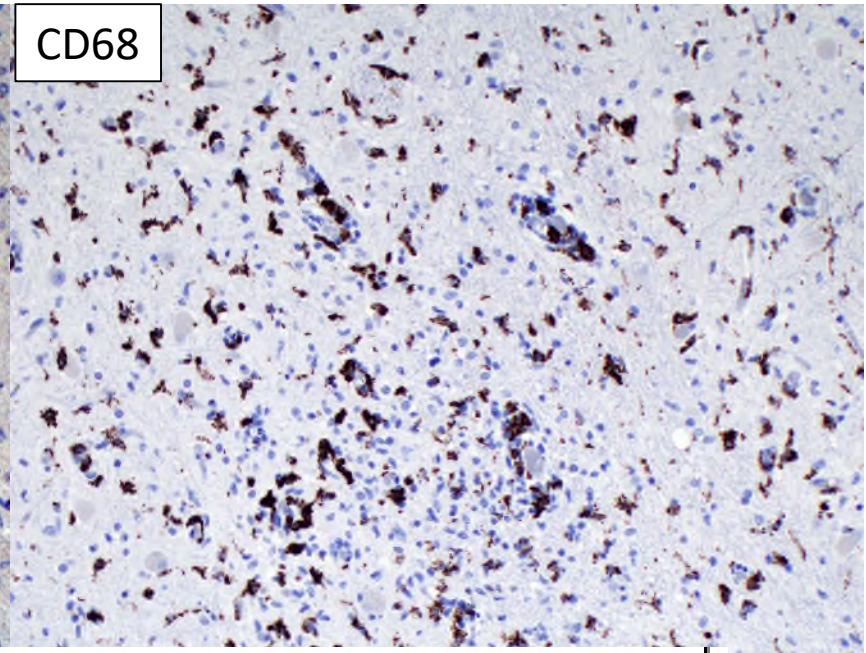












mNGS Pathogen Dx: DNA Viruses: NOT DETECTED

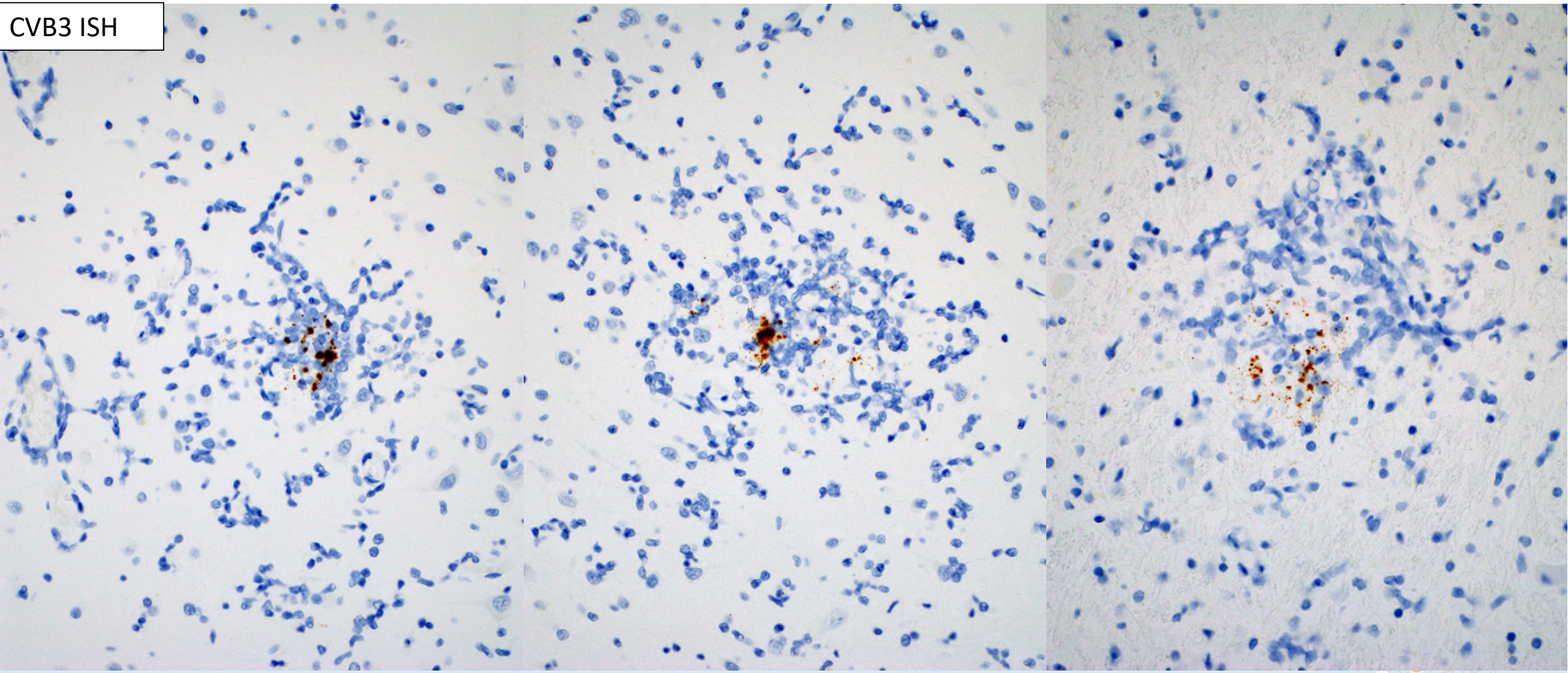
RNA viruses: DETECTED Enterovirus B. Reads align most closely to coxsackievirus B3 in the VP1 region and across the genome. Enteroviruses are common causes of aseptic meningitis and/or encephalitis, especially in children. Clinical correlation is recommended. Result may be reportable to local public health department.

Bacteria: NOT DETECTED

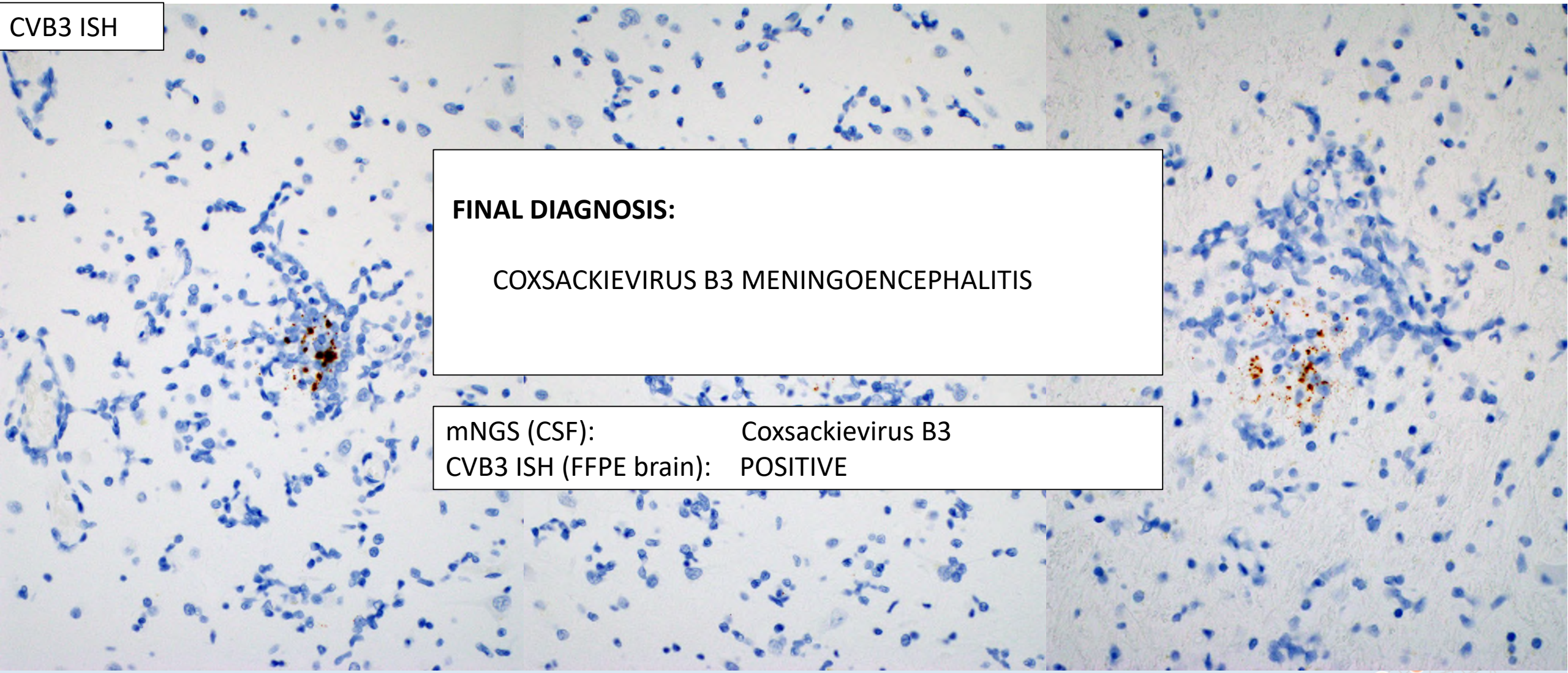
Fungi: NOT DETECTED

Parasites: NOT DETECTED

CVB3 ISH



CVB3 ISH



FINAL DIAGNOSIS:

COXSACKIEVIRUS B3 MENINGOENCEPHALITIS

mNGS (CSF):	Coxsackievirus B3
CVB3 ISH (FFPE brain):	POSITIVE

AANP

Ocrelizumab-associated meningoencephalitis

Sex	Age	PMH	Infection	Ocrelizumab exposure	Last dose prior to presentation	Survival	References
Female	50 yo	RRMS	Coxsackievirus B3	5 years 2 months	2 months	1 month after diagnosis	
Male	61 yo	RRMS	Herpes simplex virus-2	1 year	1 month	Unknown	M. I. R. Dudek et al. 2019
Male	78 yo	RRMS	PML	2 years	6 months	1 month after diagnosis	A. Patel et al. 2021
Female	61 yo	SPMS, obesity, hypertension, dislipidemia, eczema, Raynaud disease, Gastroesophageal reflux disease, Autoimmune hypothyroidism, and shingles	West Nile virus	4 years	2 months	Unknown	S. Thebault et al. 2023
Female	43 yo	RRMS, basal cell carcinoma, genital HSV, recurrent sacral shingles, and depression	West Nile virus	4 years	8 months	Unknown	S. Thebault et al. 2023
Female	41 yo	RRMS and papillary thyroid carcinoma	West Nile virus	6 months	5 months	Unknown	S. Thebault et al. 2023
Female	46 yo	RRMS, hypertension, epilepsy, breast cancer	West Nile virus	5 months	4 months	Unknown	S. Thebault et al. 2023
Female	60s	RRMS, gastroesophageal reflux disease, and hypothyroidism	West Nile virus	5 years	Unspecified	Unknown	T. Johnson et al. 2024
Female	40s	RRMS, basal cell carcinoma of the skin, and depression	West Nile virus	5 years	Unspecified	Unknown	T. Johnson et al. 2024
Female	40s	RRMS, thyroid cancer	West Nile virus	6 months	Unspecified	Unknown	T. Johnson et al. 2024
Female	57 yo	RRMS	PML	4.5 years	4 months	2 months after diagnosis	M. Puig-Casadevall et al. 2023
Female	21 yo	RRMS	MSSA and aspergillus	2 weeks	2 weeks	6 weeks after mental status changes	C. McEntire et al. 2023
Male	34 yo	RRMS	PML	1 month	1 month	Unknown	A. Toorop et al. 2021
Male	37 yo	RRMS	PML	42 days	42 days	Unknown	A. Toorop et al. 2021
Male	46 yo	RRMS	Varizella zoster virus	4 months	4 months	Unknown	P. Goyal et al. 2021
Female	70 yo	RRMS and hypothyroidism	Aseptic meningitis	1 year	6 months	Unknown	M. Theriault et al. 2020
Male	54yo	RRMS, hypertension, and hyperlipidemia	Aseptic meningitis	1 year	6 months	Unknown	M. Theriault et al. 2020

Santos *et al*. (in preparation)

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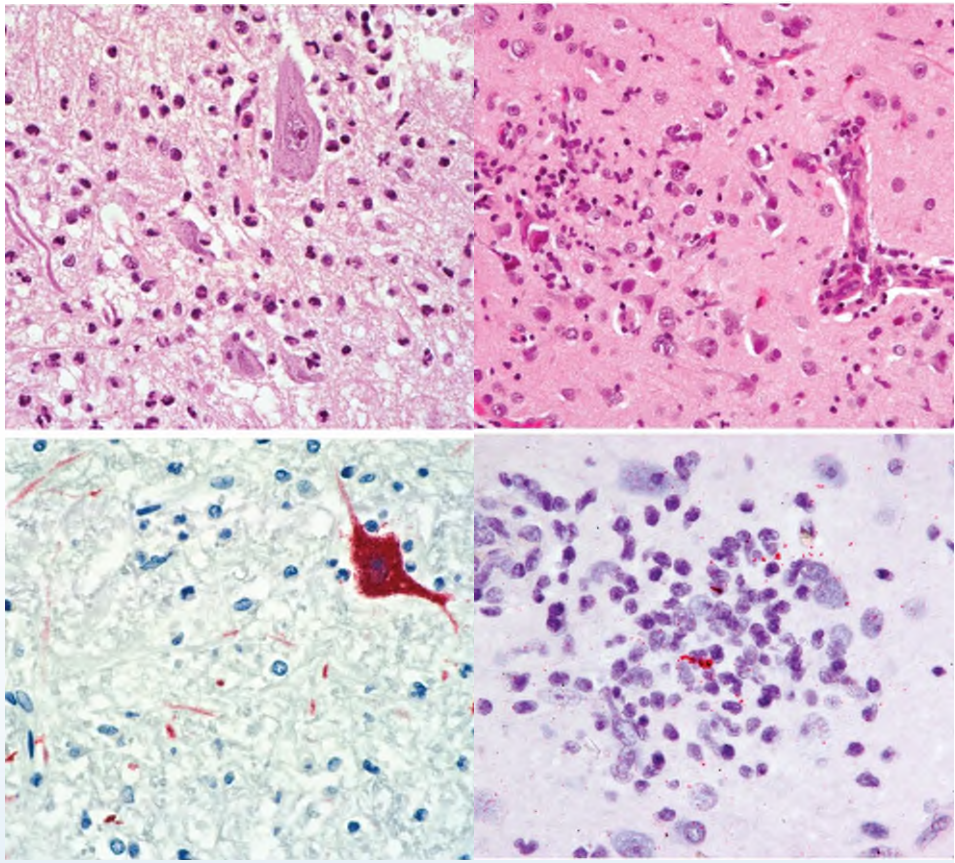
Enterovirus Infections

- Picornaviridae family: non-enveloped, icosahedral, ssRNA viruses
  - Enterovirus-A: EV-71 and several Coxsackievirus group A (CVA) viruses
  - Enterovirus-B: Coxsackievirus group B (CVB) viruses and echoviruses
  - Enterovirus-C: Polioviruses 1–3 and several CVA viruses
  - Enterovirus-D: EV-68 and EV−70
- Aseptic meningitis: leptomeningitis, edema, lymphohistiocytic infiltration; parenchyma with perivascular lymphocytic cuffing and neutrophils
- Encephalitis/encephalomyelitis: gliosis, microglial nodules, neuronophagia, neutrophilic infiltrates and necrosis; neuronal dropout in anterior and posterior horns of the spinal cord
- EV71: inflammation in the brain stem nuclei and spinal cord more severe than cortex/cerebellum; prominent neutrophils
- IHC positive in neuron cell bodies, neuronal processes, and inflammatory foci; later in illness IHC may be negative or only present as granules within microglial nodules
- 5’ UTR PCR for detection but cannot accurately identify serotypes
- VP1 capsid gene for typing due to variability of the region



Fatal EV-71 infection brainstem

EV-A encephalitis with several month-long disease course

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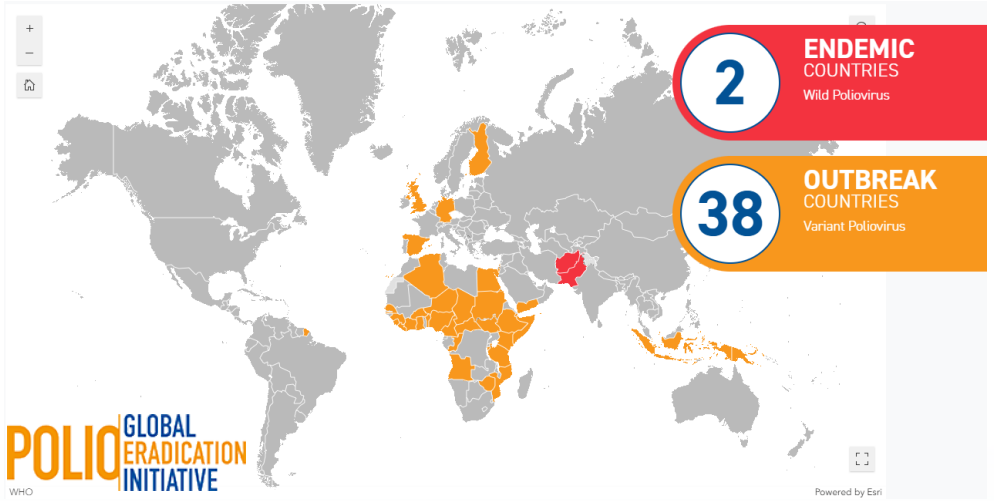
Muehlenbachs *et al*. (2015) *J Pathol*. 235(2):217-28.

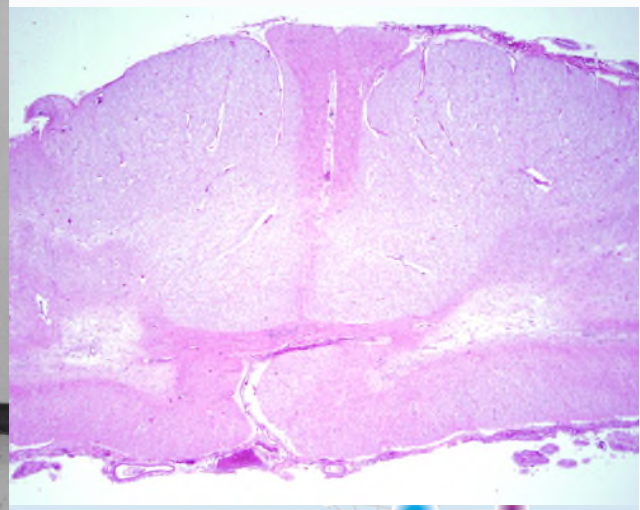
Polio

- No wild polio acquired in US since 1979 (last imported was 1993)
  - Most infections asymptomatic, 25% have self-limited flu-like symptoms
  - <1% have weakness/paralysis in their arms and/or legs, which can lead to permanent disability and death
  - 25-40% of adults with childhood paralytic polio develop post-polio syndrome (PPS) 15 to 40 years later with progressive muscle weakness and joint/muscle pain
- Non-polio enterovirus outbreaks
  - Coxsackievirus A16: most common cause of hand, foot, and mouth disease (HFMD) in US
  - Coxsackievirus A6: large outbreak of severe HFMD in 2012
  - Coxsackievirus A24 and enterovirus 70: outbreak of conjunctivitis
  - Echoviruses 13, 18, and 30: outbreaks of viral meningitis
  - Enterovirus D68: outbreaks in 2014, 2016, and 2018 with respiratory illness



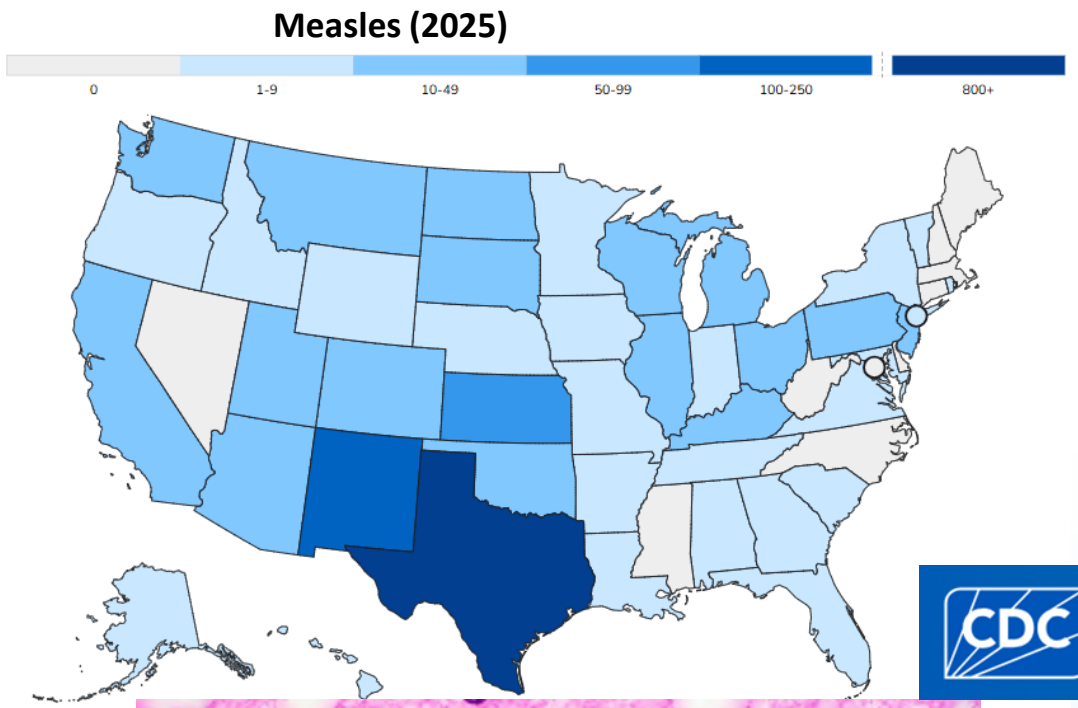
https://polioeradication.org/gisc-world-map/

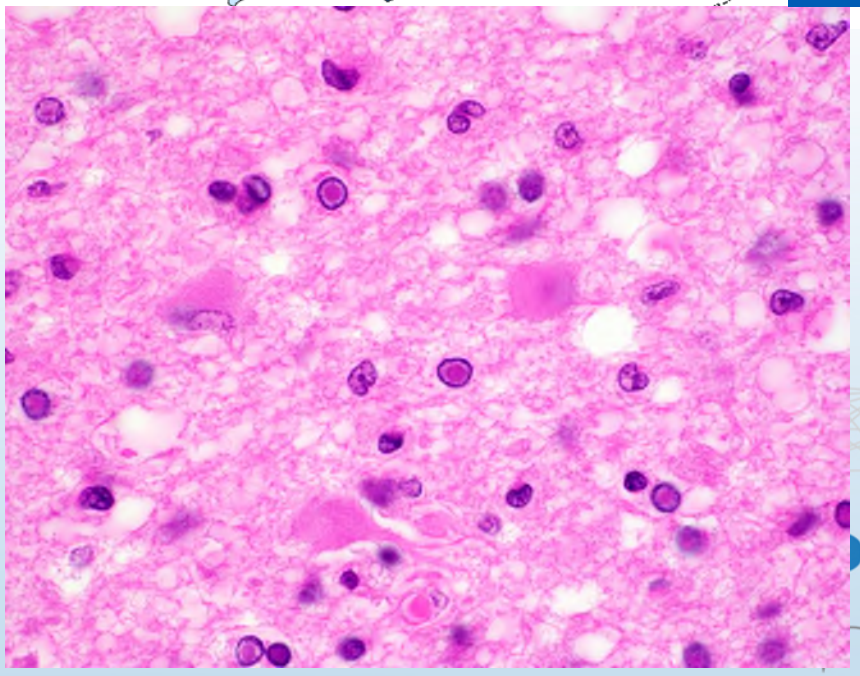
Courtesy CDC/PHIL



Measles

- Declared eradicated from US in 2000; highly contagious in non-immune population leading to clusters of cases
- Death occurs in 1-3 per 1,000 children due to respiratory and neurologic complications
- Four types of CNS involvement:
  - Primary measles encephalitis (rash phase): 1-3 per 1,000; infects endothelial cells leading to edema
  - Acute post-measles encephalitis (2-30 days): 1 per 1,000; immune-mediated demyelinating reaction
  - Measles inclusion body encephalitis (1 year): persistent viral infection in immunodeficient children
  - Subacute sclerosing panencephalitis (7-10 years): 1 per 25,000; atrophic lesions in gray matter, periventricular white matter, and brainstem, resulting in coma and death; eosinophilic intranuclear inclusion bodies within neurons and glial cells



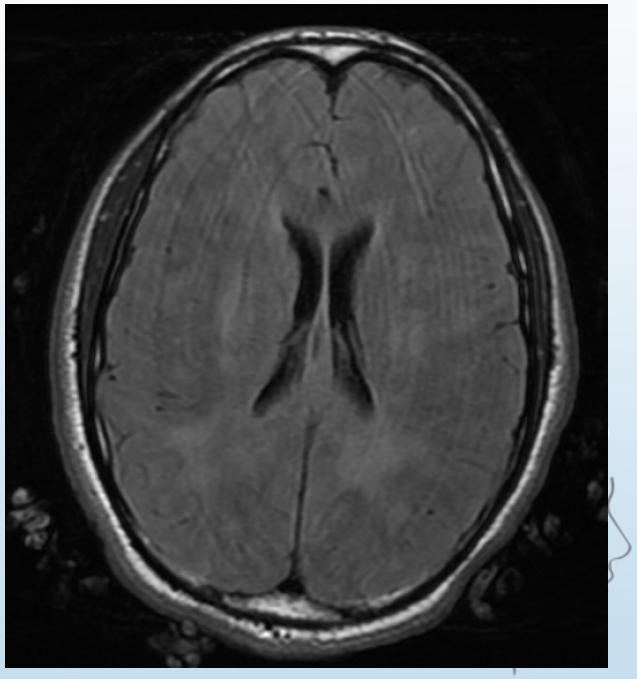


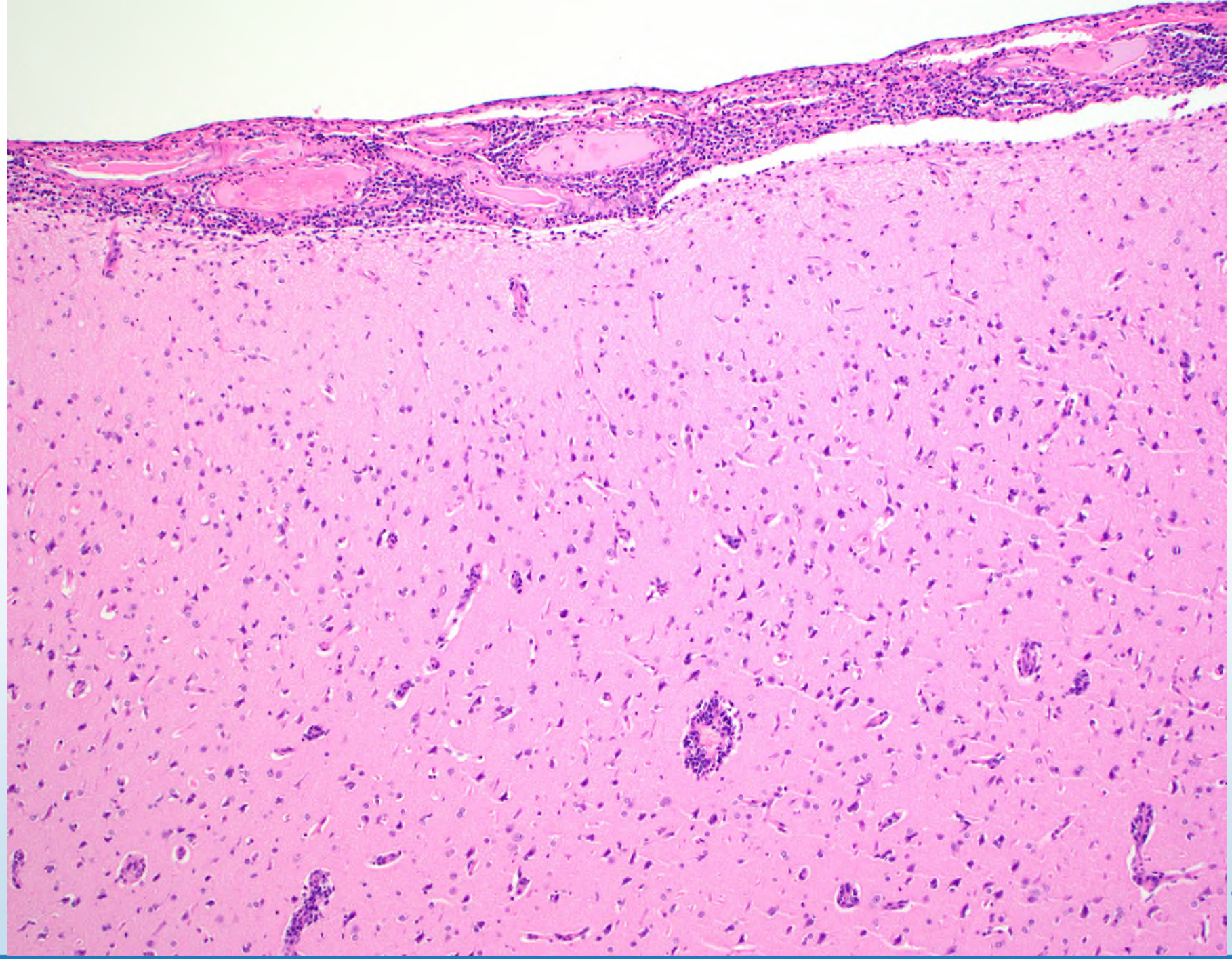
Klassen-Fischer et al. (2023) Am J Clin Pathol. 159(1):81-88.

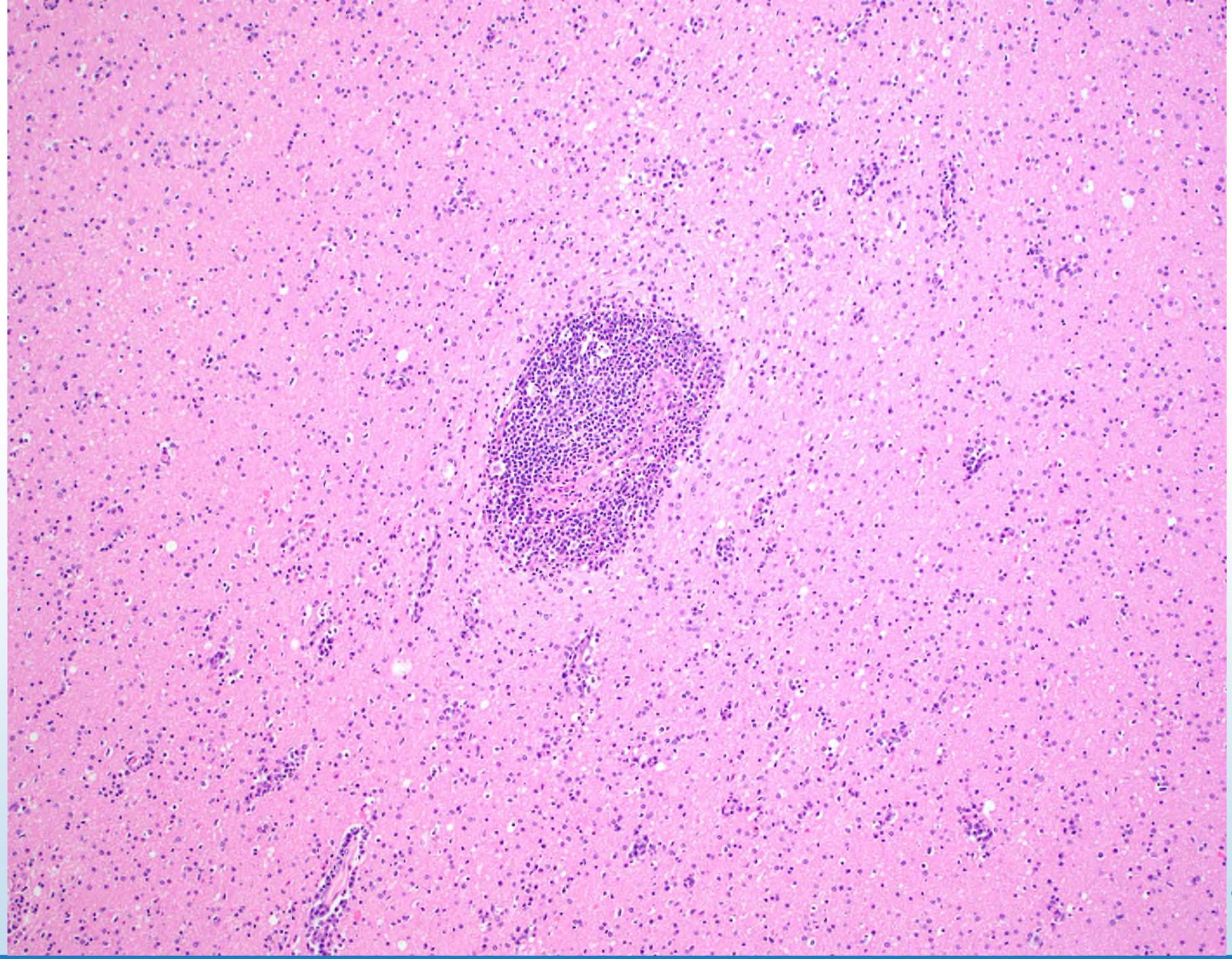
Case #3

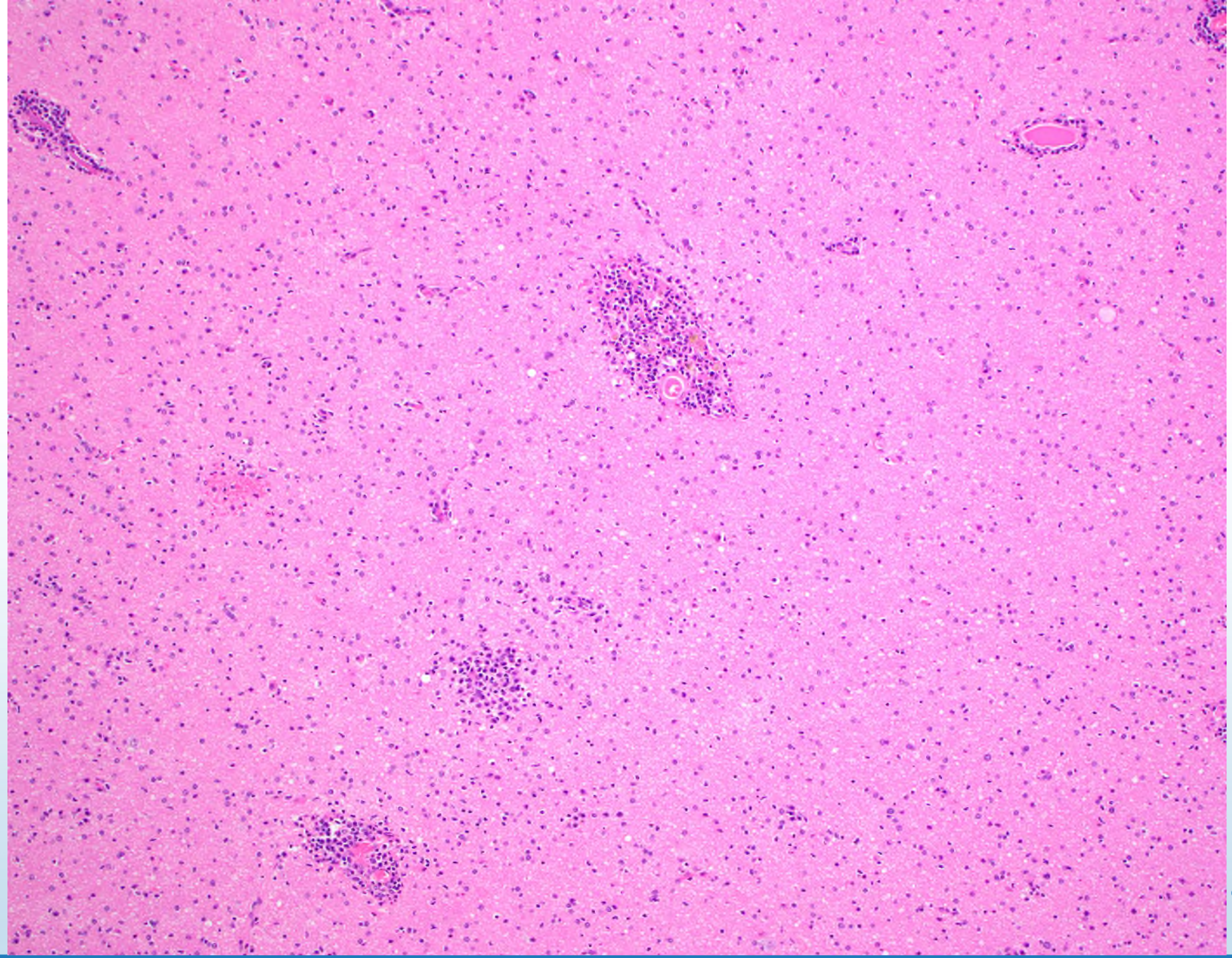
- 31-year-old man with confusion, nausea, fever
- Started on CTX/Vanc/Acyclovir meningitic coverage
- CT/MRI showed diffuse cerebral edema
- HIV screen positive, CD4 count 424, VL 182,000
- CSF HIV viral load: 586,996 cp/mL

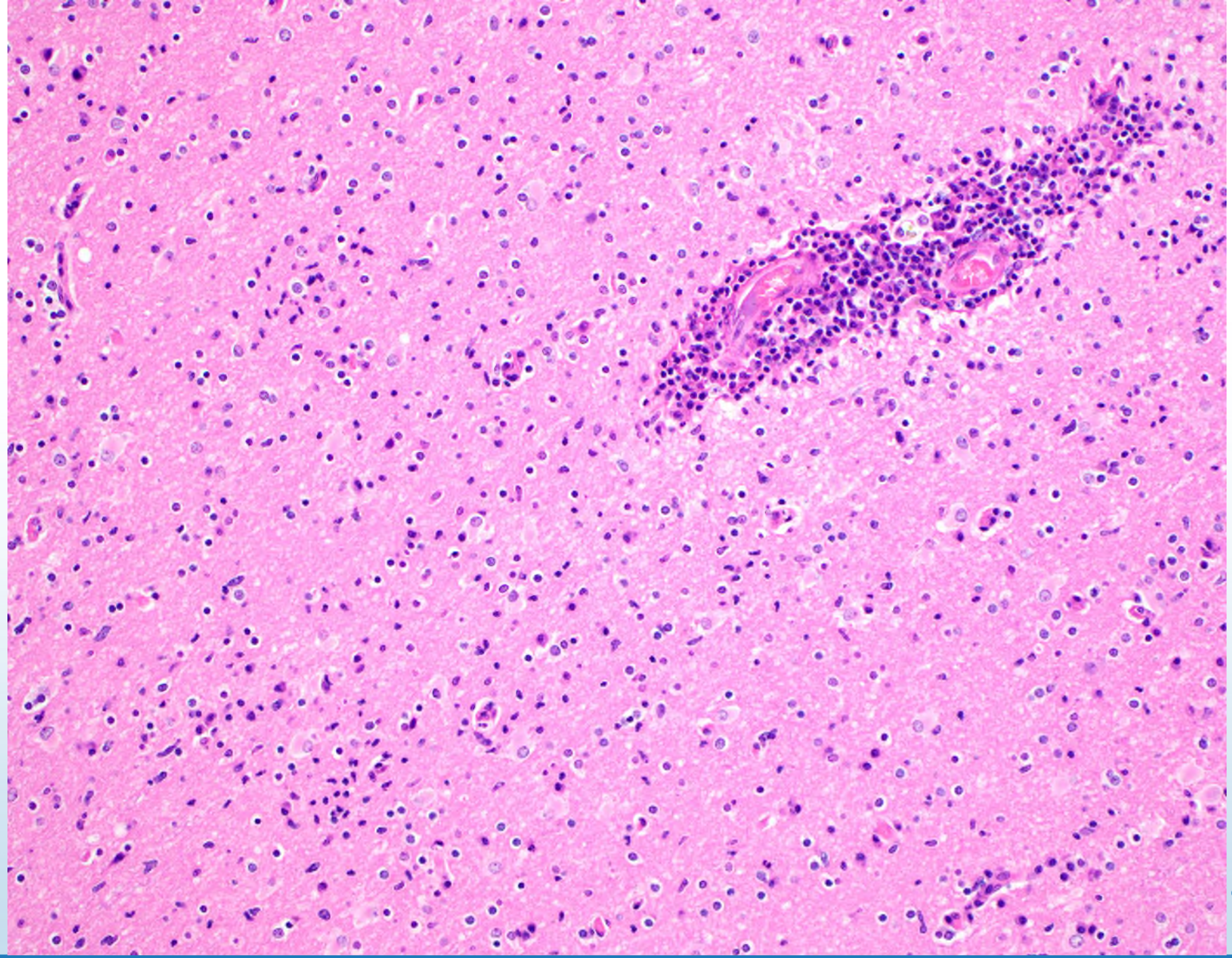
- Re-presented from rehab with worsening headache, nausea, vomiting
- Treated with methylprednisolone and plasma exchange (PLEX) -> brain biopsy

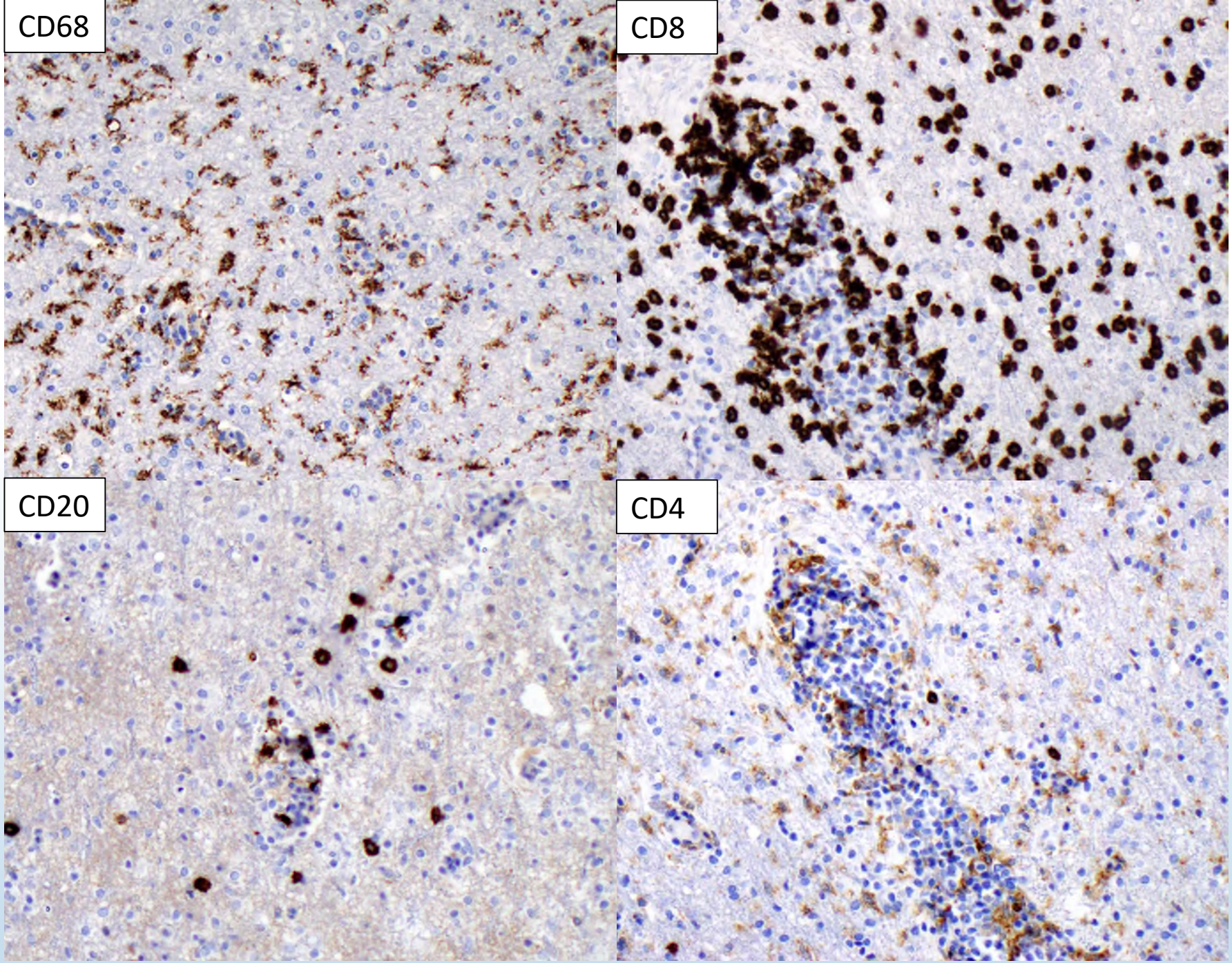


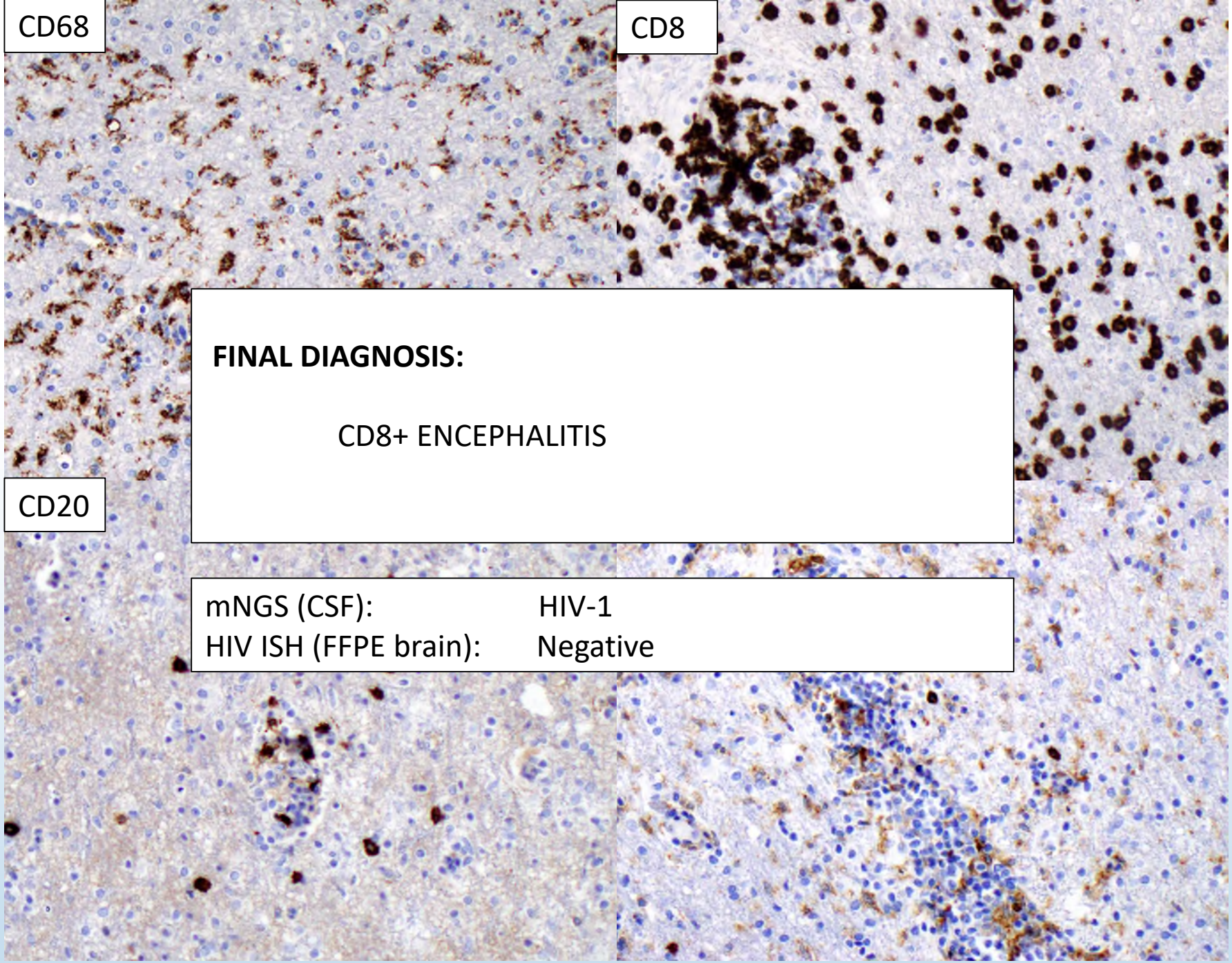









CD68
CD8
CD20
CD4



FINAL DIAGNOSIS:

CD8+ ENCEPHALITIS

mNGS (CSF):	HIV-1
HIV ISH (FFPE brain):	Negative

HIV CNS Sequelae

HIV-Associated Dementia (HAD)

HIV-Associated Neurocognitive Disorder (HAND)

HIV Encephalitis

HIV Encephalopathy

Demyelinating leukoencephalopathy

CD8+ Encephalitis

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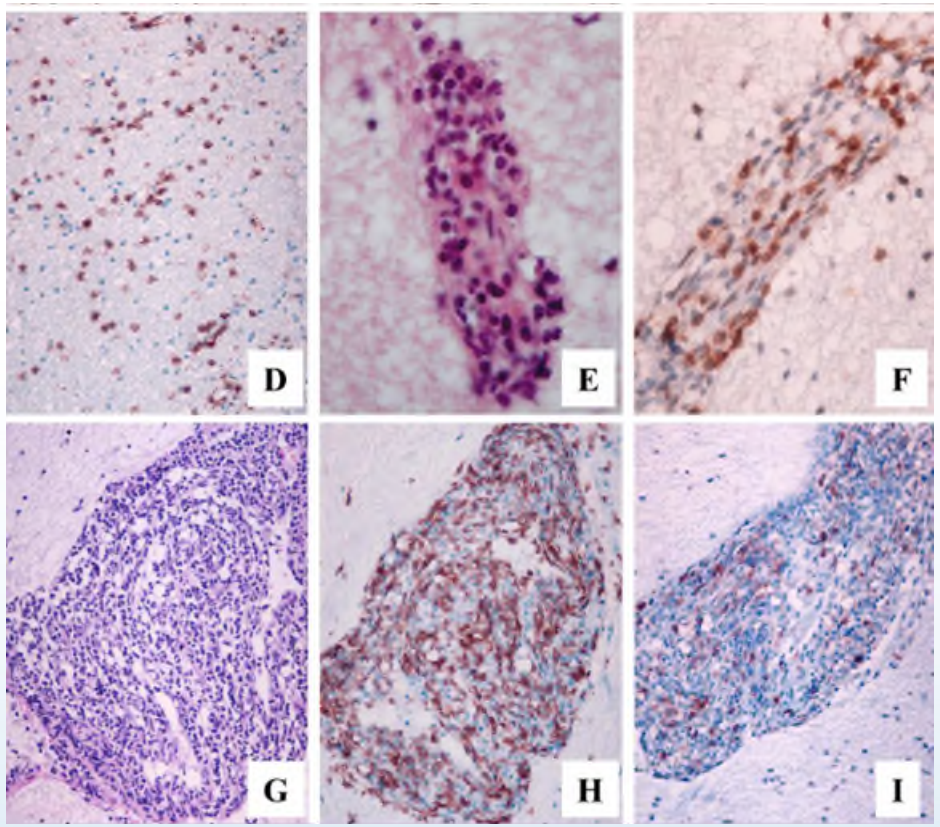
CD8+ Encephalitis

- Rapidly progressive subacute encephalopathy Biopsy shows encephalitis with diffuse, perivascular and intraparenchymal infiltration by CD8+ T-lymphocytes +/- myelin loss, slight axonal damage, weak HIV p24 expression; negative for other pathogens
- Spectrum of immune reconstitution disease in which CD8+ cytotoxic lymphocytes may cause harm by escalation of a smoldering infection and/or an immunopathological reaction similar to ADEM



Gray *et al*. (2013) *Brain Pathol*. 23(5):525-33.
Lescure *et al.* (2013) *Clin Infect Dis*. 57(1):101-8.

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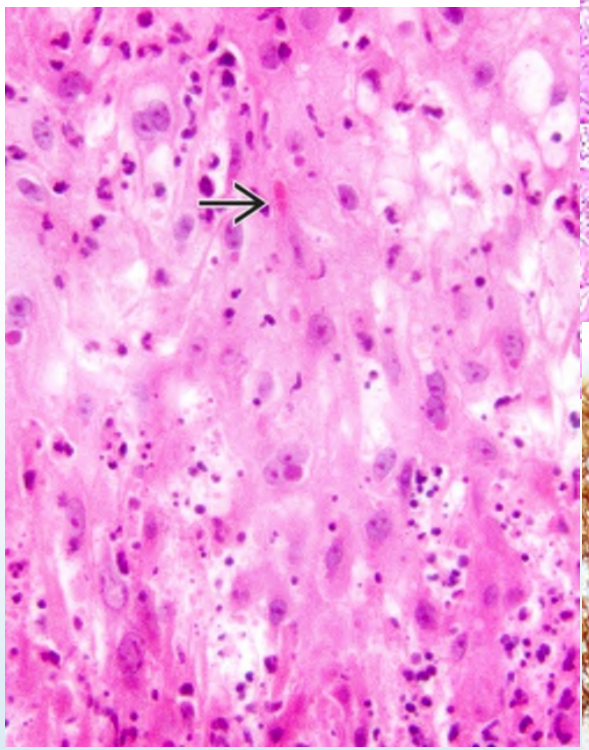
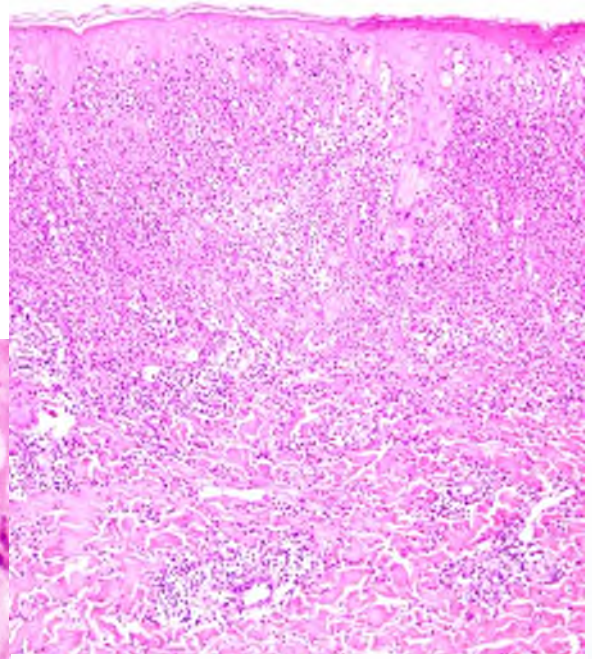
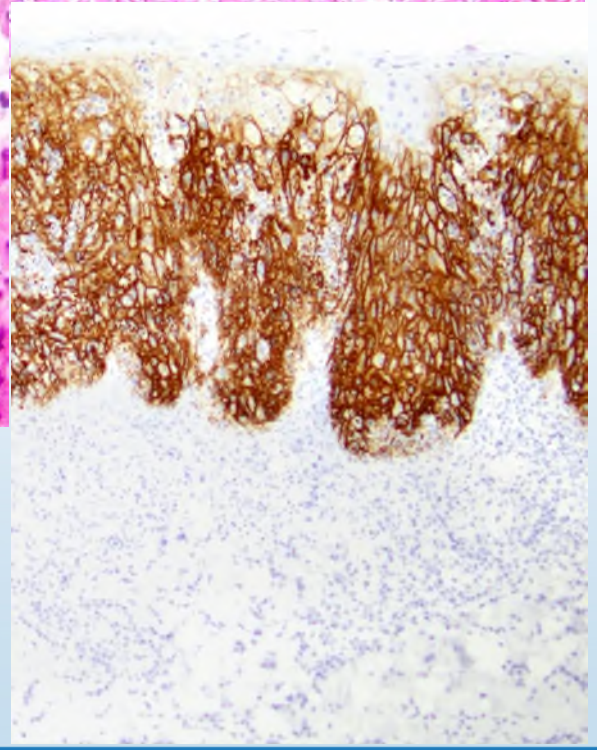
Mpox (Monkeypox virus disease)

- dsDNA orthopoxvirus transmitted through close personal contact including sexual activity
- Vaccine preventable; treated with tecovirimat and supportive care
- Epidemiology and mortality vary with clade/subclade (Ia, Ib, IIa, IIb); majority of fatal cases in immunocompromised
- Diagnosed by RT-PCR of lesional swab (occasionally by skin biopsy)

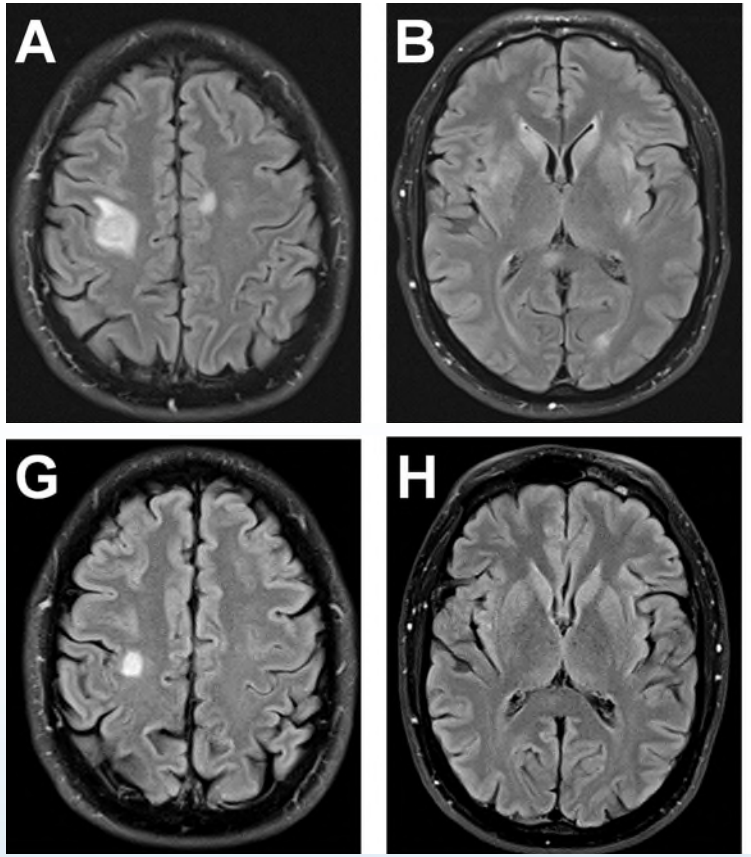






Mpox Involvement in the CNS

- Rare neurological symptoms include headaches, myalgias, seizures, altered consciousness, and encephalopathy/ encephalitis
- 3 patient case series
  - CSF lymphocytic pleocytosis, elevated CSF protein
  - Negative CSF MPXV PCR and/or serology
  - MRI with subcortical, deep gray matter, cerebellum, and/or brainstem lesions +/- multifocal spinal lesions
  - Treated with acute immunomodulatory therapies with rapid improvement and good neurologic recovery
- Hypothesized a parainfectious autoimmune process or indirect viral-mediated CNS inflammation resulting from an overexuberant host immune response



Money *et al*. (2023) *Ann Neurol*. 93(5):893-905.

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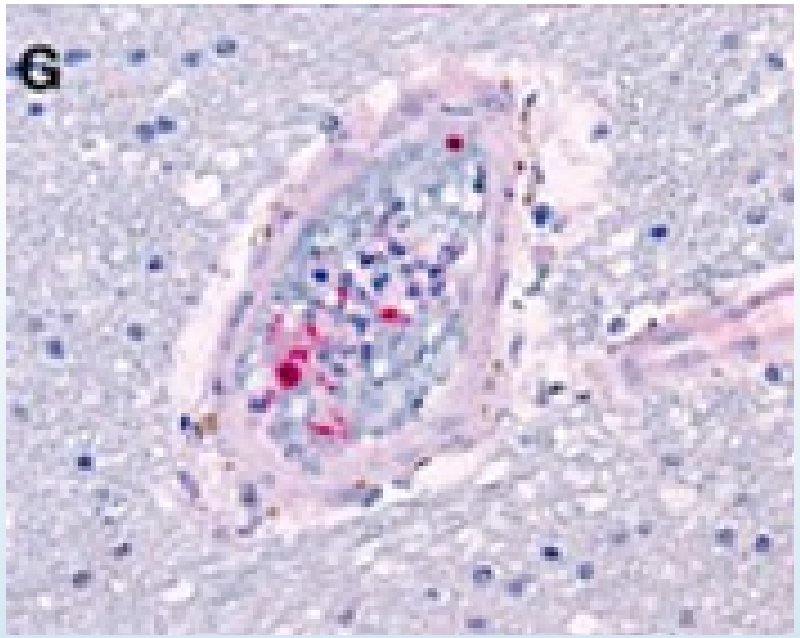
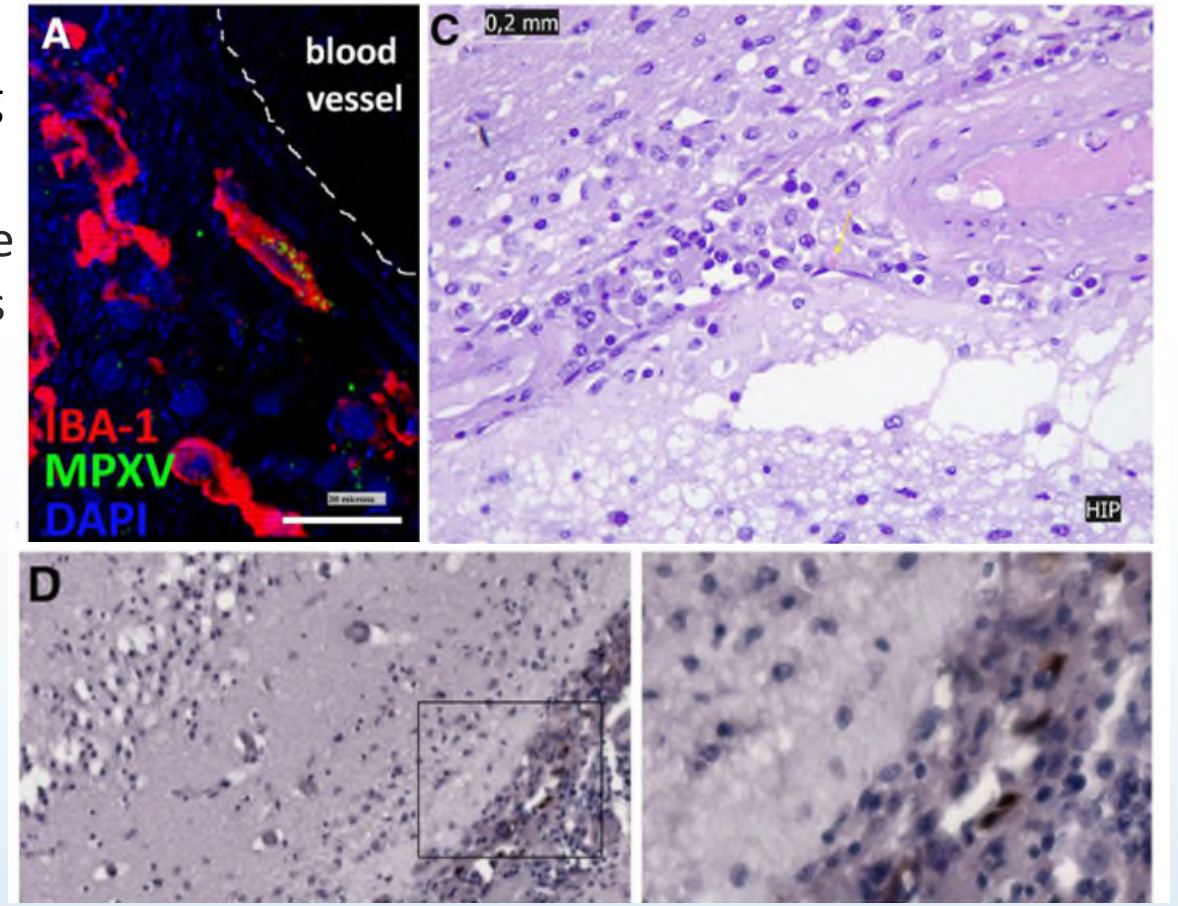
Mpox Neuropathology

- Few autopsy reports with brain examination or finding
- Research studies suggest MPXV preferentially infects human astrocytes > microglia and oligodendrocyte-like cells, activating inflammatory response and pyroptosis



Renal transplant patient. IHC staining of intravascular leukocytes in the brain.



Fatal Mpox-associated meningoencephalitis with negative CSF PCR

Ritter *et al*. (2024) *J Infect Dis*. 229(Supplement_2):S219-S228.
Miranzadeh Mahabadi *et al.* (2025) *Brain*. awaf181.

Lopez *et al.* (2005) *Crit Care Explor*;7(6):e1272.

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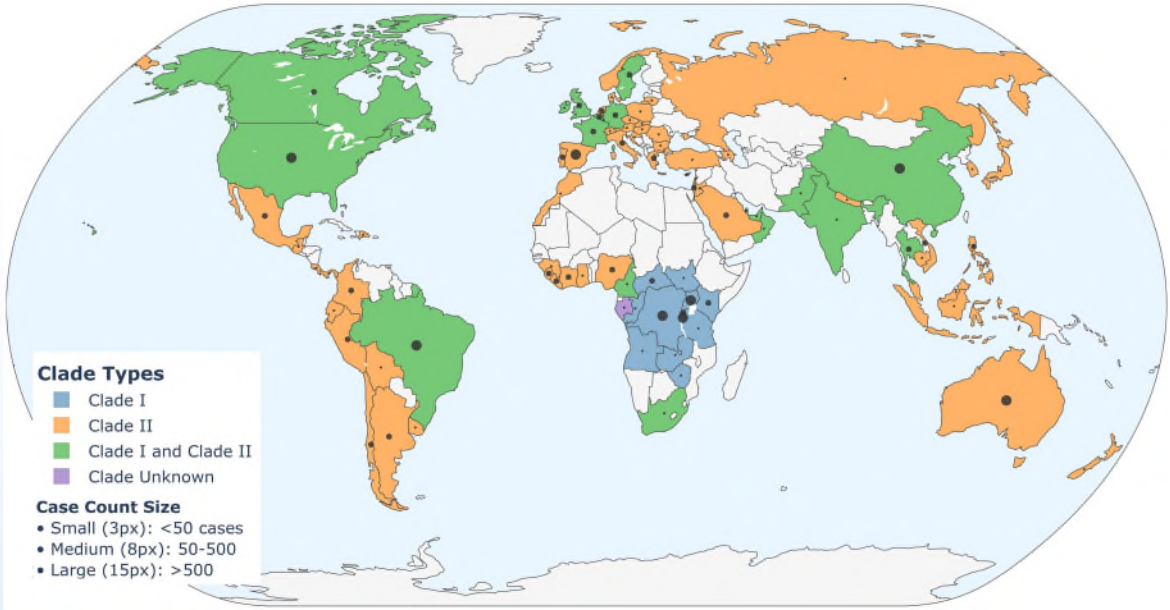
Orthopoxviruses with CNS complications

Virus	Animal to human transmission	Human to human transmission	Systemic signs and symptoms	Neurotropism and neurovirulence
Monkeypox (MPXV)	Common (e.g. rodents, primates)	Limited (e.g. close contact)	Fever, rash, lymphadenopathy, myalgia	Neurological complications (encephalitis, seizures)
Smallpox (VARV)	No	Yes (aerosol, direct contact)	Fever, rash, systemic infection	Rare (encephalitis in severe cases)
Vaccinia (VACV)	Rare (laboratory animals)	Rare (e.g. vaccine contact)	Localized rash, fever, fatigue	Rare (encephalitis, myelitis in immunocompromised), Strain dependent
Cowpox (CPXV)	Common (e.g. cats, rodents)	Not reported	Localized lesions, fever	Encephalitis reported in some cases
Camelpox (CMXV)	Rare (close contact with infected animals)	Not reported	Fever, nasal discharge, and lesions	Not reported
Ahkmeta	Rare (contact with infected animals)	Not reported	Cutaneious lesions	Not reported



Miranzadeh Mahabadi *et al.* (2025) *Brain*. awaf181.

Conclusions

- Viruses are an extremely diverse set of pathogens causing significant morbidity and mortality in humans
- Emerging viral infections may be difficult to diagnose due to non-specific symptoms or histological findings
- A high degree of clinician suspicion and appropriate use of ancillary testing is needed to confirm a specific diagnosis

Questions?

Brigham and Women's Hospital
Founding Member, Mass General Brigham

HARVARD MEDICAL SCHOOL

ihsolomon@bwh.harvard.edu

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