

From Biopsy and Necropsy to Diagnosis

Invaluable Special and Immunohistochemical Stains

Wisconsin Histology Society

Education Series

Wisconsin Veterinary Diagnostic Laboratory

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What we'll cover this morning.....

- Overview of transmissible spongiform encephalopathies and the use of immunohistochemistry in diagnostics
- Domestic cat death – Necropsy, Histopathology, and Special Staining for Diagnosis
- Mast cell tumor cases – Invaluable Special Stains for Histopathologic Interpretation and Diagnosis
- Canine oral tumor case – Diagnostic Immunohistochemistry (when special stains aren't enough for diagnosis)

Overview of Transmissible Spongiform Encephalopathies & Diagnostic Immunohistochemistry



What are TSEs?

- Diseases that affect humans and animals
- Caused by Infectious/Transmissible proteins

1. Abnormal form of normal host protein
 - Lacks nucleic acid (can't replicate on its own)
2. PrP^{res} = protease resistant prion protein

1. Normal host protein

- PrP^C = normal prion protein
 - Membrane bound glycoprotein
 - *PRNP* gene
 - Expression (though not limited to)
Neural and lymphoreticular tissue

2. Highly resistant to UV light, radiation, alcohol, disinfectants (including formalin), and heat
 - Wet heat more effective than dry heat
 - **Environmental stability**



TSE prions (PrP^{res}) differ between hosts

Animal prion diseases			
Disease	Host	Etiology	Year of Description
Scrapie	Sheep,	Infection with Prions of unknown origin	1732
	Goats		1942
TME	Mink	Infection with Prions of either sheep or cattle origin	1947
CWD	Cervids	Infection with Prions of unknown origin	1967
BSE	Cattle	Infection with Prions of unknown origin	1986
EUE	Nyala, Kudu	Infection with Prions of BSE origin	1986
FSE	Cats	Infection with prions of BSE origin	1990
NHP	Lemurs	Infection with Prions of BSE origin	1996

Plus 9 human prion diseases
3 are transmissible:

- Kuru (transmissible)
- 4 forms of CJD (2 transmissible)
- GSS (Gerstmann-Straussler-Scheinker syndrome)
- 2 forms of fatal insomnia (familial/spontaneous)
- VPSPR (variably protease-sensitive prionopathy)

Imran and Mahmood (2011) An overview of animal prion diseases. Virology Journal. 8:493

doi: [10.1186/1743-422X-8-493](https://doi.org/10.1186/1743-422X-8-493)

(MH modified)

TSE prions differ between hosts and zoonotic concern

- Sheep scrapie prions – 2 PRP^{res} forms
 1. Nonclassical or atypical scrapie – occurs sporadically in sheep and goats
 - Believed to be nontransmissible (or poorly transmissible) under natural conditions
 2. Classical scrapie – transmissible form
- **Not zoonotic** (no evidence to indicate this is transmissible to humans)
- Chronic wasting disease prion
 - No solid evidence of transmission to humans (not considered zoonotic)
- Bovine spongiform encephalopathy prion
 - Causative agent of:
 - variant Creutzfeld-Jakob disease (vCJD) in humans - **ZOONOTIC**
 - feline spongiform encephalopathy in cats
 - Possible origin of transmissible mink encephalopathy
 - **Not the same as scrapie or CWD prions**

Transmission

- Shed in milk, saliva, feces, and urine
 - Environmental contamination - **Environmental stability**
- Prion ingestion
 - Horizontal: oral
 - Placenta, colostrum/milk ingestion, environmental contamination
- Prenatal transmission
 - Transplacental or “vertical” transmission
 - Semen (?)

Pathogenesis

(how disease occurs after host acquires abnormal protein)

- PrP^{res} → abnormal prion necessary for transmission and disease development
- Two major factors that play a role in pathogenesis:
 1. Host susceptibility: *PRNP* gene and the amino acid sequence of the PrP^C protein
 2. Distribution of PrP^C in the infected host - **neural and lymphoreticular tissue**
- Function of PrP^C not fully characterized
 - Normally located in cell membrane
 - Degraded by lysosomal hydrolase (**PrP^{res} resistant to this degradation**)
- Clinical signs due to
 - Loss of normal PrP^C
 - Accumulation of PrP^{res} in neurons
 - Cytotoxic effects and vacuolation in brain (“spongiform”)

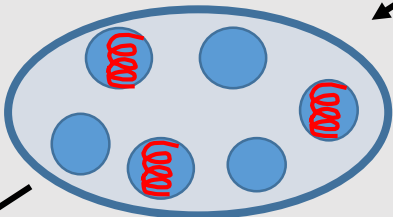
TSE Pathogenesis

Transmission

Ingested

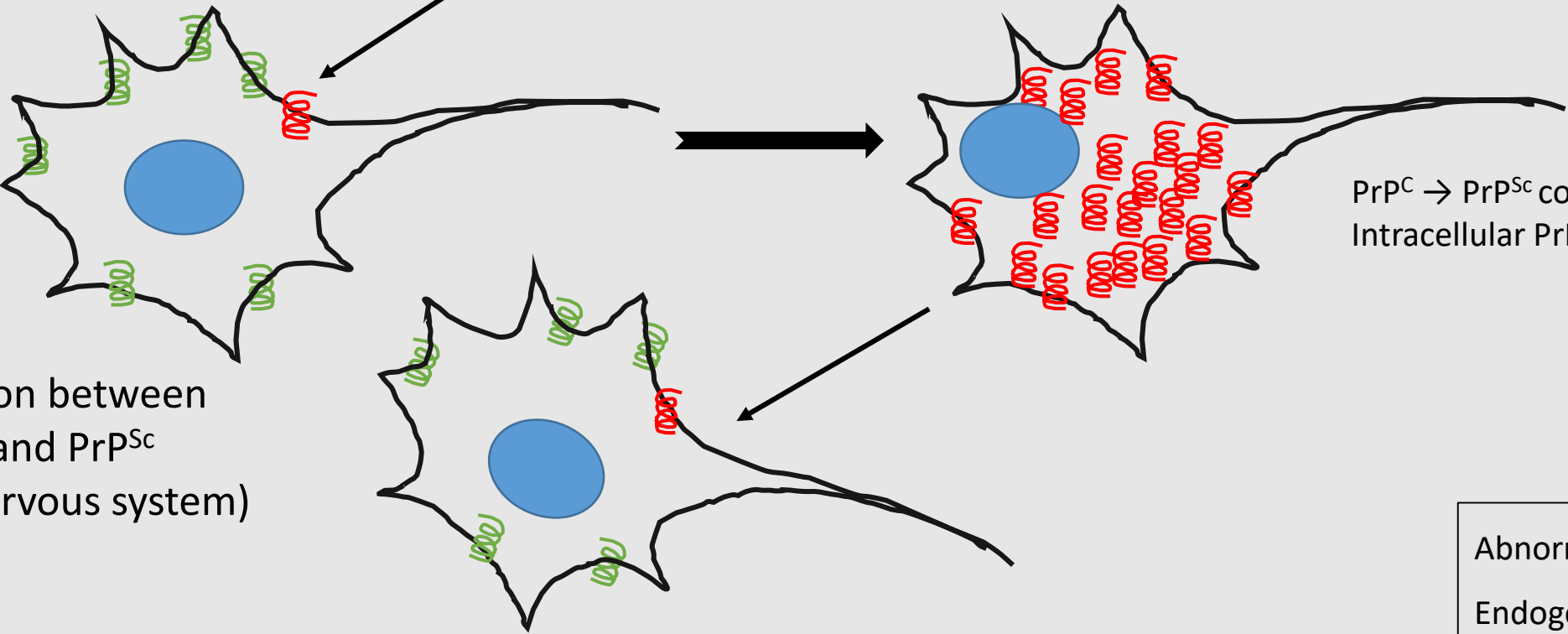
Transplacental

- PrP^{Sc} transmission (classical scrapie, CWD)
- Spontaneous generation of PrP^{Sc} (atypical scrapie)




Lymph node
(GALT/RAJ, Retropharyngeal)


Next slide



PrP^C → PrP^{Sc} conversion
Intracellular PrP^{Sc} accumulation

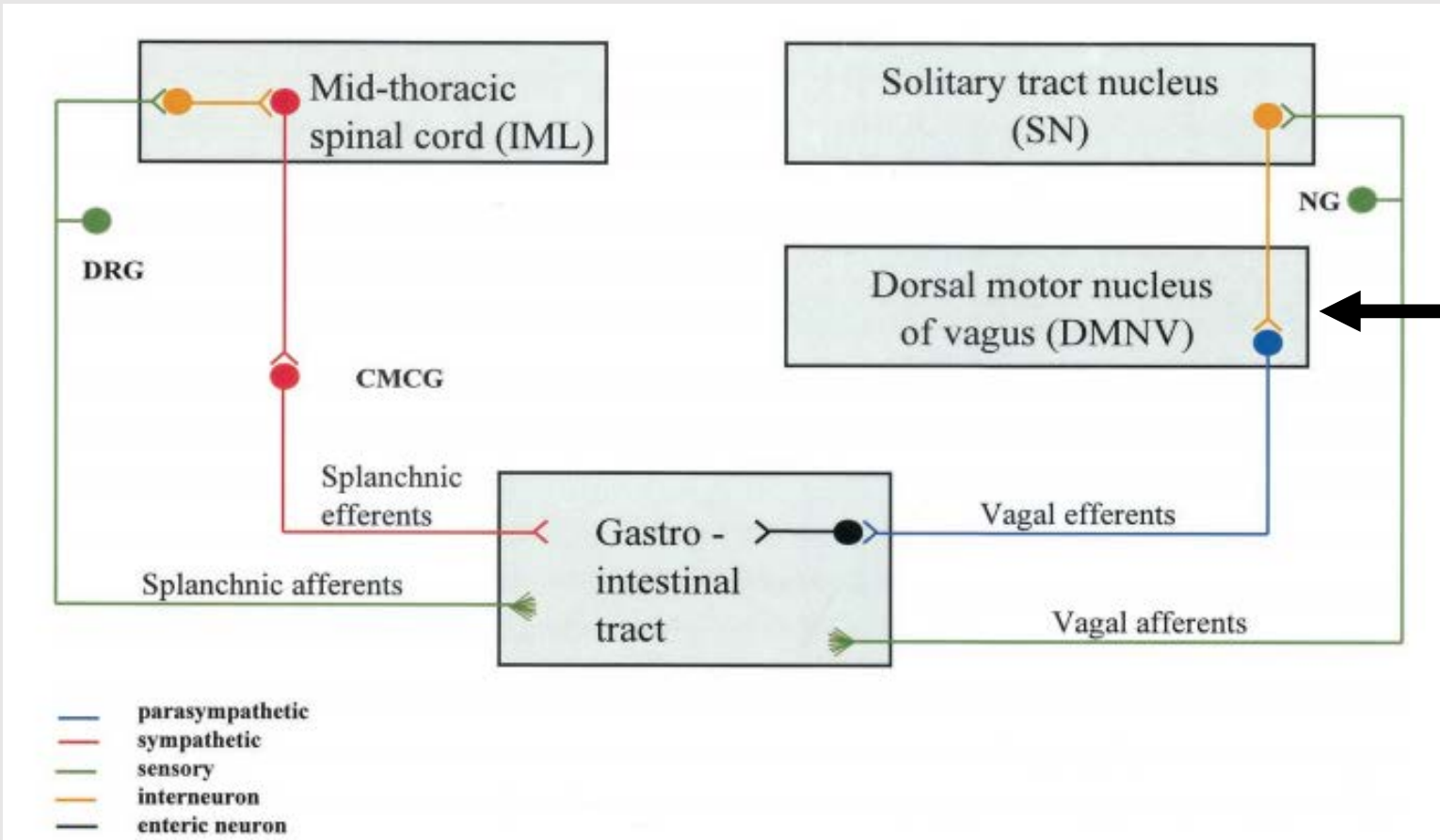
Interaction between
PrP^C and PrP^{Sc}
(central nervous system)

Abnormal PrP^{Sc} 

Endogenous PrP^C 

Lymphoid tissue → Brain

1.



2.

Blood spread → brain entry via the circumventricular organs

- 3rd and 4th ventricles
- Highly vascular structures
- Lack blood-brain barrier

Important in diagnostics (more later)

Animal TSE Testing/Diagnostics

Histopathology of brain (H&E staining) → not definitive (supplemental test only)

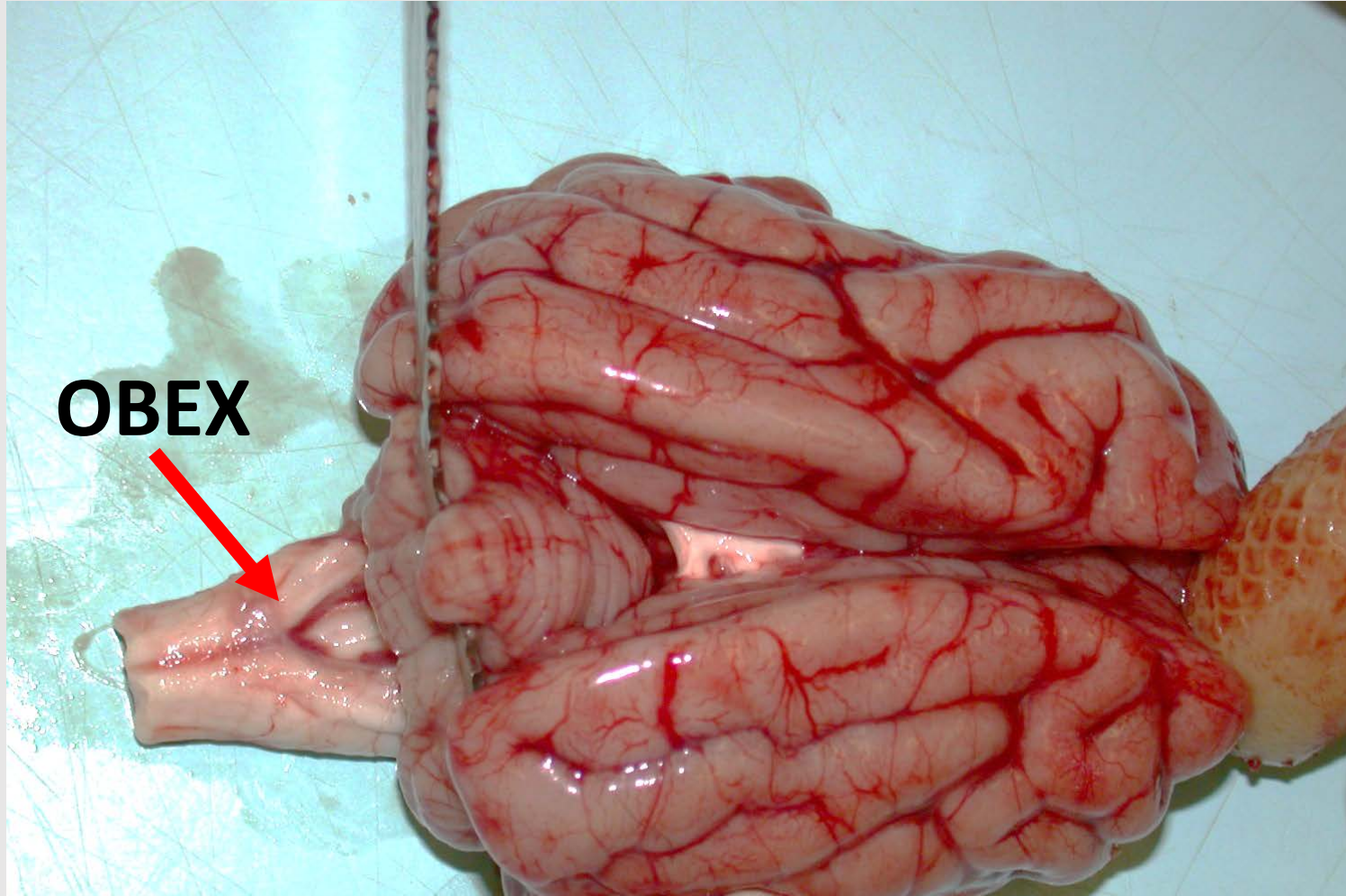
All diagnostics use antibodies that recognize PrP^{res}

(No immune response to prions)

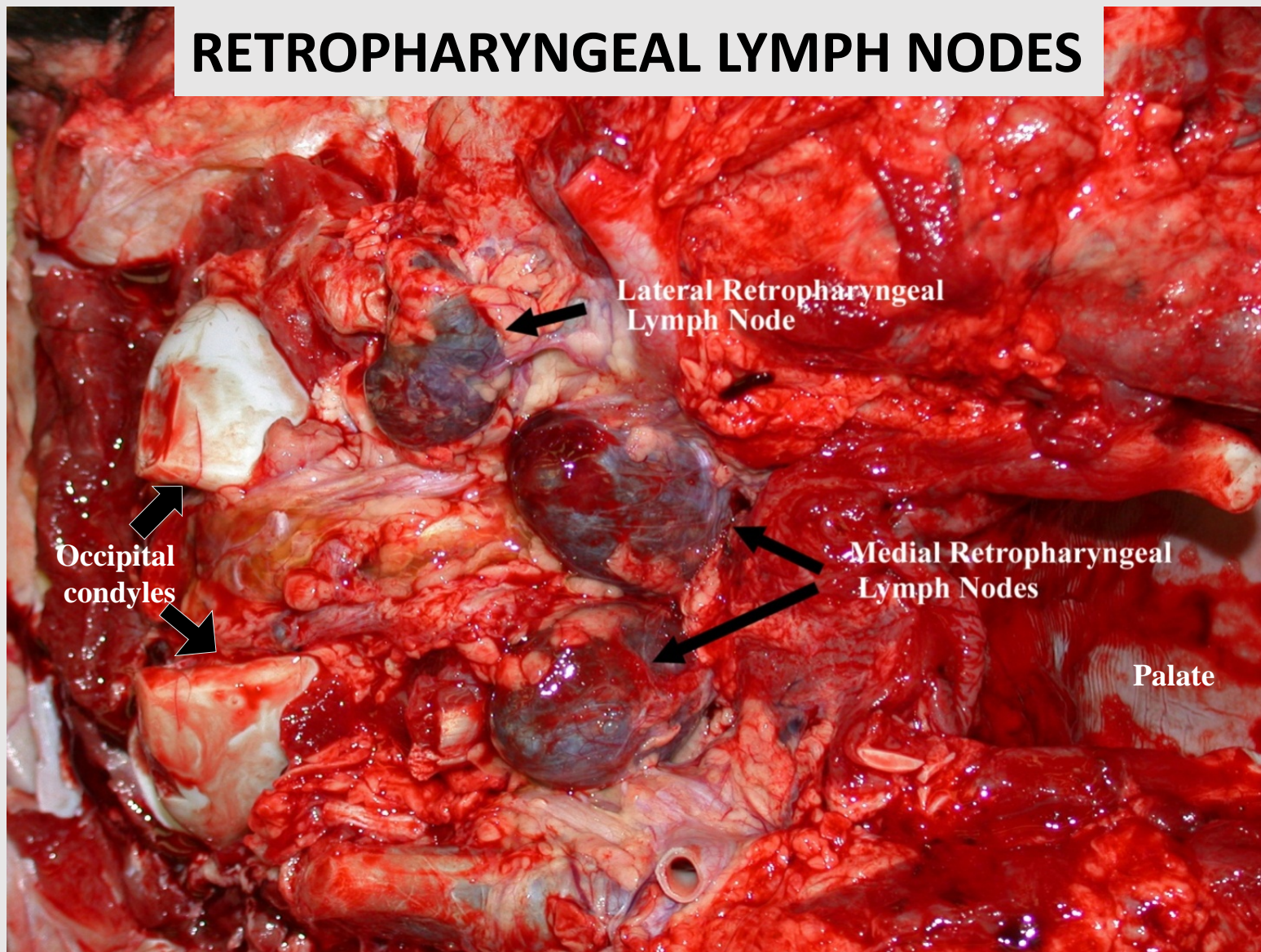
- **Immunohistochemical (IHC) staining → official or gold standard test for diagnosis**
 - Antemortem
 - Lymphoid tissue → recto-anal junction (RAJ), third eyelid, tonsil
 - Postmortem
 - Retropharyngeal lymph nodes, tonsil
 - Brain → obex (location of the dorsal motor nucleus of the vagus)
- ELISA → Screening test (tests for presence of the prion, not host antibodies)
Positive/Detect results are confirmed by IHC

TSE Diagnostics

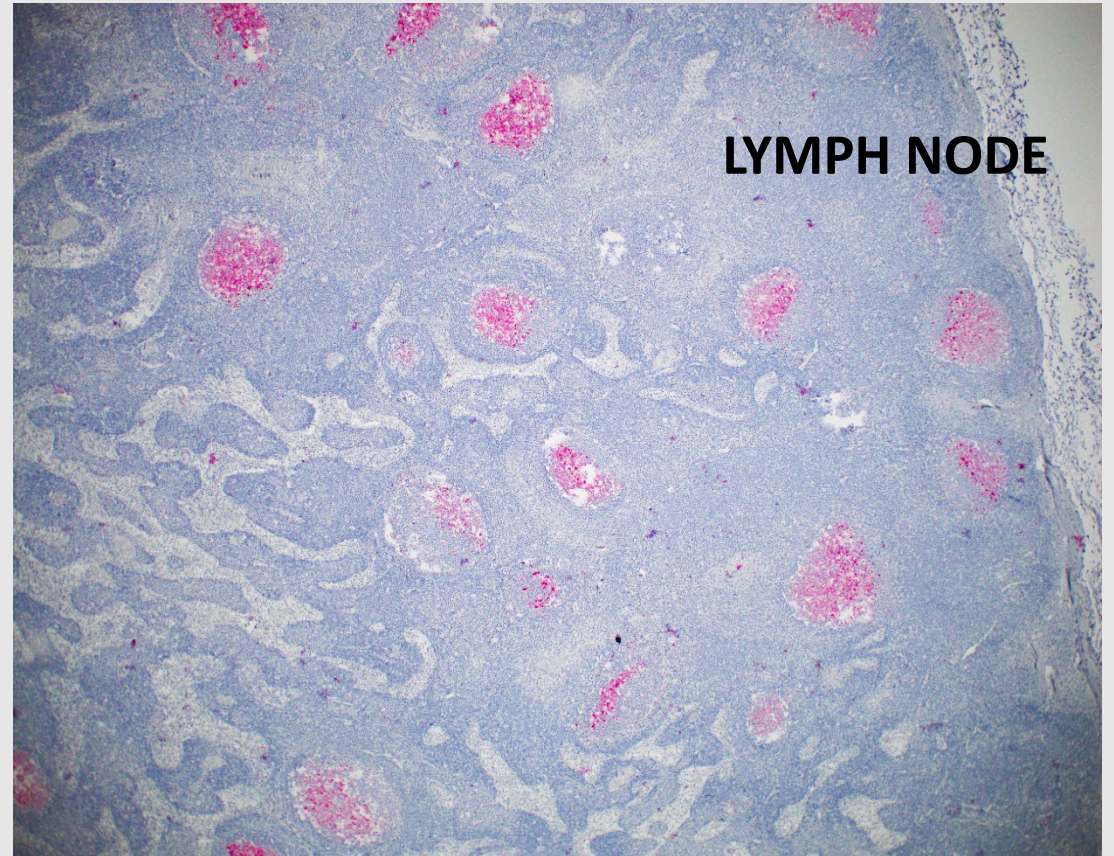
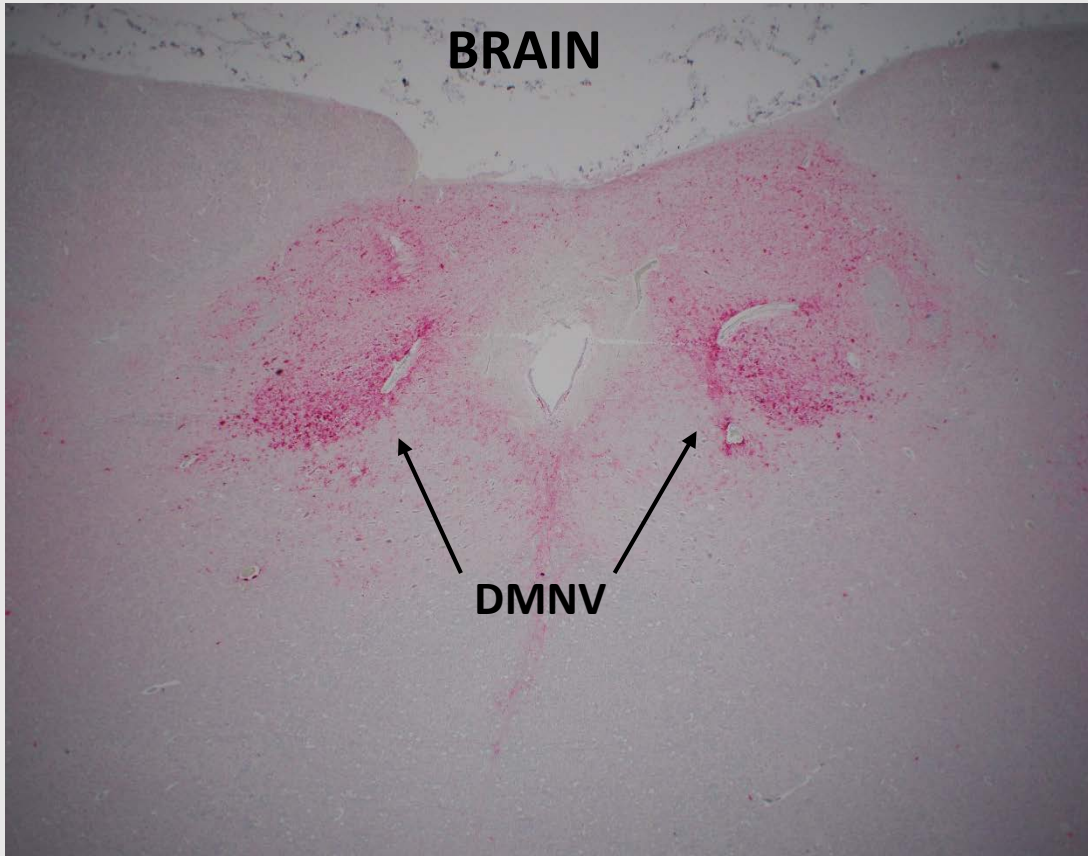
Look for the “V”



RETROPHARYNGEAL LYMPH NODES



Scrapie positive IHC staining



Testing performed in a National Animal Health Laboratory Network laboratory (currently 17 approved testing laboratories in U.S.)

https://www.aphis.usda.gov/animal_health/nahln/downloads/scrapie_lab_list.pdf

TSE Samples Tested at WVDL

<u>Test Code</u>	<u>Count FY22</u>	<u>Count FY23 through April</u>
BSE ELISA	9,431	9,086
CWD ELISA	17,106	16,428
CWD ELISA OS	27,915	29,679
CWDIHCDNR	426	561
CWDIHCOTH	10,787	18,511
CWDIHCWV2	818	1,310
CWD-OBX-LN	2,436	1,809
Scrapie	3,836	3,113
CWD Hunter	3	1
CWDIHCTX1	2	1,871
CWDIHCTX2	0	384
Total	72,760	82,753

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Signalment: 6 year old, intact male, domestic shorthair cat

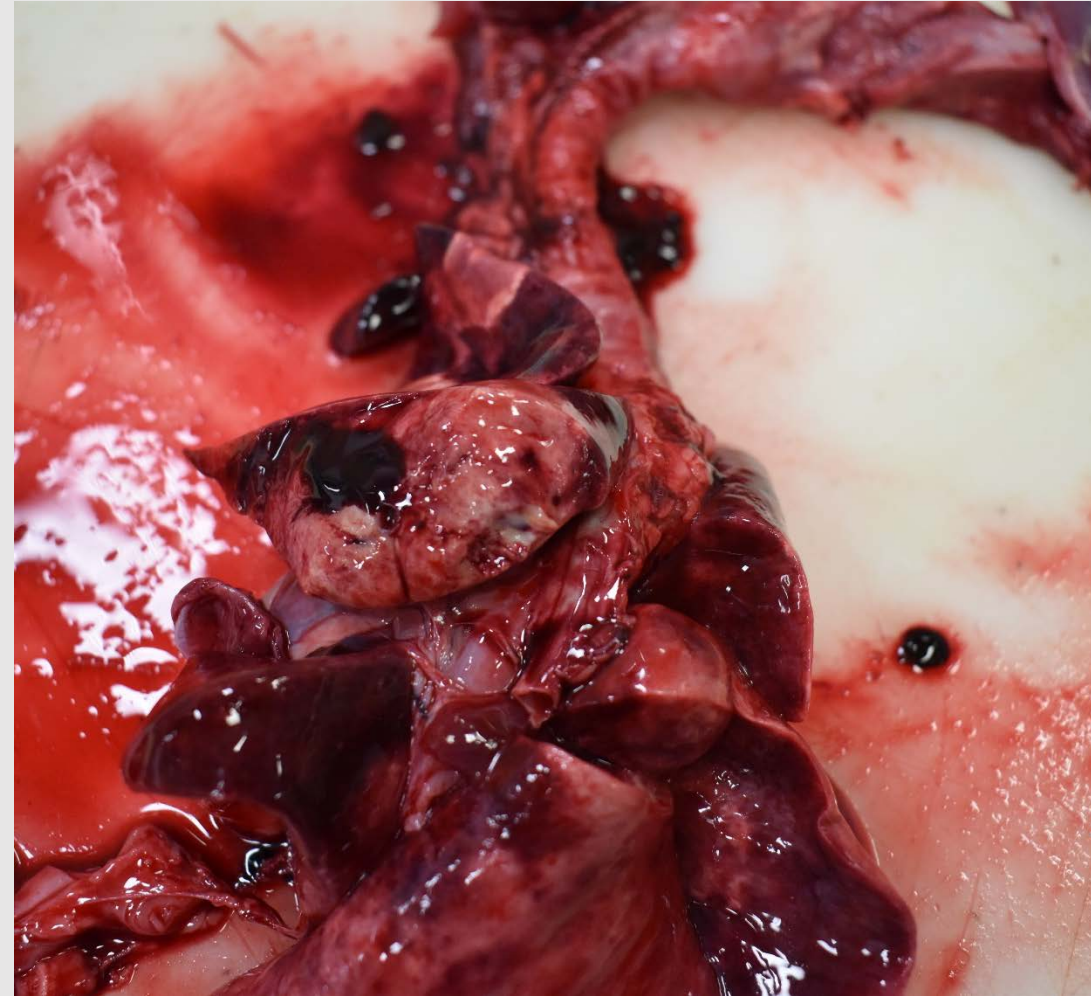
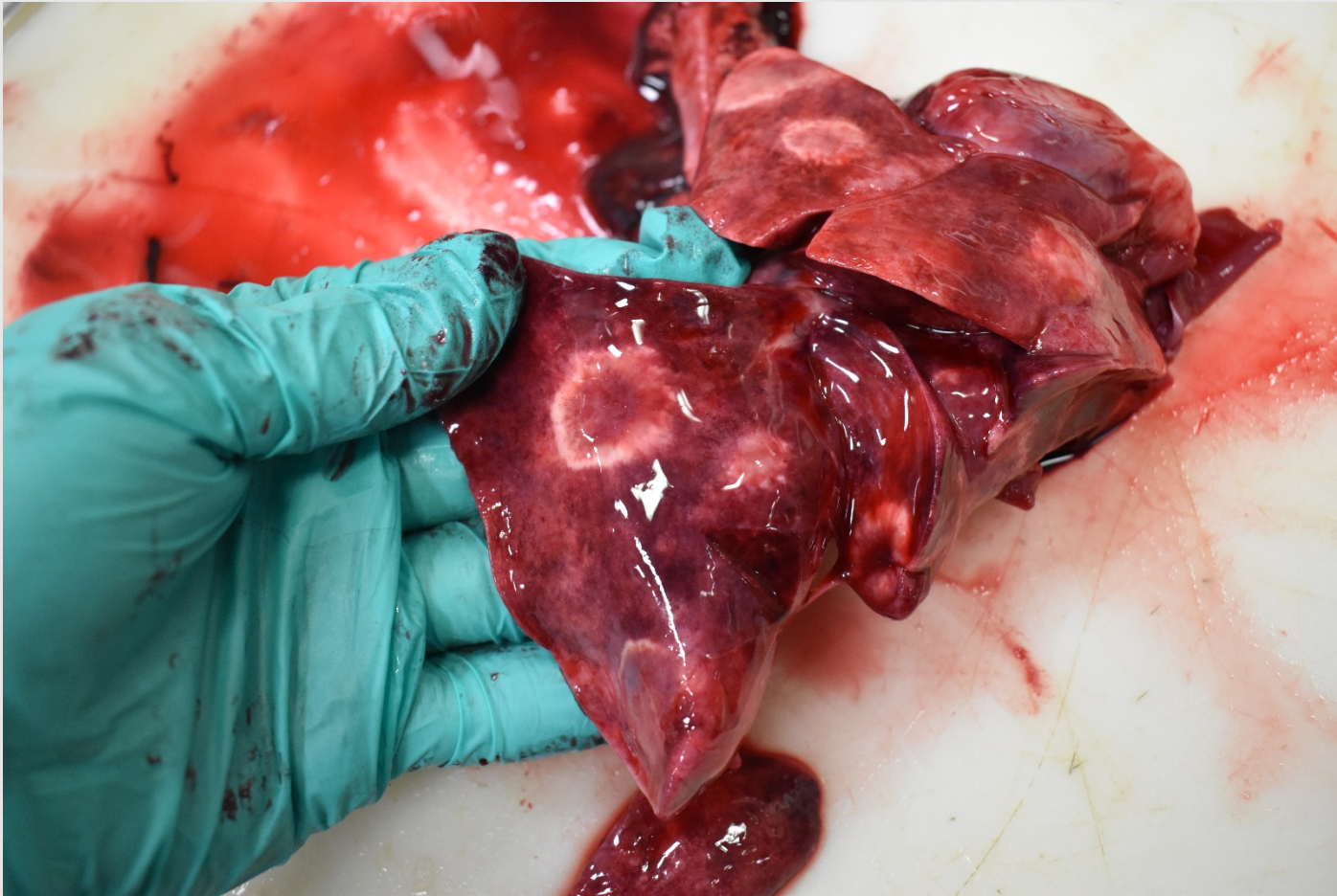
History: Indoor/Outdoor cat, recently outside for 2-3 months, returned with the following clinical signs: lethargy, cough, vomiting, decreased appetite

Progressively worse over several weeks

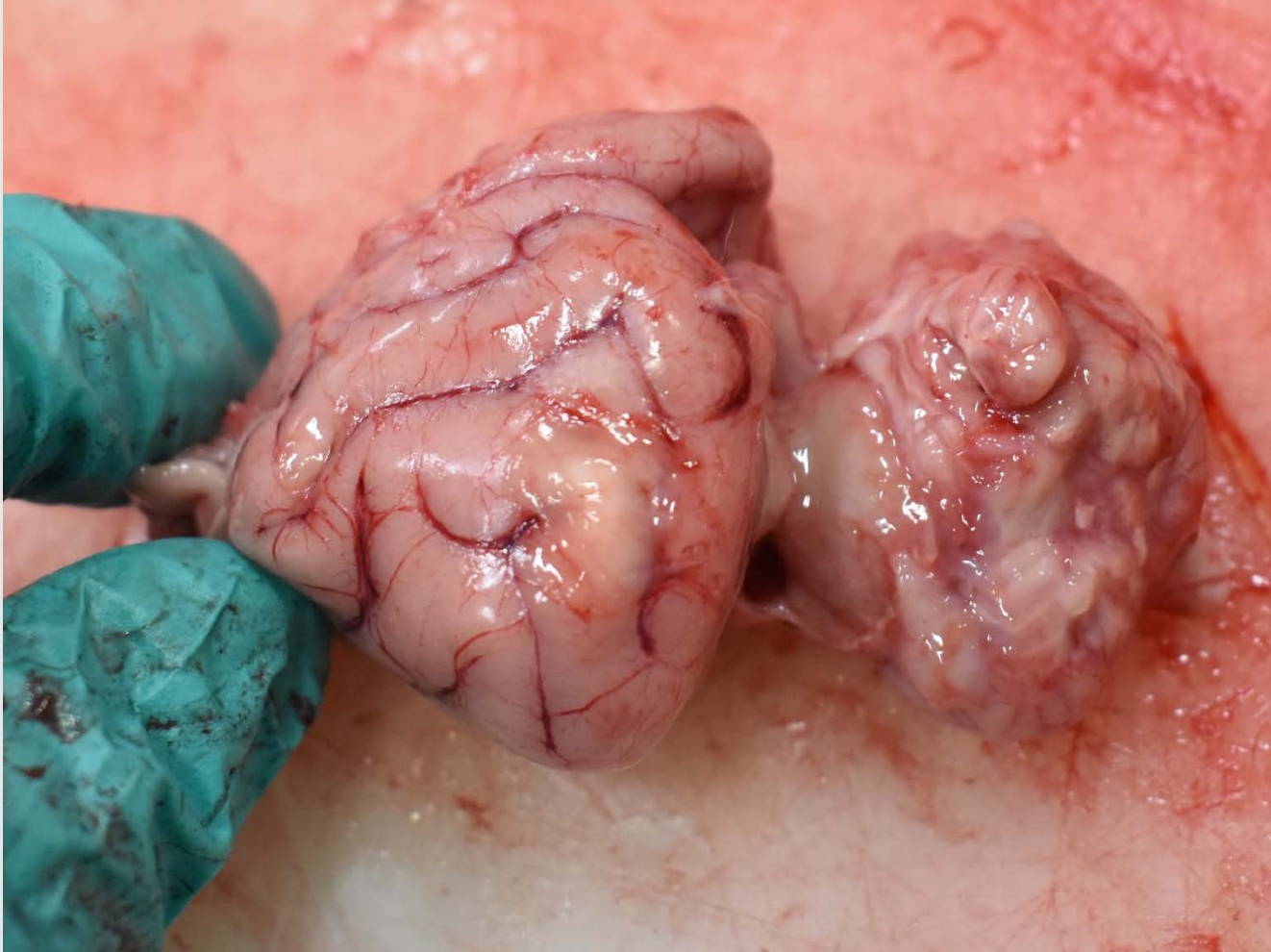
Taken to DVM, put on antibiotics

Died at home 2 days later

Necropsy findings: Lung



Necropsy findings: Brain



Histopathology with special staining...
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Case 1

Signalment: 10 year old, female spayed, Rottweiler (“Lola”)

History (provided on the submission form)

Lump at left flank, rapidly grew and patient wouldn't leave alone. At surgery peeled out easily, but seemed to be inflammatory at the ventral aspect. Couldn't remove all the inflammatory tissue and get closure.

Case 2

Signalment: 3 year old, male, Boxer (“Ruger”)

History:

- History, tissues, lesion descriptions.
- ① 3x3cm round pedunculated mast cell tumor between digit 3/4 on ⊕ hind paw. digits 3&4 amputated today.
 - ② ⊕ popliteal lymph node excised

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- Signalment: 6 year old, female spayed, Labrador Retriever (“Lily”)
- History (provided on submission form)
 - “Intra-oral mass, left buccal side of lip commissure”
 - Incisional biopsy specimen submitted.

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Thank You!

Questions/Comment?