

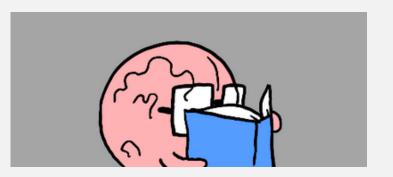
OUTLINE

- About me
- Submitting to a diagnostic lab
- Potpourri of cases









ABOUT ME



















ABOUT ME

- I am NOT an equine practitioner
 - More of an "enthusiast"
- Serve as the liaison between OADDL and the Oklahoma Horse Racing Commission (OHRC)
- Also serve as OADDL's toxicology "consultant"
 - Again, I'm NOT a toxicologist but rather an enthusiast

Veterinary Medical Diagnostic Program

January 1 - December 31, 2022

Supported by the Oklahoma Horse Racing Commission Oklahoma-Breeding & Development Program









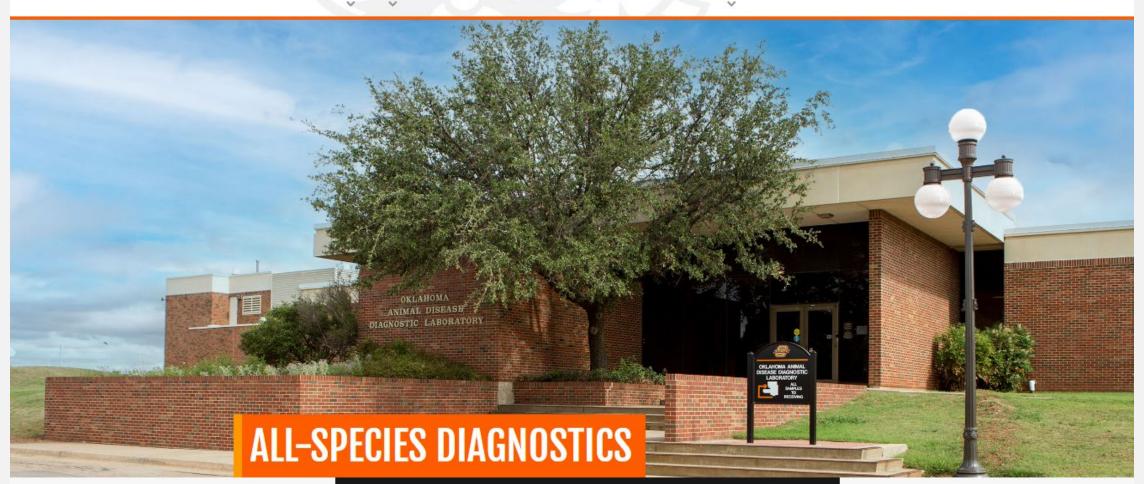


Conducted by the Oklahoma Animal Disease Diagnostic Laboratory
College of Veterinary Medicine
Oklahoma State University
Stillwater, OK
May 9, 2023





ABOUT TESTING ACCESS RESULTS CONTACT US GIVING ANIMAL CENSUS MAP DISEASE MAPS



Promoting the overall well-being of animal health through veterinary diagnostic testing, instruction of professional students, and research in diseases of economic importance to Oklahoma and beyond.

SUBMITTAL FORM

Address City Final Primary Phone # City Final Primary Phone # Email SubshitTER/BILL PARTY: Owner Clinic CINIC Owner Clinic Animal ID(s) Species Breed Sex Age 1 1 Control of the Age of	State Zip Yes Specimen Type(s) Guessing
Address Weterinarian Rep State Zip Address City Mail Remark Contact Usubmitter/BillL PARTY: Owner Clinic Connor Owner Clinic Date Specimens Collected: ZONOTIC SUSPECT? NO Animal ID(s) Species Breed Sex Age Age HISTORY/CLINICAL SIGNS In the space below, provide brief, recent, relevant information to animal's condition Please fill me out! Don't leave us getting the specimens of the space below and the specimens of the space below and the space below. The space below are specimens for the space below. The space below are specimens from the space below and the space below are specimens from the space below and the space below are specimens from the space below are specimens for the space below are specimens from the spa	Specimen Type(s) Specimen Type(s)
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a BIOPSY	
	LAB USE ONL
Short Report Long Report	
Location	
7 7 # of Sites/Lesions	
Size & Shape	
Duration	
Rate of Growth	
LAB USE ONLY	
FedEx UPS Post Mark Postage Due Vet Owner Courier/Runner	
	(only) Opened by:

For a comp		REQUESTED ements, see test catalog at https://oadd	l.okstate.edu
20000000	BACTERIOLO	GY / MYCOLOGY	S
Aerobic Culture with up to 2 Susceptit		Salmonella Culture with Susceptibility	Profile (serogrouping upon request)
Anaerobic & Aerobic Culture with up t	o 2 Susceptibility Profile	Salmonella Culture - Environmental (S	talls, barns, litter)
Fungal & Aerobic Culture with up to 2		Urine Culture with Susceptibility (cysfo	
Aerobic Culture only	Bacterial Isolate ID by MALDI-TOF	☐ Fungal Culture	TOXICOLOGY
Anaerobic Culture only	Campylobacter fetus Culture	Milk Culture & Susceptibility	Blue-Green Algae
Antibiotic Susceptibility	Clostridial Culture	Mycoplasma bovis Culture	Prussic Acid/Cyanide
	PARAS	SITOLOGY	
Baermann	Fecal Egg Count	Giardia AG	☐ Modified Knotts
Centrifugal Floatation & Direct Smear	☐ McMasters	Gross Parasite ID	Sedimentation
Coproculture (Larvae ID)	Wisconsin	Heartworm AG (Pre & Post Heat Treated)	☐ Tick ID
Centrifugal Floation	Feline Heartworm Ag	Hemoparasite Exam (Wright-Giemsa)	
	Α	VIAN	
Avian Influenza	Exotic Newcastle Disease	Mycoplasma gallisepticum/M synoviae	Salmonella pullorum-typhoid
☐ AGID ☐ PCR	☐ PCR	☐ ELISA	☐ Agglutination
☐ ELISA		☐ PCR	Microagglutination Titer
	BC	OVINE	
Diarrhea Panel - Aerobic Culture with Sus		Imonella Culture, Rotavirus Group A, Coronavirus P	CR, Fecal Float, Smear
	a, IBR, BVD SN, BVD ELISA, Brucella, Neospora	Bovine ELISA Panel - 8VD, BLV, Johne's	
Bovine Respiratory SN Profile 1 - ABR. 8		Bovine Respiratory SN Profile 2 - ABR. 8	
Bovine Respiratory Panel PCR *Basic -		Bovine Respiratory Panel PCR *Compr	
Anaplasma marginale	Bovine Respiratory Syncytial Virus	□ IBR	□ Neospora spp ELISA
☐ ELISA	□SN	☐ SN	Parainfluenza 3 (PI3) Virus SN
□ PCR	□ PCR	□ PCR	□ Pregnancy ELISA
Bluetonque	Brucella abortus / B. suis	☐ Johne's (Submit individual samples)	Rotavirus Group A Card
☐ ELISA	Bovine Viral Diarrhea (BVD)	☐ ELISA	Tritrichomonas foetus
□ PCR	ELISA (PI)	PCR - Individual	(Submit individual Samples)
Bovine Coronavirus PCR	☐ Type I SN	PCR - Pooled in Lab	Culture (InPouch TF)
Bovine Leukemia Virus	☐ Type 2 SN	Leptospira spp.	PCR (PBS or InPouch TF)
☐ ELISA	□ PCR	☐ MAT - 5 sergivers	PCR - Pooled in Lab
□ PCR	General Herpes Virus PCR	□ PCR	_
L 100	Sequencing (if Positive)	Mycoplasma bovis PCR	
		ANINE	
Diarrhea Panel - Annobic Outure with Sur		ronella Culture, Campylobacter jejuni Culture, Ranvo	wine BCR Foral Elect Steam
Brucella canis IFA	Canine Influenza PCR	Leptospira spp.	Rocky Mountain Spotted Fever IFA
Canine Distemper PCR	Canine Parvovirus PCR	MAT - 5 serovers	Tick Profile (Serology)
Canine Herpesvirus PCR	Ehrlichia sp.	□ PCR	Ehrlichia canis, RMSF, Lyme, Anaplasm
_canne respensa rex		NE / OVINE	Emilia Carac Arios , Lyma, Ampagan
Diarrhaa Banal - Asenhio Cultura with		ture, Salmonella Culture, Fecal Float, Smear	
Biosecurity Panel - C4E CL Johne's	Brucella abortus / B. suis	Johne's (Submit Individual Samples)	Leptospira spp.
Bluetonque	Brucella melitensis	ELISA	MAT - 5 sergivers
☐ ELISA	BVD PCR	□ PCR	□ PCR
□ PCR	CAE/OPP/SRLV	PCR - Pooled in Lab	Pregnancy ELISA
Пъск		DUINE	In regitality Ector
Disentes Basel Access to the contract		ronella Cultura, Rotavirus Group A, Coronavirus PCI	a discontinuo di discontinuo
Ehrlichia PCR	General Herpesvirus PCR	Piroplasmosis, Babesia caballic-ELISA Piroplasmosis, Theileria equic-ELISA	Louis.
	Sequencing (if Positive)		
Equine Herpesvirus 1 PCR Equine Herpesvirus 4 PCR	Leptospira spp. MAT – 5 serovers	Rotavirus Group A - Immunocard Test	
		Streptococcus equi PCR	
Equine Influenza PCR	□ PCR	I INF	
		LINE	
		nonella Culture, Campylobacter jejuni Culture, Parvo	
Cytauxzoon felis PCR	Feline Parvovirus/ Panleukopenia PC		Turaremia" PCR
		RCINE	
		ip A, Caronavirus PCR (TGE, PEDV, SDCaV), Fecal F	
		ture, Coronavirus PCR (TGE, PEDV, SDCoV), Fecal F	
& Brachyspira hyodysenteriae/ B. hampson		Leptospira spp.	☐ IPRRS Virus
Brucella abortus & Pseudorabies gB E	LISA Panel	MAT – 5 serovers	☐ ELISA
Brucella abortus / B. suis		☐ PCR	☐ PCR - NA/EU
Coronavirus Multiplex PCR - PEDV, TG	EV, SDCoV	Pseudorabies gB IELISA	Swine Influenza Virus PCR
		cal or chemical material derived from the submission shall b	
		samples to reference subcontractors for tests not currently o	
REC-FM-1.17	Last Mod	lified: 9/5/23	Page 2 of 2

SUBMITTAL FORM

The list is somewhat short for horses!

		EQUINE	
Diarrhea Panel - Aerobic Culture with Sus	ceptibility, Clostridium perfringens Culture, :	Salmonella Culture, Rotavirus Group A, Coronavirus PC	R, Fecal Float, Smear
Ehrlichia PCR	General Herpesvirus PCR	Piroplasmosis, Babesia caballi c-ELISA	Other:
☐ EIA ELISA	Sequencing (if Positive)	Piroplasmosis, Theileria equi c-ELISA	
Equine Herpesvirus 1 PCR	Leptospira spp.	Rotavirus Group A – Immunocard Test	
Equine Herpesvirus 4 PCR	■ MAT – 5 serovers	☐ Streptococcus equi PCR	
Equine Influenza PCR	☐ PCR		

Abortion panel

Diarrhea panel

EQUINE \$250.00	\$185.00
Necropsy with disposal (whole fetus & placenta)	Aerobic culture with antibiotic susceptibility
Placenta examination	Clostridium perfringens culture with toxin typing by PCR
	Salmonella culture with antibiotic susceptibility
Bacteriology (pooled aerobic culture on lung & stomach fluid)	Rotavirus group A, Immunocard test (only performed on animals < 6 months)
Malagulan Digunagian / DCD /FII// an liven F\/A an liven mafamal lab	Centrifugal fecal flotation; direct fecal smear with staining

FIELD NECROPSY



Fig. 1. Equipment.





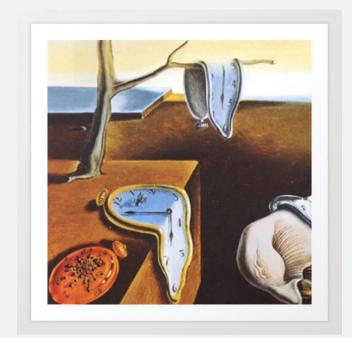
East Valley Equine Practitioners



Ok, so this is a rebreathing bag, but you get the idea...

TIME IS OF THE ESSENCE

- Autolysis (rot) is our worst enemy!
 - Precludes gross/histologic evaluation
 - Infectious agents can degrade
 - Toxicants are often stable
 - Some are NOT (e.g., cyanide)
- Autolysis is accelerated by
 - Ambient temperature
 - Obese body condition
 - Fever and inflammation



FIELD NECROPSY

THE NECROPSY BOOK

A Guide for Veterinary Students, Residents, Clinicians, Pathologists, and Biological Researchers

Revised January 2013

John M. King **Lois Roth-Johnson** David C. Dodd Marion E. Newsom

Field Necropsy of the Horse OccossMark



Chad Frank, DVM, MSc*, Dennis J. Madden, BS, Colleen Duncan, DVM, MSc, PhD

• Equine • Necropsy • Postmortem examination • Mortality • Investigation

KEY POINTS

- · Prior to initiating a necropsy, consideration should be given to equipment, location, sampling, disposal and clean up.
- . Use of a standardized approach will enable the practitioner to be better prepared to identify true pathologic lesions versus changes of minimal significance. . On completion of the necropsy, observations should be recorded and assessed in the
- context of any clinical questions. . If necessary, formalin-fixed and/or fresh tissues may be submitted to a diagnostic labora-
- tory for further evaluation.
- In addition to the biologic samples, the laboratory-specific submission form should be completed including a brief history and necropsy findings along with specific diagnostic

INTRODUCTION

This article provides an overview of the equine necropsy that can be used by veterinarians in the field. Use of a systematic process enables the practitioner to develop a familiarity with normal anatomic positioning and tissue appearance such that abnormalities are quickly identified. Although an exhaustive review of equine pathology is beyond the scope of this article, there are several excellent resources on equine pathology^{1,2} that may be used to aid in the interpretation of changes identified. Additionally, several articles elsewhere in this issue focus on disease processes in specific body systems.

Field Versus Laboratory Examination

Logistical factors often influence the decision to perform the postmortem examination in the field or have the carcass transported to a diagnostic laboratory with necropsy

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Vet Clin Equine 31 (2015) 233-245 http://dx.doi.org/10.1016/j.cveq.2015.04.002

vetequine.theclinics.com 0749-0739/15/\$ - see front matter © 2015 Elsevier Inc. All rights reserved.

MEDICINE-RESPIRATORY/CARDIOVASCULAR

How to Perform an Equine Field Necropsy

Sally L. Ness, DVM; and Fairfield T. Bain, DVM, MBA, Diplomate ACVIM, ACVP, ACVECC

> Authors' addresses: 91370 Walluski Loop, Astoria, Oregon 80127 (Ness); and Equine Sports Medicine & Surgery, PO Box 1569, Weatherford, Texas 97103 (Bain); e-mail: sally_ness@yahoo.com © 2009 AAEP.





A WORD ON SAMPLE COLLECTION

BACTERIAL

- Culture
 - Aerobic → respiratory, E. coli
 - Anaerobic → Clostridium spp.
 - Others → what do you suspect?





VIRAL (MOSTLY)

- Molecular (PCR)
 - NO media!
 - Swab +/- 0.5 ml saline
 - Fresh tissue

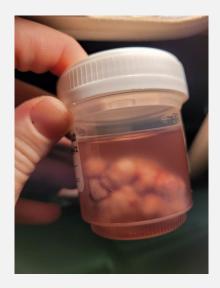




FORMALIN-FIXED SAMPLES

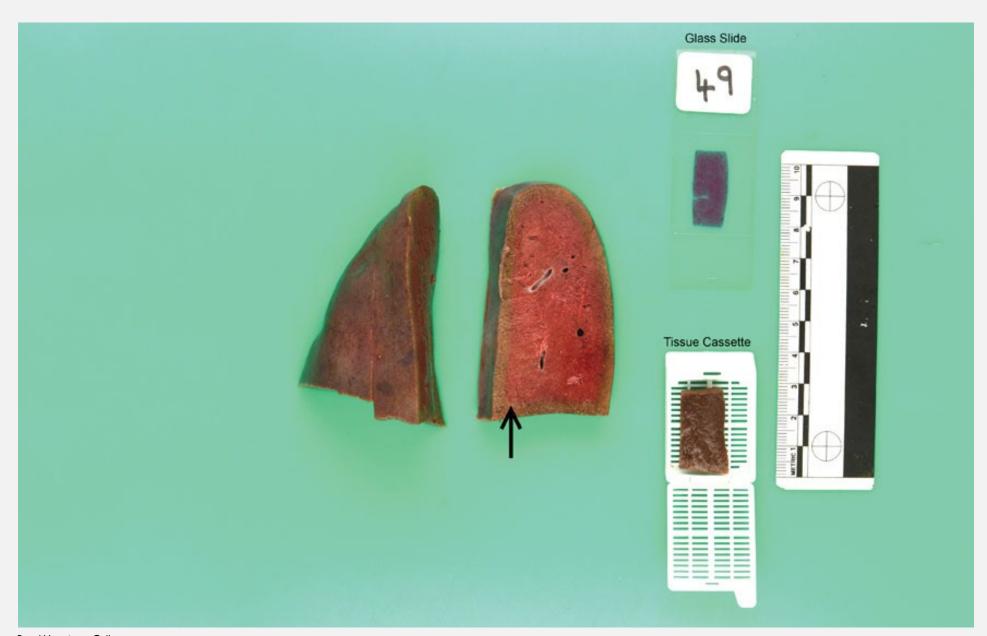
- 10% formalin
- 1:10 ratio tissue to formalin
- I cm³ –





Note: in winter months, 1:10 ratio ethanol to formalin to prevent freezing!

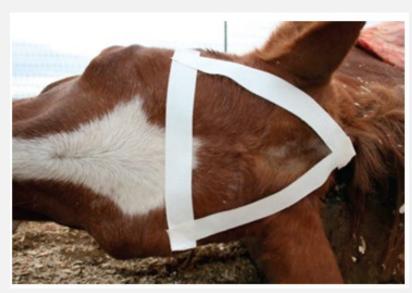




Royal Veterinary College

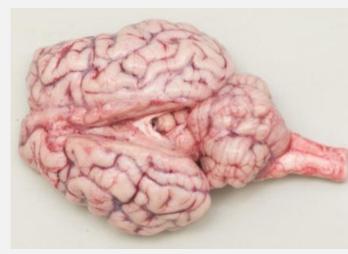


THERE'S MORE THAN ONE WAY TO SKIN A CAT GET THE BRAIN OUT

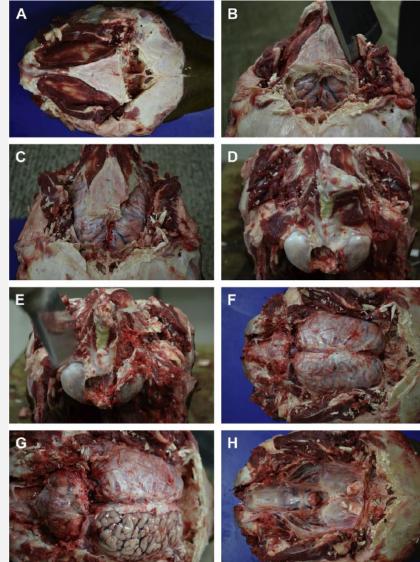


Ness SL, Bain FT (2009). How to perform an equine field necropsy. AAEP Proceedings Vol. 55.

Pretty please submit the WHOLE brain



Frank C, Madden DJ, Duncan C. Field Necropsy of the Horse. Vet Clin North Am Equine Pract. 2015 Aug;31(2):233-45.



ON THAT NOTE...

 You're called out to see an 8-year-old gelding who recently traveled to Florida for a competition

Unknown vaccine status

• Temperature: 104.5

Acute onset of severe neurologic deficits





DIFFERENTIALS?

- Viral
 - Rabies (always at the top of the list, right?)
 - West Nile Virus
 - The encephalitides: EEE, WEE, VEE
 - EHV
- Bacterial
- Parasitic
 - EPM
 - Halicephalobus gingivalis
 - Others?

- Toxic
 - ELEM
 - NPE
 - Locoweed
 - Hepatic encephalopathy
- Degenerative
 - EDM
 - EMND
- Neoplastic Neoplastic

- Do your differentials change if this were more subacute or chronic onset...?
- Are any of these zoonotic?

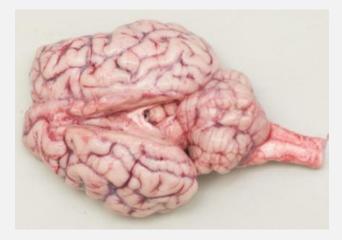
WHAT TO SUBMIT?

ANTEMORTEM





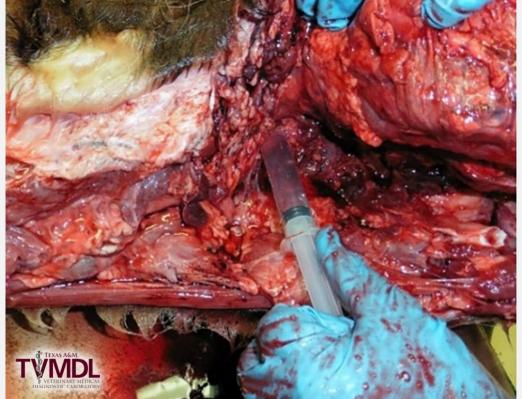
POSTMORTEM





CSF COLLECTION







Drs. Jessie Monday and Will Sims

- Rabies
- West Nile Virus
- The encephalitides
 - WEE
 - EEE
 - VEE



• EHV

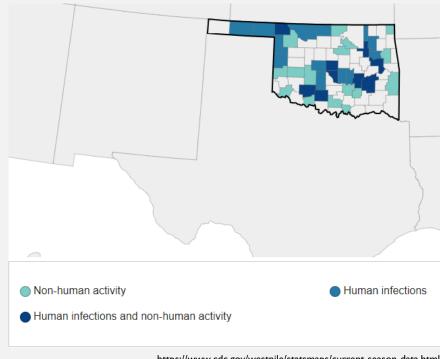


- Rabies
- West Nile Virus
- The encephalitides
 - WEE
 - EEE
 - VEE



Where the magic happens in a lot of equine neuro ©

- **Rabies**
- **West Nile Virus**
- The encephalitides
 - WEE
 - EEE
 - VEE
- EHV



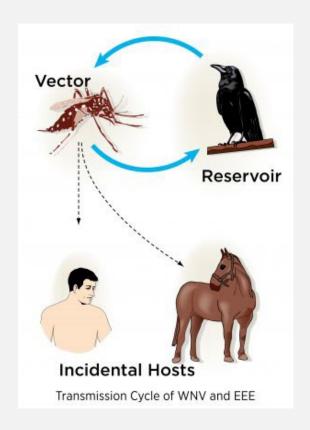
https://www.cdc.gov/westnile/statsmaps/current-season-data.html



WEST NILE VIRUS

- **Transmission**
 - Mosquito-bird lifecycle
 - Humans and horses are dead-end hosts
 - Other species (e.g. dogs) can be infected
- Incubation period: 3-15 days
- Encephalitis, myocarditis







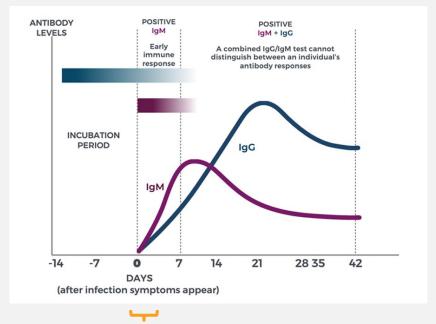
WEST NILE VIRUS



- At OADDL
 - PCR
 - I POSITIVE so far in 2023



- Serology
 - 2022: 7 IgM POSITIVES out of 41 tested
 - So far in 2023: I 0 IgM POSITIVES out of 35 tested



Small window of potential false negatives...

HOW TO DIAGNOSE

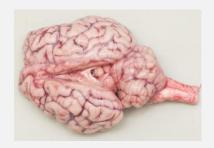
ANTEMORTEM

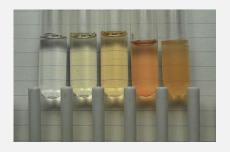
- Serum for IgM
- CSF for PCR



POSTMORTEM

Brain or CSF for PCR





- Rabies
- West Nile Virus



- The encephalitides
 - WEE
 - EEE
 - VEE



• EHV

HOW TO DIAGNOSE

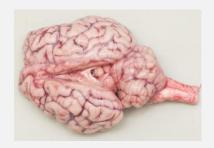
ANTEMORTEM

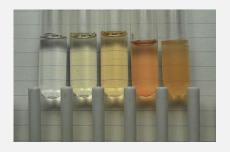
- Serum for IgM
- CSF for PCR



POSTMORTEM

Brain or CSF for PCR





SPOILER ALERT

- Remember that horse from Florida...?
- EEE positive

HHS and USDA Select Agents and Toxins

7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the regulations.

More information can be found at https://www.selectagents.gov/sat/list.htm

HHS Select Agents and Toxins

- 1) Abrin
- 2) Bacillus cereus Biovar anthracis*
- 3) Botulinum neurotoxins*
- Botulinum neurotoxin producing species of Clostridium*
- Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X,CCX,PACGX,X,X,X,CX,)
- 6) Coxiella burnetii
- Crimean-Congo haemorrhagic fever virus
- 8) Diacetoxyscirpenol
- 9) Eastern Equine Encephalitis virus
- 10) Ebola virus*
- 11) Francisella tularensis*
- 12) Lassa fever virus
- 13) Lujo virus
- 14) Marburg virus*
- 15) Mpox virus

- 16) Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
- 17) Ricin
- 18) Rickettsia prowazekii
- SARS-associated coronavirus (SARS-CoV)
- 20) SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors
- 21) Saxitoxin

South American Haemorrhagic Fever viruses:

- 22) Chapare
- 23) Guanarito
- 24) Junin
- 25) Machupo
- 26) Sabia







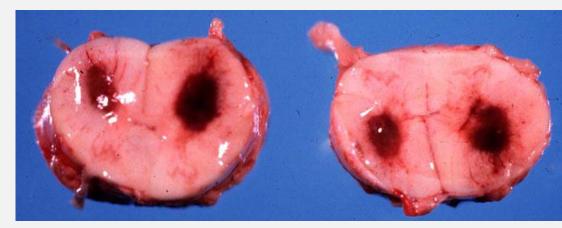


- Rabies
- West Nile Virus
- The encephalitides
 - WEE
 - EEE
 - VEE





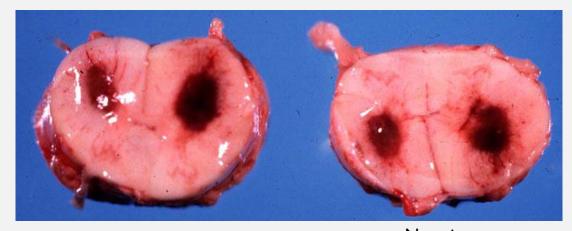
- EHV-I >>> EHV-4
 - Endotheliotropic, epitheliotropic, neurotropic
- Different strains
 - Neuropathic: G2254/D752
 - Non-neuropathic: A2254/N752
 - New variant: H752
- Incubation period is variable
 - Average 4-7 days



Not piggy snouts...



- EHM: Equine Herpesviral Myeloencephalopathy
 - 85% of cases due to mutated EHV-1
 - Unknown reason for mutation
 - Vaccination does not prevent EHM but does reduce nasal shedding of virus
 - Shedding may last up to 1 month!
 - Unvaccinated horses with EHM shed LOADS of virus in nasal/pharyngeal secretions



Not piggy snouts...



EHV DIAGNOSIS

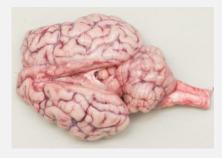
ANTEMORTEM

- Nasal swab for PCR
- EDTA blood for PCR
- CSF for PCR
- Serum for IgM





Brain or CSF for PCR









OADDL: 0 positives so far in 2023...



HERPESVIRUSES

- EHV-I
 - Neurologic disease
 - Abortion
 - Respiratory disease
- EHV-2
 - Mild pathogen?
- EHV-3
 - Equine coital exanthema

- EHV-4
 - Similar to EHV-I, but not as bad
 - Respiratory >>>> neuro or abortion
- EHV-5 and beyond...



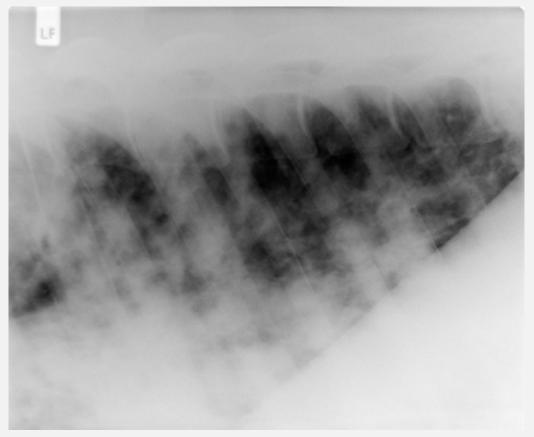
CASE WORK-UP

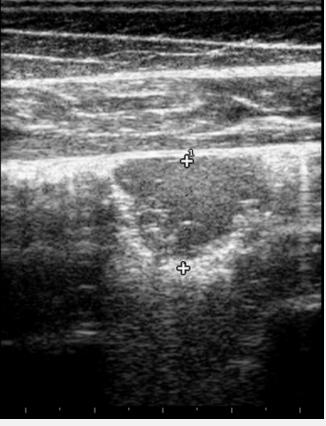
- A 14-year-old TB mare presents for chronic weight loss
- Clinical signs:
 - Mild fever
 - Mild nasal discharge
 - Increased respiratory rate and effort
 - "heave line"





CASE WORK-UP





Spelta CW, Axon JE, Begg A, Diallo IS, Carrick JB, Russell CM, Collins NM. Equine multinodular pulmonary fibrosis in three horses in Australia. Aust Vet J. 2013 Jul;91(7):274-80.

DIFFERENTIALS

- Infectious
 - Viral
 - Bacterial
 - Fungal
 - Other?
- Immune-mediated
 - Asthma
 - Aka heaves aka COPD aka RAO

- Neoplastic
- Other?



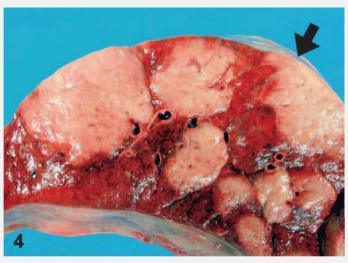


- Equine Multinodular Pulmonary Fibrosis (EMPF)
- Gammaherpesvirus
 - Proliferative rather than necrotizing (like EHV-1)
- Progressive histiocytic pneumonia and fibrosis





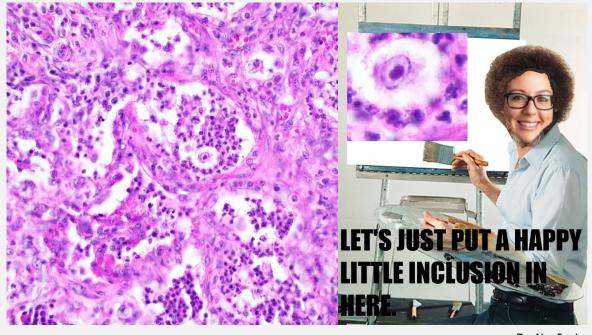
Dr. Raquel Rech, my wonderful mentor at TAMU



Williams KJ, et al. Equine multinodular pulmonary fibrosis: a newly recognized herpesvirus-associated fibrotic lung disease. Vet Pathol. 2007 Nov;44(6):849-62.

Multifocal to coalescing regions of chronic inflammation and fibrosis

- Diagnosis
 - Histopathology (gold standard)
 - PCR
 - Antemortem
 - Blood
 - Nasal swab
 - BAL
 - Postmortem
 - Fresh lung tissue



Dr. Alex Ford

- Diagnosis
 - Histopathology (gold standard)
 - PCR
 - Antemortem



- Blood
- Nasal swab
- BAL
- Postmortem
 - Fresh lung tissue

BAL or combo of blood and nasal secretions (Pusterla et al., 2015) shows high sensitivity and specificity

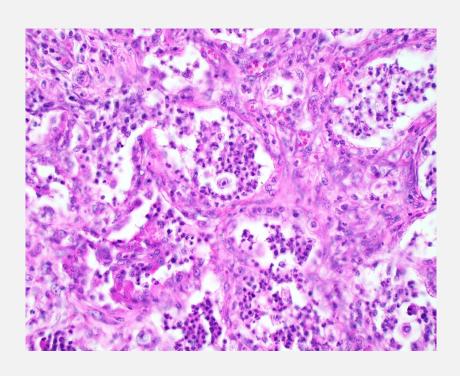
So far in 2023 at OADDL:

- 2 horses (postmortem histopath)
- "2 horses" (antemortem nasal swab)*

*Asinine herpesvirus-5 and EHV-2

- Prognosis is fair to poor 🕾
- Treatment:
 - Vancyclovir
 - +/- corticosteroids
 - Steroids alone are contraindicated**
 - Supportive care





BACK TO BRAIN

- Viral
 - Rabies (always at the top of the list, right?)
 - West Nile Virus
 - The encephalitides: EEE, WEE, VEE
 - EHV
- Bacterial
- Parasitic
 - EPM
 - Halicephalobus gingivalis
 - Others?

- Toxic?
 - ELEM
 - NPE
 - Locoweed
 - Hepatic encephalopathy
- Degenerative
 - EDM
 - EMND
- Neoplastic

BACK TO BRAIN

- Viral
 - Rabies (always at the top of the list, right?)
 - West Nile Virus
 - The encephalitides: EEE, WEE, VEE
 - EHV
- Bacterial
- Parasitic
 - EPM
 - Halicephalobus gingivalis
 - Others?

- Toxic?
 - ELEM
 - NPE
 - Locoweed
 - Hepatic encephalopathy
- Degenerative
 - EDM
 - EMND
- Neoplastic

Equine Protozoal Myeloencephalitis



- Sarcocystis neurona
 - Protozoa
 - Definitive host: opossum
- Neospora hughesi
 - Nearly identical protozoa
 - Unknown DH

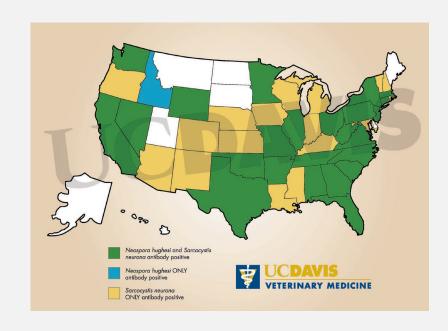


The 3 A's

- I. Asymmetry
- 2. Ataxia
- 3. Atrophy



- Over 90% of horses in the US are seropositive for S. neurona and/or N. hughesi
 - Only a small subset of horses will develop EPM
- Widely variable time of onset, clinical signs, severity
 - Most cases show progression over time
 - Uncommon to be acute onset, but can happen
- Lots of differentials
 - CVSM (spinal cord involvement)

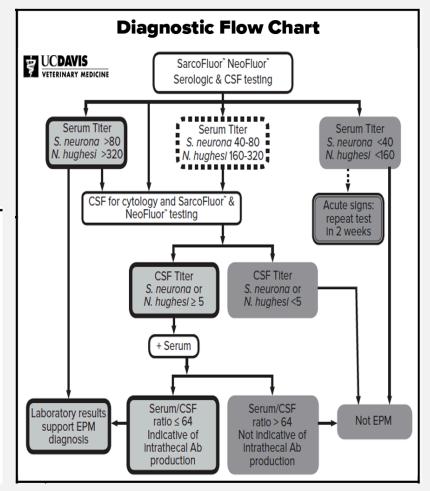


But how to diagnose...?

SarcoFluor[™] Interpretation (Sarcocystis neurona IFAT)

Serum titer result	Estimated probability of EPM due to S. neurona given the test result **
40	33%
80	55%
160	76%
320	89%
≥640	95%

CSF titer	Estimated probability of EPM due to
result	S. neurona given the test results
<5	<1%
≥5	92%



Definitive diagnosis is postmortem only



Giles, University of Kentucky



Can you imagine why this might be asymmetric...?

ANY QUESTIONS?

