Eastern Equine Encephalitis Virus in Florida
Clinical Signs, Diagnosis, Prevention

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ACVIM-Large Animal

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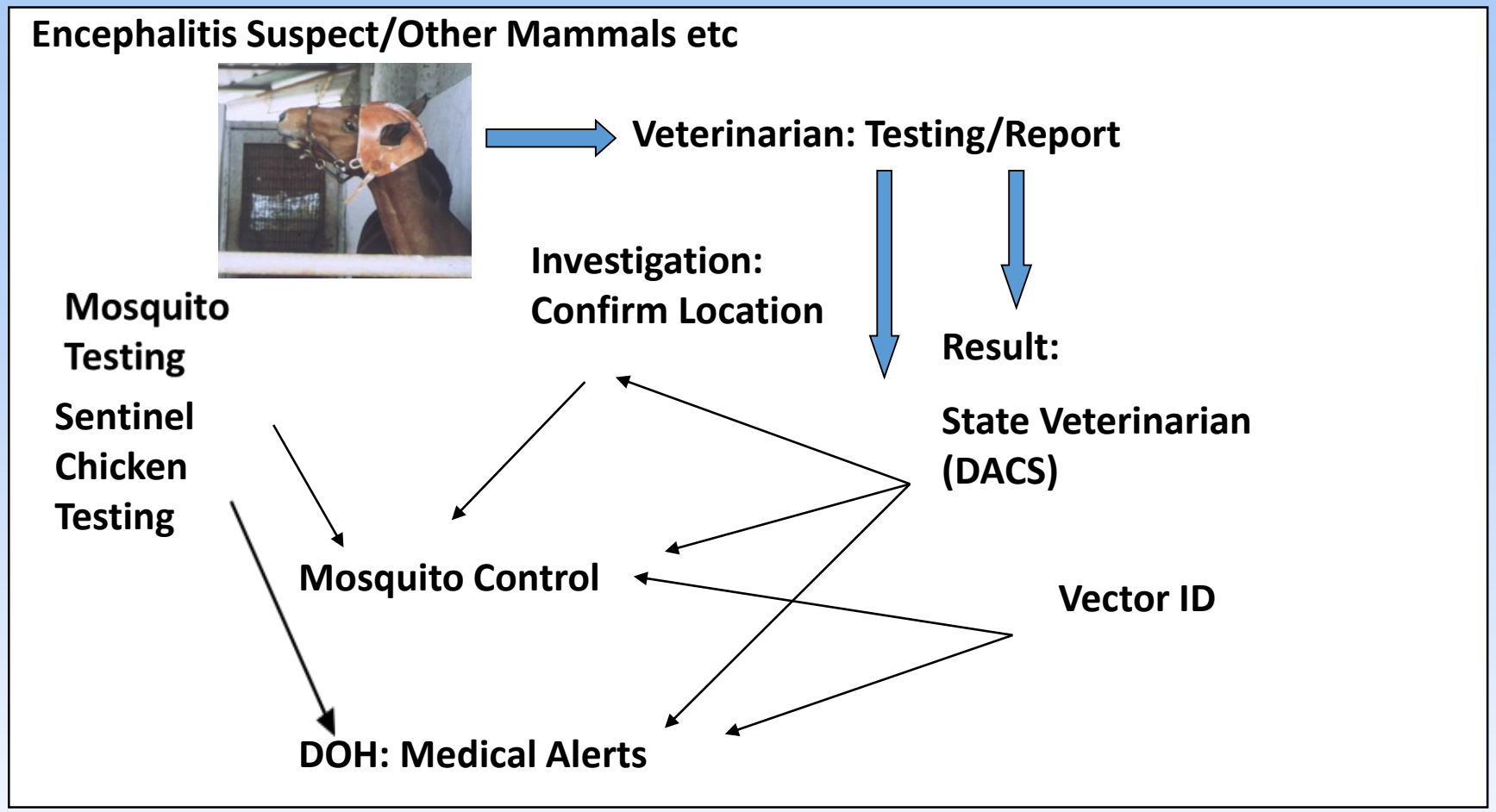
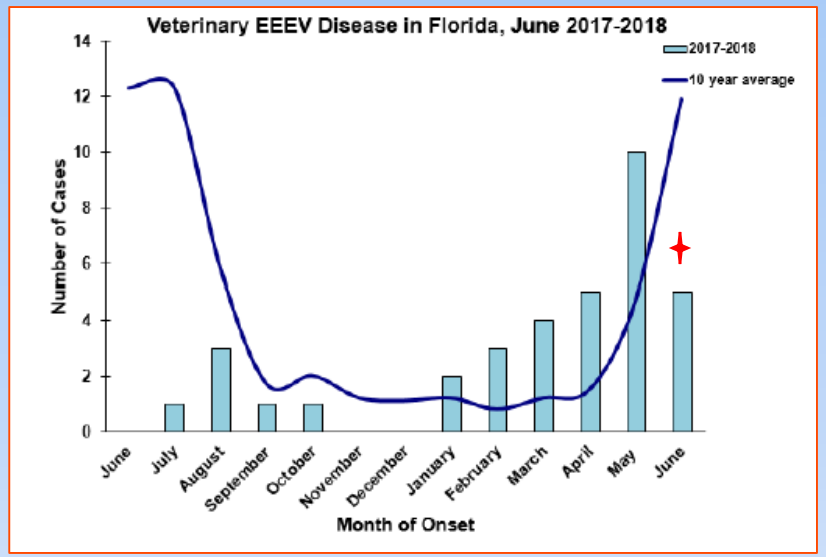
Sources of Data Sans Human Testing

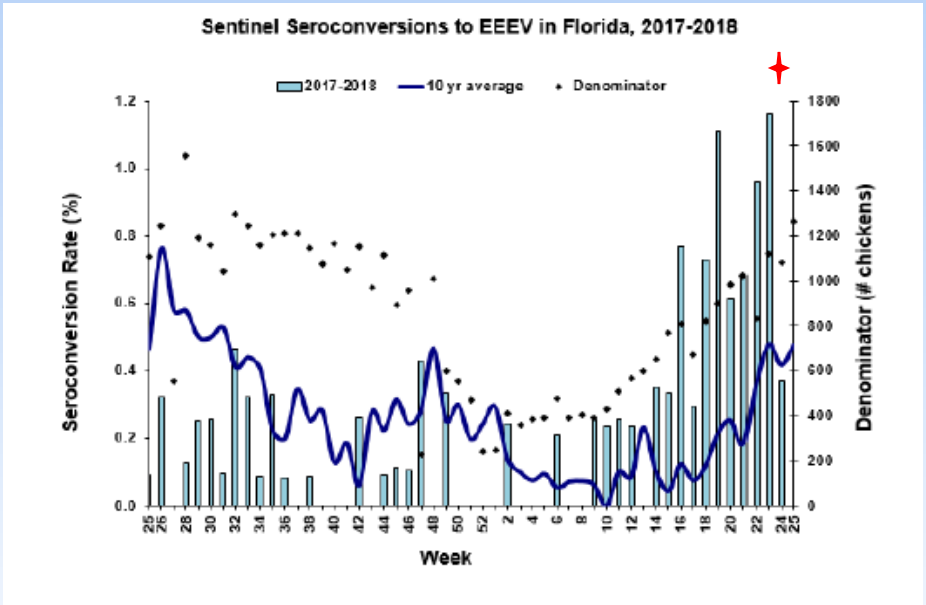


Animal Disease in Zoonotics

- Extremely important for surveillance and risk of disease in humans
- Probably contribute more regarding epidemiology
- Clinical signs, dx testing, immune response often similar
- More frequent ability to examine pathology

Alphavirus (EEEV) Surveillance





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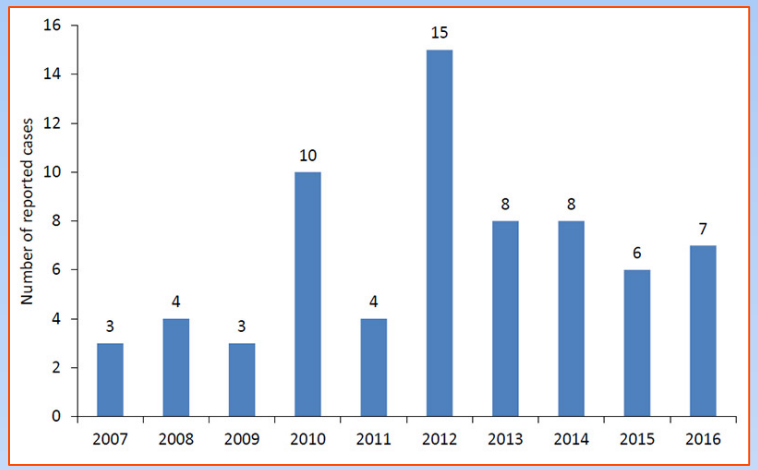
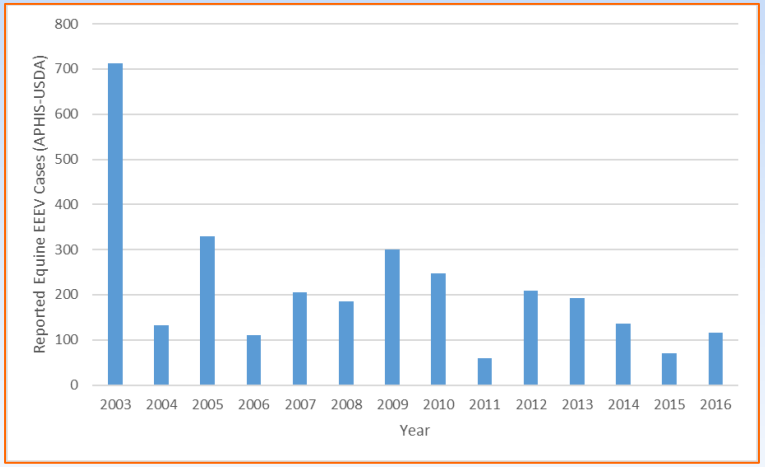
Eastern Equine Encephalitis virus

- Disease of humans and horses
  - One of the most pathogenic neurological viruses on planet
  - Mortality lower in humans
    - Horse >90%
    - Spontaneous mortality is probably equal to this





Trend of Nonhuman vs Human Cases

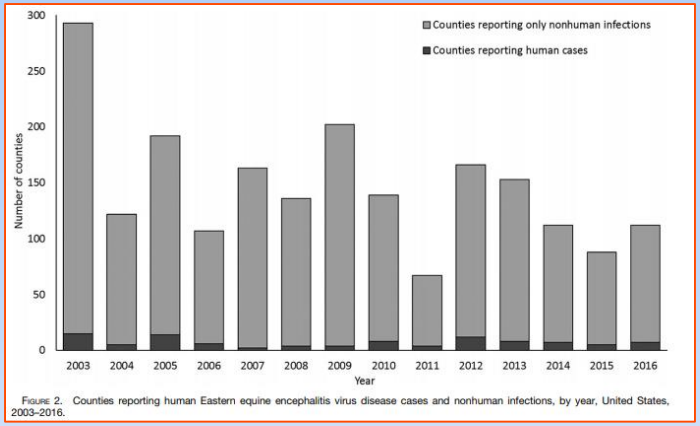


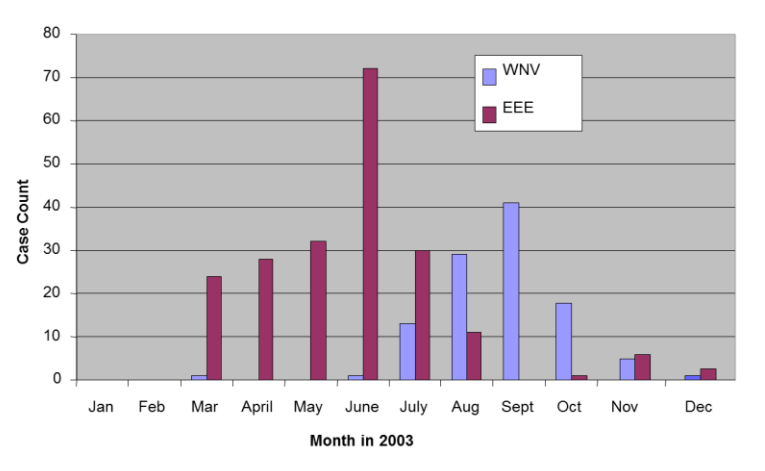
FIGURE 2. Counties reporting human Eastern equine encephalitis virus disease cases and nonhuman infections, by year, United States, 2003–2016.

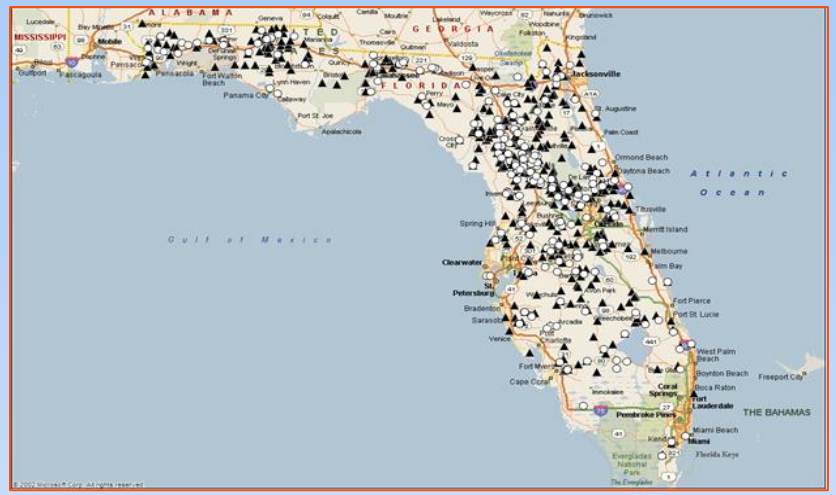
- Nonhuman:human reporting decreasing
- Vaccination
- Select Agent Status
  - Governmentally control of who works on disease
    - This includes diagnostics
  - Few veterinary diagnostic laboratories perform EEEV testing

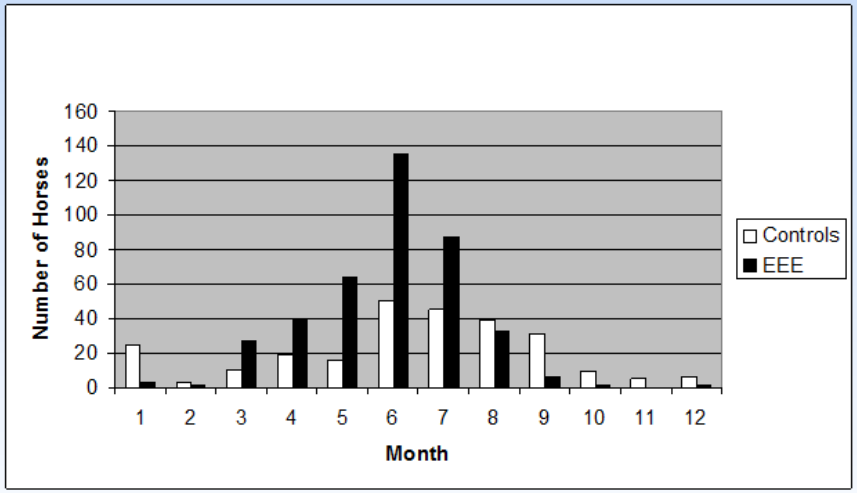
Animal Species Other Than Horses

- During active years (2003-2016; Lindsey et al):
  - Thirty-one states reported animal species
  - 88% of reported cases in nonhuman species
  - Animals
    - 97% equids (3,016)
    - 12% camelids
    - 9% canids
    - 6% cervids
    - Others: rhatites*, exotic birds, sheep, goats

Florida Analyses (Long, Gibbs, MacKay)







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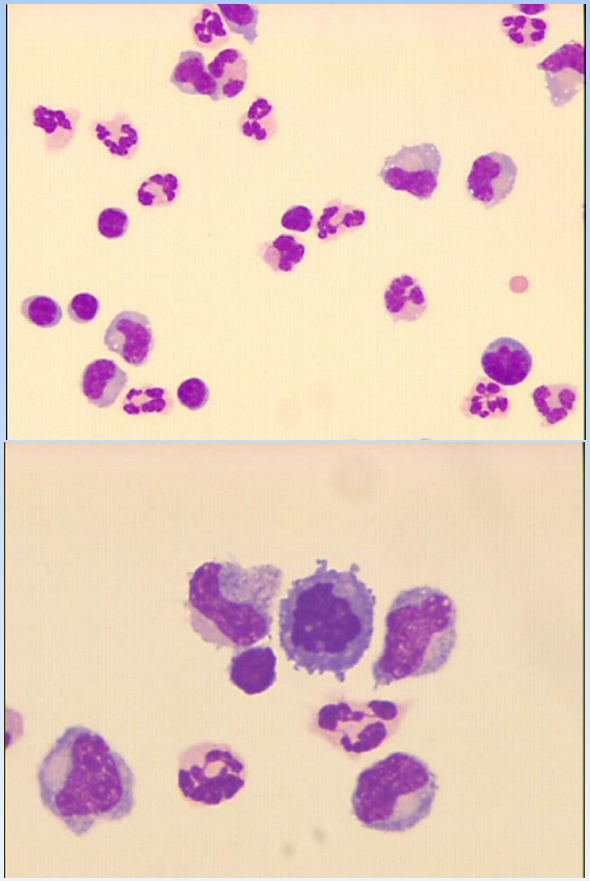
Case Example

- ~4 yrs, Ranch TW
- Dubious Vaccine Hx
- Bloodwork: hyperammonemia
  - Theiler's ?
- Eventually became comatose, recumbent
- Performed rabies postmortem
- Rabies (-) EEEV (++) WNV (-)

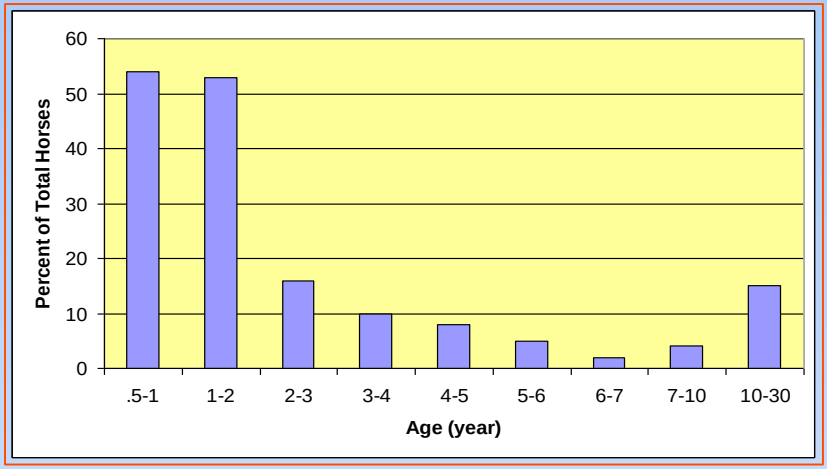
EEE

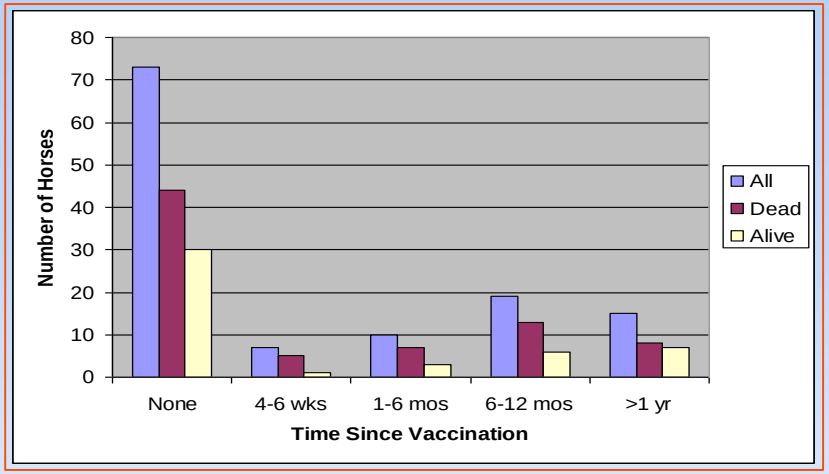
Clinical Sign	% EEE
Depression	69->90
Ataxia	63->90
Inc. R. Temp	50
Recumbence	47->90
Weakness	45
Fasciculations	30



Clinical Sign	% EEE
Aimlessness	28->75
H. Pressing	17
Hyperaesthesia	12
Blindness	8-?
Seizures	8-20
Coma	8->90

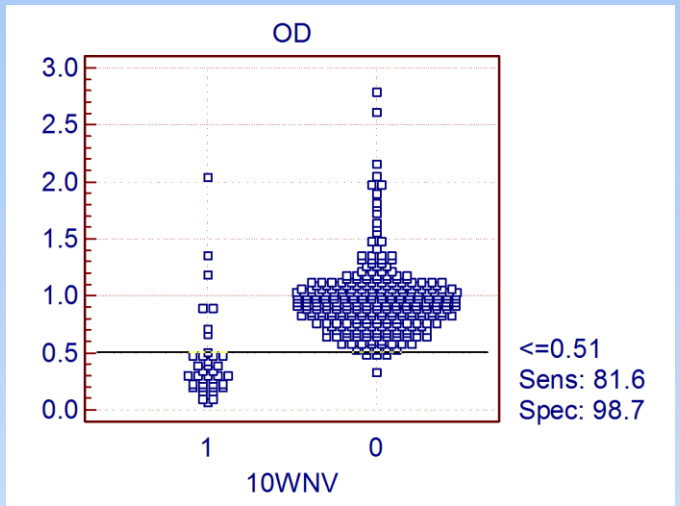
Age Distribution Similar to Humans





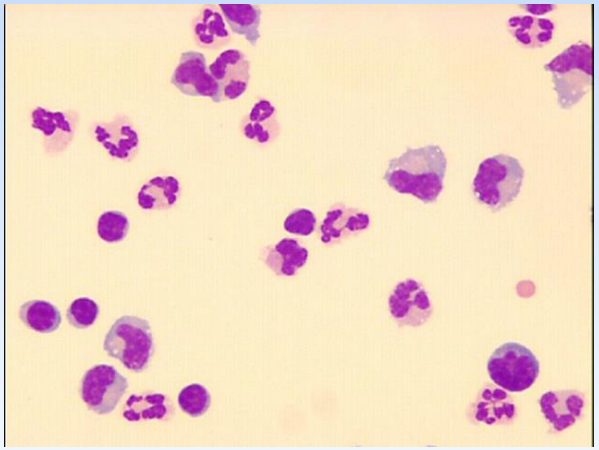
Diagnosis

- Complete under reporting!
  - Clinical Signs
  - Most by a single serum test
  - MAC ELISA
    - Mouse-brain antigen
    - Very limited distribution!
    - Need for development of non-SA antigen
  - 4-fold difference on consecutive NT
    - Rarely a horse survives for a second sample
  - 4-fold difference between EEE and WEE on single sample (see Snahu et al, Pederson et al)
  - Post-mortem
    - Many horses never reach post-mortem



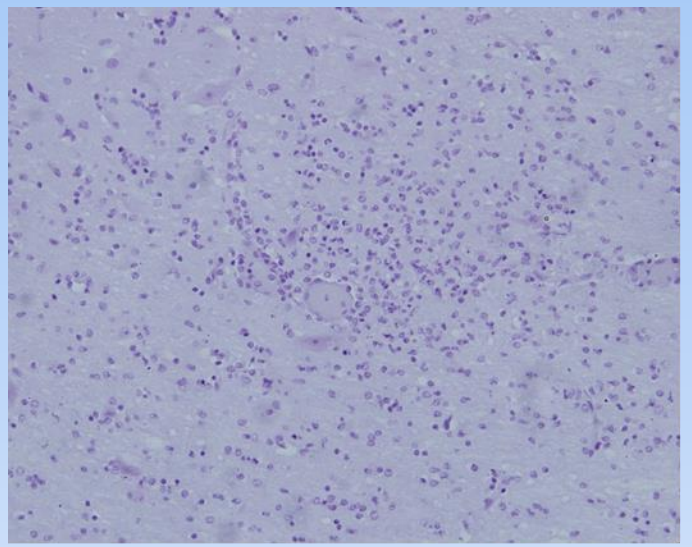
Cerebrospinal Fluid

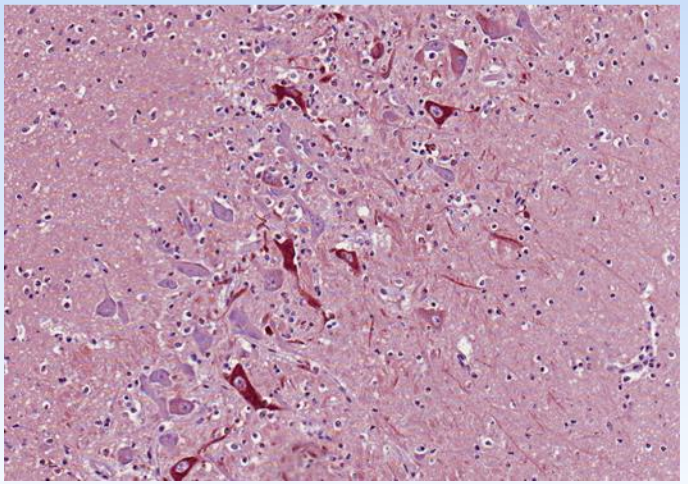
- Very specific for EEEV
  - "neutrophilic" response
- When horses are euthanized in the field
  - Can take CSF and serum
  - Will suffice for diagnosis also



Post-mortem Testing

- Must use personal protection equipment!
- Choices in Field
  - Disarticulation of head and ship to pathology/diagnostic laboratory
  - Euthanize, ship whole carcass





Prevention

- Vaccination
  - Horses:
    - All foals received three boosters 6, 7, and 9 mos.
    - Mares received a booster one month before foaling.
    - Horses aged 1-5 years should receive 3 injections per year.
    - Older than 5 years, twice per year.
  - Emus and other ratites
    - Require three injections per year.
    - Brooding females, keep vaccinated for maternal antibody.
    - Young and unvaccinated require three initial injections 3-4 weeks apart starting at 5-6 weeks of age
  - Dogs
    - Usually puppies have been reported
    - Limited number of dogs had no response to vaccines

Prevention

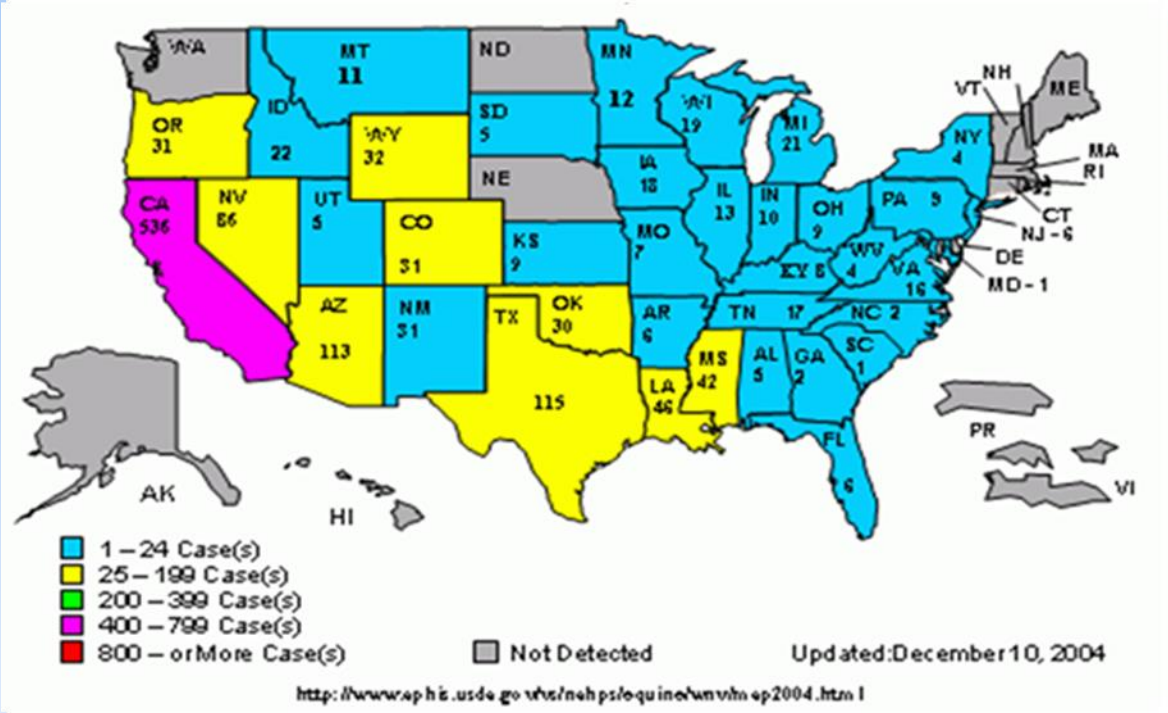
- Site cleanup
  - Standing water
  - Removal of junk
  - Removal of old tires
  - Application of "dunks"
  - Treatment of ponds-stagnant, removal of weeds
- Mosquito Control
  - Larvicide treatment
  - Adulticides
  - I use premise sprays in barns and buildings

West Nile virus

Epizootic Period: Establishment

733 equine cases detected in 19 states.
66% of cases occurred in Florida

Epizootic Period: Explosive Outbreak

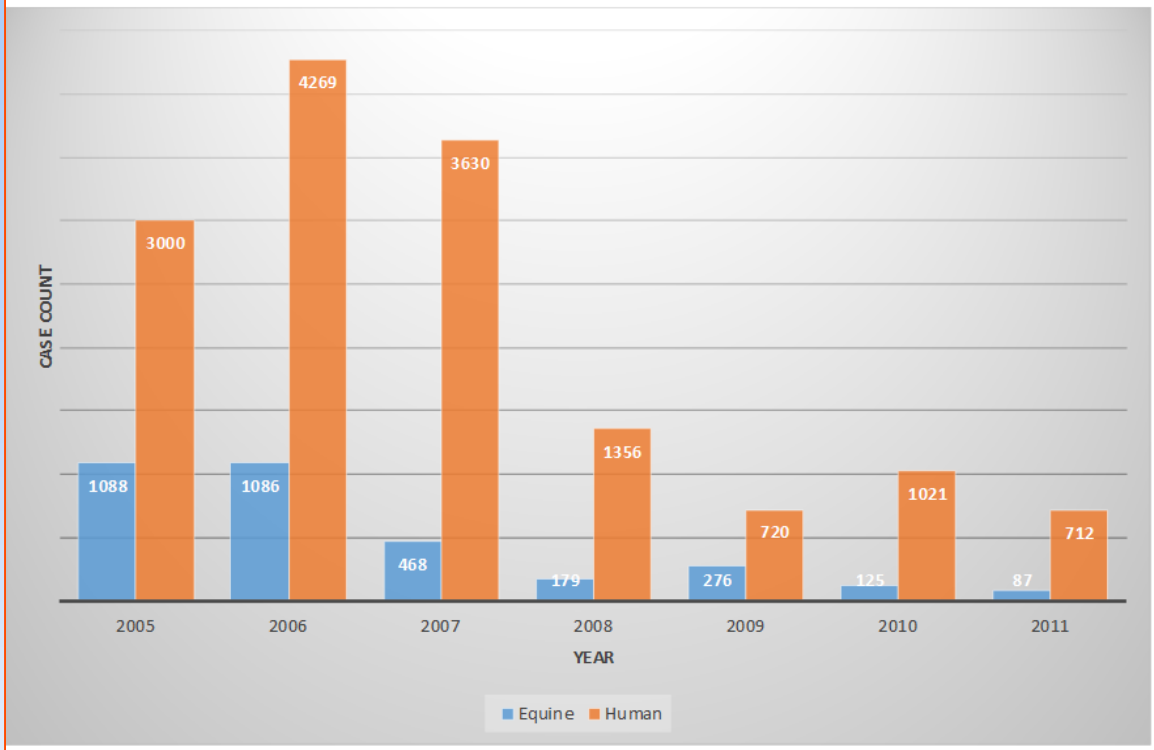


1,406 equine cases detected Most of the cases occurred in the west

2,539 human WNV cases

2004

Endemic Period



Incidence and case counts for equids lower than human case count. Dead bird counts sporadically reported. Limited mosquito testing.

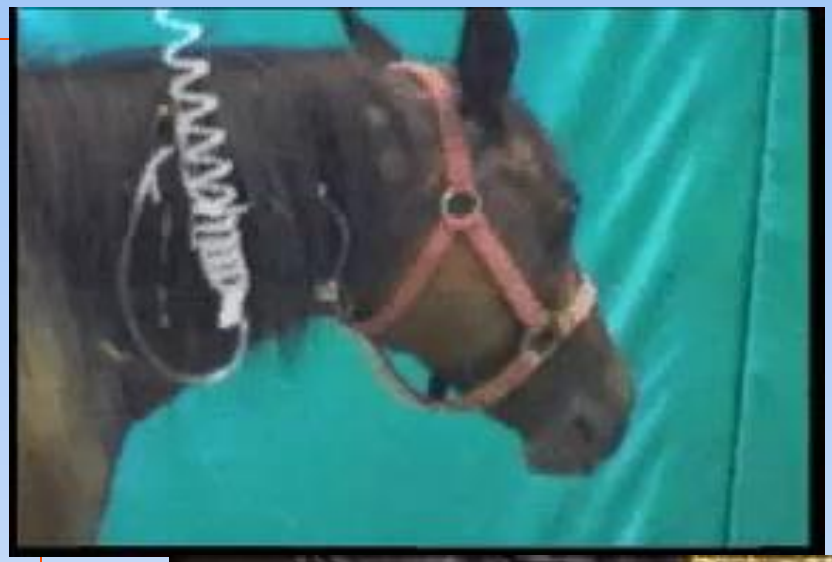
Since 2012 in re-emergent period-over 13,500 cases of WNV: 5,674 WN fever/encephalitis cases in 2012 with over 2,000 annually

Other Mammals

- Ewes: fever, abortion, rarely encephalitis
- Pigs: asymptomatic
- Dogs: asymptomatic
- Camelids: Alpaca's have relatively high virus in the brain!
- Rabbits now used for experimental infection

Clinical Signs - Horses

- 61% Fasciculation and Tremors
  - Head
  - Neck
  - Trunk
- >60% Change in Behavior
- Varies from hyperexcitability to somnolence



Clinical Signs - Horses

- Ataxia and Weakness

Paralysis

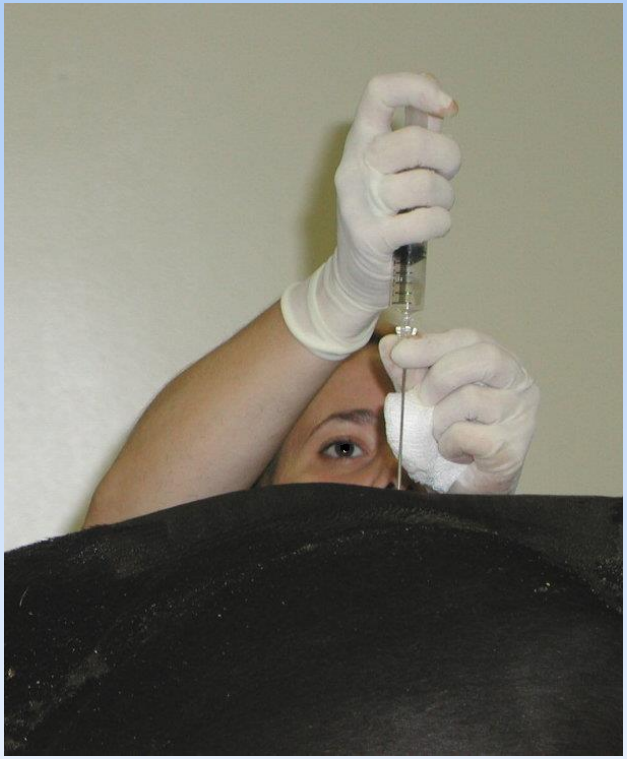
- Intermittent Weakness/ Paralysis
- Flaccid Paralysis/Recumbency

Mid- and Hindbrain Involvement

Facial paralysis	**39%**
Tongue paralysis	**20%**

Bloodwork

- CBC, Chemistry
  - No specific pattern
  - Horses early in disease show dehydration
- Spinal Tap
  - May be very helpful
  - Can indicate an encephalitis
  - Over 90% are abnormal



WNV CSF Analysis

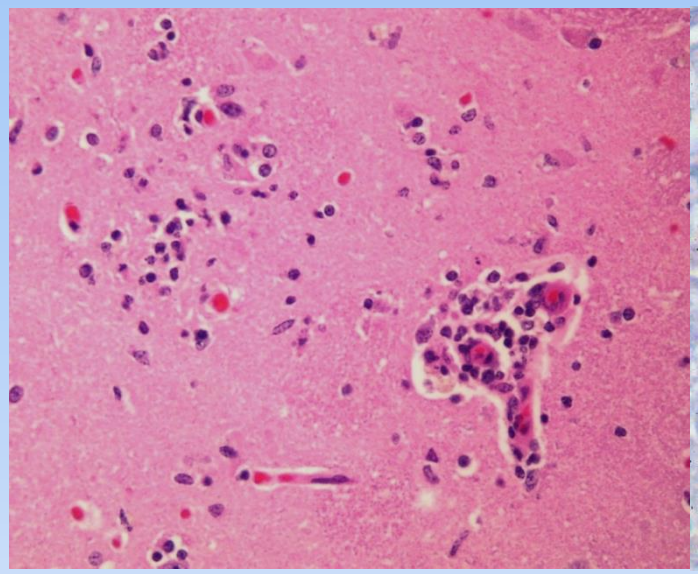
	LS		AO	
Parameter	**Median**	**Range**	**Median**	**Range**
Protein	**103 (72)**	**52-316**	**57 (72)**	**36-104**
RBC	**123**	**0-5400**	**2**	**0-376**
WBC	**14 (6)**	**0-310**	**6.5 (6)**	**0-882**
PMN (%)	**1**	**0-14**	**1.5**	**0-33**
Lymph (%)	**63**	**0-95**	**40**	**0-91**
Mono (%)	**29**	**0-81**	**54**	**0-96**

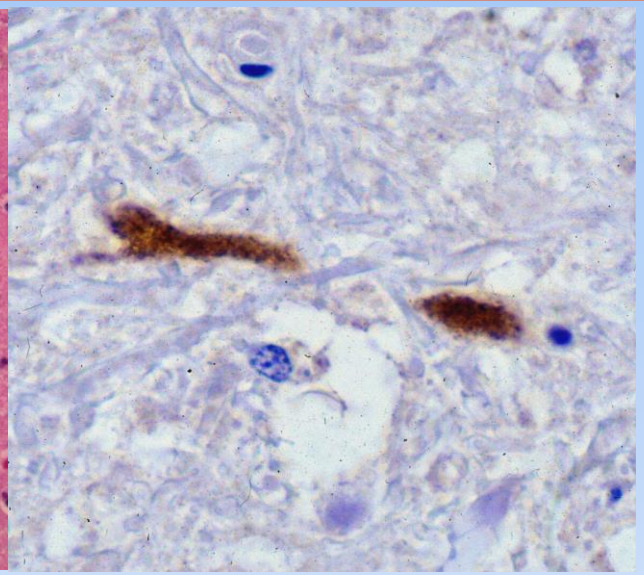
Wamsley et al, 2002 N=33

Diagnosis

- Very reliant on single IgM Capture
  - Can be confounded by recent vaccination
  - More recent publications demonstrate confonding
  - Experimental data indicates confounding
- In human testing, IgM confounded to SLE in FL (unless travel history)
  - Our study site in Pakistan of 1000 humans shows extensive cross-reaction to JEV, SLE, ZIKV, DENV 1-4

Post-mortem Confirmation More Difficult Than EEEV





Cerebrum +/-

Thalamus +++

Pons +++

Medulla +++

Cerebellum +/-

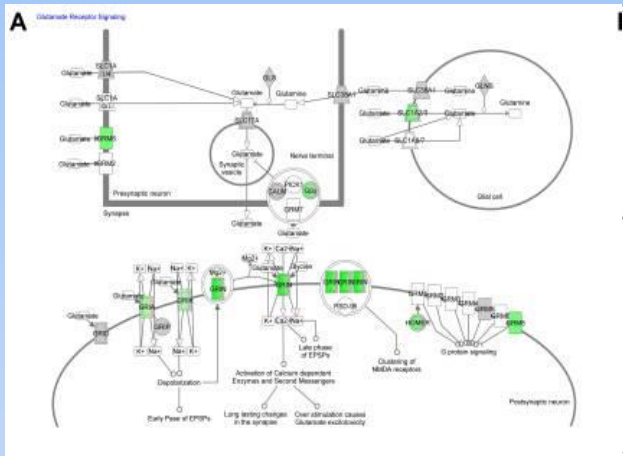
Cervical Cord +

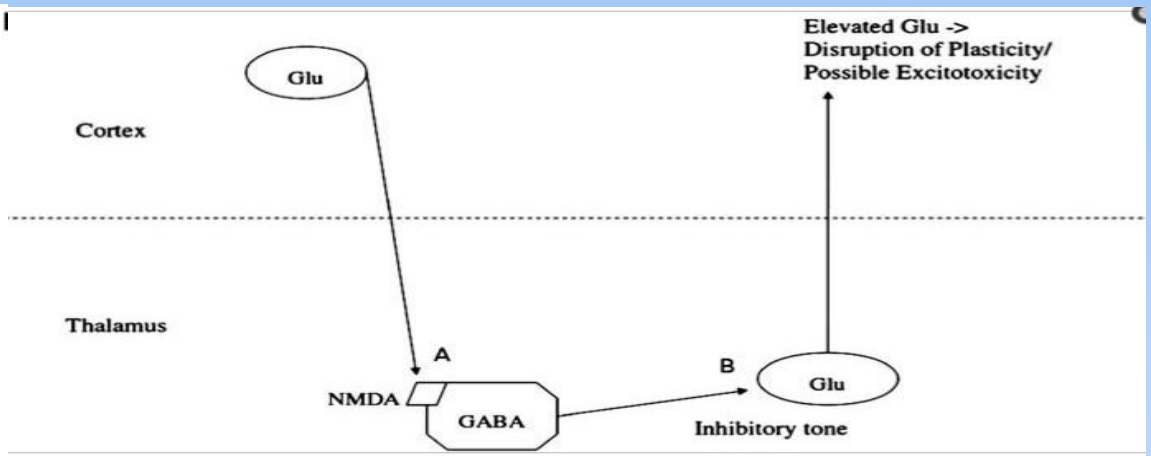
Lumbar Cord ++

All Tests Are Dependent On Receiving Sections with Lesions:
Any discoloration, multiple tissues

Nested rtPCR > Real-time rtPCR > Immunohistochemistry> Classic rtPCR

Pathogenesis=Treatment?





- Acute CNS invasion is leads to hyperexcitation:
  - Glutaminergic activation
    - Excitotoxity
    - Leads to increase CA++ in neuron, death of neuron if bad enough
- Treatments: Glutamate blocking, Glutamate Scavenging
  - Anti-psychotics?-Clonapine vs Acepromazine?
  - Glutamate receptor upregulators?-Minocycline
    - Will these blockers actually kill more neurons?

Discussion

- Are arbovirus diseases under reported in humans as they are in horses.
- How can we leverage animal/mosquito information better to inform practitioners of enhanced activity?
- Are medical practitioners similar to veterinary practitioners in under testing
  - We DVMs have a systemic bias
  - Also “no cure, no need to test”

Thank-you

4 Serologic Methods

- Presented at the AAVLD Meeting 2007
- Pederson DD, Albers SJ, Klein SM, Ostlund EN
  - USDA-APHIS, NVSL, Ames IA
- Looked at animals from 2005 season
- Compared 66 positive and 22 negative samples by IgM EEE
- Compared to HI, PRNT, CF
- All were then tested against WEE and VEE

Results-Positive IgM Capture

- 66/67 horses were positive to PRNT
  - 61/67 were only positive to EEE
  - 4/67 also positive to WEE
  - 4/67 also positive to VEE
- 59/67 horses were positive to HI
  - 48/67 horses were only positive to EEE
- 7/67 were positive to CF

Results-IgM Negative

- 17 were positive to the PRNT
  - 4 reacted to EEE only
  - 9 reacted to EEE and WEE
  - 3 reacted to VEE-all were from South America
- 16 were positive on the HI
- 8 were positive to the CF

Discussion

- IgM is highly useful on single serum sample
  - Especially for rapid screening
  - In EDART-if IgM positive to EEE, will be low positive to VEE but not WEE
- Those samples with a high suspicion and IgM, PRNT would be next useful
  - MUST have prior vaccine history
- Do our testing guidelines agree with this?

Serology Results

Table 4. Equine encephalomyelitis antibody titers in horses that yielded eastern equine encephalomyelitis (EEE) virus.

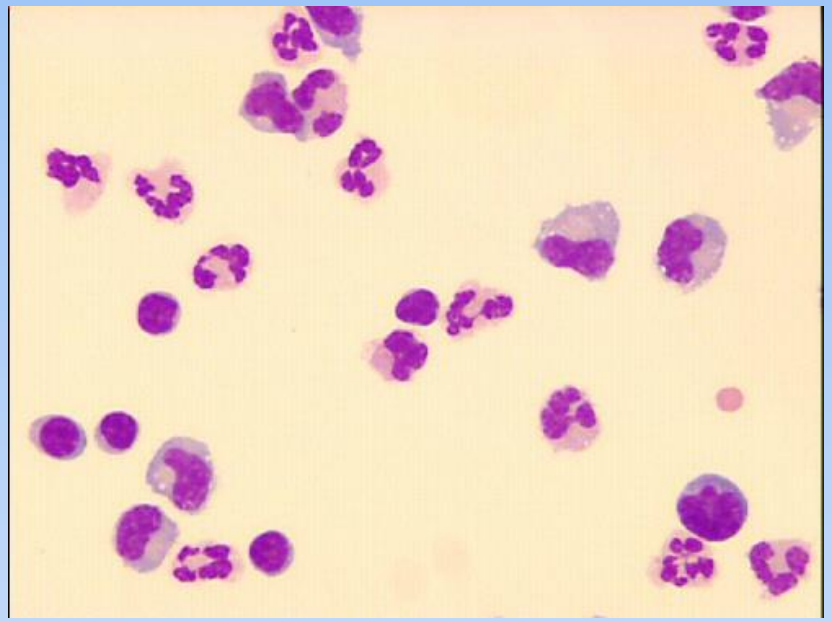
Sample no.	ELISA titer to EEE		Hemagglutination inhibition titers			Virus neutralization titers		
	IgM	IgG	EEE	WEE*	VEE†	EEE	WEE	VEE
17	<100	<100	160	160	80	<10	<10	<10
18	<100	<100	40	80	<10	<10	<10	<10
19	<100	<100	40	80	40	<10	<10	<10
20	<100	<100	640	640	640	<10	<10	<10
21	>1,000	<100	160	40	80	10	<10	<10
22	>1,000	<100	160	80	80	<10	<10	<10
23	>1,000	<100	40	80	40	10	<10	<10
24	>1,000	<100	<10	<10	<10	ND‡	ND	ND
25	>1,000	<100	10	10	10	<10	<10	<10
26	>1,000	<100	40	10	<10	>100	10	<10
27	>1,000	<100	>1,280	160	40	100	<10	<10
28	>1,000	1,000	40	160	40	100	<10	<10
29	>1,000	1,000	40	40	10	10	<10	<10
30	>1,000	100	160	160	40	>100	<10	<10
31	>1,000	100	80	320	20	>100	<10	<10

* Western equine encephalomyelitis.
† Venezuelan equine encephalomyelitis.
‡ ND = not done.

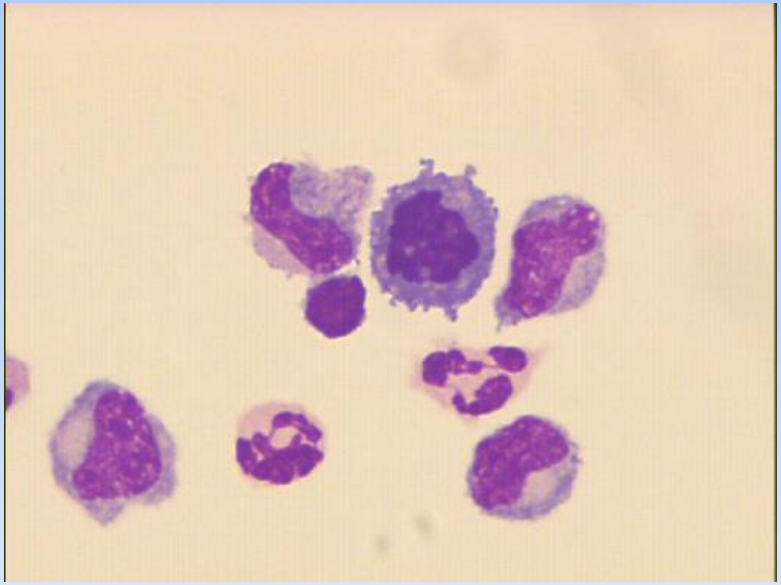
Cerebrospinal Fluid

	EEE	WEE	WNV	Rabies	EPM	EHV	Parasite	Bot
Protein	⇑	⇑	**N to** ⇑⇑	**N to** ⇑	**Moderate >80 mg/dl**	⇑⇑⇑	⇑⇑	**N**
Cells	⇑⇑	**N or** ⇑	**N to** ⇑ ⇑	**N to** ⇑	**N to** ⇑	**N**	⇑⇑	**N**
Cell Types	**P, LM**	**LM**	**LM**	**LM**	**LM**	**LM**	**P, E**	**N**
Color	-------	--------	(Xantho	(Xantho)	(Xantho)	**Xantho**	----	**N**

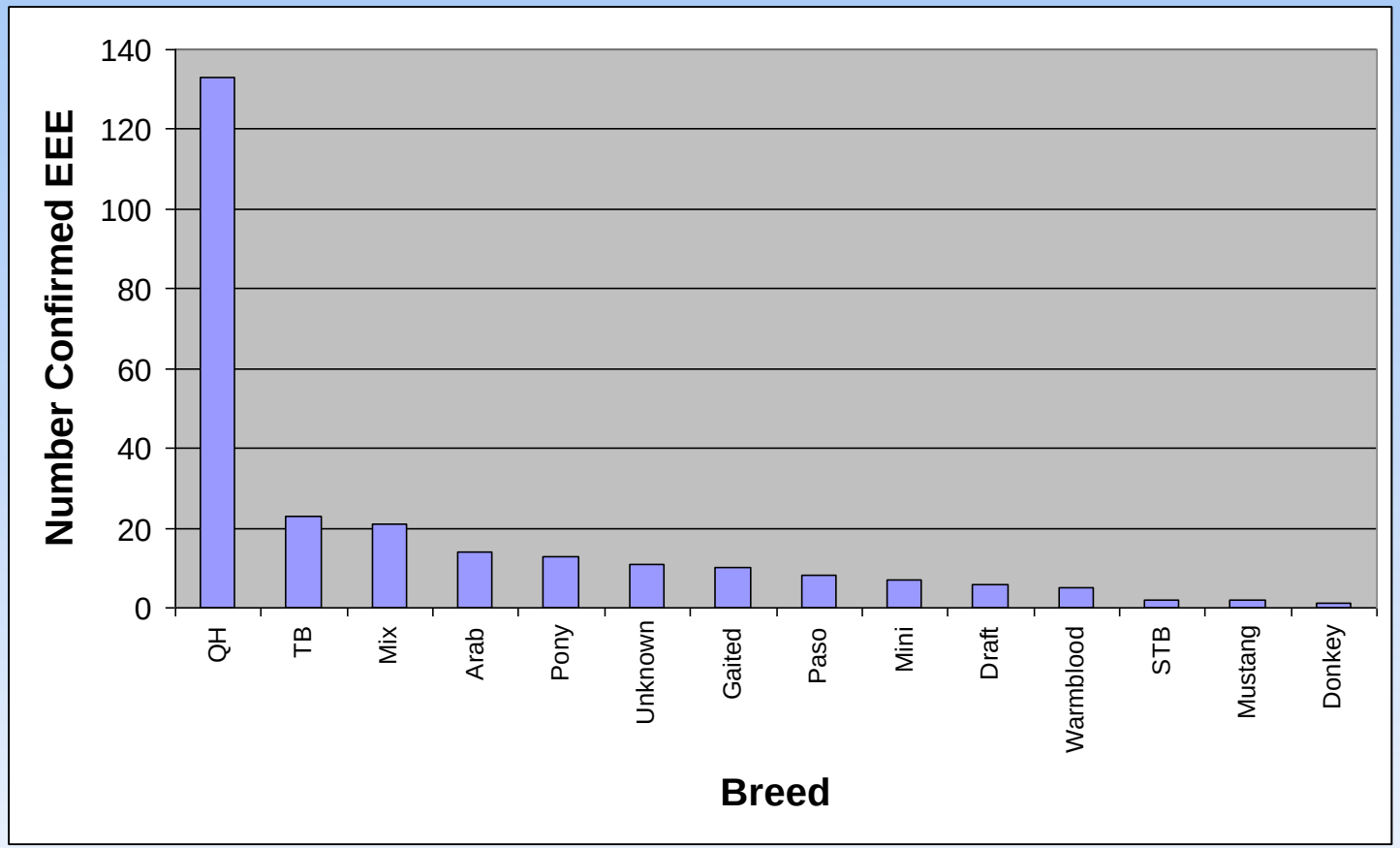
P=PMN LM=Lymphs, Monos



Eastern Equine: Hypersegmented Neutrophils



Confirmed EEE Breeds



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EEE & WNV Clinical Signs

Clinical Sign	% EEE	% WNV
Aimlessness	28	---
H. Pressing	17	1-2
Hyperaesthesia	12	63
Blindness	8	1-2
Seizures	8	1-2
Coma	8	---