EPM WORKSHOP 2014
Hosted by the EPM Society

Thursday, October 23

8:00-8:30 REGISTRATION AND CONTINENTAL BREAKFAST

8:30-8:45 Greetings, program overview and goals, *Steve Reed*

8:45-12:30 Morning topics with presenter/discussion moderator: summary of current knowledge followed by open discussion of attendee submitted questions

- 8:45-9:15 Biology, *Dave Granstrom*
- 9:15-10:15 Infection models, *Bill Saville and Siobhan Ellison*

10:15-10:30 MORNING BREAK

- 10:30-11:30 Functional biology, genetics, *Dan Howe and Mike Grigg*
- 11:30-12:30 Immunology, vaccine, *Sharon Witonsky and Martin Furr*

12:30-1:30 CATERED LUNCH

1:30-4:30 Afternoon topics with presenter/discussion moderator: summary of current knowledge followed by open discussion of attendee submitted questions

- 1:30-2:30 Laboratory diagnostics, *Jennifer Morrow and Amy Johnson*
- 2:30-3:30 Treatment, prevention, *Nicola Pusterla and Steve Reed*

3:30-3:45 AFTERNOON BREAK

3:45-4:30 Thursday summary and Friday preview, *Moderated by Steve Reed*

4:30-6:30 Oral abstract presentations (10 minute talk with 5 minute Q&A) with appetizers and drinks, *Moderated by Rob MacKay*

6:30 CATERED DINNER

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Friday, October 24

8:00-8:30	CONTINENTAL BREAKFAST
8:30-9:30	10-minute topic summaries (by presenter/discussion moderators), *Moderated by Martin Furr*
9:30-10:30	Genetic approaches to tracking immunity to toxoplasma, *Chris Hunter, University of Pennsylvania*
10:30-10:45	MORNING BREAK
10:45-noon	Open discussion, project proposals, wrap-up and adjourn, *Moderated by Steve Reed*
Afternoon	Races at Keeneland or return home

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Welcome to the EPM Workshop

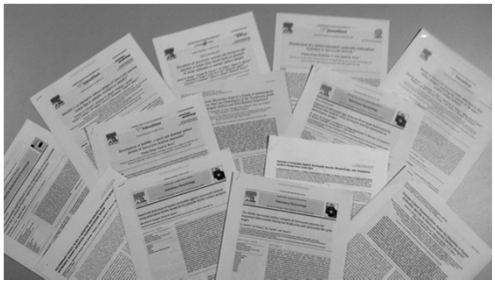
Stephen M. Reed, DVM, Diplomate ACVIM
Rood and Riddle Equine Hospital Lexington, KY
Emeritus Professor The Ohio State University
Adjunct Professor University of Kentucky

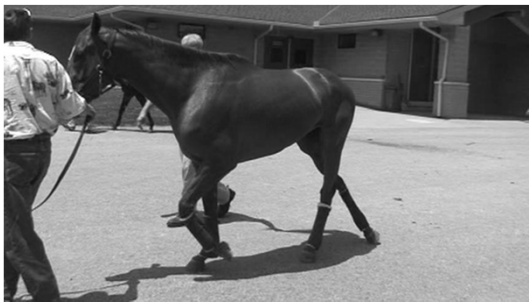
Acknowledge and Thank our sponsors

Why are we here?

To better understand EPM and where we are with this disease

Belmont Opossum





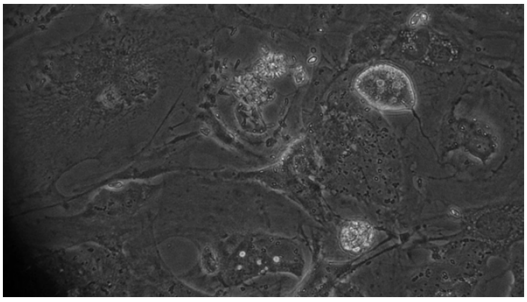
- **EPM**
  - **Unresolved issues:**
    - **How do you diagnose a horse with EPM ?**
    - **Pathogenesis – why do many horses become infected yet so few develop signs of disease ?**
      - **What accounts for the difference ?**
        - **Strain variation**
        - **Parasite virulence**
        - **Horse immune system**
        - **Level of exposure**
    - **What level of immunity is protective?**
    - **Would current chemotherapies be effective against latent forms?**
    - **Could a vaccine ever be developed?**

- **EPM**
  - **Despite many unresolved issues we have made progress on this disease and have come along way since "Segmental Myelitis" of the 60's**

Overview: What We Know

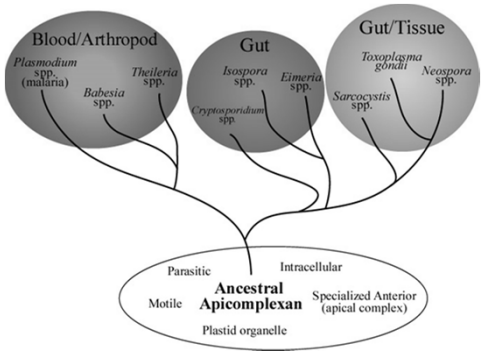
- **Introduction to *Sarcocystis neurona* and EPM**
  - **Biology, epidemiology, and pathogenesis**
- **Recent basic research**
  - **Sequencing projects, parasite genes/proteins, and antigen variation**
- **Improved EPM diagnosis**
- **Available EPM treatments**
- **Prospects for EPM prevention**

Sarcocystis neurona in cell culture



Sriveny Dangoudoubiyam, M.H. Gluck Center

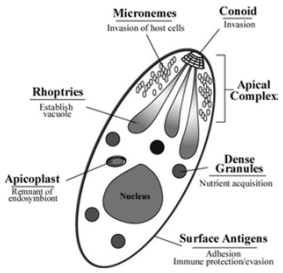
Phylum Apicomplexa



Ultrastructure of an apicomplexan

- **Eukaryotic organisms**
- **Numerous specialized organelles and virulence factors**
  - **Mostly novel molecules**
  - **Candidate targets for:**
    - **Chemotherapeutics**
    - **Diagnosis**
    - **Vaccines**

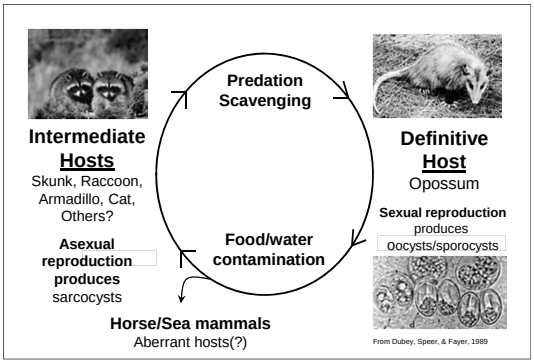
Cartoon apicomplexan cell (zoite)



Genus *Sarcocystis*

- **>100 described species that infect mammals, birds, reptiles, fish**
- **Very host specific, two-host life cycle**
- **Develop "sarcocysts" in tissues (muscles) of intermediate host animals**

Sarcocystis neurona life cycle



- **EPM - Unresolved issues:**
  - **How do you diagnose a horse with EPM ?**
  - **Pathogenesis – why do many horses become infected yet so few develop signs of disease ?**
    - **What accounts for the difference ?**
      - **Strain variation**
      - **Parasite virulence**
      - **Horse immune system**
      - **Level of exposure**
  - **What level of immunity is protective?**
  - **Could a vaccine ever be developed?**

- Is There a Best Test
- Regardless of what test:
  - Should include a neurological examination
  - Along with Testing on Blood and CSF
  - What's available?
    - Western Blot (EDS, Neogen, MSU, IDEXX, UC- Davis)
    - SAG 1/ ELISA (Pathogenes, Antech)
    - IFA-UC Davis
    - SAG 2 & SAG 4/3 Elisa's- EDS

Diagnosis- Why look for a new test?

- Lack of consistent findings when submitting split samples to multiple labs
- Difficulty interpreting results
- Desire to not over treat
- Desire to not miss true EPM cases
- Belief that CSF increases accuracy of diagnosis

Main Take-Home Points

- Basic research will provide the foundation for developing clinical applications
  - Genome project, characterization of parasite genes/proteins
- Improved EPM diagnosis
  - SnSAG2+4/3 serum:CSF ratios
- EPM treatment options
  - Effective anti-coccidials are available
- Prospects for EPM prevention
  - Chemotherapeutic – logical and most likely safe
  - Vaccination – seemingly feasible, but a long term proposition

Questions?

Equine Protozoal Myeloencephalitis: History and Biology

David E. Granstrom, DVM, PhD, DACVM
(Parasitology)

Overview

- Brief History of EPM
- General Biology Review

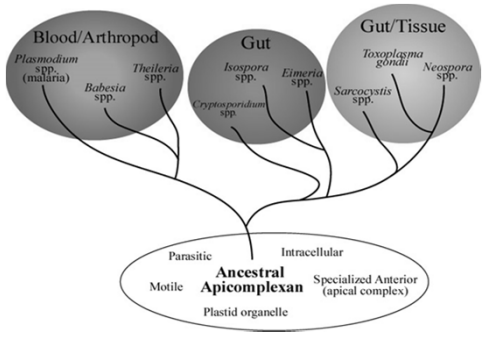
History

- Segmental Myelitis, Rooney 1964
- "Toxoplasma-like" protozoan recognized - EPM, Beech; Cusick; Dubey, 1974
- *Sarcocystis* ?, Dubey, 1976
- *Sarcocystis neurona* cultured and named, Dubey, et. al, 1991

History

- Opossum identified as definitive host, Fenger, et. al, 1995
- *Neospora caninum* / *N. hughesi*, Daft, et. al., 1996
- Intermediate hosts identified, Dubey, et. al., 2000, 2001; Cheadle, et. al., 2001

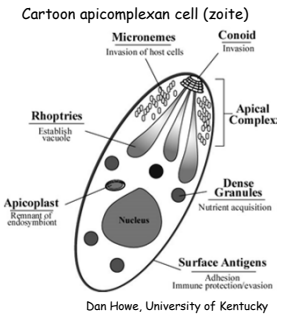
Phylum Apicomplexa



Dan Howe, University of Kentucky

Apicomplexan Ultrastructure

- Eukaryotic organisms
- Numerous specialized organelles and virulence factors
  - Mostly novel molecules
  - Candidate targets for:
    - Chemotherapeutics
    - Diagnosis
    - Vaccines



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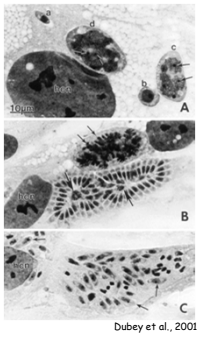
Dan Howe, University of Kentucky

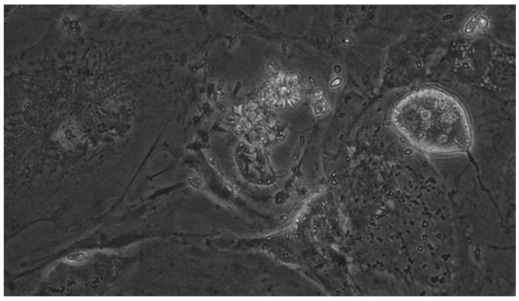
Intracellular development of *S. neurona*

- Endopolygeny
- Chromosome duplication dissociated from nuclear and cell division
  - Large cell body with polyploid nucleus
- Sixth duplication concurrent with nuclear division and budding of daughter cells
  - Produces 64 haploid merozoites



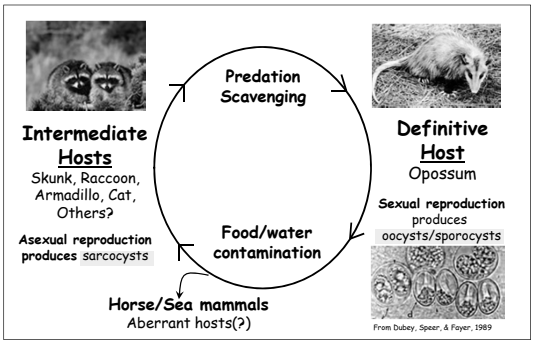
Dubey et al., 2001

Sarcocystis neurona in cell culture



Sriveny Dangoudoubiyam, M.H. Gluck Center

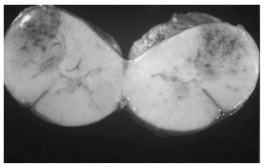
Sarcocystis neurona life cycle



Dan Howe, University of Kentucky

S. neurona infection of horses and marine mammals

- Normal propagation in lymphatics and vascular endothelium
- Invasion of central nervous system
  - Parasites often not apparent
  - Pathology due to inflammatory response



Neil Williams, University of Kentucky VDL

Geographic Distribution

- Occurs in North, Central, and South America
- Geographic range depends on presence of competent definitive host (opossums) for *S. neurona*

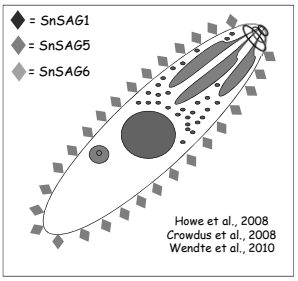
Dan Howe, University of Kentucky

Seroprevalence vs. Incidence

- *S. neurona* infection ≠ disease
  - High seroprevalence (30-50%)
  - Low disease incidence (<1%)
  - Latency
- Factors influencing disease remain unclear
  - Immune competence? Inoculum size? Strain Variation?

At least 3 alternative major surface antigens are found in *S. neurona* strains

- SnSAG1, SnSAG5, and SnSAG6 are mutually exclusive
  - Only one gene present in any given strain



Dan Howe, University of Kentucky

The OSU Team

Bill Saville – Team Lead
Steve Reed - Neurology
Mike Oglesbee - Pathology
CD Sofaly – Neurology
JP Dubey – Life Cycle
DE Granstrom – Life Cycle
AE Marsh – Life Cycle

Epidemiology

- High exposure rates to the parasite in some parts of the country
- Ohio - ~54%; Oregon 22 to 65%; Colorado ~34%
- Low incidence of clinical disease – 14 cases/10,000 horses/yr (<0.5%)

Epidemiology

- Farm/ranch horses – 1 ± 1 cases/10,000 horses/yr
- Pleasure horses – 6 ± 5 cases
- Breeding horses – 17 ± 12 cases
- Racing horses – 38 ± 16 cases
- Show/competition horses – 51 ± 39 cases

NAHMS Report, 2001: EPM in the US horse population

Epidemiology

- Risk Factors for exposure
- Age – increased age = increased risk
- Season effects – exposure rates lower with increased freezing days
- Climate may affect parasite transmission
- May also be related to definitive host activity

Saville *et al*, 1997. JAVMA 210(4): 519-524.

Epidemiology

- Risk factors for disease
- Increased risk – age (1-5; >13; <1-1/20; 6-13 yr – 1/3); season of admission (Sp 3X; Su 3X; Fa 6X); woods on premises (2X); prior EPM diagnosed on premises (2.5X)

Saville *et al*, 2000. JAVMA 217(8): 1174-1180

Epidemiology

- Risk factors for disease
- Increased risk – racing (5X) or showing (11X) as primary use; opossums on premises (2.5X); health events prior to diagnosis (<15 – 2X; >15 – 30d – 3X; >30 – 90d – 10X; >90d – 16X)
- Decreased risk – creek or river on premises (1/2); feed security (1/2 – 1/3)

Epidemiology

- Risk Factor
- Transport stress is an important risk factor
- Equine model of disease study completed in fall of 1999 at OSU, horses that received sporocysts immediately on arrival, were more severely affected and seroconverted much earlier than groups that were acclimatized for 2 wks

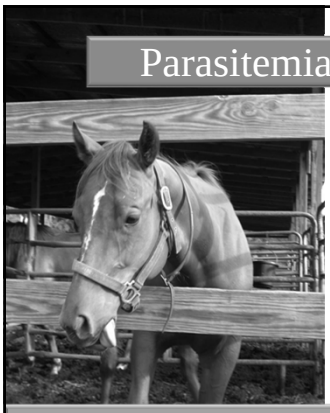
Saville *et al*, 2001. Vet Parasitology, 95: 211 - 222

Recent Developments

- IFA test developed at UCDavis demonstrated cross reactivity with *Sarcocystis fayeri* (Saville et al, 2004)
- SAG 1 test developed in Florida will not detect the raccoon strain (Ellison et al, 2002)
- Recent study by Hoane et al (2005) demonstrates excellent SN and SP using recombinant surface antigen antibodies SAG 2 - 4

Recent Developments

- 1st time *S. neurona* isolated from MLN, Liver and Lung
- Quick infection rate
- -- parasites in organ tissue by 1 dpi
- -- lesions in CNS by 7 dpi



Parasitemia to Paresis

How Why What
of the
Trojan Horse Model

Siobhan Ellison DVM PhD
Tom Kennedy PhD
Karen Brown PhD

A model of *S. neurona* infection that induces EPM

We learned that SN infections are biphasic in horses. CNS inflammation precedes ataxia in a sequential fashion in all animals. There are quantifiable changes in innate and adaptive immune responses.

- Examiner experience can make a difference in diagnosis
- Neuroinflammation (early signs, CNS inflammation) precedes ataxia
- Degree of proliferation responses (PMI/I WITONSKY) correlate with outcome
- CSF antibody peaks then declines decreasing the diagnostic value of CSF
- Specific antibodies are more valuable for diagnosis of *S. neurona* sarcocystiasis
- Drugs can modify the course of disease

All studies were blinded, placeo controlled. The primary outcome variable was gait assessment by ACVIM veterinarians.

- Animal evaluations from challenge
  - **7 board certified veterinarians evaluated 74 horses**
  - **Minimum of 2 veterinarians per study**
  - **Blinded to challenge, always placebo controlled**
  - Always had sentinel, unchallenged animals on farm
  - Histopathology to validate model in 4 horses

Merozoite infected WBC's are horse-adapted and allowed similar challenge material between studies.

Why use merozoites?

- Pathogenesis of *Sarcocystis* is via parasitemia.
- Repeatability
  - technically difficult to identify sporocysts
  - difficult to produce sporocysts for multiple studies.
- Avoid unintended consequences of intermediate hosts
  - intermediate host bias sporocyst population.
- Archive merozoites for infections (consistency)
  - USDA compliant validation assays.

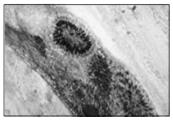
Isolation of parasites was accomplished by SAG 1 immunoaffinity beads. Successful isolation using this challenge may illustrate the importance of strain or stage.

- How the model differs from other models:
  - Challenge with SAG 1 merozoite
    - **isolated from 4 horses**
    - **same organism in all experiments**
- Repeatable
  - Multiple studies
  - Multiple investigators
  - Multiple sites

Demonstrated that *S. neurona* was recovered from CNS tissues (4/4)

***S. neurona* is an obligate intracellular parasite. Egress or purification allows extrusion of the conoid with exposure of microneme proteins, and may suggest that (stage and strain dependent) binding of microneme proteins on cell receptors is important to breach B/CSF/B.**

- Principle of infection
  - Obligate intracellular parasite
  - Parasitemia
  - *neurona* infects equine
  - leukocytes
- Critical issues
  - Pass through intended host
  - Monolayer selection
  - Stage of merozoite
- Method of infection
  - Enrich leukocytes
  - Infect leukocytes *in vitro*
  - Return infected cells to horse



S. Neurona infects any horse. Infections can be identified by serum antibodies.

- Horses that get *S. neurona* in CNS are not immune deficient
  - We can infect any untreated, unvaccinated horse.
  - Clinical signs are dose dependent
- 24 horse study, 3 blinded examiners, statistics
  - CSF testing is not meaningful to diagnose EPM
    - No statistical significance using CSF antibody to identify EPM horses
    - CNS signs are dose dependent; parasites in CNS are not
  - Statistically significant increase in antibody titer with increased duration of infection
    - Seroconversion supports challenge

Study assessments of model infections were clinical observations and immune responses. Outcome can be altered with drugs.

Serial neurological exams ……*Early signs of EPM*
Blinded gait exams ……..... *Gait deficits-ataxia*
Specific IgG (SAG 1)……..…. *adaptive immune response*
Specific T cell responses…….. *adaptive immune response*
non-specific T cell responses……...*innate immune response*

Outcome can be altered by specific treatments

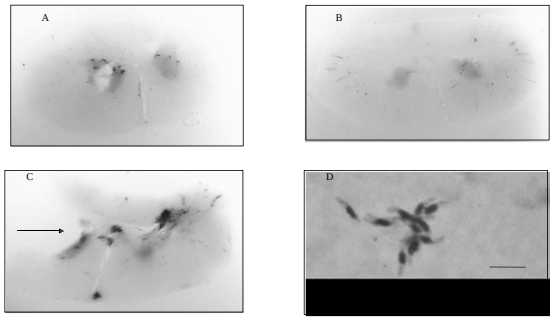
Sensitivity and Specificity of clinical exam may vary with examiner experience

Infection defined as clinical signs and ataxia after challenge

Blinded exams every 30 days

		Examiner 1		University Exam		Immunoblot (WB)		SAG1 ELISA	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Test+	Positive	35	3	5	4	34	3	38	1
Test -	Negative	5	2	2	1	6	2	2	4
		40	5	7	5	40	5	40	5
	sens	0.88		0.71		0.85		0.95	
	spec	0.4		0.20		0.4		0.8	
	PPV	0.92		0.56		0.92		0.97	
	NPV	0.29		0.33		0.38		0.67	

Areas of necrosis caused by *S. neurona* infection are shown. Parasites were recovered from CNS tissues by culture from area shown by the arrow (C).



Ataxia was observed in 94% of untreated challenged horses.

False negative WB results were observed in 21% of the animals.

Infection defined as ataxia determined by blinded exams

	N=	NA	ataxia	signs	SAG Serum	SAG AI	Neg WB	Pos WB
Challenged	66						21%	79%
No treatment	33	2	31	32	33	32	5	28
Treated	33	19	14	19	33	33	9	10
No treatment	0.50	0.06	0.94	0.97	1.00	0.97	0.15	0.85
Treated	0.50	0.58	0.42	0.61	1.00	1.00	0.47	0.53

Ataxia was present in 90% (challenged untreated horses) by 23 DPC

Infection defined as ataxia determined by blinded exams

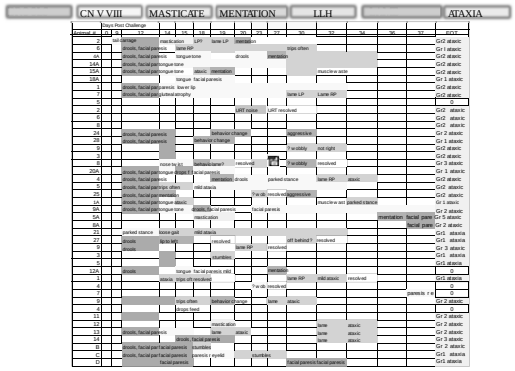
Days to ataxia	0-23	24-30	31-60	61-90	NA	
No treatment	27	2	1	1	2	31 ataxic/33 challenged
Treated	0	10	3	1	19	14 ataxic/33 challenged
No treatment	0.9	0.1	0.0	0.0	0.1	
Treated	0.0	0.7	0.2	0.1	0.6	

Neuroinflammation was present in 90% (challenged untreated) horses by 23 DPC

Infection defined as ataxia determined by blinded exams

Days to signs	0-23	24-30	31-60	61-90	NA	
No treatment	30	2			1	32 w signs/33 challenged
Treated	15	3	1		14	19 w signs/33 challenged
No treatment	0.9	0.1			0.0	
Treated	0.8	0.2	0.1		0.4	

CNS inflammation was sequential, quantifiable, and preceded ataxia in all horses suggesting pathogenesis of disease.



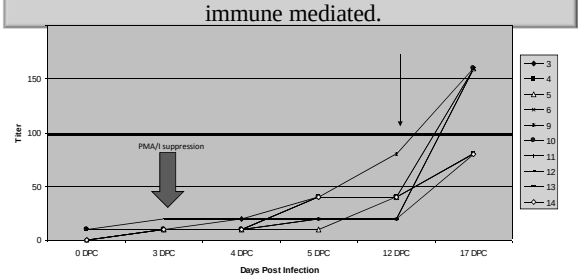
The sequential incidence of clinical signs, by occurrence, in 37 challenged horses that became ataxic indicates a mechanism of disease that is not random.

EPM in challenged	Drops feed	Drools	Slack lips	Facial paresis	Mastication	Ptosis
3	3	3	3	3	0	1
9	9	8	7	7	1	1
21	15	15	18	6	1	1
4	3	3	3	3	1	1
37	30	29	31	19	3	4
88%	81%	78%	84%	51%	10%	11%
Mentation change	Parked stance	Weakness	Muscle w aste	Limb paresis	LLH	Ataxia
2	2	2	1	2	nd	3
4	5	5	4	5	1	9
11	5	16	4	3	11	21
3	2	4	2	2	nd	4
20	14	27	11	12	12	37
54%	38%	73%	30%	32%	40%	100%

Relevance of cumulative (weighted) scores in disease outcome in a blinded, placebo controlled 10 horse study is that disease is progressive.

	1	2	3	4	5	6	7	8	9	10
Onset signs Days Post Challenge	52	6	13	6	11	6	83	6	12	6
Early signs	no	yes	yes	yes	yes	yes	no	yes	yes	yes
Ataxia		**Grade 3**		**Grade 3**	**Grade 3**			**Grade 1**		**Grade 2**
Paresis	**Grade 1**	**Grade 3**		**Grade 3**	**Grade 3**			**Grade 1**		**Grade 3**
Cumulative Weighted Score	35	472	14	610	442	12	13	217	5	228

Immune suppression, detected by PMA/I, detected at 72 hours, followed by simultaneous appearance of clinical signs and antibodies suggest the disease is biphasic and immune mediated.

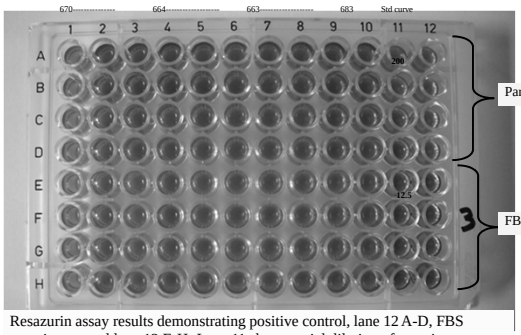


The antibody detected by SAG1 ELISA correlated with clinical signs of disease when blinded observers evaluated horses before and after infection.

Resazurin assay in challenged horses is useful to assess the model

- Assay that quantitatively evaluates cell viability
  - proliferation of mammalian cell lines, fungi, bacteria
  - Commonly used in literature
- Resazurin measures innate cellular metabolic activity
  - Resazurin is reduced and measures (visually or optically) the viable cells that are present in a test sample.
  - Dye is reduced by NADPH, reduced flavin adenine dinucleotide, reduced flavin mononucleotide, and the cytochromes produced inside the cells
- Uses of Resazurin dye assay in *S. neurona* infections
  - Viability of challenge organism
  - Quantitate parasitemia during and following challenge
  - Quantitate serum neutralization by strain

Serum neutralization by resazurin assay

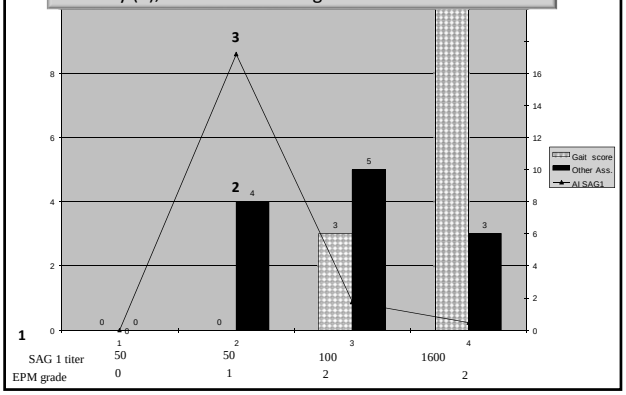


Resazurin assay results demonstrating positive control, lane 12 A-D, FBS negative control lane 12 E-H; Lane 11 shows serial dilution of parasites to obtain a standard curve to quantatively determine neutralization.

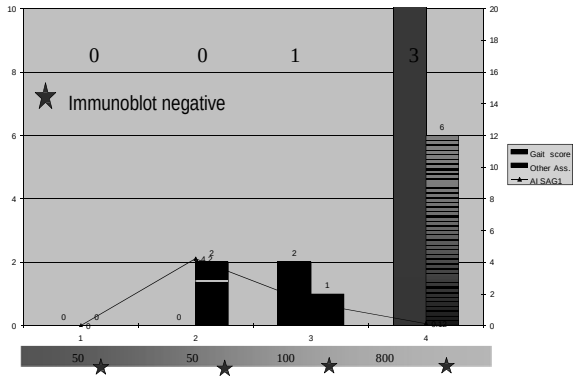
Calculation of AI SAG1 to evaluate CNS antibody post-challenge

- AI=AQ/Alb Q
- AI≥1 is significant
- Titer is the reciprocal of the last dilution with a positive OD_{405} rSAG1 ELISA
- AQ=(CSF titer)x 1000/serum titer
- AlbQ= (CSF alb/serum alb) x 1000

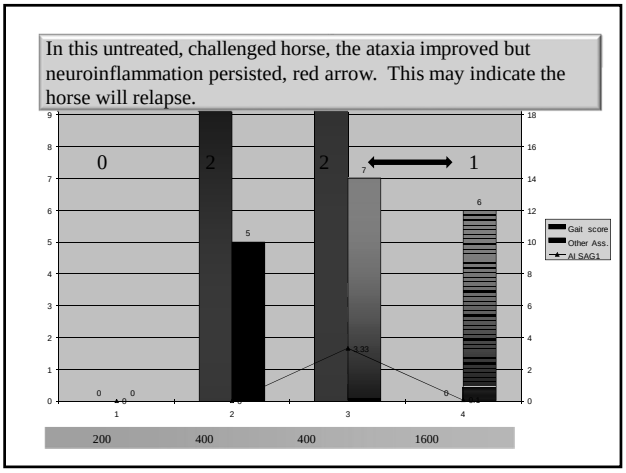
Typical EPM horse showing increasing SAG titer (1), early signs precede gait deficits (2), and rise and fall of CSF antibody (3), untreated challenged horse.



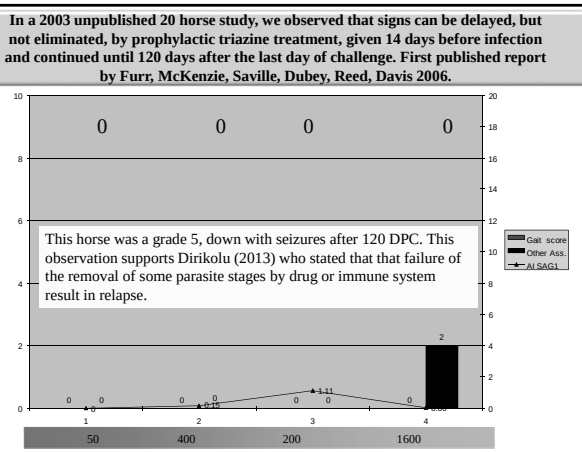
In this typical untreated, challenged horse, EBI immunoblot (serial serum and CSF samples) did not detect immunoconversion.



In this untreated, challenged horse, the ataxia improved but neuroinflammation persisted, red arrow. This may indicate the horse will relapse.



In a 2003 unpublished 20 horse study, we observed that signs can be delayed, but not eliminated, by prophylactic triazine treatment, given 14 days before infection and continued until 120 days after the last day of challenge. First published report by Furr, McKenzie, Saville, Dubey, Reed, Davis 2006.



Levamisole HCl supports the pathogenesis of EPM is immune mediated because it can alter clinical signs.

- Levamisole HCl prevents clinical signs in challenged horses.
  - A parasitemia of 1 million parasites was detected by blood smear
  - No clinical signs were observed during and after challenge
  - Speculate that Levamisole HCl modulates the PMA/I pathway
- Levamisole HCl alleviates clinical signs of EPM in field infections.
  - Levamisole HCl regulates JAK/Stat pathways by down regulating IL6 production.

A change in innate and adaptive immune responses were measured in the studies

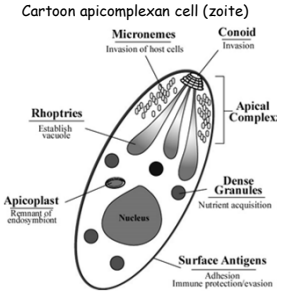
- Significant changes in immune system after challenge
  - IFN γ, IL6, C-reactive protein, PMA/I suppression of WBC proliferation
- Treatment decreased IgG response in challenged group
  - Modification of disease by specific treatment supports model
- Serum antibody levels increased from 30 to 150 DPC
  - Statistically significant antibody increases with duration of infection
  - CSF antibody and clinical disease were not statistically significant
- Immunoblot does not detect antibody in SAG 1 challenge horses.

The Trojan Horse model indicates EPM is a biphasic, immune mediated, inflammatory disease. CSF antibody is not directly related to clinical course of EPM.

•Questions?

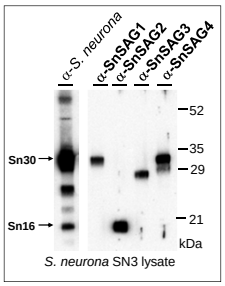
Molecular Composition of *Sarcocystis neurona*

- Specialized organelles & virulence factors
  - Study to better understand intracellular parasitism and pathogenesis
  - Exploit for:
    - Diagnosis
    - Chemotherapeutics
    - Vaccines



S. neurona major surface antigens (SnSAGs)

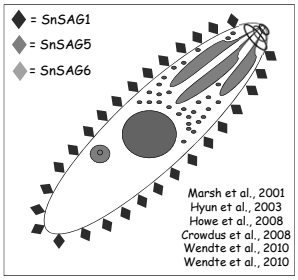
- ID based on homology to SAG/SRS gene family in *Toxoplasma*
- Elicit robust immune responses in horses
  - Logical candidates for serodiagnosis and vaccine development



Howe et al., 2005

SnSAG variation in *S. neurona* strains

- Alternative major surface antigen
  - SnSAG1, SnSAG5, and SnSAG6
- Mutually exclusive
  - Only one gene present in any given strain



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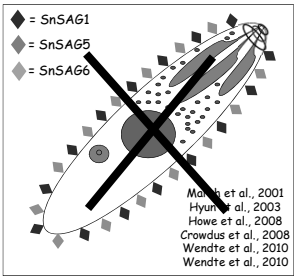
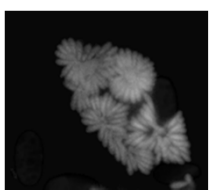


Molecular Genetic Tools for *S. neurona*

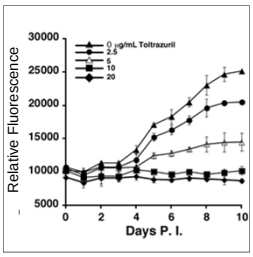
- Reporter molecules
  - Luciferase, β-galactosidase, yellow fluorescent protein (YFP)
- Selection of stable transformants
  - Mutant DHFR for Pyr^r
  - HXGPRT knockout for 6-TX/MPA positive and negative selection
  - FACS



Transgenic *S. neurona* expressing YFP

Molecular Genetic Tools for *S. neurona*

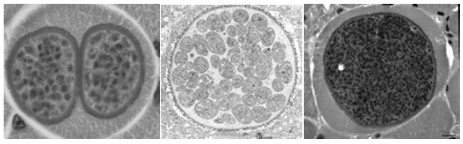
- Enables new experimental approaches
  - gene knockouts
  - complementation studies
  - gene regulation assays
  - etc.



In-vitro growth assay with SN3(YFP)

National Institute of Allergy and Infectious Diseases
Leading research to understand, treat, and prevent infectious, immunologic, and allergic diseases.

Sarcocystis neurona, an emerging parasite infecting marine mammal sentinels



Molecular Parasitology Section

Aim: Investigate the molecular origins and genetic basis of protozoal pathogen emergence and disease

NIAID

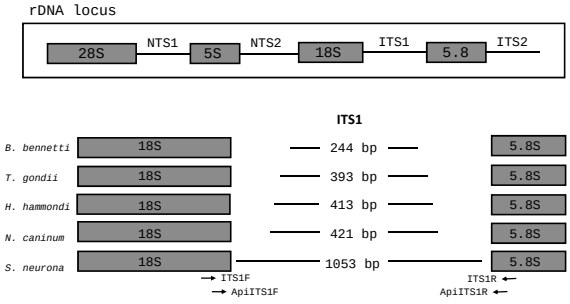
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Michael E. Grigg, Ph.D.
Laboratory of Parasitic Diseases/ NIAID / NIH

S. neurona Population Genetics

- Develop genetic markers to qualitatively and/or quantitatively describe the population genetic diversity of *S. neurona* in the USA
- Determine factors that account for the diversity (or lack thereof) present
- Applications to disease:
  - Trace the emergence and transmission of strains
  - Determine strain associations with disease

Barcoding for Coccidian Biodiversity



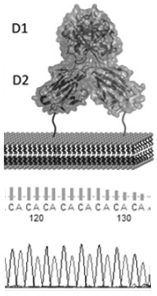
Typing strategy: PCR-DNA sequencing

Surface antigen genes (SnSAGs)

- Host pathogen interface
  - Focus of antibody response
  - 6 known SnSAGs (SnSAG1-6)
- Successful as markers for *T. gondii*

Microsatellite markers

- Previously demonstrated intra-strain variation

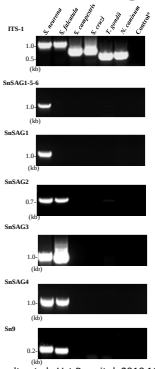


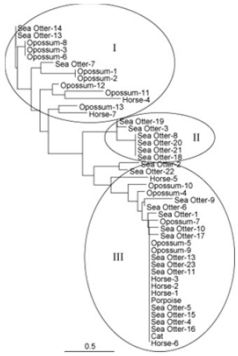
Vet Parasitol. 2008 Nov 25;158(1-2):36-43
Infect Immun. 2005 Feb;73(2):1023-33

Infect Genet Evol. 2006 Sep;6(5):352-60.
Vet Parasitol. 2008 Mar 25;152(1-2):8-15

Rejmanek et al., Vet. Parasitol. 2010 170:20-29
Wendte et al., Vet Parasitol. 2010 169:37-44

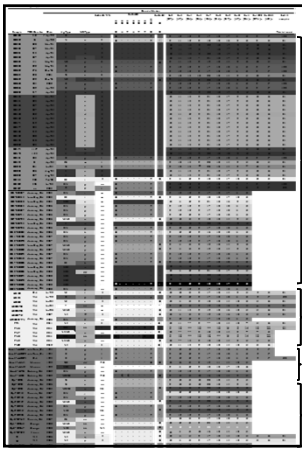
S. neurona has a simple population genetic structure



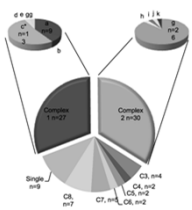


Wendte et al., Vet Parasitol. 2010 169:37-44

Rejmanek et al., Vet. Parasitol. 2010 170:20-29



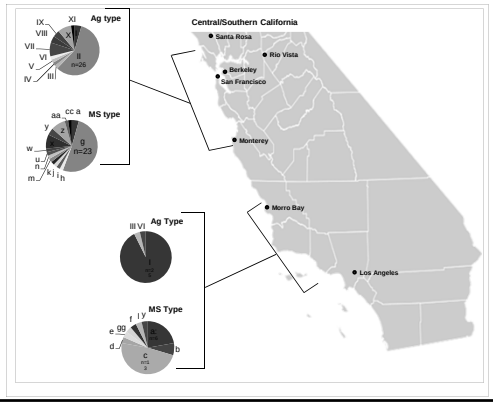
13 distinct *S. neurona* genotypes are circulating in the USA



Two *S. neurona* clonotypes comprise the majority of strains

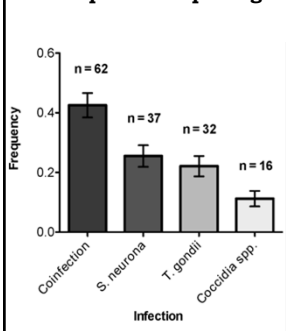
Wendte et al. PLoS Genetics 2010

S. neurona strain diversity partitions geographically

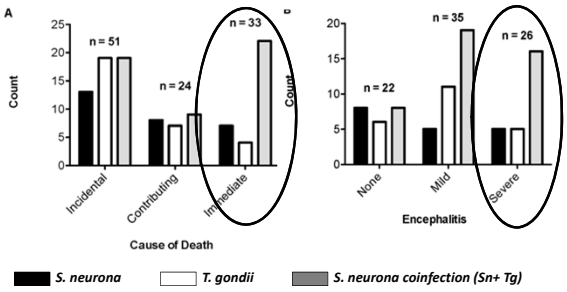


Patterns of *S. neurona* infection in the Pacific Northwest

What protozoal pathogens associated with disease?

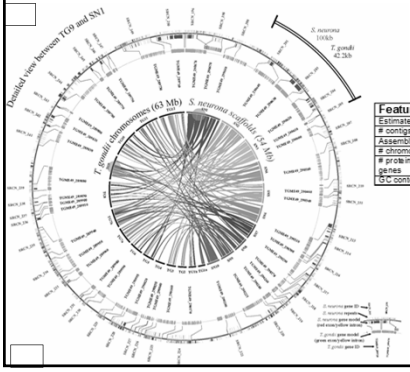


Coinfection (polyparasitism) is associated with increased disease severity



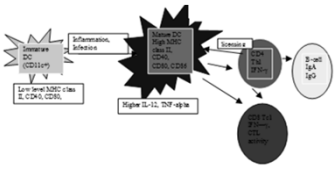
Gibson et al, PLoS Neg Trop Dis, 2011

Sequencing of the SN1 genome identifies synteny with other coccidia (*Toxoplasma)*



Feature	S. neurona	T. gondii
Estimated size	~127 Mb	~ 65 Mb
# contigs	11,452	2,511
Assembly length	117,871,271	65,464,221
# chromosomes	n/a	14
# protein coding genes	7,093	8,322
GC content	51.5%	52.2%

EPM: Immunity/vaccine developments



Sharon Witonsky Martin Furr

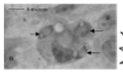
Host pathogen balance

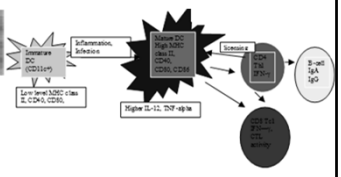
National seroprevalance >50%

0.5-1% of horses get disease

Factors

- Stress (shipping, showing, training, pregnancy)
- Season
- Location
- Age
- Other diseases





Role Cell Mediated Immunity

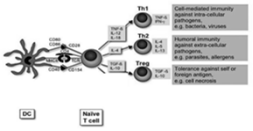
EPM horses have decreased immune responses

VIRGINIA-MARYLAND REGIONAL COLLEGE OF VETERINARY MEDICINE

Factors affecting exposure vs. disease

Immune response regulated by

- Antigen processing/presentation
- DC/macrophage (innate) response
- Antibodies
- Cell mediated immune response



Innate directs T-cell response

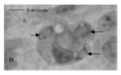
Factors influencing outcome/immune response

- Overall health status of horse/exposure
- *S. neurona* is intracellular:"hide"
- Ability to inhibit/limit immune response
- Time to develop immune response

Dubey et al., 2001

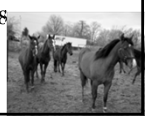
VIRGINIA-MARYLAND REGIONAL COLLEGE OF VETERINARY MEDICINE

What is the protective immune response in horses?



What is the protective immune response in mice?

- Nude mice lacking T-cells develop disease.
- Interferon gamma knock out (GKO) mice develop encephalitis, but have increased CD_4 and CD8 memory cells (Witonsky et al., 2003).
- B-cell deficient mice: no disease; increased CD_4 and CD8 effector and increased CD8 memory cells (Witonsky et al., 2005).
- CD_4 knockout (KO) mice: no disease (Witonsky et al., 2005)
- CD8 KO mice: develop encephalitis (Witonsky et al., 2005)
- Infected immunocompetent mice: CD_4 and CD8 response (Witonsky et al., 2003)



What happens in horses with EPM?

- Tornquist: Decreased CD_4 and antigen specific immune response
- Naturally and experimentally infected horses: decreased proliferation response (PMA/I)(Yang et al., 2006; Witonsky et al., 2008).

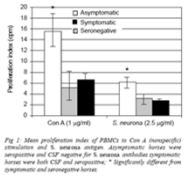


Fig 1. Mean proliferation index of PBMCs to Con A (nonspecific) stimulation and S. neurona antigen. Asymptomatic horses were seropositive and CSF negative for S. neurona antibodies symptomatic horses were both CSF and seropositive. * Significantly different from symptomatic and seronegative horses.

Tornquist et al., 2001

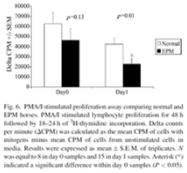


Fig. 6. PMA/I-stimulated proliferation assay comparing normal and EPM horses. PMA/I stimulated lymphocyte proliferation for 48 h followed by 18–24 h of ^{3}H-thymidine incorporation. Delta counts per minute (ΔCPM) was calculated as the mean CPM of cells with mitogens minus mean CPM of cells from unstimulated cells in media. Results were expressed as mean ± S.E.M. of triplicates. N was equal to 8 in day 0 samples and 15 in day 1 samples. Asterisk (*) indicated a significant difference within day 0 samples ($P < 0.05$).

Yang et al., 2006

Changes in immune responses

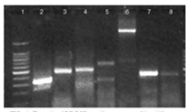


FIG. 1. Example of RT-PCR amplicons separated in a 1% agarose gel and stained with ethidium bromide. PCR products were produced by lymphocytes in response to SnSAG1 incubation. Lane 1, 1-kb molecular weight marker; lane 2, RT-PCR positive control; lane 3, β-actin amplicon; lane 4, IFN-γ amplicon; lane 5, TNF-α amplicon; lane 6, IL-2 amplicon; lane 7, IL-4 amplicon; lane 8, IL-6 amplicon.

TABLE 1. Presence of gene transcripts in response to coculture with SnSAG1 for 48 or 72 h as visualized on a 1.5% agarose gel

Cytokine	No. of horses[a]			
	48 h		72 h	
	EPM neg	EPM pos	EPM neg	EPM pos
IFN-γ	10	0	0	4
TNF-α	3	0	1	2
IL-2	3	1	2	5
IL-4	4	4	3	7
IL-6	3	3	1	3

[a] Results are expressed as the total number out of 21 horses examined. neg, negative; pos, positive.

Spencer et al., (2004)
n=43 EPM negative horses, no neurologic signs (CSF negative for *S. neurona* antibodies);
n=28 horses with EPM, W. blot positive antibodies in CSF.
Decreased SAG1 proliferation and SAG1 induced IFN-gamma expression in EPM horses .

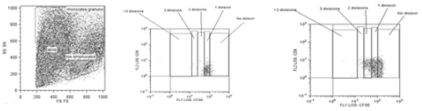
Spencer et al., (2005)
n=21 EPM negative
n=21 EPM positive
EPM horses decreased IFN gamma and increased IL-4 expression when stimulated with SAG1.

Decreased response (CFSE)(Lewis et al., accepted 2014)

PHA	Percent CD4	Percent CD4 0 division	Percent CD4 1 division	Percent CD4 2 divisions	Percent CD4 3 divisions	Percent CD4 >3 divisions
Day 2 CD4 Control	35.8 +/-4.68	52 +/- 5.67	39.23 +/- 5.65*	11.47 +/- 0.86*	1.32 +/- 0.32*	0.19 +/- 0.09=
Day 2 CD4 Infected	43.74 +/- 4.37	33.44 +/- 7.08	49.8 +/- 4.86	13.86 +/- 2.15	2.892 +/- 0.59	0.442 +/- 0.18
Day 28 CD4 Control	34.7 +/- 0.81	51.3 +/- 13.02	46.63 +/- 13	9.22 +/- 0.90*	1.62 +/- 0.28*	2.50 +/- 0.08*
Day 28 CD4 Infected	33.04 +/- 3.79	50.44 +/- 6.7	40.12 +/- 6.49	8.134 +/- 0.52	0.424 +/- 0.11	1.82 +/- 0.37

PHA/I	Percent CD8	Percent CD8 0 division	Percent CD8 1 division	Percent CD8 2 division	Percent CD8 3 division	Percent CD8 > 3 divisions
Day 2 CD8 Control	10.48 +/-1.3	94.27 +/- 2.39*	38.4 +/- 3.7*	9.40 +/- 1.01*	1.44 +/- 1.56*	0.44 +/- 0.08=
Day 2 CD8 Infected	10.08 +/- 1.16	36.78 +/-7.97	44.62 +/- 4.54	20.7 +/- 3.26	2.948 +/- 2.91	0.636 +/-0.12
Merozoites						
Day 28 CD8 Control	26.38 +/- 0.65	29.26 +/- 2.36*	63.05 +/- 2.26*	10.11 +/- 0.8*	1.185 +/- 0.26*	1.23 +/- 0.01
Day 28 CD8 Infected	23.32 +/- 1.88	18.78 +/-1.69	76.22 +/- 1.03	8.41 +/- 0.77	0.88 +/- 0.26	1.89 +/- 0.33
Day 56 CD8 Control	17.62 +/-2.26	65.37 +/- 23.2*	28.46 +/- 16.61*	7.28 +/- 6.36*	0.12 +/- 0.09	0 +/- 0
Day 56 CD8 Infected	19.52 +/- 3.17	81.72 +/- 10.61	18.14 +/- 10.87	1.16 +/- 0.74	0.12 +/- 0.02	0.13 +/- 0.08
Day 70 CD8 Control	23.1 +/- 2.04	64.67 +/- 14.5*	37.53 +/- 14.16*	1.78 +/- 1.09*	0.09 +/- 0.09*	0.14 +/- 0.07*
Day 70 CD8 Infected	21.06 +/- 2.3	82.62 +/- 5.57	19.75 /- 6.27	0.62 +/- 0.13	0.12 +/- 0.06	0.04 +/- 0

N= 5 infected, n=3 controls
Horses given 6,000 *S. neurona* SAG1 organisms/day in autologous blood



Local immune response

- Furr et al., (2001): CSF cells (n=7 normal; n=4 EPM horses) no difference total cell numbers normal vs. EPM horses. Furr et al., (2001): $TGFB_2$ decreased in CSF from EPM affected horses (n=9 normal and 9 EPM horses).
- Furr et al., (2002) n=4 EPM horses, n=1 control;
  T-cells predominated based on immunohistochemistry (IHC).

	Normal Horses (n = 7)			*EPM-affected Horses (n = 4)*	
Cell Label	*Blood*	*CSF*	*P-value†*	*Blood*	*CSF*
CD5+	67.6 ± 9.6	79.0 ± 16.8	.14	73.2 ± 6.3	93.2 ± 3.6
CD4+	51.4 ± 7.9	59.6 ± 15.6	.25	50.5 ±14.4	60.9 ± 8.3
CD8+	13.6 ± 3.7	23.4 ± 4.9	.001	18.2 ± 5.3	26.9 ± 8.4
CD11a/CD18	83.4 ± 13.1	98.2 ± 1.6	.06	86.6 ± 7.2	94.8 ± 5.4
CD4 /CD8	3.9 ± 0.8	2.6 ± 0.8	.01	2.9 ± 0.9	2.4 ±0.9

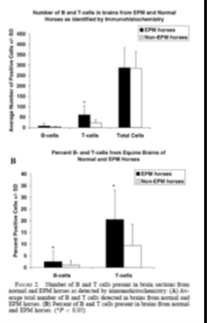
TABLE 2: Concentration of TGF-β_2 in serum and CSF of normal and EPM-affected horses. Data reported in pg/ml

	Serum		CSF	
	Normal	EPM	Normal	EPM
	2429	2492	260	374
	1781	1127	341	163
	4644	1094	311	173
	1935	2057	339	163
	1182	887	241	51
	837	1993	269	0
	641	887	51	76
	1329	1921	223	241
	1025	886	269	57
Mean ± s.e.	1756 ± 408*	1482 ± 209*	256 ± 29†	144 ± 38†

*P = 0.56; †P = 0.03

Local immune response

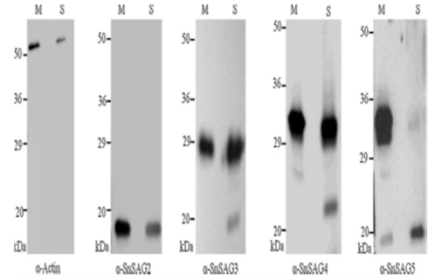
Scott et al., (2005): EPM horses (n=17) all had histologic lesions consistent with EPM;
15/17 euthanized due to neurologic signs;
2/17 euthanized due to fractures;
non-EPM (n=8) horses controls from NSAID clinical trial;
none of controls had neurologic signs.
T-cell (CD3); B-cell (CD36); DAB conjugate for CD3; Fast red for CD36.
Increased number of T-cells present in EPM horses.



Summary

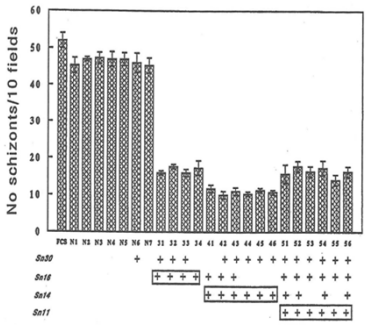
- Balance host/pathogen
- Role for Cell Mediated immune response
- Some horses with EPM have some defects in immune responses

Stage specific expression of surface proteins



Gautam, et al. Stage specific Vet Para 2013

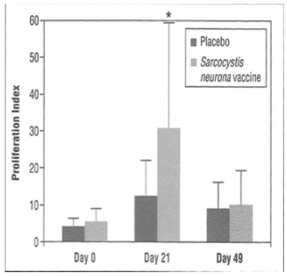
Infectivity determined by *in-vitro* neutralization



From: Llian, Granstrom, et al, Inf Immunity 1998

Killed *S. neurona* vaccine

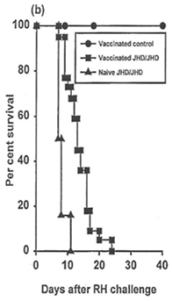
- Sn-UCD1 isolate
- Meta-Stim adjuvant (Fort Dodge)
- Demonstrated
  - Seroconversion (IFAT)
  - Enhanced PBMC proliferation
  - No adverse clinical signs
- Protection tests not performed



From: Marsh, A. Vet Ther 2004

B cells are important

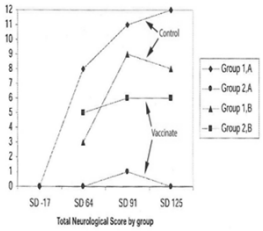
- B cell deficient mice
- Attenuated tachyzoites of *T. gondii*
- Vaccination protective in control not B cell deficient mice
- IFN-γ production preserved



Sayles et al, Inf Immunity 2000.

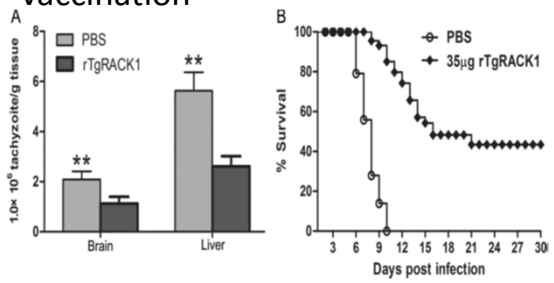
SnSAG1 Vaccine trial

- 10 horses
- Vaccinated with rSnSAG1
- IV merozoite challenge
- Substantial variation in neurologic scores
- Small numbers preclude conclusions



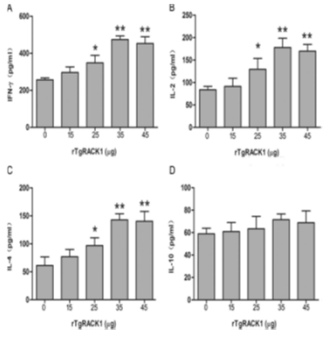
From: Ellison and Witonsky, Can Vet J, 2009

T gondii -Activated C Kinase vaccination



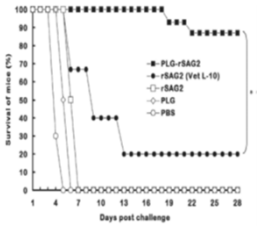
Wang , 2014

T. gondii - Activated C Kinase Vaccination



T. gondii SAG2 (with PLG microparticles)

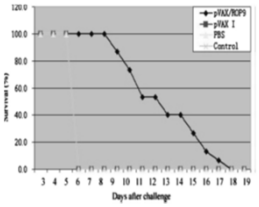
- Prolonged (10 wks) lymphoblast transformation and IFN-γ response
- Increased survival (86%) following lethal challenge (TG tachyzoites RH strain)



Chuang, et al. 2014, Parasitology

T. gondii Rhoptry Protein 9 vaccination

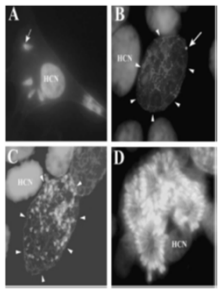
- DNA vaccine rhoptry protein
- Challenged with virulent TG
- Increased survival
- IFN and IL2 prod
- Mixed CD4 CD8 response
- Strong humoral and cellular response



Chen, et al. Exp Parasitol 2014

Microneme protein (SnMIC10) vaccination(?)

- Important for host cell invasion (?)
- Expressed protein is available



From: Hoane, et al. Int J Parasitol 2003

Possible future studies

- Identification of immunodominant proteins/protective antigens
- What are the defects in immune response in EPM affected horses
- Vaccine development

Referencess

- **Veterinary Parasitology**, 2012, 183, 1/2, pp 37-42, 44 ref.
- **Parasites and Vectors**, 2014, 7, 431, pp (8 September 2014), 80 ref.
- **Acta Tropica**, 2014, 137, pp 58-66, many ref.(Wang)
- **Parasitology**, 2014, 141, 12, pp 1657-1666, 34 ref.
- **Experimental Parasitology**, 2014, 139, pp 42-48, 43 ref. T gondii rhopty protein 9
- **Canadian Journal of Veterinary Research**, 2009, 73, 3, pp 176-183, 30 ref.

EPM Workshop 2014 – Diagnostic Testing

Features of clinical laboratory testing

- Use of good lab practices
- Validation using "gold standard" samples
- Evaluation of test performance:
  intra-assay and inter-assay variability
- Determination of sensitivity (false negative) and specificity (false positive)
- Advice on appropriate testing
- Use of frequent and appropriate quality controls

EPM Workshop 2014 – Diagnostic Testing

General differences between the current EPM tests

- The *S. neurona* strain used for the test
- Test format
- Dilution factor of samples as tested
- Validation samples and gold standard samples used
- Recommended samples for testing
- Result interpretation
- Sensitivity and specificity
- Quality control measures

EPM Workshop 2014 – Diagnostic tests

Western blot (WB):

- Standard WB – versions of SWB offered by UC Davis, Equine Diagnostic Solutions and IDEXX

  Granstrom *et al.* (1993) J Vet Diag Invest 5:88-90

  Validated with paired serum and CSF from necropsy cases. Respectively, serum and CSF sensitivities were 90% and 83%; specificities were 42% and 86% (EDS updated data 2010).
- Modified WB – offered by Michigan State University

  Rossano *et al.* (2000) J Vet Diag Invest 12:28-32

  Validated with sera from 57 horses native to India & Germany ("true" negatives) and 6 EPM positive necropsy cases (true positives). Respectively, sensitivity = 100% and specificity = 98%.

EPM Workshop 2014- Diagnostic tests

Test: Indirect (immunofluorescent) antibody test (IFAT) (Duarte); also available as a sendout from IDEXX

Lab: University of California at Davis

***S. neurona* strain:** UCD1, expresses SAG1

Validation samples: 110 general necropsy cases (none prospective) with paired serum and CSF samples; 8 had an EPM Dx

Result interpretation: A subsequent group of 182 (102 with paired serum & CSF) horses with natural infections, experimental infections or enrolled in a vaccine study were used to generate mathematical probabilities of having EPM. The likelihood of EPM was calculated based on a pre-test probability of 10%. Serum titers (reciprocal)of ≤40 have ≤33% probabilities of EPM and ≥80 have ≥55% probabilities. CSF titers of≥5 have a 92% probability of EPM.

Test performance:

The necropsy cases had sensitivities of 83% (serum) and 100% (CSF) and specificities of 97% (serum) and 99% (CSF)

EPM Workshop 2014 – Diagnostic tests

Recommended samples: serum and CSF are encouraged, but serum is a good screening sample (high negative predictive value) to support the clinical Dx

Quality control: positive and negative controls on each slide; difficult interpretations are retested in Dr. Conrad's research lab

References:

Duarte *et al.* (2003) Comparison of a serum indirect fluorescent antibody test with two western blot tests for the diagnosis of equine protozoal myeloencephalitis. J Vet Diag Invest 15:8-13

Duarte *et al.* (2004) Evaluation and comparison of an indirect fluorescent antibody test for detection of antibodies to *Sarcocystis neurona*, using serum and cerebrospinal fluid of naturally and experimentally infected, and vaccinated horses. J Parasitol 90:379-386

Duarte *et al.* (2006) Indirect fluorescent antibody testing of cerebrospinal fluid for diagnosis of equine protozoal myeloencephalitis. Amer J Vet Res 67:869-876

Other tests offered:

N. hughesi IFAT

S. neurona western blot

EPM Workshop 2014 - Diagnostic tests

Tests: SnSAG 1,5,6 peptide ELISA (Ellison); also available as a sendout from Antech

Lab: Pathogenes, Inc.

***S. neurona* strains:** information not provided

Validation samples:

SAG1: 75 horses (non-naïve) experimentally infected with undisclosed strain

SAG5: 20 horses (naïve) vaccinated with undisclosed strain

SAG6: (# not provided) horses (naïve) vaccinated with undisclosed strain

Result interpretation: serum antibody titers (reciprocal) of ≥8 are positive for antibody, indicating past or present infection.

Test performance serum only: information not provided

EPM Workshop 2014 – Diagnostic tests

Recommended sample: serum is adequate to support the clinical diagnosis

Quality control: positive controls on each plate, same standard run on every plate, daily review of positive values, outside reviewer of SOPs.

References:

Ellison *et al.* (2003) Development of an ELISA to detect antibodies to rSAG1 in the horse. Intern J Appl Res Vet Med 1: 318-327

Ellison and Lindsay (2012) Decoquinate combined with levamisole reduce the clinical signs and serum SAG 1, 5, 6 antibodies in horses with suspected equine protozoal myeloencephalitis. Intern J Appl Res Vet Med 10: 1-7

Other tests offered:

C Reactive protein ELISA

SAG 1, 5, 6 ELISA for dogs and cats

EPM Workshop 2014 – Diagnostic tests

Test: SnSAG 2, 4/3 ELISA (Howe)

Lab: Equine Diagnostic Solutions, LLC

***S. neurona* strain:** SN2 which expresses SAG1

Validation samples: 128 necropsy cases (mostly prospective) with paired serum and CSF samples; 44 had an EPM Dx

Result interpretation:

Serum titer only = exposure if ≥1:250; <1:250 is negative

CSF titer only = correlates well with EPM Dx if ≥1:40

Serum/CSF titer ratio = very predictive of EPM Dx if ≤100

Test performance of the serum/CSF titer ratio:

using ratio <100 as cutoff, sensitivity = 86% & specificity = 96%

using ratio ≤100 as cutoff, sensitivity = 93% & specificity = 83%

EPM Workshop 2014 – Diagnostic tests

Recommended samples: serum **and** CSF preferred

Quality control: positive & negative samples every test plate, repeat testing of previous samples, CSF albumin levels

References:

Hoane *et al.* (2005) Enzyme-linked immunosorbent assays for the detection of equine antibodies specific to *Sarcocystis neurona* surface antigens. Clin and Diag Lab Immunol 12:1050-1056

Yeargan and Howe (2011) Improved detection of equine antibodies against *Sarcocystis neurona* using polyvalent ELISAs based on the parasite SnSAG surface antigens. Vet Parasitology 176:16-22

Reed *et al.* (2013) Accurate antemortem diagnosis of equine protozoal myeloencephalitis (EPM) based on detecting intrathecal antibodies against *Sarcocystis neurona* using theSnSAG2 and SnSAG4/3 ELISAs. J Vet Intern Med 27: 1193-1200

Other tests offered:

N. hughesi ELISA (Howe)

Specific antibody index & C value (MacKay & Furr)

S. neurona standard western blot (Granstrom)

Test comparisons

- Duarte *et al*. 2003 *J Vet Diagn Invest*
  - WB, mWB, IFAT
- Saville 2007 ACVIM EPM SIG
  - WB, mWB, IFAT, SAG1
- Johnson *et al*. 2010 *J Vet Intern Med*
  - IFAT, SAG1
- Reed *et al*. 2010 ACVIM
  - WB, IFAT, SAG1, SAG2, 4/3
- Renier *et al*. 2012 ACVIM EPM SIG
  - IFAT, SAG2, 4/3
- Johnson *et al*. 2013 *J Vet Intern Med*
  - IFAT, SAG2, 4/3

Test comparisons

- Duarte *et al*. 2003 *J Vet Diagn Invest*
  - **WB (serum), mWB (serum), IFAT (serum)**
  - Samples: 9 positive, 39 negative (necropsy)
  - Results: similar Se (88.9%) but Sp varied (IFAT 100%, WB 87.2%, mWB 69.2%)
  - Author conclusions: IFAT accuracy better than WBs

Test comparisons

- Saville 2007 ACVIM SIG
  - **WB (serum), mWB (serum), SAG1 (serum), IFAT (serum)**

Sample Description	EBI	MSU	Pathogenes	UC Davis
2 European born	Negative	Negative	Negative	Negative
Experimental infection:				
2 uninfected controls	Negative	Positive	Negative	Negative
	Negative	Suspect	Negative	Negative
1 *S. neurona* infected	Positive	QNS	Negative	Positive 1:80 (55%)
1 *S. fayeri* infected	Negative	QNS	Negative	Positive
8 with Wobbler Dx	3 Negative	3 Suspect	2 Negative	3 Negative
				1 Pos 1:80
	1 Equivocal	1 Positive	1 Negative	1 Negative
	1 Low positive	1 Positive	1 Negative	1 Negative
	1 Positive	1 Suspect	1 Negative	1 Negative
	2 Positive	2 Positive	2 Negative	1 Negative
				1 Positive 1:160 (76%)
4 with EPM Dx	1 Negative	1 Positive	1 Negative	1 Negative
	1 Low positive	1 Positive	1 Negative	1 Negative
	1 Positive	1 Suspect	1 Negative	1 Positive 1:160 (76%)
1 necropsy confirmed	Strong positive	Positive	Negative	Positive 1:1280 (95%)

Test comparisons

- Saville 2007 ACVIM SIG
  - **WB (serum), mWB (serum), SAG1 (serum), IFAT (serum)**
  - Samples: 5 positive, 13 negative (4 experimental, 13 clinical, 1 necropsy)
  - Results: as shown on previous page
  - Author conclusions: WB, IFAT most accurate (IFAT cross-reactive with *S. fayeri*); mWB – false positives; SAG1 – false negatives

Test comparisons

- Johnson *et al*. 2010 *J Vet Intern Med*
  - **IFAT (serum, CSF), SAG1 (serum)**
  - Samples: 9 positive, 17 negative (necropsy) + 10 positive, 29 negative (clinical)
  - Results: Se differed markedly (IFAT serum 94.4%, IFAT CSF 92.3%, SAG1 serum 12.5%), Sp comparable (IFAT serum 85.2%, IFAT CSF 89.7%, SAG1 serum 97.1%)
  - Author conclusions: low Se limited usefulness SAG1

Test comparisons

- Reed *et al*. 2010 ACVIM
  - **WB (CSF), IFAT (serum), SAG1 (serum), SAG2, 4/3 (ratio)**
  - Samples: 7 positive, 5 negative (necropsy) + 6 positive, 2 negative (clinical)
  - Results: variable Se (SAG2, 4/3 90%, WB 90%, IFAT 70%, SAG1 55%) and Sp (SAG2, 4/3 100%, WB 95%, SAG1 90%, IFAT 85%)
  - Author conclusions: SAG 2, 4/3 ratio most accurate

Test comparisons

- Renier *et al*. 2012 ACVIM SIG
  - **IFAT (CSF), SAG2, 4/3 (ratio)**
  - Samples: 6 positive, 17 negative (necropsy)
  - Results: IFAT Se (6/6, 100%) higher than SAG2, 4/3 (5/6, 83.3%); SAG2, 4/3 Sp (100%) higher than IFAT (82.4%)
  - Author conclusions: IFAT advantages include testing for *N. hughesi* (and use as serum stand-alone test)

Test comparisons

- Johnson *et al*. 2013 *J Vet Intern Med*
  - **IFAT (serum, CSF, ratio), SAG2, 4/3 (serum, CSF, ratio)**
  - Samples: 11 positive, 28 negative (necropsy) + 6 positive, 14 negative (clinical)
  - Results: SAG2, 4/3 ratio most accurate (97%); IFAT CSF and ratio also had high accuracy (88%)
  - Author conclusions: serum results alone were least accurate; more accurate methods should be used

Summary test comparisons

- Serum tests less accurate
  - Generally due to low specificity
  - SAG1 showed poor sensitivity
- Poor to fair test agreement
- SAG2, 4/3 ratio most accurate (3/6 studies)
  - Compared to WB, SAG1, IFAT
- No comparison studies for SAG1, 5, 6

EPM Workshop 2014 – Diagnostic testing

Questions for discussion part one:

- Diagnostic value of serum only
- When to recommend *N. hughesi* testing
- Sequential testing – usefulness for diagnosing an active infection or for evaluating effectiveness of treatment
- Effect of blood contamination of CSF on testing and how is blood contamination of CSFs evaluated for each test
- What is effect of previous treatment (within 1-4 months prior to workup) on testing

EPM Workshop 2014 – Diagnostic testing

Questions for Discussion part two:

- Knowledge about biomarkers such as serum amyloid A, C reactive protein and heavy chain neurofilament
- Using the SAG 1/5/6 peptide ELISA, what is the frequency of mixed infections
- Is there evidence for SAG6 strains infecting horses in nature
- Are there any new tests being developed and should there be

Current Therapeutic and Preventive Approaches for EPM

Steve Reed and Nicola Pusterla

Rood and Riddle Equine Hospital, Lexington, KY
School of Veterinary Medicine, University of California, Davis, CA

Goals of Therapeutic Agents

- **First generation therapeutic agents based on classic *anti-Toxoplasma gondii* pyrimethamine-sulfonamide combination**
- **Second generation therapeutic agents based on diclazuril and related triazine agents**
- **Properties of ideal anti-protozoal therapeutic agent**
  - **High efficacy**
  - **Therapeutic levels in CNS (neurons)**
  - **Convenient administration route**
  - **Low toxicity**

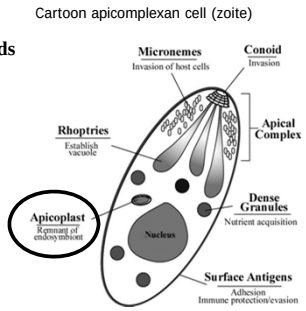
Sulfonamides and Pyrimethamine

- **Inhibit folic acid metabolism and nucleotide biosynthesis**
- **FDA approved oral suspension (Rebalance)**
  20 mg/kg sulfadiazine + 1 mg/kg pyrimethamine
  90-270 days
- **Rapid CSF steady-state (no need for loading dose)**
- **Efficacy 16/26 horses (61.5%)**
- **Side effects include bone marrow suppression**
- **Relapse rate up to 10%**

Triazines Mode of Action

Benzeneacetonitrile compounds
Related to Atrazine

Specific action not known
Presumed to target plant-like characteristics



Diclazuril

- **Precise mode of action of anticoccidial triazines unclear**
- **FDA approved pellets (Protazil)**
  1 mg/kg diclazuril for 28 days
- **Steady-state reached after 9 days**
  (plasma 800-1,000; CSF 19-26 ng/ml)
  Levels ≥ 100 ng/ml after 6-8 hours single dose
- **Efficacy 28/42 horses (66.7%)**
- **Little toxicity due to selective action mode**
- **Relapse rate up to 5%**

Toltrazuril

- **Precise mode of action of anticoccidial triazines unclear**
- **FDA approved pellets (Marquis)**
  5 mg/kg diclazuril for 28 days
- **Steady-state plasma 4,300 ng/ml, CSF 162 ng/ml)**
  Long ½ live requires loading dose (15 mg/kg)
  DMSO/vegetable oil increase bioavailability
- **Efficacy 28/47 horses (59.6%)**
- **Little toxicity due to selective action mode**
- **Relapse rate unknown**

Nitazoxanide

- **Interferes with pyruvate:ferredoxin oxidoreductase enzyme**
- **Formerly approved FDA paste (Navigator)**
  50 mg/kg diclazuril for 28 days
- **Peak concentration reached rapidly (1-4 hours)**
  Vegetable oil increase bioavailability, ⇓ side effects
- **Efficacy 44/63 horses (69.8%)**
- **High toxicity rate (depression, anorexia, diarrhea)**
- **Relapse rate 7%**

Supportive Treatment

- **Additional treatment in EPM cases:**
  - **Anti-inflammatory drugs (dexamethasone, banamine, phenylbutazone, DMSO)**
  - **Antimicrobials in animals at risk for infections**
  - **Vitamin E (antioxidant)**
  - **Immune modifying agents such as Levamisole, Vital stress formula, Zylexis**
- **Other measures may include rectal and bladder evacuation, mineral oil administration, feeding changes**

Prevention Strategies

- **Vaccination is controversial**
- **Reduce risk factors (stress, transportation)**
- **Keep wild animals away**
- **Eliminate carcasses - can be consumed by opossums**
- **There is no justification to eliminate definitive hosts but keeping them away from equine feed and common water sources is important**
- **Prophylactic treatment**

Imaging the immune response to infection

Tracking host-pathogen interactions

Toxoplasma as an endothelial pathogen

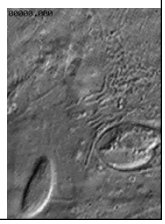
T cell behavior

Treg behavior

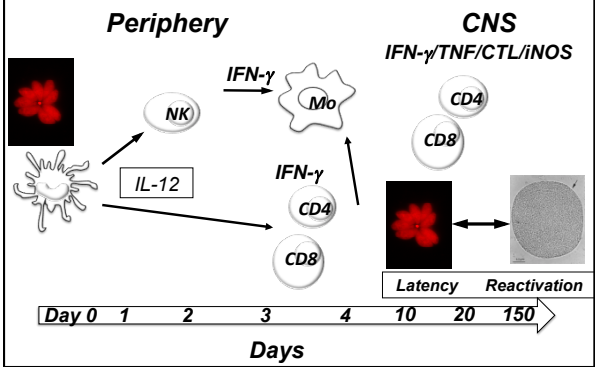
"All models are wrong, some are useful" George Box

What you need to know

- Toxoplasma infects the CNS as part of its lifecycle.
- 3-70% of you are infected!
- Little is known about how microbes enter the brain and aberrant replication of microorganisms in the CNS is typically catastrophic
- T cells operate in CNS and are beneficial/pathological

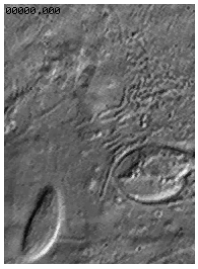


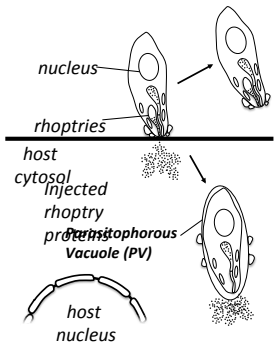
Acute and chronic phases of infection



Toxoplasma gondii Infection – Active Invasion

T. gondii inject proteins into host cytosol during invasion or Kiss & Run



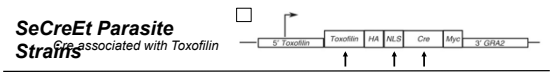


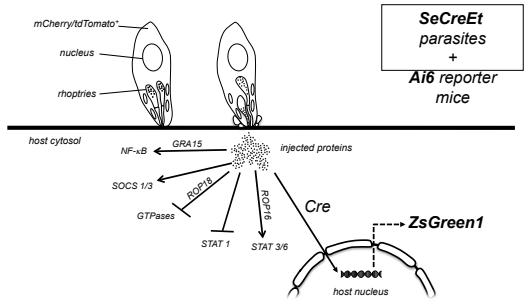
Boothroyd & Dubremetz, Nature Reviews Microbiology (2008)

Tools for Tracking All Host-Pathogen Interactions

SeCreEt Parasite Strains

Cre associated with Toxofilin

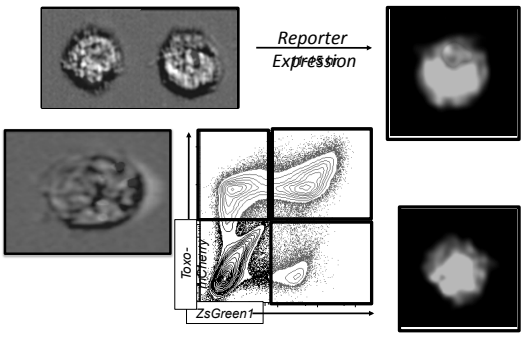




SeCreEt *parasites* + ***Ai6*** *reporter mice*

Koshy AA, et al., Nature Methods (2010)

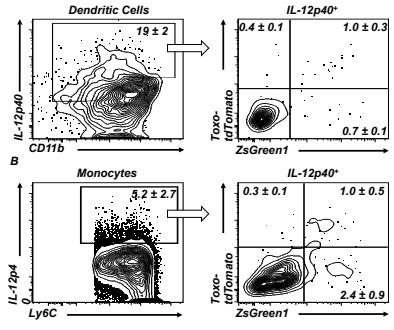
Delineating Host-Parasite Interactions

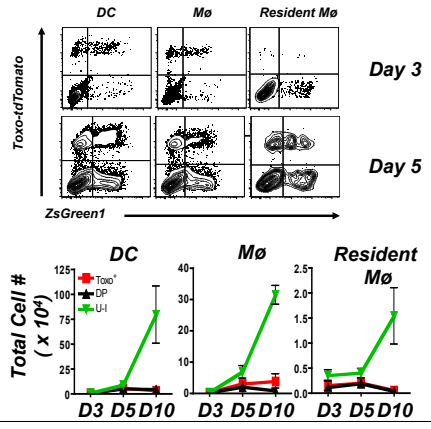


Ai6 BMMφ
MOI = 1
20 hr p.i.

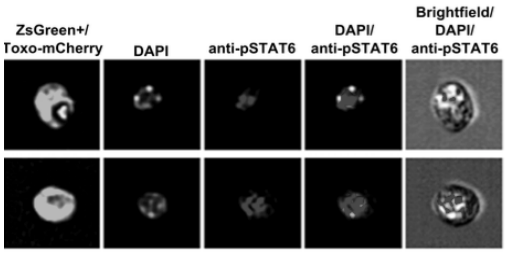
Uninfected-Injected (U-I) cells

What interactions lead to IL-12 production?

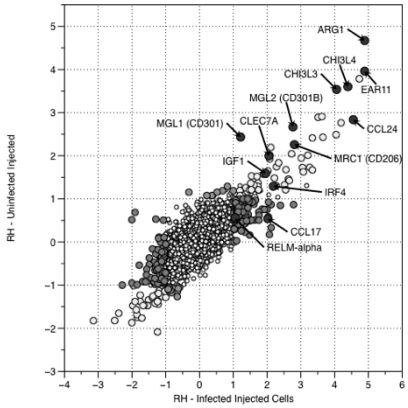


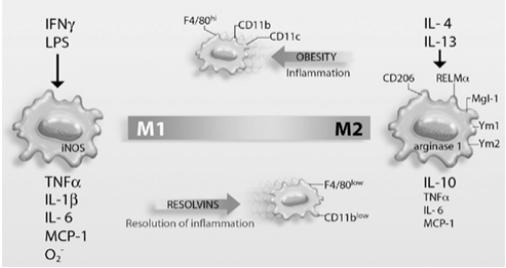


Infection-induced phosphorylation and nuclear translocation of STAT6.

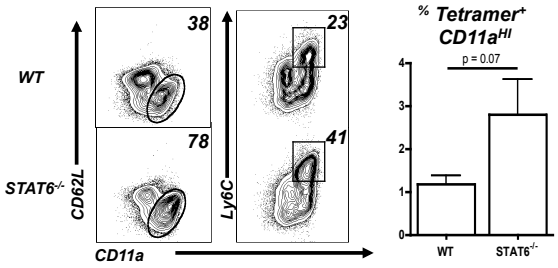


Koshy AA, Dietrich HK, Christian DA, Melehani JH, et al. (2012) Toxoplasma Co-opts Host Cells It Does Not Invade. PLoS Pathog 8(7): e1002825. doi:10.1371/journal.ppat.1002825 PLoS PATHOGENS

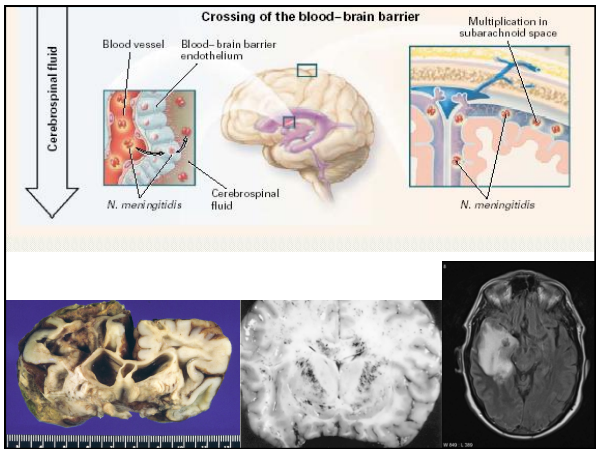




Impact of STAT6 on T cell Activation

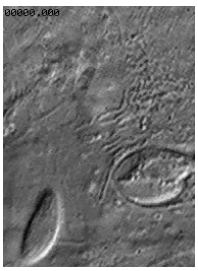


LN 10 dpi
10^5 Type III, i.p.

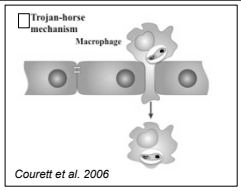


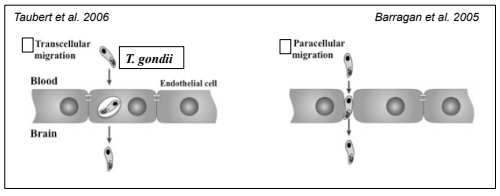
Toxoplasma and the CNS

- Toxoplasma is a superb cell biologist and immunologist – that has already taught us a lot about the immune system!
- How does this common pathogen access the CNS?
- What functions do astrocytes have during this infection?
- Modifiers of STAT1-functions

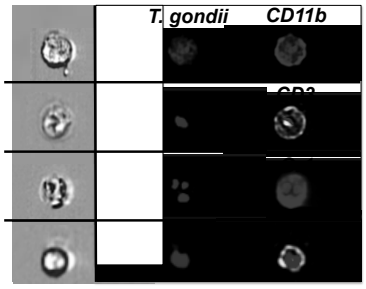


Proposed mechanisms for crossing the BBB

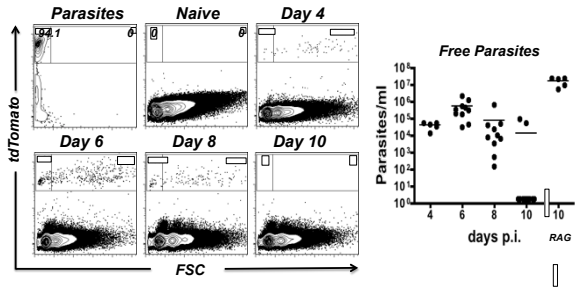




Identification of infected populations in the blood during acute toxoplasmosis

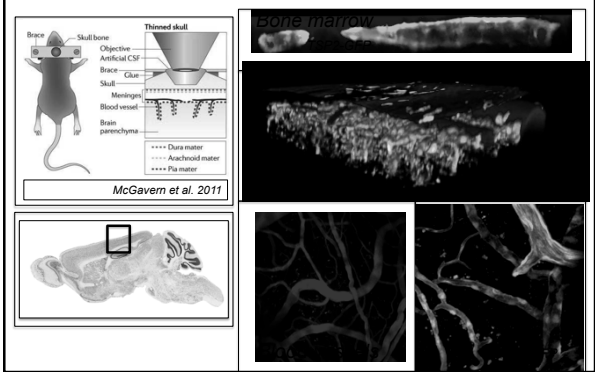


Detection of free parasites in the blood of infected mice

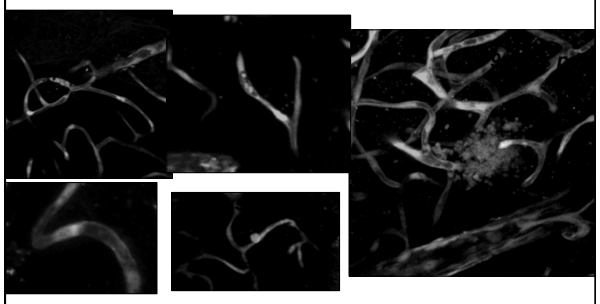


*Free parasites were not expected for this **obligate** intracellular parasite!*

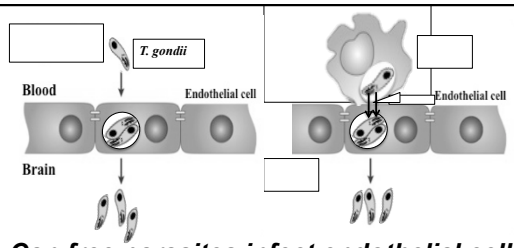
Imaging different compartments of the CNS



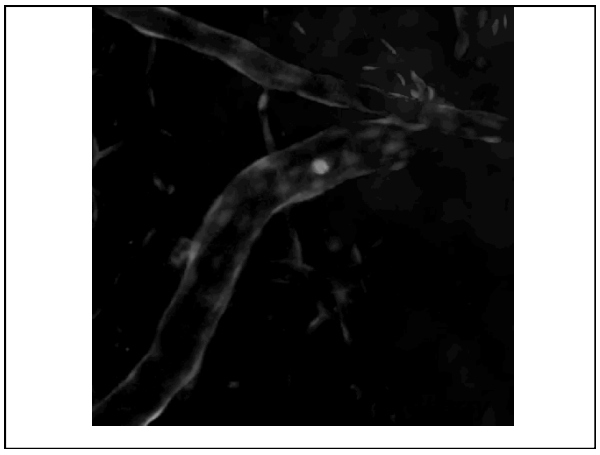
Detecting T. gondii in endothelial cells of the brain vasculature (not such a rare event!)

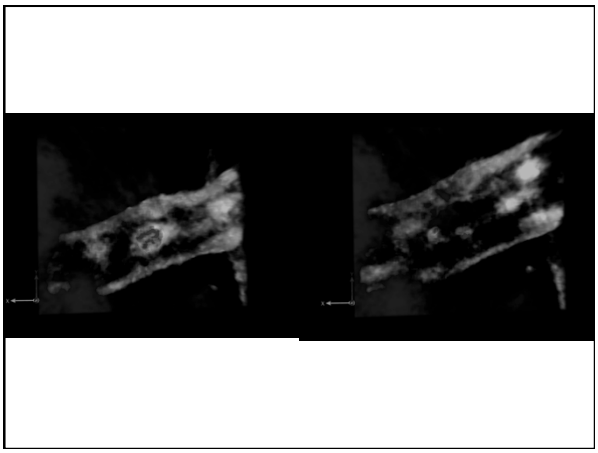


Tie2-GFP mouse 7-9 days after i.p infection with Pru-tdTomato



Can free parasites infect endothelial cells (why would they not – immunity – shear stress)
Is replication essential? Would be useful to identify cells that have interacted with T. gondii

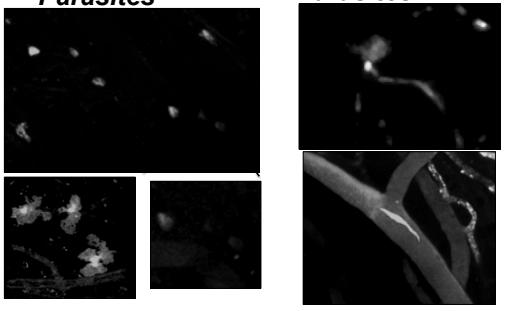




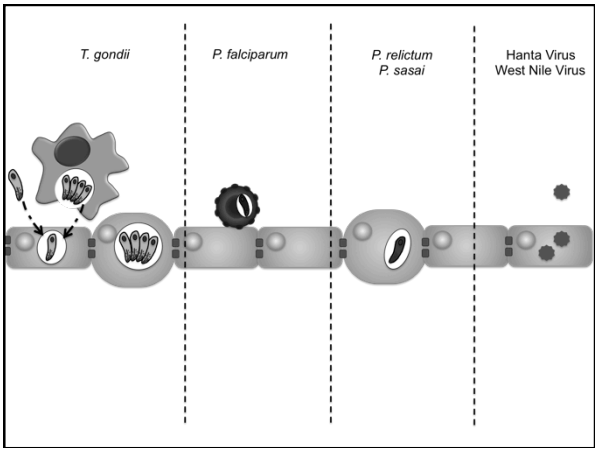
Tracking T. gondii-host cell interaction after iv infection

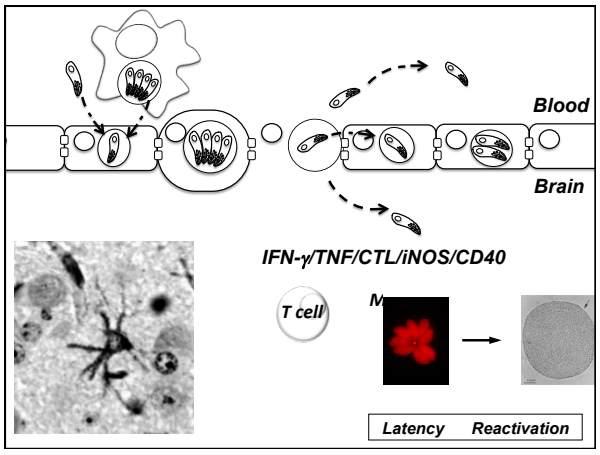
Replicating Parasites

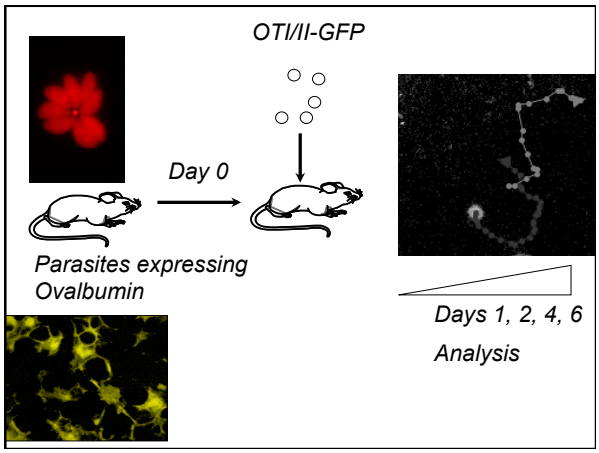
Non-replicating Parasites

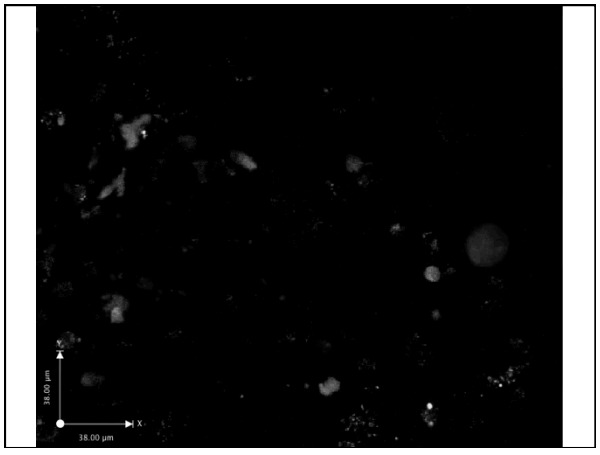


Green: ZsGreen+ Cell Red: T. gondii White: Vasculature

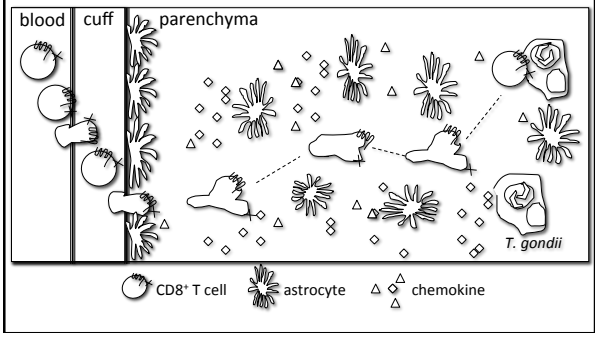




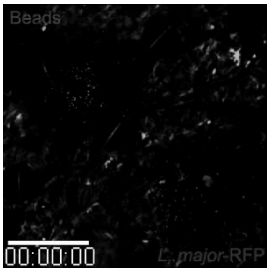




How do T cells protect against chronic toxoplasmosis

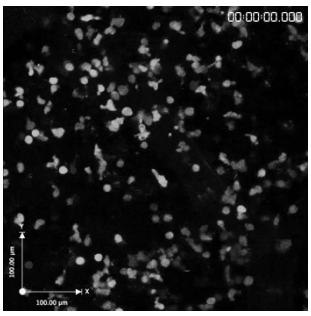


Directed migration of neutrophils



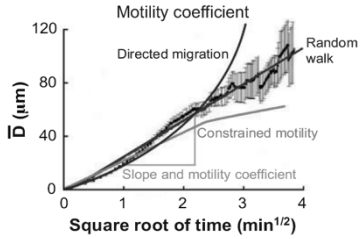
Peters et al Science 2011

Imaging "parasite-specific" OT1 CD8 T cells in the CNS



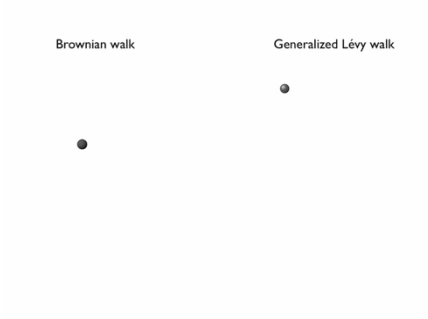
On the scale imaged – no signs of directionality

Mean Squared Displacement Analysis: Random Walk

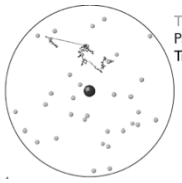


Many different underlying types of random motion can produce identical displacement curves – how to extract this information from these data!

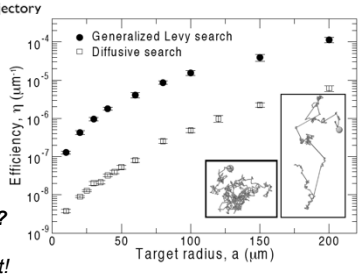
Generalized Levy type random walk (This is not: bimodal correlated, simple or Gaussian random walk or various permutations of Standard Levy walks)



Generalized Lévy walks are more efficient than diffusive walks



What questions can we address with this model?



What is the effect of chemokine blockade?

Approx 2x less efficient!

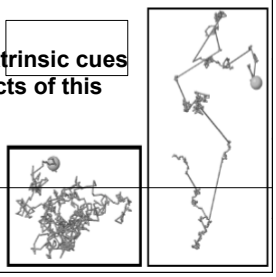
Why do effector T cells utilize this type of behavior?

Probably not the most efficient strategy!

Persistent infections depend on their ability to "hide"

What are the intrinsic and extrinsic cues that influence different aspects of this search pattern?

What about other T cells?



Things I forget to say

- "You can observe a lot just by looking"
- Imaging and hypothesis generation

- **Does behavior underlie function in the immune system?**
- Neutrophils responding to tissue damage
- Predatory T cells searching for rare targets (that know how to hide)
- Regulatory T cells searching for abundant DC (that are not hiding)

Acknowledgements

The School, Beena John, Tajie Harris, Christoph Konradt, David Christian

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University of Pennsylvania

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Today's Discoveries – Tomorrow's Cures

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Paul Mrass

McGill

Florence Dzierszinski

Yasmine Belkaid
David Roos
***Andrea Liu**
Ed Bannigan
Carl June/Mike Kalos
John Boothroyd
Anita Koshy

EPM WORKSHOP 2014: ABSTRACTS

***SARCOCYSTIS FALCATULA* DISPLAYS A HIGHLY SIMILAR ORTHOLOG TO SNSAG 6.** **S Ellison**, Pathogenes, Fairfield, FL., M Grigg, National Institutes of Health, Bethesda, MD.

Clinical signs associated equine protozoal myeloencephalitis (EPM) can be due to the presence of protozoa (*Sarcocystis. neurona*) or the inflammatory responses they stimulate. All pre-mortem immunodiagnostic tests for sarcocystosis (EPM) are based on proteins displayed on the surface of *S. neurona*. Three mutually exclusive surface proteins (SAG's), SnSAG's 1, 5, and 6, have been described for *S. neurona* and the strains can be phenotyped by antibodies. Three additional SAG's are described for *S. neurona*, they are SnSAG's 2, 3, and 4. The SAG's 2, 3, and 4 are highly conserved gene sequences (orthologs) among *S. neurona* and *S. falcatula* and prevent antibody distinction between these species. *Sarcocystis falcatula* does not infect horses based on a study in which sporocysts from opossums fed *S. falcatula* infected cowbird muscle tissues (bioassay in cowbirds) failed to cause disease. (Cutler 1999). The purpose of this study was provide *S. falcatula* (used in an equine infection study) for genetic analysis using a battery of high resolution, polymorphic microsatellite and gene-coding markers. Genetic markers were used to identify and type virulent *S. neurona* strains (Grigg and Wendte, NIH, Bethesda, MD) responsible for fatal neurological disease in the southern sea otter. The results of the genetic analysis (Figure 1) show that the SfSAG 6 protein is nearly identical to SnSAG 6 surface antigen proving at least one strain of *S. falcatula* displays a *S. neurona* antigen that can confound immunodiagnostics. *Sarcocystis falcatula* and *S. neurona* are differentiated by high level genetic analysis or bioassay. Strains of *S. falcatula* displaying SAG 6 may confound tests that rely on highly conserved orthologs but may be differentiated by detection with equine antibodies due to host selectivity. The significance of this report is that bioassay in conjunction with molecular sequence analysis are critical aspects of understanding EPM syndrome in horses.

Figure 1. Comparison of the putative amino acid sequence between SnSAG 6 and SfSAG 6 illustrating the similarity between the proteins.

Score 494 bits(1271)	Expect 5e-174	Method Compositional matrix adjust.	Identities 259/283 (92%)	Positives 267/283 (94%)	Gaps 2/283 (0%)
SnSAG6 1	MTRAVLLTILLTLCSARVSLVKAANPRQATCANGQKTATKVENPG ALQLVCPQQYQLNPA				60
SnSAG6 61	PANDAAGDMQVFGTEAADNAVALRGVLPAATYINANGATTLTVP QLPPKPVSVFIQCRQA				120
SnSAG6 121	AQGAQQAGQCIIEVQVAGSPRLGLGPNTCAAQQSRIDFEIKAANE AAVFSCGAGLALLQQ				180
SnSAG6 181	ASDDTCSKDQALPSGVALAAKEAGAVQLAFPQLPQNPLKICYICT PNGQRAEAAQRCEIH				240
SnSAG6 241	VTVAGSGDGGNPGPTGAAPVGPAARSASALVLAVVAAGFFHFW VTVAGSGDGGNPGPTGAAPVGPAARSASALVLAVVAAGFFHFW				283

EPM WORKSHOP 2014: ABSTRACTS

EFFECTS OF EQUINE CEREBROSPINAL FLUID ON LYMPHOBLAST PROLIFERATION

Goehring, L* and **Furr, M**. Marion duPont Scott Equine Medical Center. Virginia-Maryland Regional College of Veterinary Medicine. (*-Current address Ludwig Maximilian University, Munich Germany)

Equine Protozoal Myeloencephalitis (EPM) is the most frequently diagnosed neurologic disorder of horses in the United States, and it is caused by the protozoan organism *Sarcocystis neurona.* The disease has a profound impact on the American Horse Industry. This impact includes prolonged and expensive treatment without a guaranteed return to a previous level of exercise for the individual horse. Poor response to, and the prolonged duration of treatment, may suggest an immune mediated impairment of host response. There is limited information about the direct interaction between the pathogen and the host. In two *in vitro* experiments we investigated a) whether the presence of the protozoan organism can influence mitogen-stimulated peripheral blood mononuclear cells (PBMCs), suggesting a direct influence of the protozoan organism on cells of the immune system, and b) if cerebrospinal fluid (CSF) from horses with EPM has an effect on mitogen stimulated PBMCs, suggesting that the Central Nervous System microenvironment may influence the course of the disease.

Experiment 1

Mitogen-stimulated PBMCs from EPM affected and control horses were co-cultured with fragments of freeze-thawed bovine turbinate cells that were infected with *S. neurona* merozoites. Compared to controls PBMCs co-cultured with *S. neurona* fragment VI were the only cells that showed a decreased proliferative ($p \square 0.05$). A difference between EPM affected and control horses could not be detected ($p>0.05$).These results may suggest that the persistence of *S. neurona* infection in the horse's CNS is, in part, due to a pathogen-derived mechanism that attenuates the host's immune response.

Experiment 2

Mitogen-stimulated PBMCs from a horse affected with EPM and a control were co-cultured in the presence of cerebrospinal fluid (CSF) from EPM affected horses and uninfected controls. Prior to co-culture the CSF was fractionated by a filtration process over two microfilter units. An identical volume of NaCl (0.9%) served as control for the volume of CSF that was added. The proliferation assay revealed a deviation of the response depending on cell donor and CSF fraction that was used. The effect was independent from the protein concentration of the CSF fractions, and a decrease in lymphocyte proliferation was not caused by increased cellular death. This suggests the presence of substances within the CSF which have a stimulatory or suppressive influence on the cells in culture. The effect was cell donor dependent which implies a difference in lymphocyte subsets between the two horses that were used.

EPM WORKSHOP 2014: ABSTRACTS

ABILITY OF *SARCOCYSTIS NEURONA* TO INFECT EQUINE PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO. B. Heid[1], D. Howe[2], S. Ellison[3], D. Lindsay[1], N. Surendran[1], **S. Witonsky[1]**. 1. Virginia Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. 2. Gluck Equine Research Center, University of Kentucky, Lexington, KY. 3. Pathogenes, Fairfield, FL.

The mechanisms by which *Sarcocystis neurona* infects the horse and migrates into the central nervous system to cause disease are not well understood. It is known that *S. neurona* can infect endothelial cells. Additionally both Lindsay and Ellison have demonstrated the ability of *S. neurona* to infect leukocytes. However, the preferential ability of *S. neurona* to infect leukocytes was not known, nor its impact on viability and apoptosis. Therefore, the objectives of this study were to determine 1) the bias in infectivity of *S. neurona* leukocytes in normal horses 2) if *S. neurona* altered viability of infected cells 3) if *S. neurona* altered the viability of resident non-infected cells in vitro.

For this study, equine peripheral blood mononuclear cells were isolated from clinically normal horses (n=8). Cells were infected with *S. neurona* expressing yellow fluorescence protein (YFP). At 1 and 24 hrs post-infection, infected cells were identified based on YFP expression. Percentages of infected and uninfected cells were determined as well as alterations in apoptosis of infected and resident cell populations. When possible, 10,000 events were collected by flow cytometry (FACS-Aria). At both 1 and 24 hr, there was a significant increase in the percentage of mononuclear cells infected with *S. neurona*. At 24 hr, the percentage of infected B-cells was greater than CD4 cells. At 1hr, monocytes had increased number of cells in apoptotic group. In comparing apoptosis of infected vs. uninfected cells, at 1 and 24 hr, there was a pattern for CD4 and CD8 infected cells to have an increase in viable, early and late apoptotic cells than uninfected cells. By contrast, infected monocytes, at 1hr, had a decrease in late apoptotic cells whereas at 24 hr, there was an increase in infected late vs. uninfected late apoptotic cells. These data support that *S. neurona* does preferentially infect specific subsets, and infection could affect cell viability.

EPM WORKSHOP 2014: ABSTRACTS

EFFECTS OF EXPERIMENTAL *SARCOCYSTIS NEURONA* INDUCED INFECTION ON IMMUNITY IN AN EQUINE MODEL. SR Lewis[12], SP Ellison[3], JJ Dascanio[4], DS Lindsay[1], R M Gogal Jr[8], SR Werre[1], N Surendran[6], ME Breen[17], BM Heid1, FM Andrews5, VA Buechner-Maxwell1, **SG Witonsky1**. 1. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, 2. Rangoria Equine Services, New Zealand. 3. Pathogenes Inc, Fairfield, FL. 4. Lincoln Memorial College of Veterinary Medicine, Harrogate, TN. 5. School of Veterinary Medicine, Veterinary Teaching Hospital and Clinics, Louisiana State University, Baton Rouge, LA 6. Rochester General Hospital Research Institute, University of Rochester, Rochester, N. 7 Natural Vet Palm Beach, Juno, FL.8. College of Veterinary Medicine, University of Georgia.

Sarcocystis neurona is the most common cause of Equine Protozoal Myeloencephalitis (EPM), affecting 0.5-1% horses in the United States during their lifetimes. The objective of this study was to evaluate the equine immune responses in an experimentally induced *Sarcocystis neurona* infection model. Neurologic parameters were recorded prior to and throughout the 70 day study by blinded investigators. Recombinant SnSAG1 ELISA for serum and CSF were used to confirm and track disease progression. All experimentally-infected horses displayed neurologic signs post infection. Neutrophils, monocytes and lymphocytes from infected horses displayed significantly delayed apoptosis at some time points. Cell proliferation was significantly increased in *S. neurona* -infected horses when stimulated non-specifically with PMA/I, but significantly decreased when stimulated with *S. neurona* compared to controls. Collectively, our results suggest that horses experimentally-infected with *S. neurona* manifest impaired antigen specific response to *S. neurona,* which could be a function of altered antigen presentation, lack of antigen recognition or both.

EPM WORKSHOP 2014: ABSTRACTS

PRE-COLOSTRAL ANTIBODY DETECTION TO *NEOSPORA HUGHESI* AND *SARCOCYSTIS NEURONA* IN FOALS SUPPORTS TRANSPLACENTAL INFECTION

N. Pusterla[1], S. Mackie[2], A. Packham[1], P. Conrad[1]. 1. School of Veterinary Medicine, University of California, Davis, CA. 2. Elkton, MD.

The goal of this study was to investigate the likelihood of *Neospora hughesi* and *Sarcocystis neurona* transplacental transmission in foals born from seropositive mares.

The study population consisted of two unrelated groups of adult broodmares. The first group was composed of two Percheron mares originating from the same farm in northern California. These two mares had previously been reported to be latently infected with *N. hughesi*, based on persistently elevated titers measured using the indirect fluorescent antibody test (IFAT). The same mares had given birth between 2006 and 2007 to a total of 3 healthy, congenitally infected foals which all had high titers to *N. hughesi* prior to colostrum ingestion. The two mares were followed from 2008 to 2012 to study transplacental transmission of *N. hughesi* in their subsequent 4 offsprings, with one of them giving birth to a foal in 2012. The second group of horses was composed of 174 Standardbred broodmares originating from a large farm in Maryland. The farm was selected because of a high seroprevalence to *S. neurona* in their population of horses $\geq$ 2 years of age. Mare and foal pairs from this farm were enrolled in the study from March to June of 2014. Whole blood was collected from each mare and foal pair shortly after birth and from every foal at 24-48 hours of age Serum samples were tested for antibodies to *N. hughesi* and *S. neurona* using an IFAT as previously described.

From 2008 to 2012, the two mares with persistent *N. hughesi* infections gave birth to 4 healthy foals with pre-colostral antibody titers to *N. hughesi* ranging from 640 to 2,560. At the time of birth, both these mares had antibody titers to *N. hughesi* in serum ranging from 320 to 5,120. One of the initial seropositive foals was bred as a 3-year-old broodmare and gave birth to a filly born with a pre-colostrum titer to *N. hughesi* of 640. One-hundred fifty-eight of the 174 broodmares (90.8%) tested seropositive to *S. neurona* with titers ranging from 40 to 1,280 (median titer 80). Pre-colostrum serum samples were collected from all 174 foals, 173 of which had no detectable antibodies to *S. neurona* in serum (titer <40). One foal born from a seropositive mare had a pre-colostrum titer to *S. neurona* of 80. The titer of that foal increased to 320 at 24 hours of age. After ingestion of colostrum, 147 foals had positive titers to *S. neurona* ranging from 40 to 320 (median titer 80).

The study results showed endogenous transplacental infection in 100% of the foals born from *N. hughesi* latently infected broodmares and the transmission of *N. hughesi* from one of the congenitally infected filly to her progeny as she became a broodmare. A previous study performed on 4 California farms during 3 foaling seasons showed that there was no serological or histological evidence of *in utero* infection with *S. neurona*. In our study, one out of 158 foals (0.63%) born from a *S. neurona* seropositive broodmare had serological evidence of transplacental infection based on the presence of specific antibodies in pre-colostrum blood. The discrepancy in pre-colostrum antibody titers to *N. hughesi* and *S. neurona* likely relates to differences in the biology of these organisms and modes of transmission.

EPM WORKSHOP 2014: ABSTRACTS

USE OF DAILY DICLAZURIL PELLETED TOP DRESS FOR THE PREVENTION OF *SARCOCYSTIS NEURONA* INFECTION IN FOALS. **N. Pusterla[1]**, A. Packham[1], P. Conrad[1], P. Kass[1], S. Mackie[2]. 1. School of Veterinary Medicine, University of California, Davis, CA. 2. Elkton, MD.

Therapeutic treatment strategies for the prevention of *Sarcocystis neurona* infection in horses have been empirical. A pelleted top dress 1.56% diclazuril anti-protozoal drug labeled for the treatment of EPM has the potential to be used for the prevention of *S. neurona* infection due to its convenient formulation. A low dose of diclazuril given at 0.5 mg/kg body weight has been shown to reach plasma and CSF concentrations at steady-state in excess of the minimal concentration known to be inhibitory to *S. neurona* merozoite production in cell culture. The purpose of this study was to evaluate the temporal serological response against *S. neurona* in foals receiving daily diclazuril and in untreated herd mates.

Thirty-three foals from a farm with a high seroprevalence to *S. neurona* were randomly assigned to either an untreated control group or a diclazuril treated group. Foals in the treatment group received 0.5 mg/kg body weight of diclazuril administered as a pelleted top dress starting at 4 weeks of age and continued until foals were 12 months of age. Whole blood was collected from every dam and foal 24 hours post-foaling and monthly thereafter from each of the 33 foals for the duration of the study. The blood was tested for IgG against *S. neurona* using an indirect fluorescent antibody test.

Following ingestion of colostral antibodies to *S. neurona*, there was a steady and continuous decline in antibody titers to *S. neurona* until the foals from both groups reached weaning age. Thereafter, untreated foals showed an increase in monthly seroprevalence ranging from 53 to 88%. Diclazuril treated foals showed significantly lower monthly seroprevalences ranging from 6 to 37%.

The daily supplementation of diclazuril pelleted top dress at 0.5 mg/kg body weight demonstrated a significant reduction in seroconversion against *S. neurona* in foals treated up to 12 months of age when compared to untreated control foals. To the author's knowledge this is the first report evaluating dose and duration of treatment for the prevention of seroconversion in foals originating from a farm with high exposure rate to *S. neurona*.

Equine Protozoal Myeloencephalitis Society
Founded 1999

Membership Application

The objectives of the EPMS and the requirements for membership (Articles of Incorporation, Article IV; Bylaws, Article III) are:

Objectives: "The Corporation shall organize individuals whose interests in equine protozoal myeloencephalitis ("EPM") research contribute to a greater understanding of the disease; encourage cooperative EPM research; promote awareness of current EPM research and new developments in the field; and evaluate and disseminate guidelines for the diagnosis, treatment, and prevention of EPM. In all events the assets of the corporation, both principal and income, shall be applied exclusively for charitable, religious, testing for public safety, prevention of cruelty to children or animals, scientific, literary, or educational purposes within the meaning of Section 501(c)(3) of the Code (or corresponding provisions of any future United States Internal Revenue law)..."

Membership: Active. Any veterinarian or scientist who is interested in Equine Protozoal Myeloencephalitis ("EPM") research and whose application for admission as an Active Member has been approved by the corporation and who has paid the dues assessed for such category of membership for the then current fiscal year shall be an Active Member during such fiscal year. Active Members shall be entitled to receive all member communications and notices from the corporation. Active Members shall be entitled to vote on all matters submitted to a vote of the members who have voting rights in accordance with the provisions of the Articles of Incorporation of the corporation or these bylaws. Active Members shall be eligible to hold office in the corporation. Sustaining Members. Any entity other than an individual interested in supporting the objectives of the corporation and approved by the corporation and who has paid the dues assessed for such category of membership for the then current fiscal year shall be a Sustaining Member during such fiscal year. An entity with a sustaining membership shall be entitled to designate two employees to represent the company for that fiscal year. Each employee shall be eligible to vote on all matters submitted to a vote of the members who have voting rights in accordance with the provisions of the Articles of Incorporation of the corporation or these bylaws. Each employee shall be eligible to hold office in the corporation. Subscriber. Any individual who is not eligible to be an Active Member of the corporation and whose application for admission as a Subscriber Member has been approved by the corporation and who has paid the dues assessed for such category of membership for the then current fiscal year shall be a Subscriber Member during such fiscal year. Subscriber Members shall not be entitled to vote and shall not be eligible to hold office in or serve as a director or a member of a committee of the corporation, but shall receive all other benefits accorded to Active Members. Student. Any student enrolled in a college of veterinary medicine or in a graduate program who has an interest in EPM and whose application for admission as a Student Member has been approved by the corporation shall be a Student Member. Student Members shall not be entitled to vote and shall not be eligible to hold office in or serve as a director or a member of a committee of the corporation, but shall receive all other benefits accorded to Active members. Student members shall be exempt from the payment of dues.

The dues year runs from January 1- December 31. In order to be considered for membership, complete the attached application and submit it with a check (Active or Subscriber $40.00; Sustaining $250.00, US currency) payable to the EPM Society, to the Secretary-Treasurer:

Dr. Jennifer Morrow
1501 Bull Lea Road, Suite 104
Lexington, KY 40511
(859) 288-5255
jmorrow@edslabky.com

Application for Membership/Renewal

Membership Classification:

Active: _____ Sustaining: _____ Subscriber: _____ Student: _____

Member Information:

Name: _____ Title: _____

Academic Degree(s): _____

Institutional/Business Affiliation: _____

Mailing Address (Lab/Office): _____

_____

_____

Phone: _____ Fax: _____

E-mail: _____

Signature: _____ Date: _____

Sustaining Membership – Information:

Company Name: _____

Company Address: _____

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Contact Name: _____ Contact Phone: _____

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