Molecular testing for bacterial diseases transmitted by ticks

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RICKETTSIOSIS

TEXAS A&M AGRILIFE RESEARCH

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VETERINARY MEDICINE & BIOMEDICAL SCIENCES

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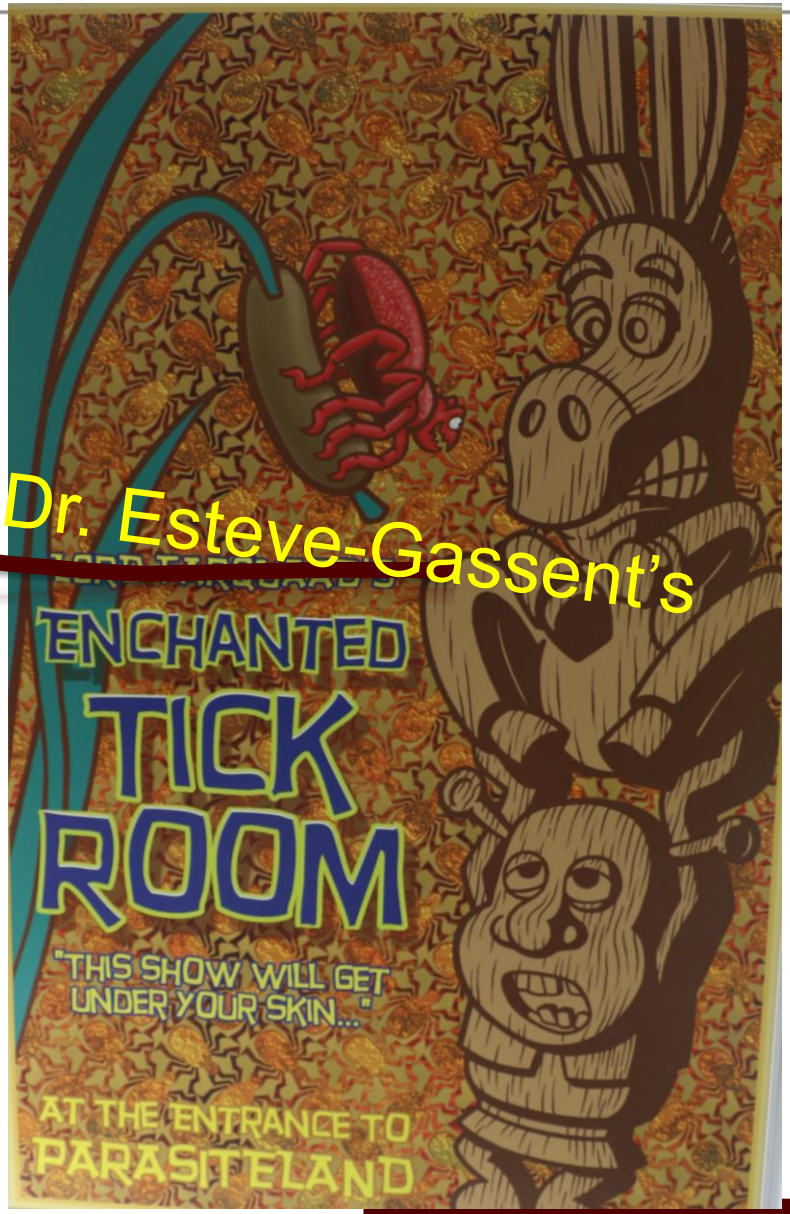
Testing ticks and animals

Development of a multiplex PCR for the detection of:

- *B. burgdorferi,*
- *Ehrlichia canis, E. chaffeensis,*
- *Anaplasma phagocytophilum* and
- *Rickettsia rickettsii*



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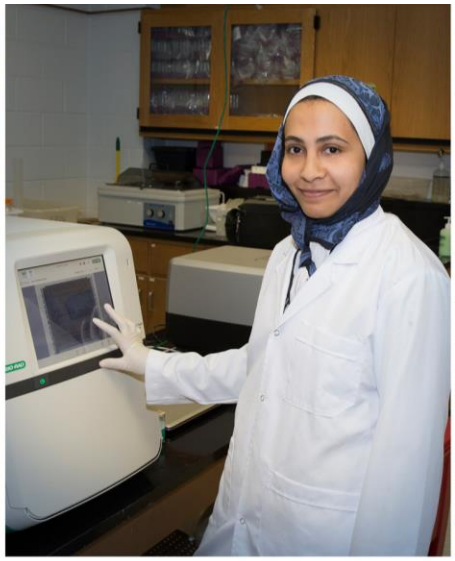

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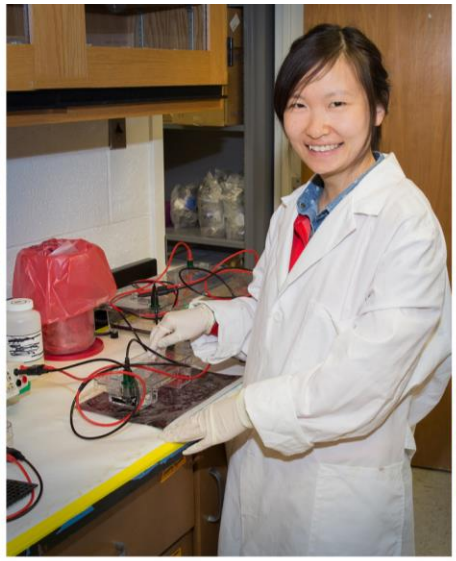

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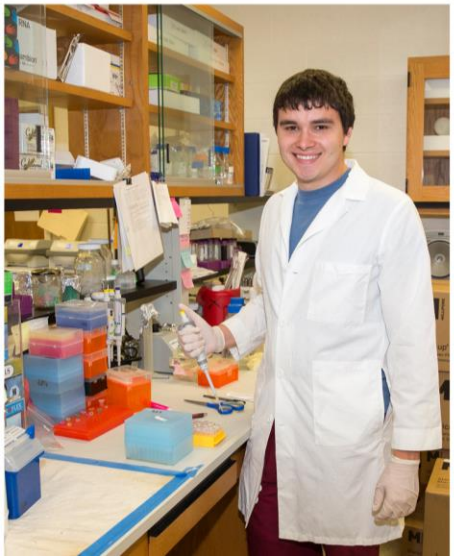

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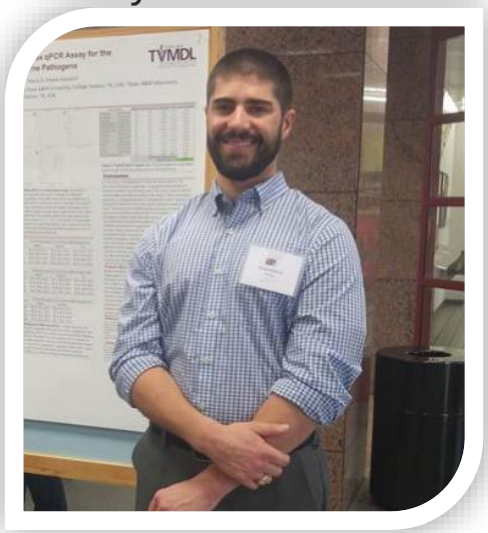
Undergraduate research team:
Brian, Annie, Thomas and Brittany

Jaqueline: visiting scholar (BZ)
Mackenzie: PhD student



Joseph: PhD student and TVMDL Fulltime employee

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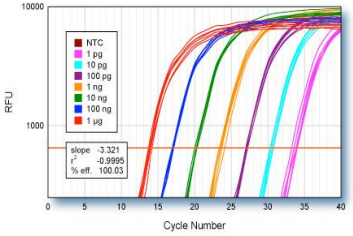
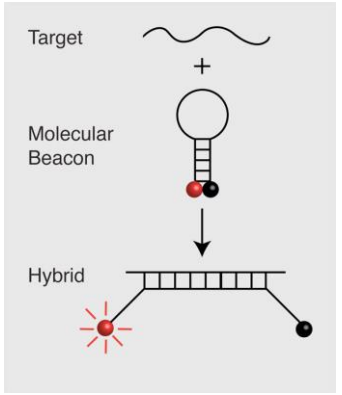
Background

- Tick-borne diseases are becoming a serious problem worldwide
- Lyme disease, Human Anaplasmosis and Rocky Mountain Spotted fever have emerged as the most common vector born bacterial illnesses in the US and Mexico.

Aim of the study

- Develop a Multiplex PCR technique
  - Multi pathogen detection
  - Compatible with Sequencing
    - Species confirmation
    - Molecular epidemiology
  - Compatible with diagnostic platforms
    - Real time PCR
    - Molecular Beacon PCR technology
    - Others





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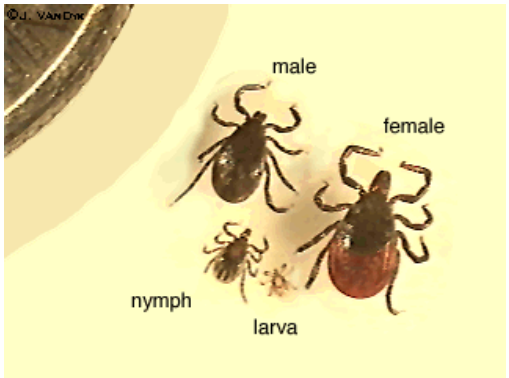
Lyme disease (LD)

- Most prevalent arthropod borne disease in the US
  - Over 30,000 cases reported yearly to CDC
  - Transmitted by *Ixodes scapularis* and *I. pacificus* tick bite (in the US)
  - Mammalian reservoirs are small rodents
- Caused by the spirochetal pathogen *Borrelia Burgdorferi*
- Multi-phase disorder in humans
  - Early LD (70% Erythema migrans)
  - Early disseminated LD (Flu like symptoms)
  - Chronic LD (Arthritis and carditis)



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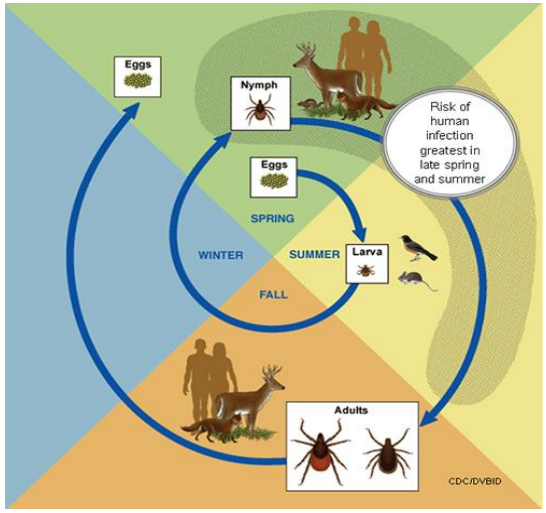
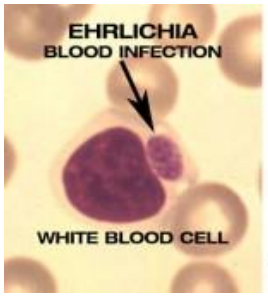
Ehrlichiosis

- Small, gram-negative bacteria, round or ellipsoidal in shape.
- Symptoms in humans: fever, headache, fatigue, and muscle aches.
- These symptoms occur within 1-2 weeks following a tick bite.





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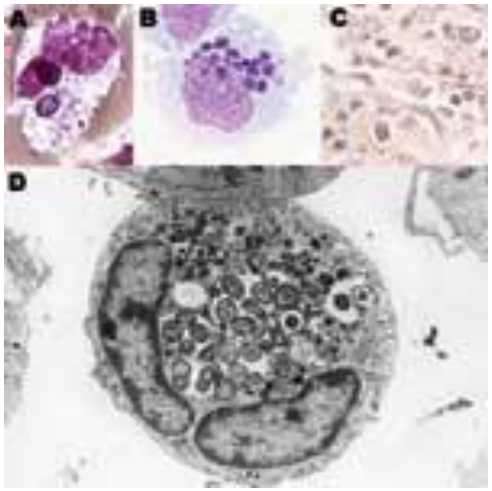
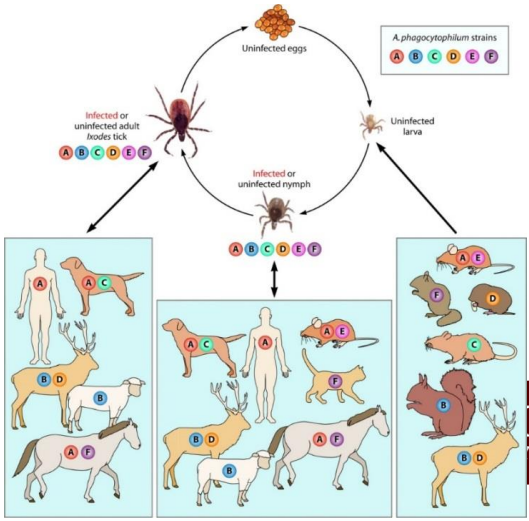
Anaplasma phagocytophilum

- Gram-negative and intracellular: targets neutrophils, alters their function in the host, and forms morulae within vacuoles.
- Symptoms in humans: fever, headache, muscle pain, malaise, chills, nausea, abdominal pain, cough, and confusion.
- Severe clinical presentations may include difficulty breathing, hemorrhage, renal failure or neurological problems.





Rocky Mountain Spotted Fever (RMSF)

- Gram-negative, intracellular, coccobacillus bacterium
- Typical symptoms include: fever, lethargy, abdominal pain, vomiting, and muscle pain
- Rash found on 90% of patients
  - Classic RMSF rash - 2 to 5 days post fever
    - small, flat, pink macules - develops on distal extremities
  - Varies greatly and is unreliable at times
- Pathogen of interest: *Rickettsia rickettsii*

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Previous Rickettsiosis Forum

- Tijuana May 2015
- Discussed the possibility of testing
  - *Rhipicephalus sanguinus* from Baja California
  - Associated with canids in areas where severe human cases were reported
  - Test an initial submission of ticks at UTSA
  - Test further specimens by qPCR methodology

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Baja California Ticks

- Our team has developed a Multiplex qPCR protocol under revision for patent application

- Detects:
  - *B. burgdorferi*
  - *Ehrlichia canis*
  - *Rickettsia rickettsii*
  - Canine internal control

  - Additional targets
    - *Anaplasma phagocytophilum, E. chaffeensis*

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Sensitivity

Borrelia burgdorferi			*Rickettsia rickettsii*			*Anaplasma phagocytophilum*			*Ehrlichia canis*		
DNA ng/µl	**Mean C_t**	**Copy Number**	**DNA ng/µl**	**Mean C_t**	**Copy Number**	**DNA ng/µl**	**Mean C_t**	**Copy Number**	**DNA ng/µl**	**Mean C_t**	**Copy Number**
4.95	N/A	1.05E+09	4.95	N/A	1.05E+09	4.97	N/A	6.90E+09	4.97	N/A	6.90E+09
4.95E-07	28.6	840	4.95E-07	27.7	840	4.97E-06	25.4	6900	4.97E-06	25.1	6900
1.24E-07	30.6	210	1.24E-07	29.8	210	4.97E-07	28.8	690	4.97E-07	28.6	690
3.09E-08	32.8	53	3.09E-08	32.0	53	4.97E-08	32.1	69	4.97E-08	31.6	69
7.73E-09	34.2	13.13	7.73E-09	33.8	13.13	1.24E-08	34.3	17.20	1.24E-08	34.0	17.20
3.87E-09	35.9	6.56	3.87E-09	35.0	6.56	3.11E-09	36.1	4.31	3.11E-09	36.4	**4.31***
1.93E-09	37.6	**3.28***	1.93E-09	36.8	**3.28***	1.55E-09	37.2	**2.16***	1.55E-09	39.1	2.16
4.83E-10	0.0	<1	4.83E-10	37.7	<1	7.77E-10	0.0	<1	7.77E-10	0.0	<1

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Specificity

Substrates	*B. burgdorferi* qPCR C_t	*A. phagocytophilum* qPCR C_t	*R. rickettsii* qPCR C_t	*E. Canis* qPCR C_t	*E. Chaffeensis* qPCR C_t
B. burgdorferi	30.1	0.0	0.0	0.0	0.0
A. phagocytophilum	0.0	25.1	0.0	0.0	0.0
R. rickettsii	0.0	0.0	27.3	0.0	0.0
E. canis	0.0	0.0	0.0	27.0	0.0
E. chaffeensis	0.0	0.0	0.0	0.0	26.2
Babesia canis	0.0	0.0	0.0	0.0	0.0
B. gibsoni	0.0	0.0	0.0	0.0	0.0
A. marginale	0.0	0.0	0.0	0.0	0.0
R. typhi	0.0	0.0	0.0	0.0	0.0

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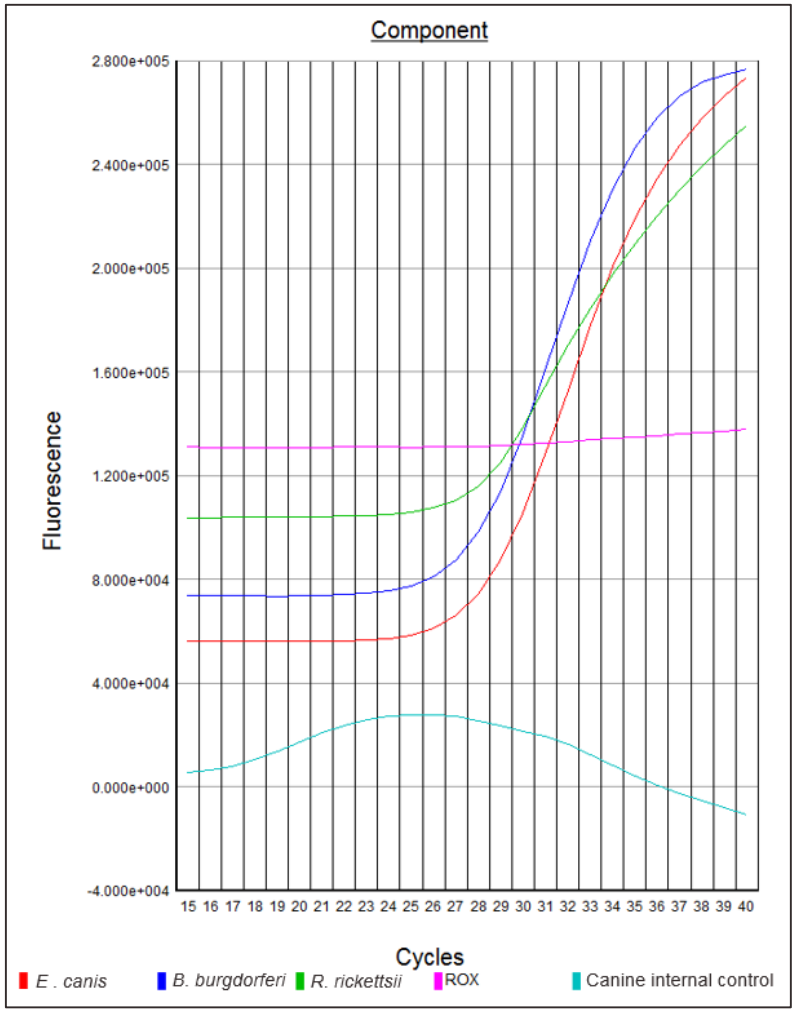
Canine internal control



Specific to	Validated Against
Canis lupus familiaris	Equine
Canis lupus	Feline
Canis latrans	Caprine
	Ovine
	Cervine
	Bovine
	Porcine
	Avian
	Procyonine
	Vulpine
	Mephitidae

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Baja California samples

- We evaluated a total of 211 *R. sanguineus* ticks
  - Collected from dogs/environmental locations (n=160)
- Samples were processed for:
  - Confirmation of tick species, sex, age
  - Detection of pathogens
- All experiments were conducted at Texas A&M Veterinary Medical Diagnostic Lab (TVMDL)

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Texas A&M Veterinary Diagnostic Laboratory

TEXAS A&M TVMDL VETERINARY MEDICAL DIAGNOSTIC LABORATORY

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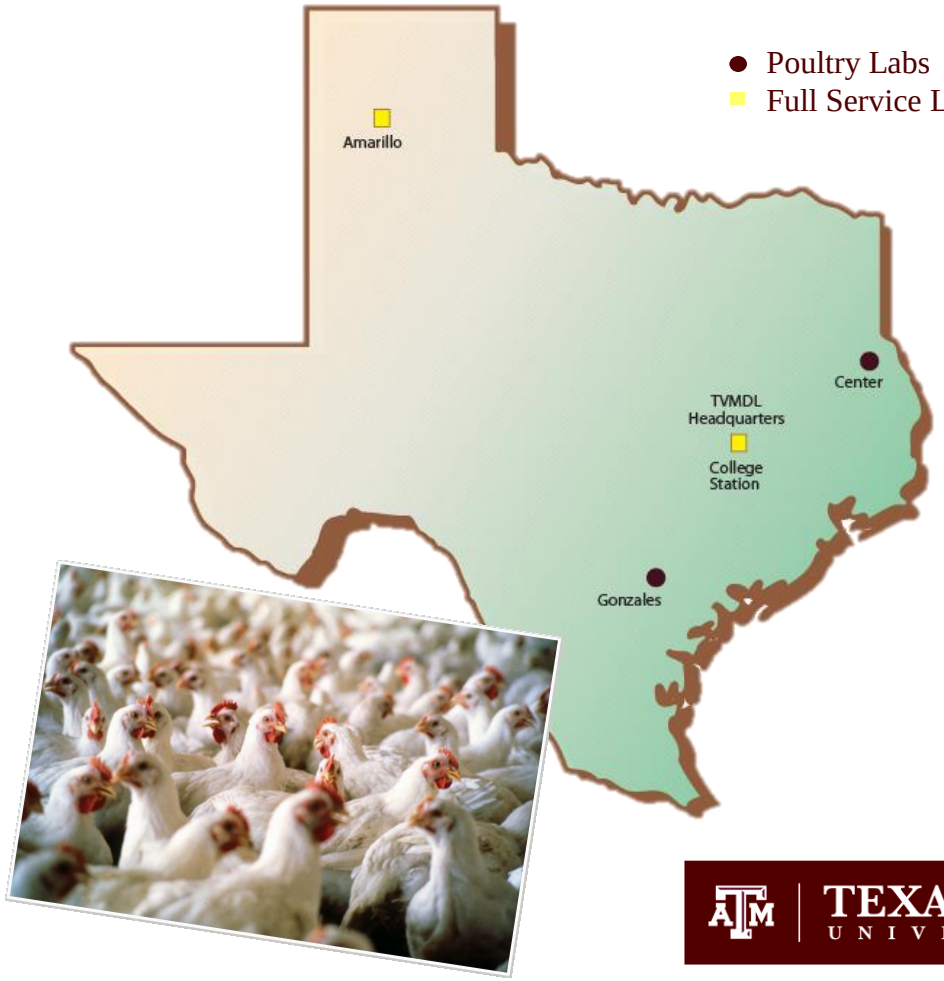
Locations

- 165 staff
- Over 30 professional staff who hold a DVM and/or PhD
- 21 professionals with board certifications in their specialty
- Strategically located in the livestock and poultry rich regions of Texas



Vision and Mission

Vision

To be the global leader in providing innovative and state-of-the-art veterinary diagnostic services

Mission

To promote animal health and protect agricultural, companion animal, and public health interests in Texas and beyond by providing excellence in veterinary diagnostic service

Clientele

- Veterinarians and animal owners from Texas and other states
- Local, state and national agencies
- International clientele
- Commercial and state diagnostic laboratories

Strategic Partnerships

Texas A&M TVMDL Veterinary Medical Diagnostic Laboratory

Texas A&M University College of Veterinary Medicine & Biomedical Sciences

Texas Animal Health Commission · Serving Texas Animal Agriculture · Since 1893

USDA

Texas Parks & Wildlife

Texas Department of Criminal Justice

AgriLIFE RESEARCH
Texas A&M System

TVMA
Texas Veterinary Medical Association
A Legacy of Service

AgriLIFE EXTENSION
Texas A&M System

APHIS

CSREES Education · Research · Extension · USDA

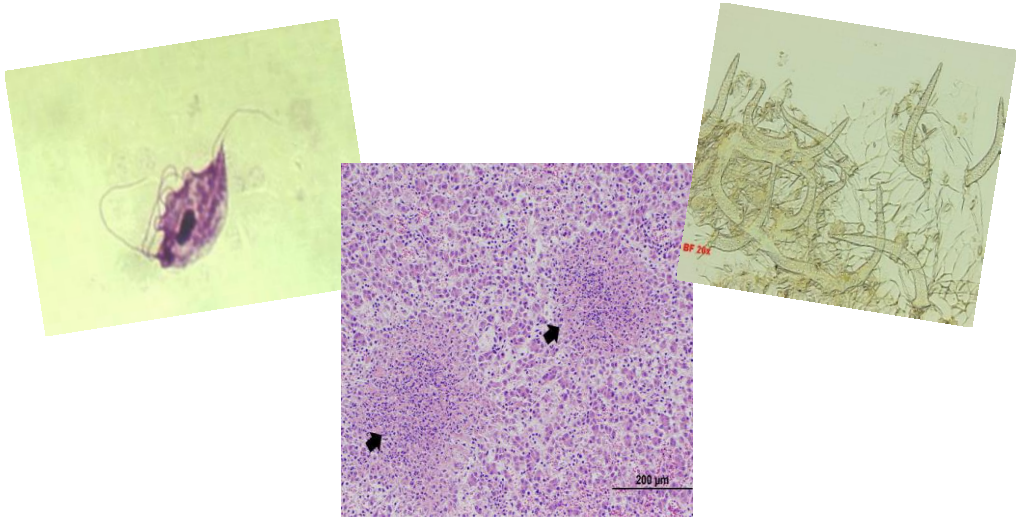
AAVLD

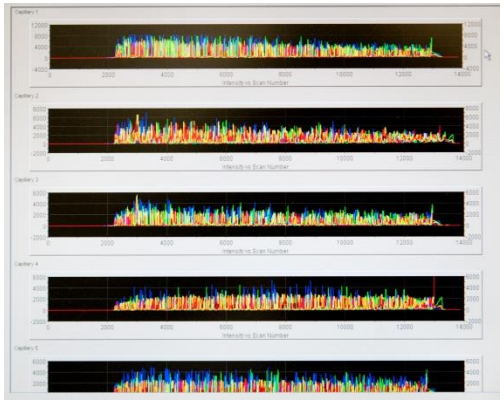
Texas Department of State Health Services

Institute for Infectious Animal Diseases
A Department of Homeland Security Science & Technology Center of Excellence

Disciplines

- Bacteriology
- Virology
- Endocrinology
- Parasitology
- Serology
- Toxicology
- **Molecular Genetics**
- Clinical Pathology
- Histopathology
- Necropsy
- Poultry Diagnostics
- Epidemiology
- Drug Testing





Molecular Diagnostics

- Maceration of tick samples: Omni Bead Ruptor

- DNA extraction: KingFisher™ Flex

- qPCR amplification and analysis:
  - ABI® 7500 qPCR System

Results

Patógenos	Positivos (%)*
Borrelia burgdorferi	0
Anaplasma phagocytophilum	0
Ehrlichia canis	18 (8.5)
Ehrlichia chaffeensis	0
Rickettsia rickettsii	2 (0.9%)
Total	**20 (9.5%)**

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Conclusions

- *E. canis* positive ticks were confirmed by sequencing
- *R. rickettsia* positives are under study
- Zoonotic pathogens are present in *R. sanguineus* ticks of Baja California
- Epidemiological studies will certainly provide relevant information for the implementation of control programs

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Take home message

- Tick borne diseases are circulating in the Texas-Mexico transboundary region
- Bi-national efforts can
  - Generate distribution maps
  - Assess Human risk
- Multiplex technology has been developed:
  - Eco-epidemiology (surveillance)
  - Molecular epidemiology (surveillance)
  - Diagnostics

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    - Emy Hassan
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- CENAPRECE
- Secretaría de Salud

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Thank you!
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