



**MINISTRY OF BUSINESS,  
INNOVATION & EMPLOYMENT**  
HIKINA WHAKATUTUKI



**LANDCARE RESEARCH**  
MANAAKI WHENUA

# Vaccine development to prevent growing impacts of tick-borne diseases to New Zealand's livestock industries

Dan Tompkins & Felix Guerrero



USDA  
Agricultural  
Research  
Service

CENTRE FOR  
COMPARATIVE GENOMICS

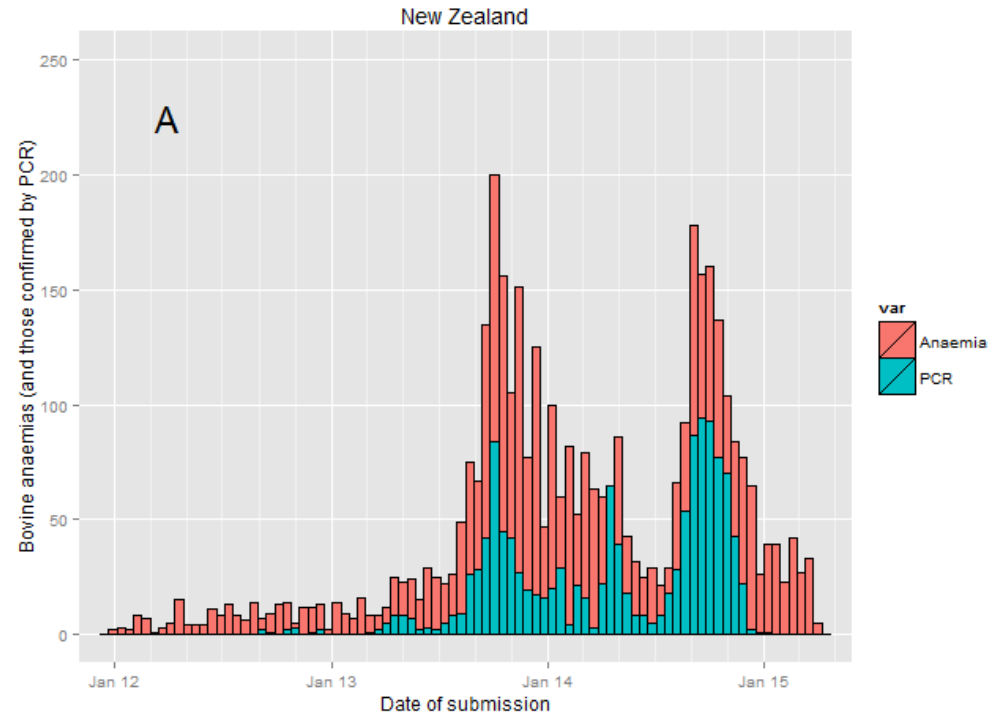
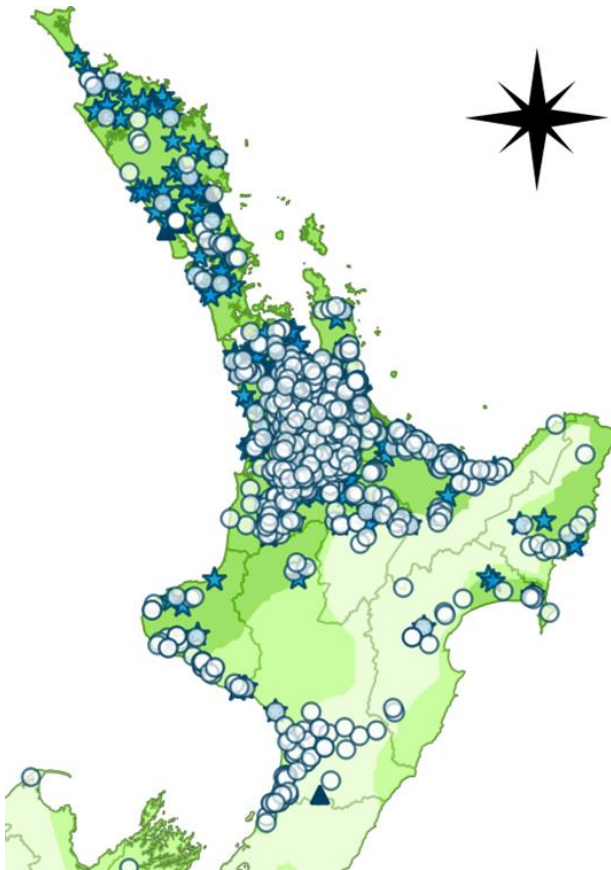
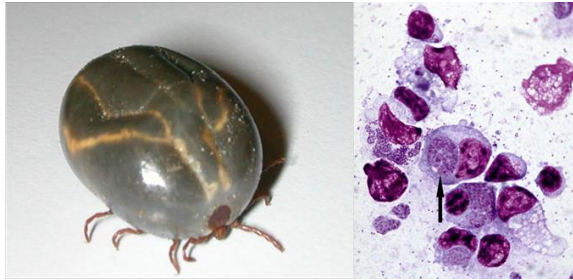


**ag**research



**MASSEY**  
UNIVERSITY

# *Theileria orientalis* Ikeda emergence



## Index case:

- Approximately 20 cows died over the calving period of “milk fever like disease”.
- Reproduction: 6 week in calf rate of 44% (Target 78%)
- Milk production: 230 milk solids per cow (compared to regional average of 315).

# Other threats...

Table 8. Summary table of medium and high priority agents

Agent type		Present in New Zealand	Absent from New Zealand
Virus	Flavivirus	Unidentified Group B virus?	West Nile virus
			Yellow Fever virus
	Alphavirus	Whataroa virus	Murray valley encephalitis virus
		Unidentified Group A virus?	Ross river virus
			Sinbis virus
Quaranfil	Johnston Atoll virus. Introduced.	Barmah forest virus	
Lyssavirus		Bovine Ephemeral fever virus	
Haemosporidia	Plasmodium	<i>Plasmodium relictum</i>	
		<i>P. cathemerium</i>	
		<i>P. spp.</i>	
	Haemoproteus	<i>Haemoproteus spp.</i>	
Leucocytozoon	<i>Leucocytozoon tawaki</i>		
		<i>Leucocytozoon spp.</i>	
Piroplasms	Babesia	<i>Babesia kiviensis</i>	<i>Babesia bigemina</i> ●
			<i>Babesia gibsoni</i> ●
			<i>Babesia ovata</i> ●
			<i>Babesia major</i> ●
			<i>Babesia ovis</i> ●
			<i>Babesia shortii</i>
			<i>Babesia pierci</i>
Rickettsias	Anaplasma	<i>Anaplasma sp.</i> unidentified. Introduced.	
	Rickettsia	<i>Rickettsia typhi</i> (murine typhus). Introduced.	<i>Rickettsia japonica</i> ●
		<i>Bartonella henselae</i> . Introduced.	<i>Rickettsia tsutsugamushi</i> (scrub typhus). ●
		<i>Bartonella claridgeiae</i> . Introduced.	<i>Rickettsia spp.</i> ●
		<i>Rickettsia felis</i> . Introduced.	<i>Coxiella burnetii</i> (Q fever). ●
		<i>Bartonella spp.</i> ●	
	Theileria	<i>Theileria orientalis</i> . Introduced. ●	<i>Theileria sergenti</i> ●
<i>Theileria sp.</i> Introduced. ●		<i>Theileria buffeli</i> ●	
Bacteria	Yersinia		<i>Yersinia pestis</i>
	Spirochete	<i>Borrelia garinii</i> ? Introduced.	<i>Borrelia burgdorferi</i> (Lyme disease), also <i>B. afzelii</i> , <i>B. japonica</i>
			<i>Borrelia recurrentis</i> (louse-borne relapsing fever).
Nematodes	Filaroid		<i>Dirofilaria immitis</i>
			<i>Wuchereria bancrofti</i>
			<i>Brugia malayi</i>

# Leverage overseas developments in tick vaccinology



## **MBIE Catalyst: Seeding**

- Hold an NZ workshop;
- Circulate the first draft proposal;
- Expand the project team;
- Engage partners as appropriate;
- Finalise project application

## **Benefits to NZ**

- Improve current livestock productivity;
- Prevent future losses;
- Overseas vaccine sales;
- Build capability (preparedness);
- Safeguard human and animal health



# A Genome-based Approach To Deliver New Pest Control Technology

**Dr. Felix D. Guerrero**

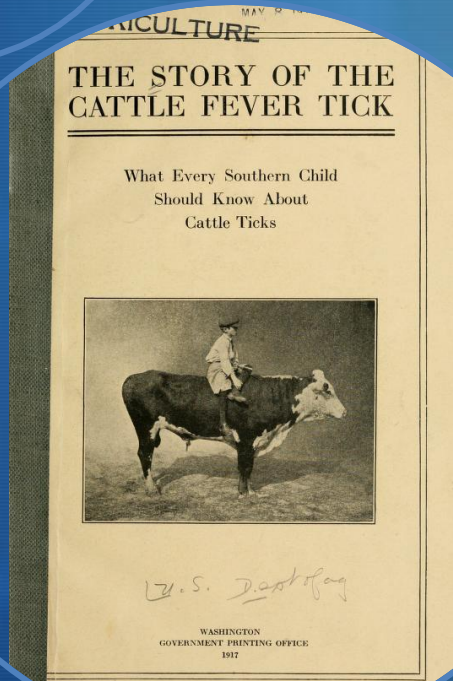
U. S. Department of Agriculture  
Agricultural Research Service  
U. S. Livestock Insects Research Laboratory  
Kerrville, TX USA

# USDA-ARS Kerrville Lab Research Mission

The mission of the Knipling-Bushland U.S. Livestock Insects Research Laboratory is to **provide** the Cattle Fever Tick Eradication Program of APHIS/VS, the U.S. cattle industry, and the public, **technology for eradicating or controlling ticks and blood-feeding flies of veterinary and medical importance.**

# My Specific Research

- ✧ Genome-Based Cattle Fever Tick Studies
  - ✧ Molecular basis of pesticide resistance
  - ✧ Development of an anti-cattle tick vaccine
- ✧ Biting Flies
  - ✧ DNA-based diagnosis of pesticide resistance
  - ✧ Anti-biting fly vaccine research
  - ✧ Maintenance of Screwworm eradication status of U.S.
    - ✧ Led development of transgenic female conditional lethal strain



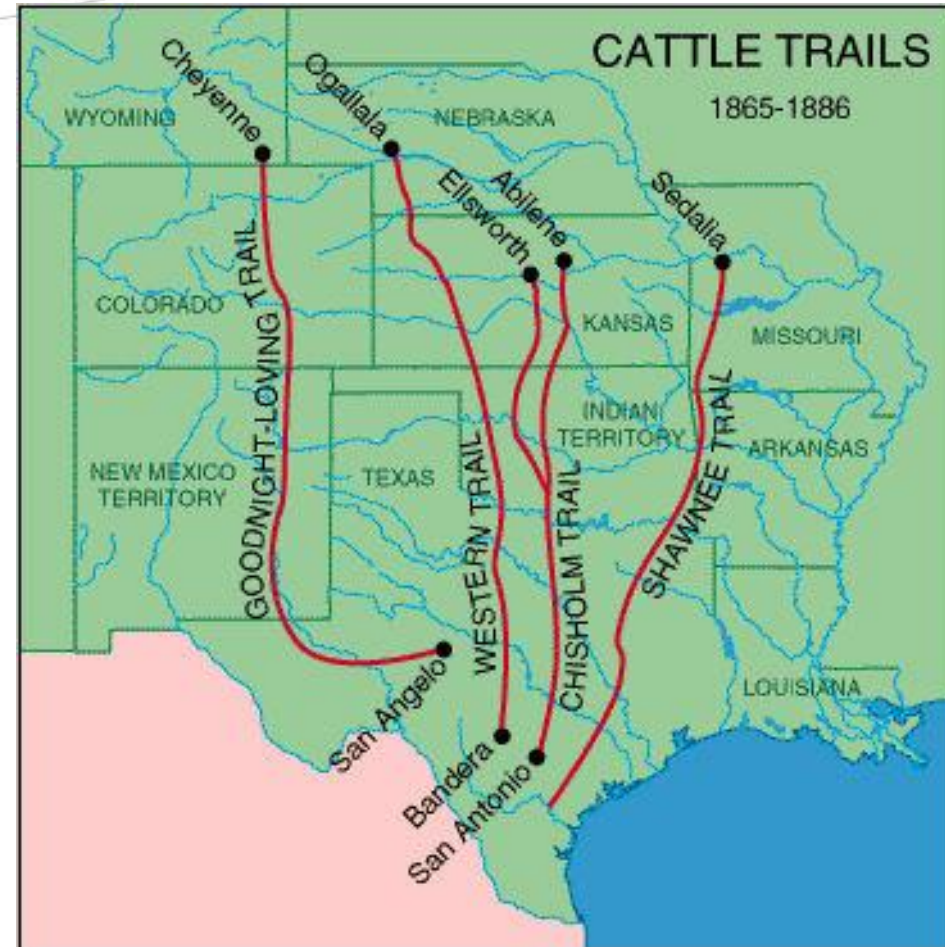
# Today's Focus: Cattle Ticks

- ❖ Relevance to U. S. agriculture
- ❖ Genome-based discovery
- ❖ Discovery to application

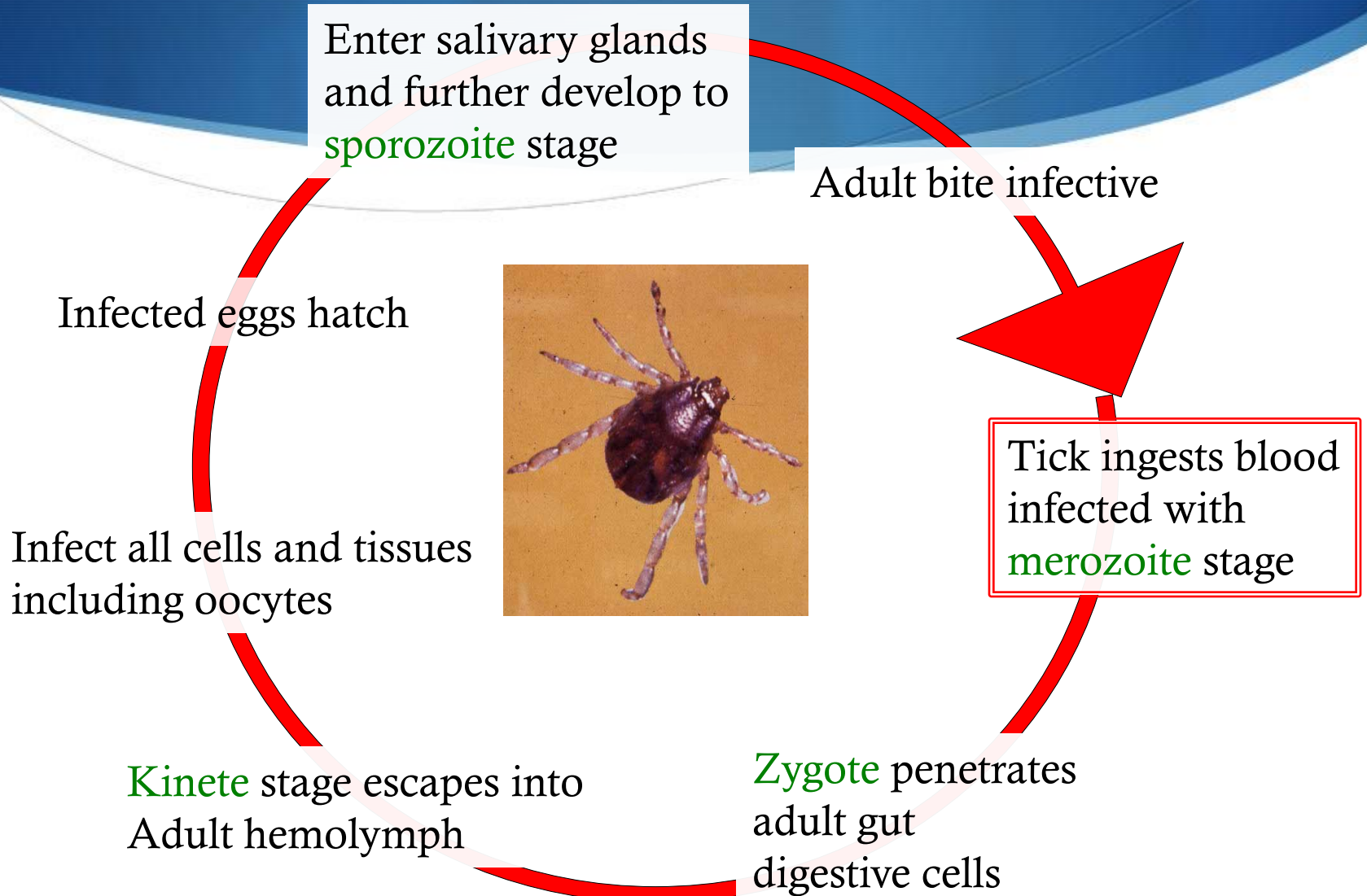


# Historical Background of the Texas Cattle Fever Problem

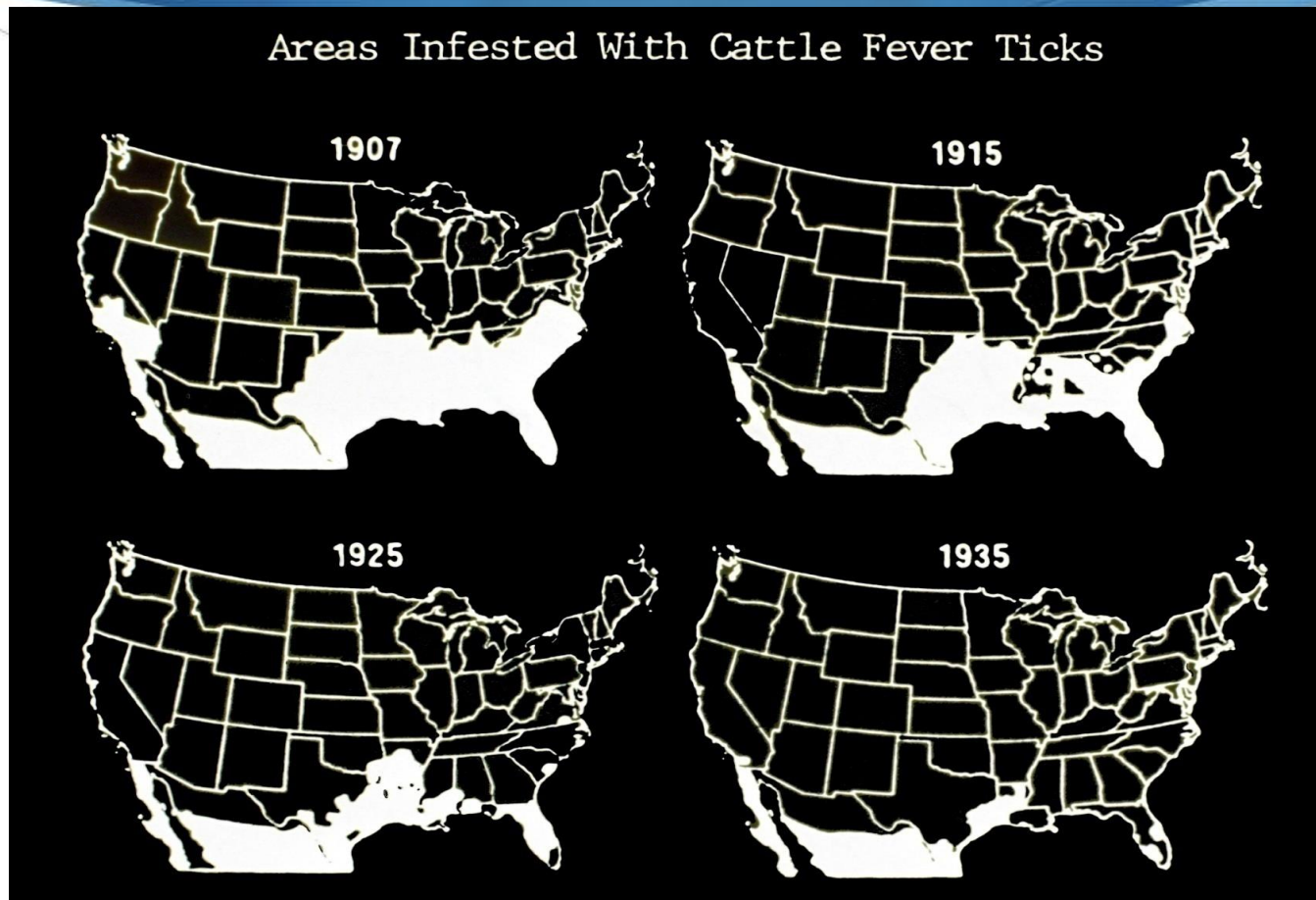
- Cattle tick was endemic in US
- Problems for cattle industry
- 1795 & 1796: Severe outbreaks of disease in cattle following a shipment of cattle northward from the south
- 1850 – Texas cattle began to be driven through AK, MO, KS & western states: **50-90 % death** in cattle
- 1868: disasters in IL & IN left **15,000 cattle dead**



# Cattle Tick-*Babesia bovis* Interaction



# CFT Officially Declared Eradicated from USA in 1943

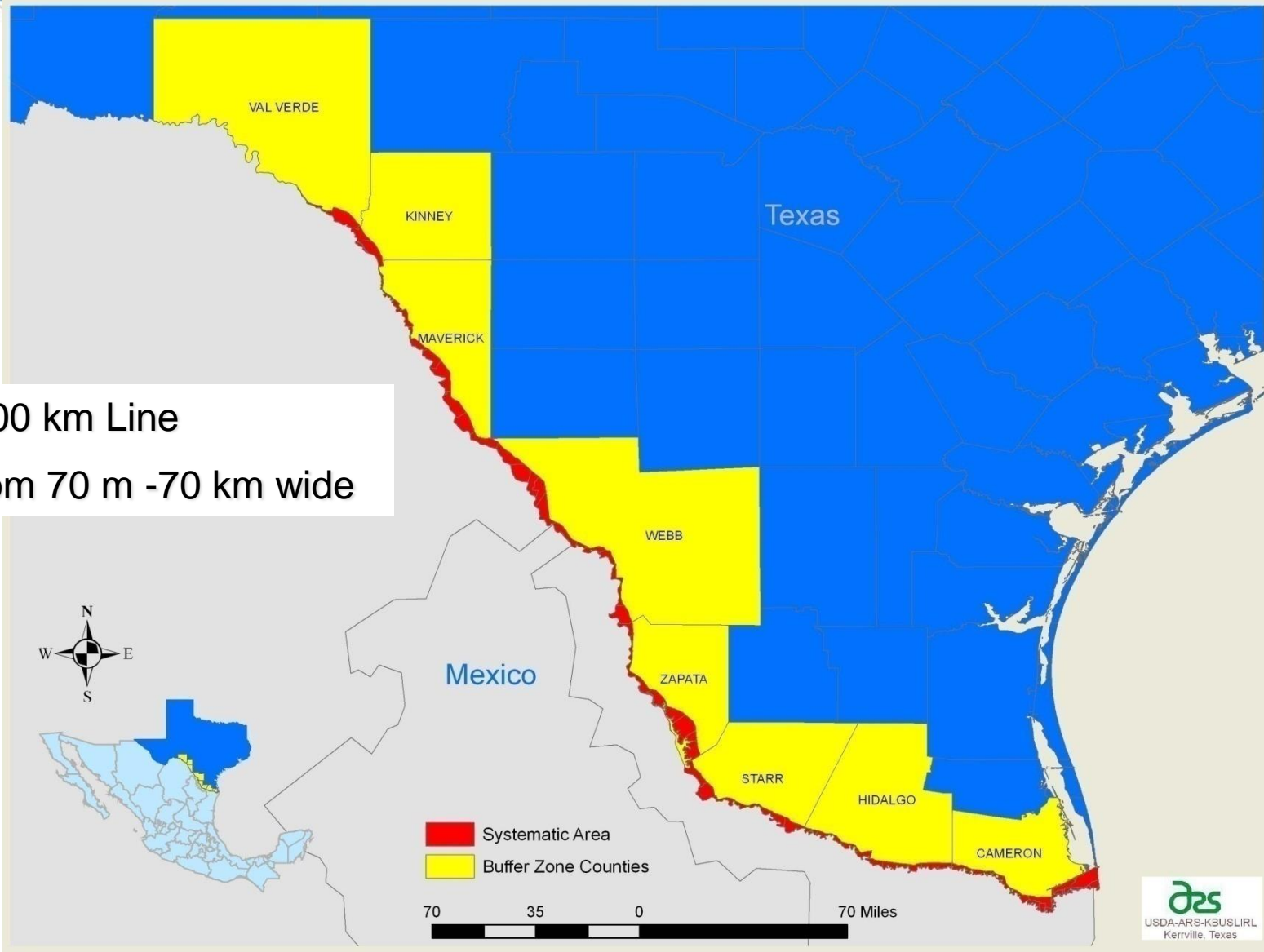


# Tick Eradication Saved \$\$\$



\$3 billion estimated  
annual savings in  
today's dollars realized  
by livestock industry

# CFTEP Permanent Quarantine Area



- 1200 km Line
- From 70 m -70 km wide

# Ticks are Common in Mexico

## U.S.-Mexico Border

- ✦ Import >1,000,000 head annually
- ✦ Mandatory dipping in OP
- ✦ OP resistant ticks in Mexico



**With pesticide resistance and increased infestations within and outside of the quarantine zone.....**

**Is reinfestation of the US inevitable?**





# **Genome-Based Approach to Discover New Control Technology**



# Cattle Tick Genome Project 2004-2016

- ✧ Based on our belief that the **genome holds the key to development of new control technologies!**
- ✧ Critical factors drove us to a **2-phase approach**
  - ✧ Huge genome: 2.4 X larger than human genome
  - ✧ Difficult genome: due to >70% repetitive DNA content
- ✧ **Phase 1** focused on sequencing **gene-rich regions**
- ✧ **Phase 2** focused on sequencing **complex genome regions**

# Tick Genome Fractions and 2004 Technology



↗ **Highly repetitive:** Sequencing technology not available

→ **Moderately repetitive:** Available technology too expensive

↘ **Unique:** → Appropriate technology existed  
Isolate and sequence

↓  
Assembly into database

# Phase Ia Approach: 2004-2009

- ✧ Collaboration with TIGR (2004-7)
  - ✧ 50,000 Sanger ESTs sequenced and assembled 13,800 gene coding regions into a Gene Index
- ✧ Collaboration with Murdoch Univ. initiated **CattleTickBase**
  - ✧ <http://cattletickbase.ccgapps.com.au>

The screenshot shows the CattleTickBase website for *Rhipicephalus microplus*. The header includes the Centre for Comparative Genomics logo and Murdoch University logo. The main navigation area features several buttons: 'View Analyses' (with a list of options like 'Browse Genome Annotations GBrowse'), 'Analysis Tools' (with options like 'Create your own bioinformatics pipelines'), 'Download Data' (with a link to 'cattle\_tick\_data'), and 'Publications' (with a link to 'tick\_publications'). On the right, there are informational sections: 'Introduction to CCG Rhipicephalus microplus Bioinformatics' (describing the collaborative project), 'Research Aims' (integration of novel vector sequences), and 'Project site contains' (listing data types like EST, BAC, and Cot DNA sequences).

CENTRE FOR COMPARATIVE GENOMICS  
Western Australia

Murdoch UNIVERSITY

**CattleTickBase - *Rhipicephalus microplus***

Queensland Government Agricultural Research Service

**View Analyses**  
Browse Genome Annotations GBrowse  
Select a BAC sequence to view in GBrowse  
Select a BmiGI Cot extended transcript to view in GBrowse  
View gene annotations for larval transcript library  
View gene annotations for tick gut transcript library  
Go back to organism portal

**Analysis Tools**  
Create your own bioinformatics pipelines to analyse data - YABI  
Browse Genome sequence and add your own tracks - GBrowse  
Blast your data against our data sets

**Download Data**  
• [Link to download cattle\\_tick\\_data](#)

**Publications**  
• [Live feed search for cattle tick publications](#)

**Introduction to CCG Rhipicephalus microplus Bioinformatics**  
A collaborative project between the State of Queensland (Department of Employment Economic Development and Innovation), Murdoch University's Centre for Comparative Genomics, and the United States Department of Agriculture Agricultural Research Service has generated a large amount of novel data and led to the creation of this online *R. microplus* genome resource. *Rhipicephalus microplus*. The cattle tick (formerly known as *Boophilus microplus*) is the most significant ectoparasite of cattle worldwide causing production losses and hide damage. Ticks also rapidly develop resistance to the chemicals (acaricides) used to treat cattle during heavy tick burden. Ticks are also vectors for highly pathogenic organisms including protozoan parasites *Babesia bovis* and *B. bigemina* causing bovine babesiosis and rickettsia *Anaplasma marginale* causing bovine anaplasmosis.

**Research Aims**  
Integration of novel vector sequences and analyses to identify the genomic mechanisms to effectively control vector *R. microplus* livestock infestations.

**Project site contains**  
This site contains access to data, analysis and to tools specific to the analysis of *Rhipicephalus microplus*. Data types include EST, BAC, and Cot DNA sequences, microarray data and miRNA studies direct link table can be found under "Download Data". Access to tools specific to the analysis of *Rhipicephalus microplus*, this includes interactive Bioinformatics pipeline analysis construction (YABI). Get started using our online tutorial (link) and online help. Or go direct to View Analysis tools links.

© 2010 Centre for Comparative Genomics, Murdoch University

# Phase Ib Approach: 2009-2012

- ✧ Sequence "unique" **gene-rich regions** of genomic DNA through a reassociation kinetics-based approach
- ✧ Collaboration with Mississippi State University and Molecular Research LP.

# Phase 1 Product: Gene-rich Dataset



- ✧ **CattleTickBase:** Website hosts the dataset of 28,900 cattle tick gene-coding regions
- ✧ **<http://cattletickbase.ccgapps.com.au>**
  - ✧ Reassociation kinetics-based approach for partial genome sequencing of the cattle tick, *Rhipicephalus (Boophilus) microplus*. FD Guerrero, P Moolhuijzen, et al., BMC Genomics 11: 374, 2010.
  - ✧ CattleTickBase: An integrated Internet-based bioinformatics resource for *Rhipicephalus (Boophilus) microplus*. MI Bellgard, PM Moolhuijzen, et al. Intl J Parasitol 42: 161-169, 2012.

# Phase 2: The Difficult Genome Region 2012-13

- ✧ Collaboration with **National Center for Genome Resources** in Santa Fe, New Mexico.
- ✧ Utilizing Next-Next Gen technology: **Pac Bio** in long-read mode
  - ✧ 177 Pac Bio SMRT cells
  - ✧ >200 million reads per SMRT cell
    - ✧ Version 3 cells
    - ✧ Pac Bio XL enzyme
    - ✧ Avg. sequence read length ~5,000 bp

# Phase 2 Product: Draft Quality Genome Sequence

- ✧ ~12X genome coverage
  - ✧  $52 \times 10^9$  bp from Illumina/454 (**7X**)
  - ✧  $33 \times 10^9$  bp from Pac Bio (**5X**)
  - ✧  $2 \times 10^9$  bp from BAC library clone sequencing (**0.3X**)
- ✧ Assembled at Murdoch University

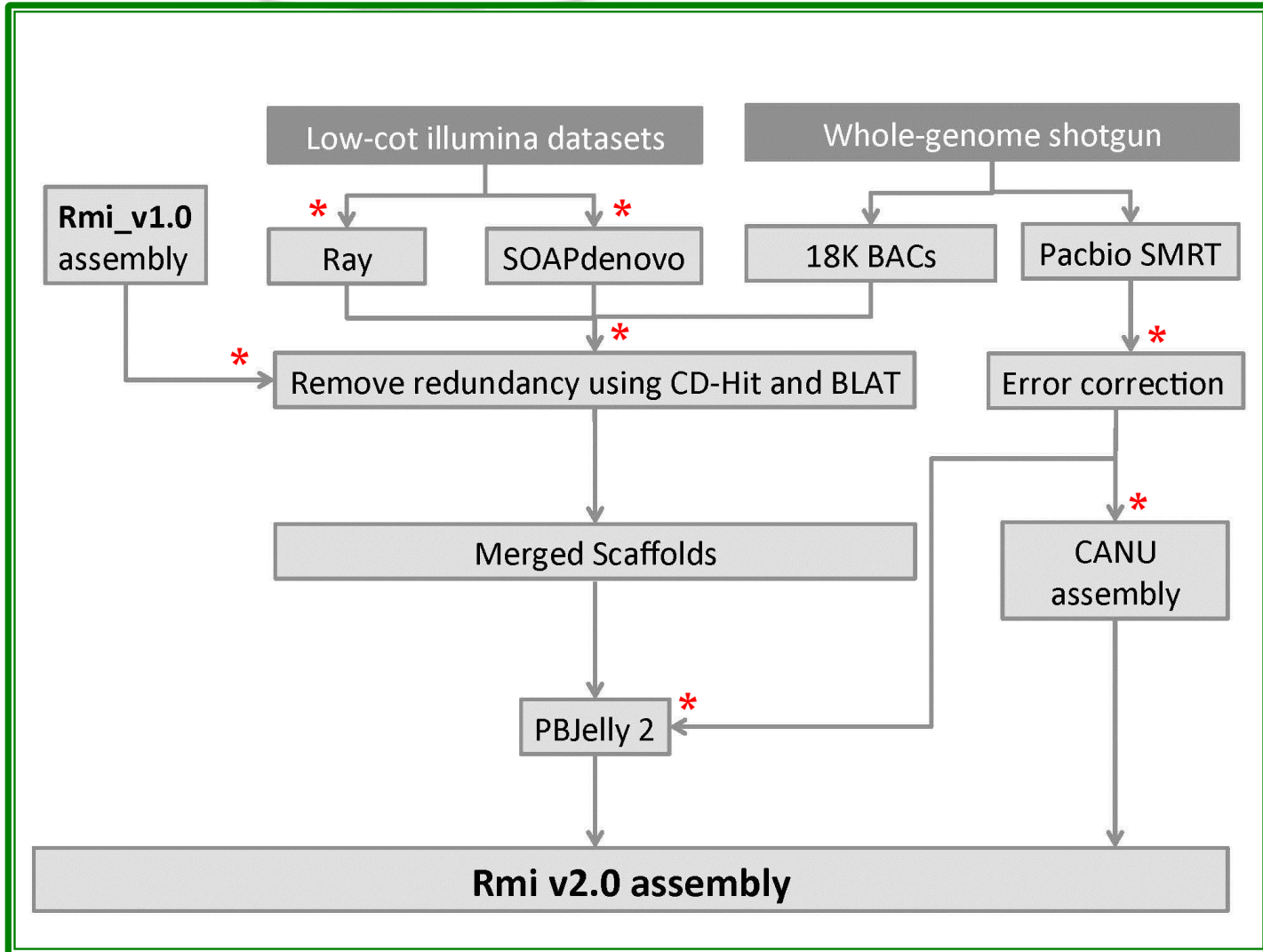
# The Size of the Problem

- **Statistics:**
  - 14,000,000 Pac Bio sequences
  - Average read insert length = 2,371
  - Maximum read insert length = 26,364
  - Total number of tick genome basepairs =  $33 \times 10^9$  (5X coverage)
- **Must error correct PacBio reads before assembly.**
- **Such a large PacBio read set covering an entire complex eukaryotic genome had never been reported as assembled.**

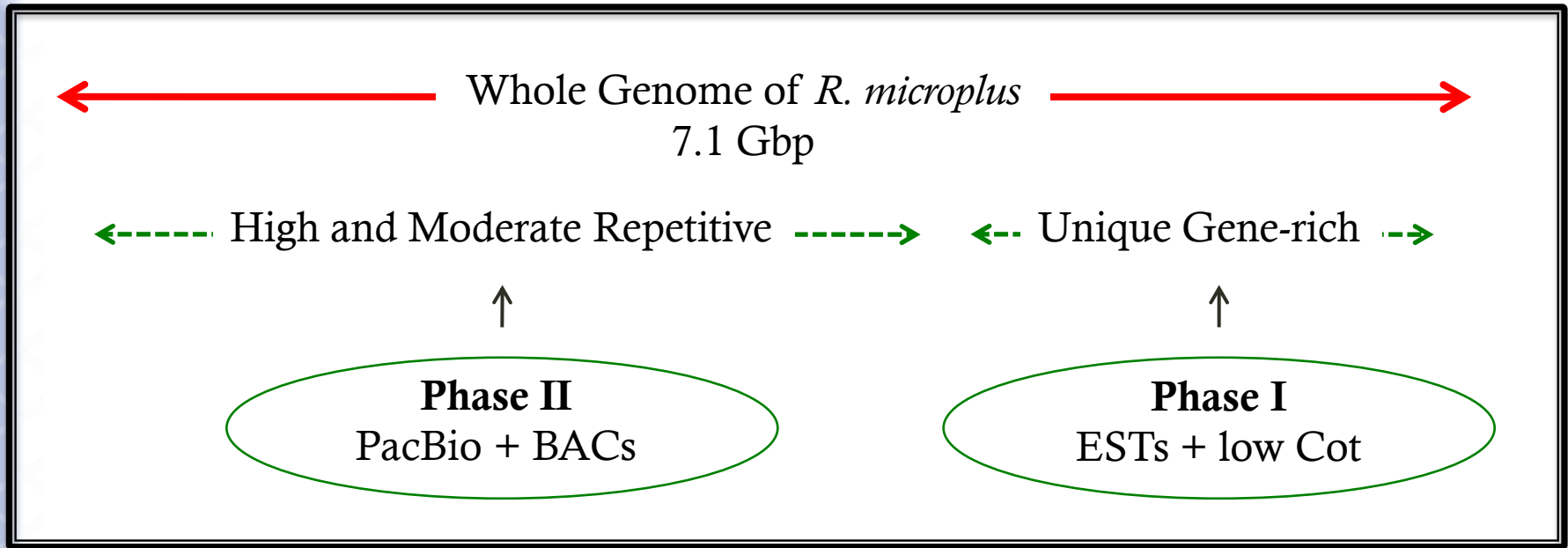




# Assembly Overview



# 12X-Coverage in CattleTickBase



*By comparison >>>*

Human Genome  
3.2 Gbp

# Route to Anti-Cattle Tick Vaccine

- ✧ **Genome studies** identified tick genes and sequence
- ✧ **Bioinformatics** analysis identified gene function
- ✧ **For vaccine development, we focus on tick genes/proteins with critical function**
  - ✧ Development
  - ✧ Feeding
  - ✧ Pathogen transmission

# Vaccine Antigen Candidates

## Selection Criteria

### **Selected from tissue-specific gene datasets**

- ❖ Verified expression in gut or ovary membrane.
- ❖ Low sequence similarity to mammalian proteins.
- ❖ Exclude members of large gene families.
  - ❖ Prefer single or low copy genes to avoid functional redundancy among gene family members.
- ❖ Critical function to the tick.

# Datasets Available to Mine for Vaccine Antigens

- ✧ Various treated larvae
- ✧ Babesia-infected vs. uninfected
  - ✧ Engorged female ovary
  - ✧ Engorged female gut
  - ✧ Larvae
- ✧ Synganglia
- ✧ Haller's Organ
- ✧ 0, 3 hour fed female adults
- ✧ 3 hour host-exposed unfed female adults

# Genome Project-Selected Vaccine Candidates

Name	Basis for Selection	Status
Antigen 1 (Aquaporin)	<i>In silico</i> structure and function	In cattle trials
Antigen 2	Midgut/saliva protein Upregulated w/Babesia inf.	In cattle trials
Antigen 4	Midgut upregulated w/Babesia inf. + outperformed Bm86 during <i>in vitro</i> tick feeding	In cattle trials
Antigen 5	Upregulated in adult gut, receptor binding function	Lab scale up
Antigen 6	Adult gut protein upregulated after Babesia infection	Lab scale up
Antigen 7	Adult ovary protein upregulated after Babesia infection	Lab scale up
Antigen 8	Protein upregulated in ovary and salivary glands after Babesia infection	Lab scale up

# Vaccine antigen production

- ✧ Protein coding region cloned into DNA expression plasmid
  - ✧ Histidine tag on C-terminus to aid in purification
  - ✧ *myc* epitope recognition sequence at C-terminus to aid in protein detection
  - ✧ Methanol-inducible gene promoter incorporated into plasmid
- ✧ Production vector is a *Pichia pastoris* commercial strain amenable to methanol-inducible growth using standard media

# Antigen Production Process Timeline

Example of Rm86Texas

From Gene Discovery



Antigen

**9 months**

Identified Bm86 in database

May 2011



Prepared sequence for transfer into yeast

July 2011



Yeast expressing Bm86

September 2011



Growth and purification optimized

December 2011



First doses shipped

February 2012

Production capacity:

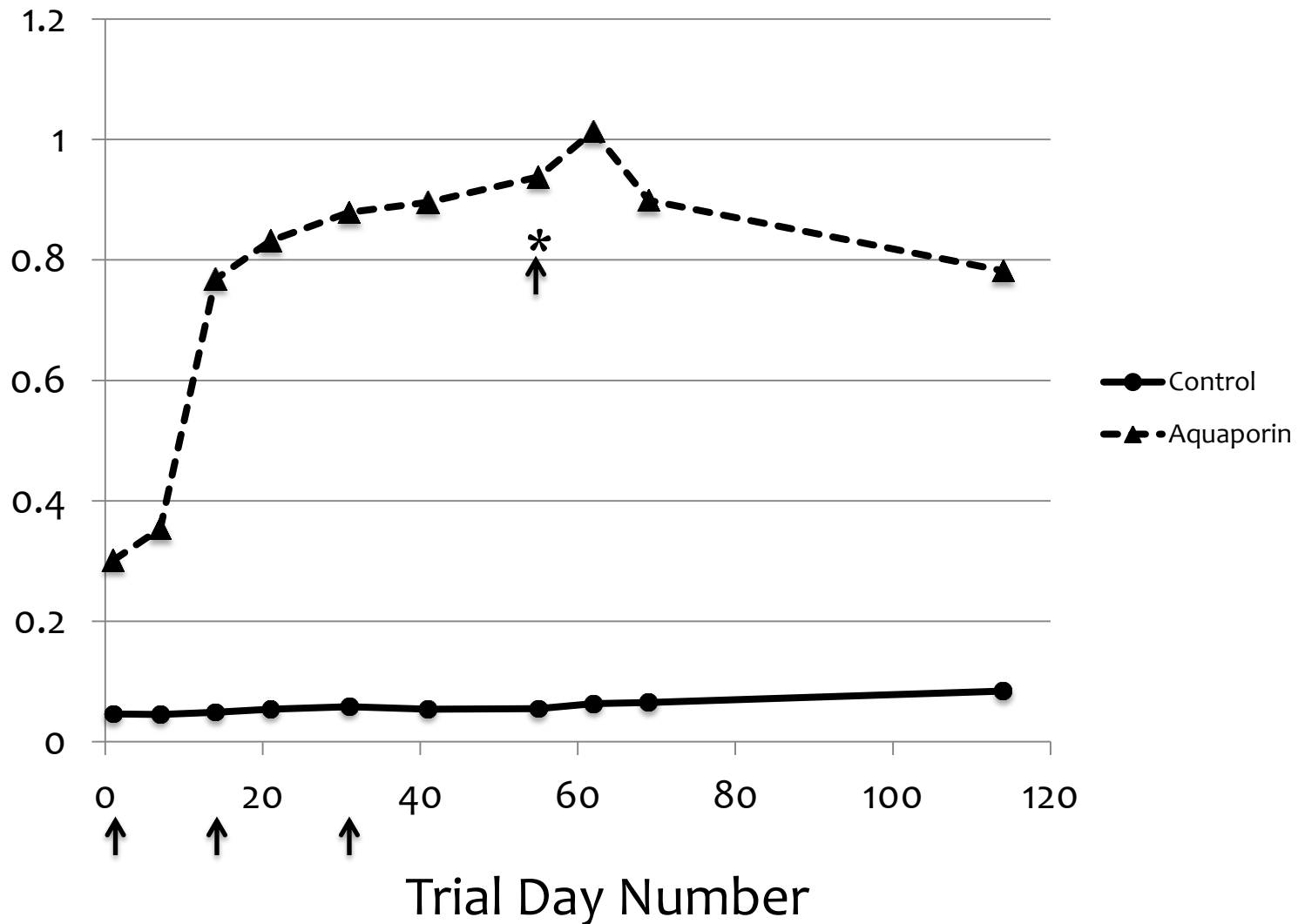
3 week process to produce 300 doses



# Antigen 1 Vaccine Trial Brazil EMBRAPA

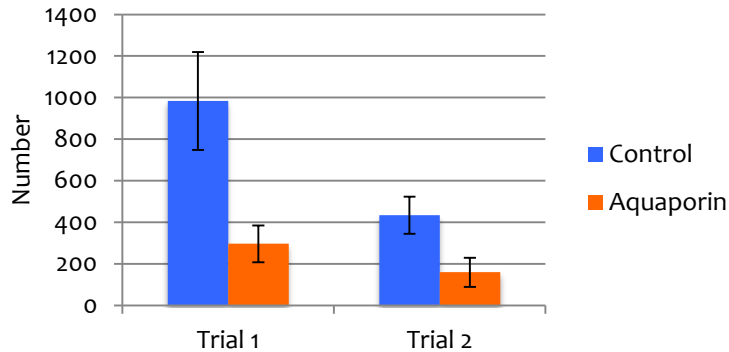
- ✧ Vaccine Antigen: *R. microplus* aquaporin
  - ✧ Water channel membrane protein
  - ✧ Highly expressed in tick neural tissues
  - ✧ Produced as recombinant protein in *Pichia pastoris*
- ✧ Vaccine Adjuvant: Montanide 61

# ELISA results



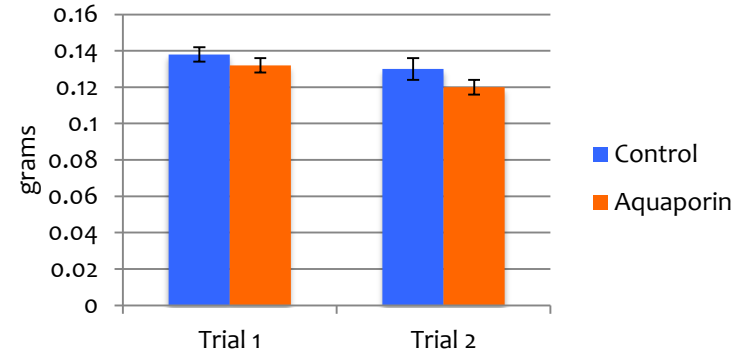
# Vaccine Efficacy Data

## Avg. No. Ticks/Animal



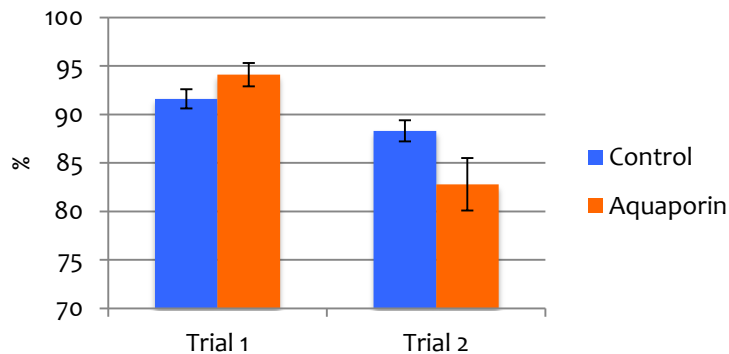
Mean = 31% of control

## Avg. Egg Mass Wt./Female



Mean = 94% of control

## Avg. % Egg Hatch



Mean = 98% of control

Overall Efficacy = 72% tick control

# Followup and Technology Transfer

- Patented Antigens 1 (aquaporin) and 2
- CRADA for cattle pen trial in Texas
  - Product development with commercial partner
- Evaluation of Antigens 5-8 in Brazil cattle trials
- Evaluating efficacy against ticks on dogs

## 2011 Vaccine Evaluations in Brazil

Name	Basis for Selection	% Efficacy
Antigen 1	<i>In silico</i> structure and function	76%
Antigen 2	Midgut/saliva protein Upregulated w/Babesia inf.	63%
Antigen 4	Midgut upregulated w/Babesia inf. + <i>In vitro</i> vs Bm86	70%
6-peptide cocktail	Epitopes from 6 candidates	86%

In comparison, Bm86, the antigen in the only commercially available tick vaccine had 39% efficacy

# Recent Vaccine Candidate Evaluations

- ✧ **ARS Cattle Fever Tick Research Lab, Moore Field, TX**
  - ✧ Ag 1 and Ag 2 DNA vaccine trials in cattle (2015)
- ✧ **Louisiana State University AgCenter**
  - ✧ Ag 1 and Rm86Texas vaccine trial in white tailed deer (2012-3)
- ✧ **ARS-Brazil partnerships**
  - ✧ CRADA partner to evaluate Ag 5-8 in Parana state (in progress)

# Evaluations (Cont.)

- ❖ **Animal health industry partner**
  - ❖ Pen and pasture trials planned for USA and Brazil (2017)
- ❖ **Single peptides cattle trials in Australia (2014)**
- ❖ **Two patent applications w/USPO**
  - ❖ Ag 1 and Ag 2 for use in cattle (filed 2012, granted 2014 and 2016)
  - ❖ Ag 1 for use in companion animals (filed 2013, granted 2016)
- ❖ **Patent applications w/Brazil, Mexico, and Australia**

# Reverse Vaccinology Approach to Vaccine

Vaccine development starts with  
bioinformatics genome sequence analysis



**Dr. Rino Rappuoli**  
Pioneer of  
Reverse Vaccinology

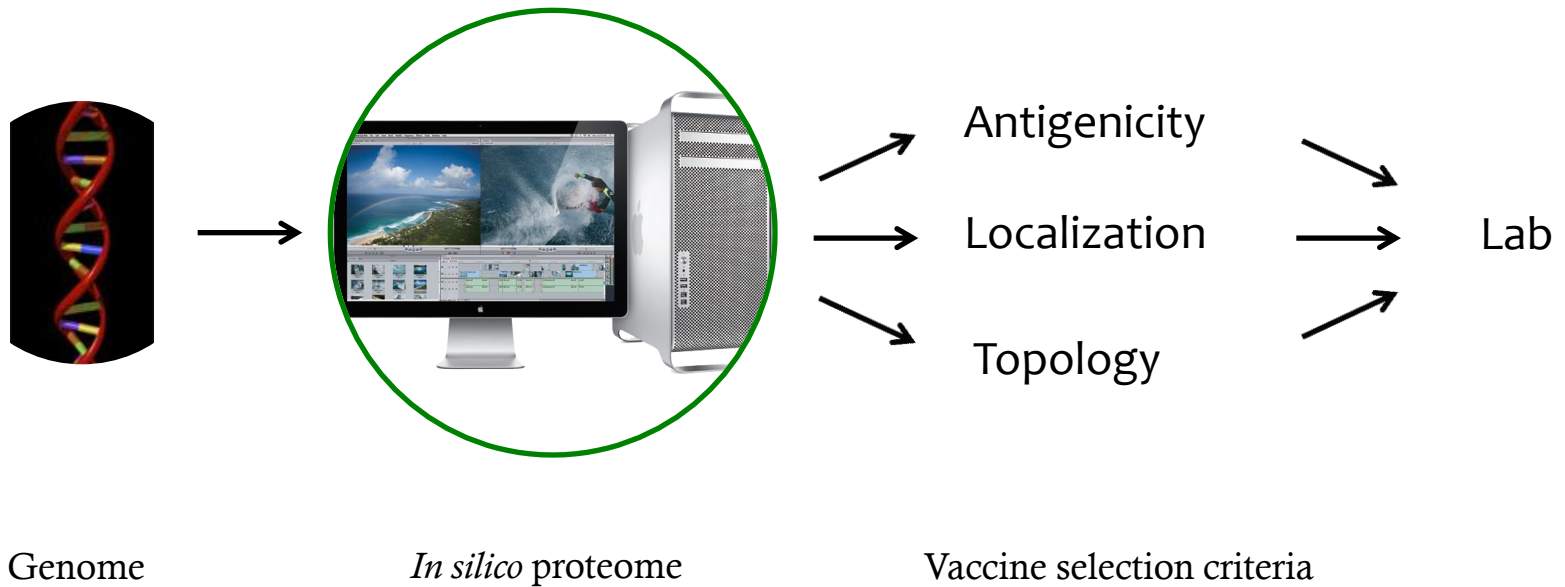
*Neisseria meningitidis* serogroup B  
→ meningococcal B (MenB) disease

Pizza M, *et al. Science.*  
2000 10;287(5459):1816-20.

Milestone:  
**Novartis's vaccine Bexsero**  
approved by Europe & USA



# Reverse Vaccinology Process



# Relevance to Brown Cattle Tick?

**Sequence genome and transcriptome**



**Predict and verify protein sequences**



**Bioinformatic pipeline (Vaxign, Vacceed) candidate predictions**



**Laboratory production of selected proteins**



**Vaccine efficacy trials in groups of 6 cattle**



**Government/Industry partnership**



**IP protection + Field tests**



# Control the ticks



# Acknowledgements

## **Consortium Leadership Team**

- + Felix D. Guerrero, USDA-ARS Kerrville, TX, USA
- + Matthew I. Bellgard, Murdoch University, Murdoch, WA, Australia
- + Robert J. Miller, USDA-ARS Edinburg, TX, USA

## **Collaborating Organizations**

- + U. S. Dept. of Agriculture, Agricultural Research Service
  - + Kerrville, TX, Edinburg, TX, and Beltsville, MD
- + Murdoch University, Murdoch, WA, Australia
- + National Center for Genome Resources, Santa Fe, NM, USA
- + Purdue University, West Lafayette, IN, USA
- + Mississippi State University, Starkville, MS, USA
- + J. Craig Venter Institute, Rockville, MD, USA
- + Queensland Alliance for Ag. and Food Innovation, Brisbane, QLD, Australia
- + Amplicon Express, Pullman, WA, USA
- + Molecular Research LP, Shallowater, TX, USA

# Acknowledgements

## **Individuals from each organization (apologies to any that might have been left off)**

### +U. S. Dept. of Agriculture, Agricultural Research Service

- + Felix D. Guerrero
- + Robert J. Miller
- + Adalberto Perez de Leon
- + Kylie G. Bendele
- + John E. George
- + Daniel Strickman

### +Murdoch University, Murdoch, WA, Australia

- + Matthew I Bellgard
- + Roberto Barrero
- + Paula Moolhuijzen
- + Adam Hunter
- + John McCook
- + Michael Black

### +National Center for Genome Resources, Santa Fe, NM, USA

- + **Ernie Retzel**
- + Callum Bell
- + Andrew Farmer
- + Jennifer Jacobi
- + Nico Devitt
- + Peter Ngam
- + Patricia Mena
- + Faye Schilkey

# Acknowledgements

- + Purdue University, West Lafayette, IN, USA
  - + Catherine Hill
- + Mississippi State University, Starkville, MS, USA
  - + Daniel G. Peterson
- + J. Craig Venter Institute/TIGR, Rockville, MD, USA
  - + Vish Nene
  - + Appolinaire Djikeng
  - + Shelby Bidwell
  - + Lis Caler
  - + Mathangi Thiagarajan
  - + Linda Hannick
  - + Vinita Joardar
- + Queensland Alliance for Ag. and Food Innovation, Brisbane, QLD, Australia
  - + Ala Lew-Tabor
  - + Manuel Rodriguez Valle
- + Amplicon Express, Pullman, WA, USA
  - + Robert Bogden
  - + Evan Hart
  - + Suresh Iyer
  - + Amy Mraz
  - + Bandie Harrison
  - + Travis Ruff
- + Molecular Research LP, Shallowater, TX, USA
  - + Scot E. Dowd

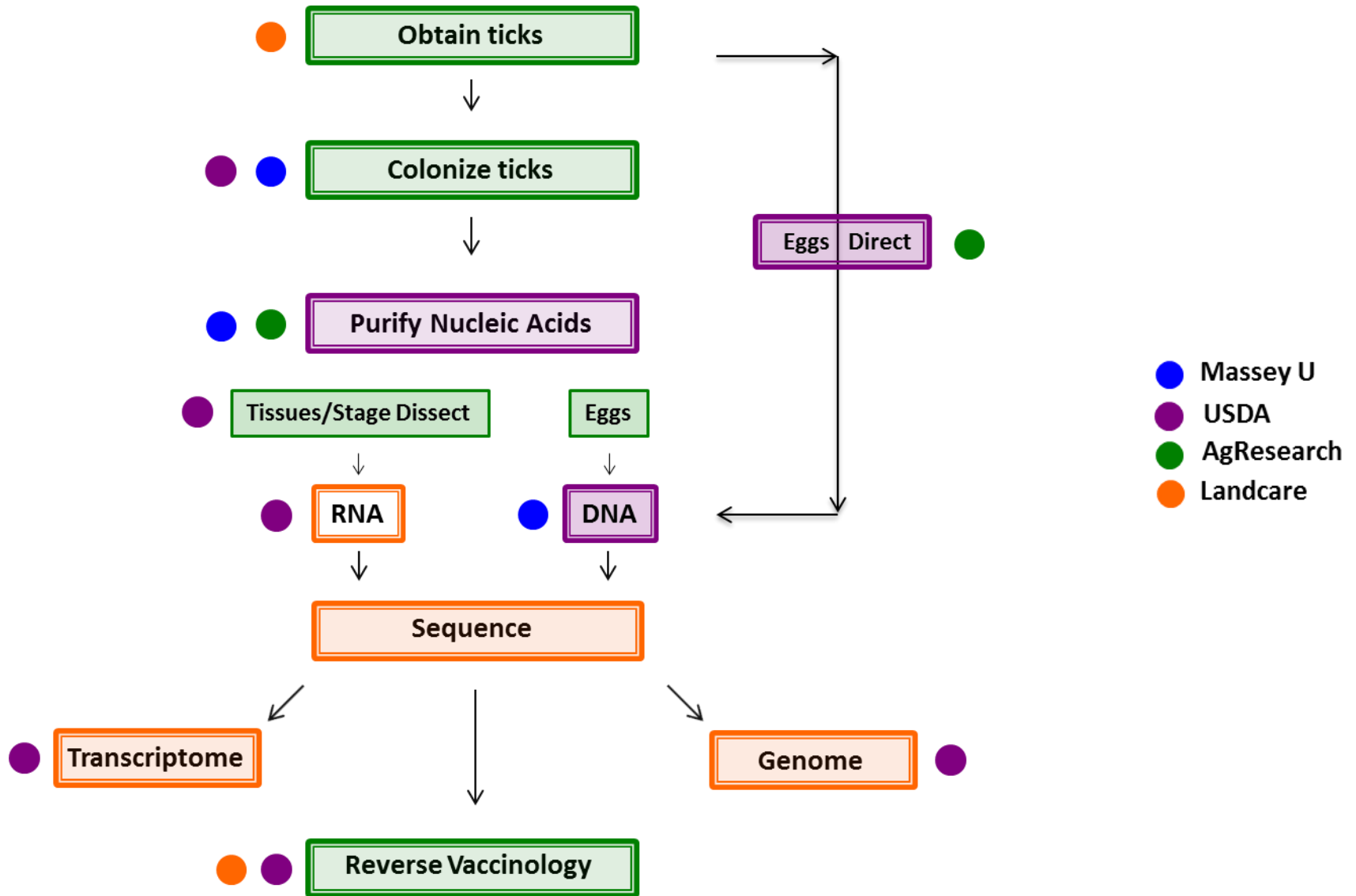
# Acknowledgements







# Ticks to DNA Database



# DNA to Vaccine

