# CANINE BABESIOSIS

## A Continued Threat to Canine Health

Adam Birkenheuer

DVM, PhD, DACVIM

Professor - North Carolina State University - College of Veterinary Medicine

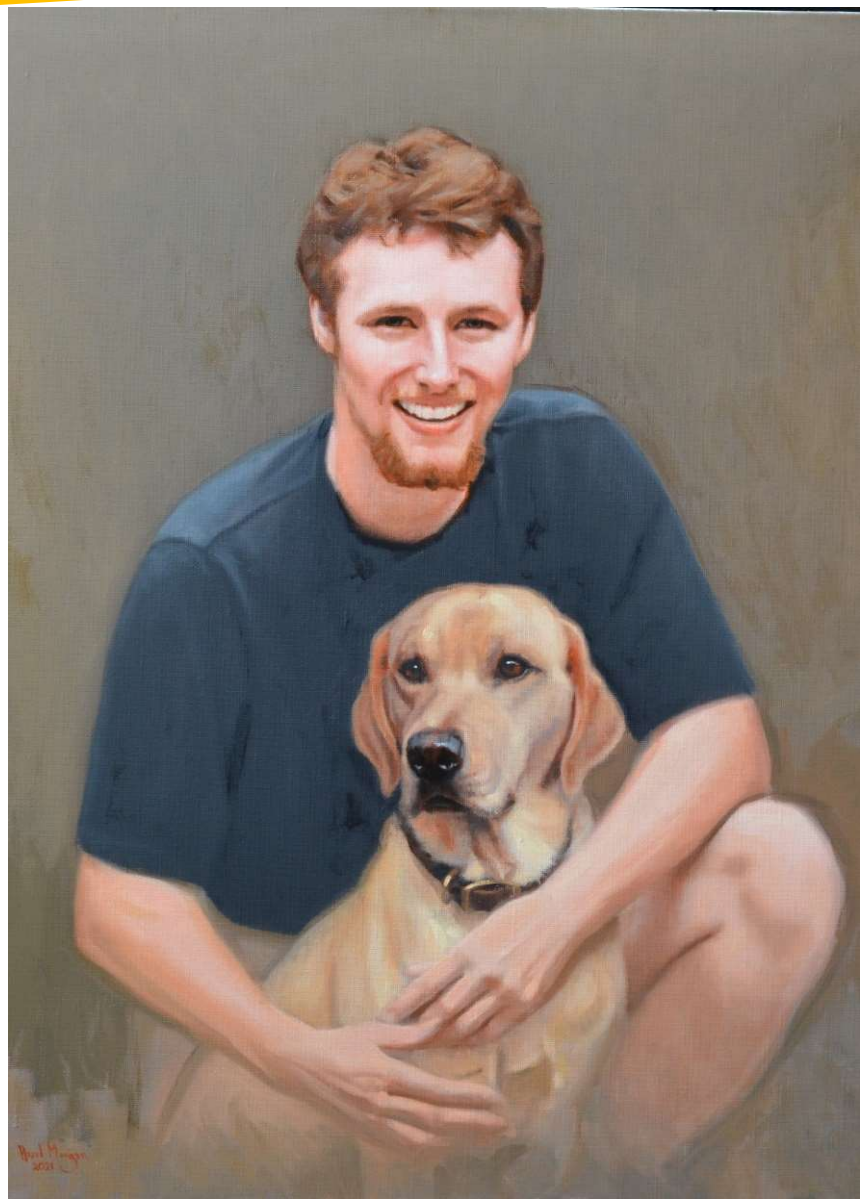
# Outline

Brief history of canine babesiosis…
Including some of my life history

Where are we now?

What's next?

- Andy Quattlebaum Distinguished Chair of Infectious Disease Research
- Companion Animal Research Endowment
- Purpose: **Advance the understanding of companion infectious diseases (etiology, diagnosis, treatment, etc.) for the benefit of dogs and cats.**
- https://cvm.ncsu.edu/alumni-and-donors/foundation/



# My Life

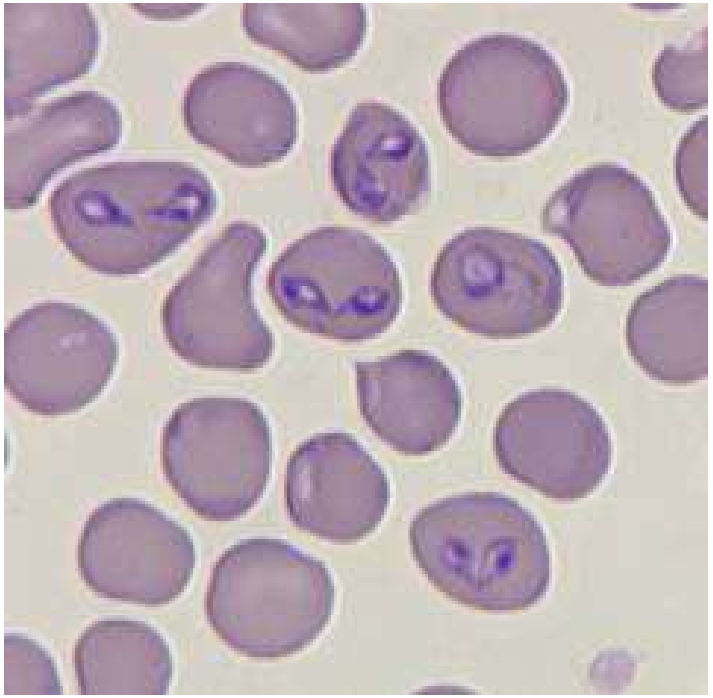
# My Life

# Lady Anne Barnard ca. 1779

In a letter she wrote from the Cape of South Africa:

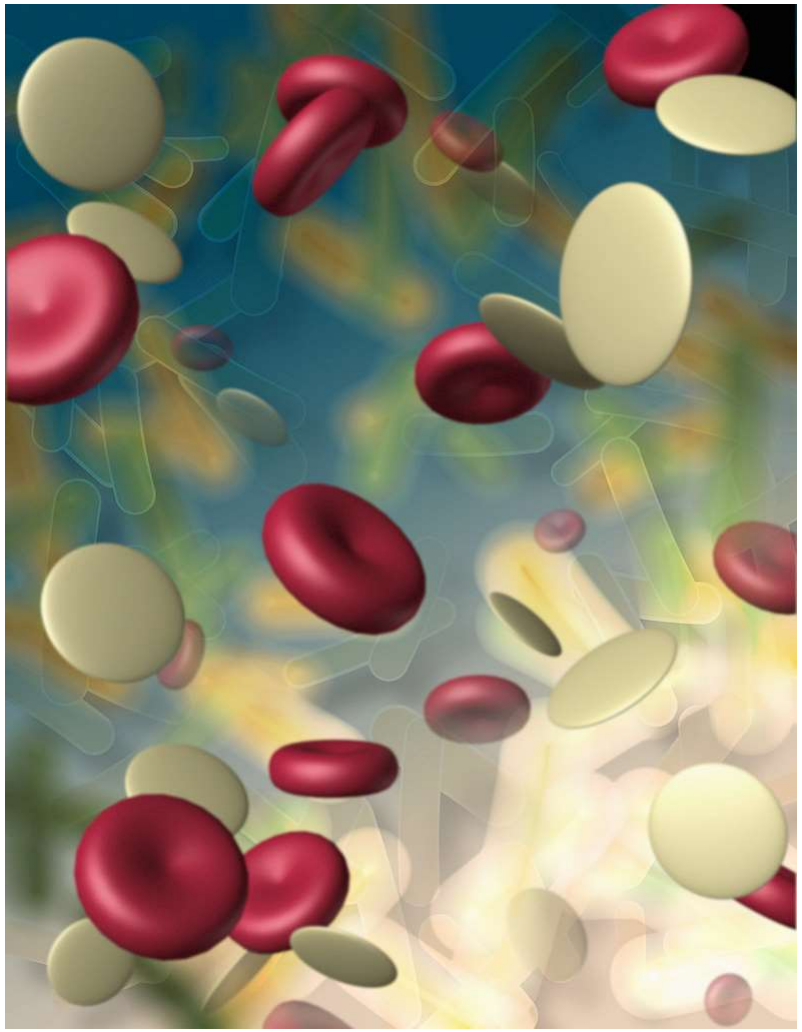
*"…we saw our only English dog who has survived the ailment which attacks all who arrive here…"*

# What I learned in veterinary school



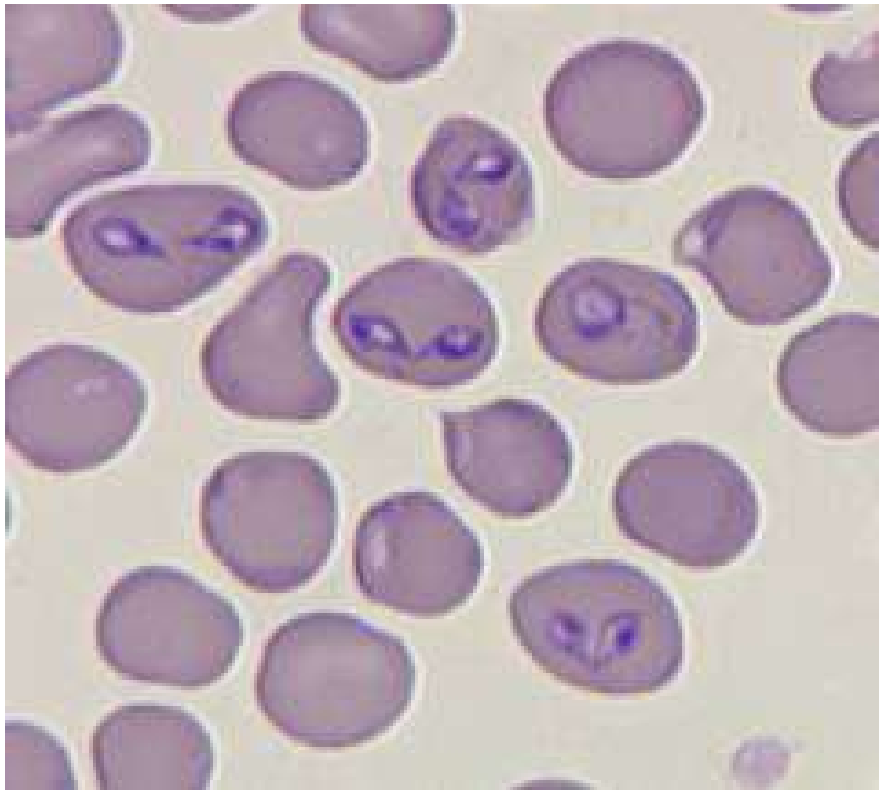
# Babesiosis: Typical Signs

- Anemia
  - Hemolytic
  - +/- Coombs' positive
  - Regenerative



# Babesiosis:

- Large canine *Babesia* = *Babesia canis*
- Small canine *Babesia* = *Babesia gibsoni*…Don’t worry we don’t have that here in the USA

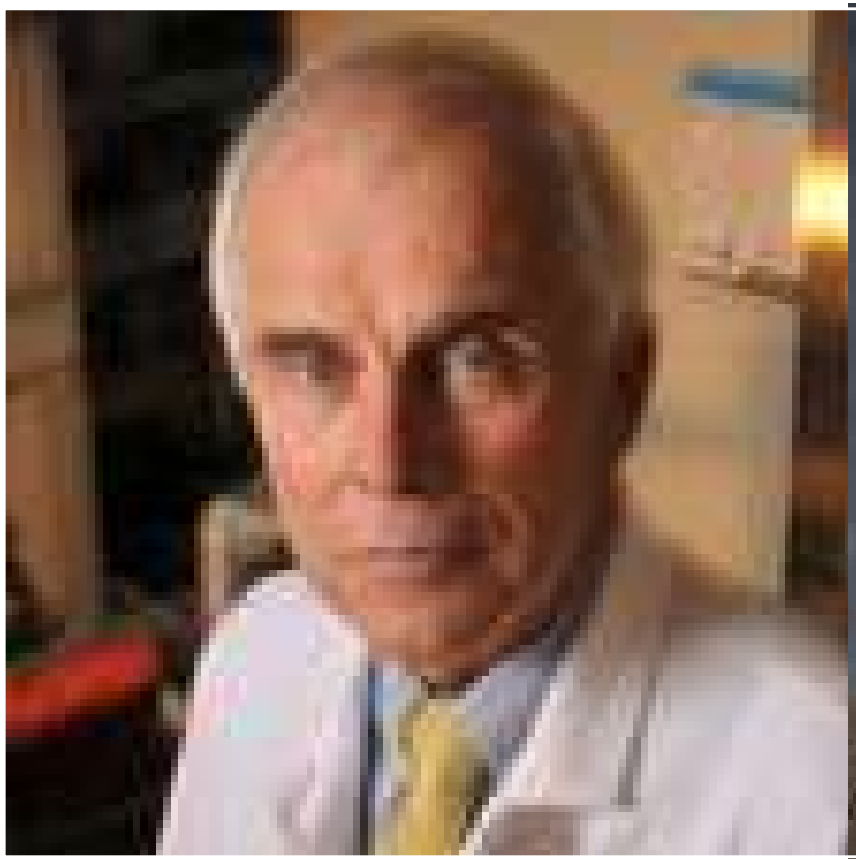


# July 1, 1995:
# My first day as a veterinarian: I'll take the Pit Bull….

# I barely knew what *Babesia* was!

- I just learned “we don’t have that here”
- …and I definitely HATED research!
- …but I had a lot of questions…
- What are we dealing with?
- How many animals are affected?
- How is it transmitted?
- How do we treat it?
- And so on

# It could have all ended right there..



# And it almost did…It took me years to publish my first paper…

## *Babesia gibsoni* Infections in Dogs From North Carolina

The recognition of canine babesiosis in North Carolina caused by *Babesia gibsoni* documents the expansion of the previously reported endemic area of this disease. Clinical signs ranged from severe hemolytic anemia and thrombocytopenia to subclinical infections. No infected dogs had traveled to endemic areas. Antibabesial treatment failed to eradicate the organism from infected dogs. **J Am Anim Hosp Asso[illegible] 1999; [illegible]5:125–8.**

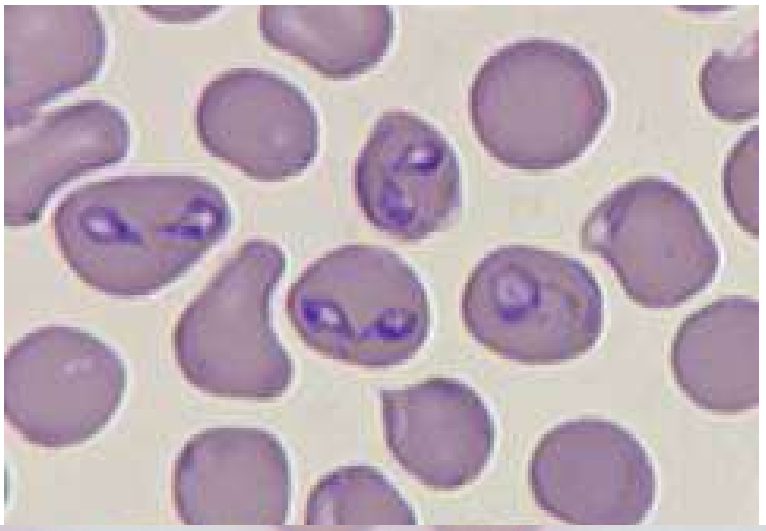
# Flashback…
# 1983

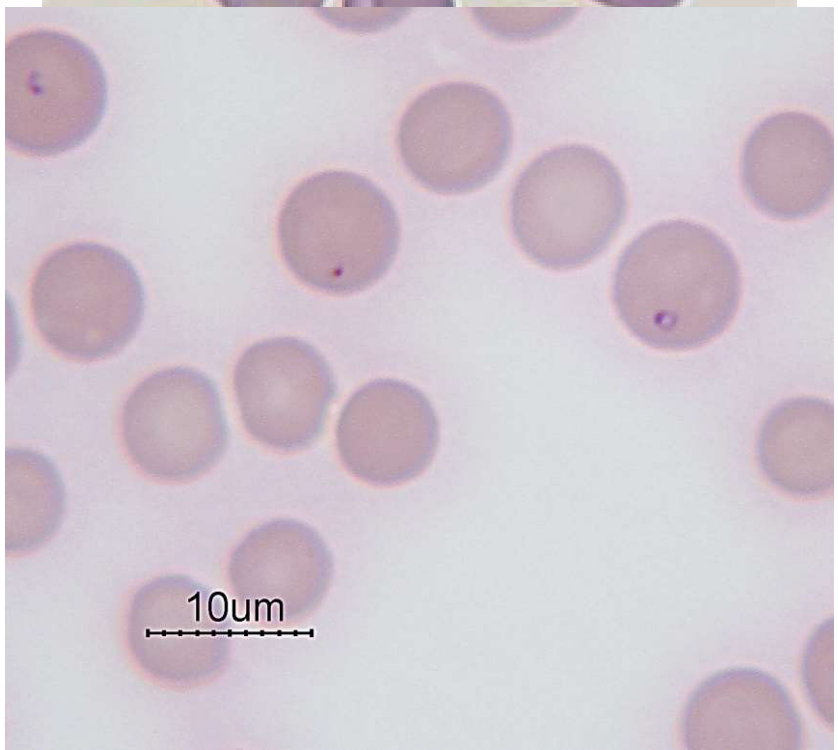
This guy changed everything…

# We Went from This…

# To this….

1. *B. gibsoni*
2. *B. vogeli*
3. *Babesia sp. Coco*
4. *B. conradae*
5. *B. canis*
6. *B. rossi*
7. *B. vulpes (T. annae)*
8. *B. negevi*
9. Novel sp. in England
10. *T. equi*
11. There will be more!
12. Good new! None of these have yet to be convincingly demonstrated to infect humans





# Back to the 90s

- We had found B. gibsoni…
- But the DNA sequence didn't match what was in Genbank

# We started to learn that genotype did not always follow phenotype

ELSEVIER

International Journal for Parasitology 30 (2000) 1501–1505

The Australian Society for Parasitology Inc.
INTERNATIONAL Journal for PARASITOLOGY
www.parasitology-online.com

## There are at least three genetically distinct small piroplasms from dogs☆

A.M. Kjemtrup[a], A.A. Kocan[b], L. Whitworth[b], J. Meinkoth[b], A.J. Birkenheuer[c], J. Cummings[d], M.K. Boudreaux[e], S.L. Stockham[f], A. Irizarry-Rovira[g], P.A. Conrad[a,*]

# Needed a better "mousetrap"

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2003, p. 4172–4177 Vol. 41, No. 9
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.9.4172–4177.2003


## Development and Evaluation of a Seminested PCR for Detection and Differentiation of *Babesia gibsoni* (Asian Genotype) and *B. canis* DNA in Canine Blood Samples

Adam J. Birkenheuer,[1] Michael G. Levy,[2] and Edward B. Breitschwerdt[1]*

*Departments of Clinical Sciences[1] and Food Animal and Health Resource Management,[2] College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606-1428*

- We made an important "decision" to develop "broad-range" diagnostics
- Is there ANY *Babesia* present?
- Then drill down to species level in secondary assays

# Helped us understand who was infected: APBT in Kennels

# Typical living conditions of the kennel dogs

# How is it being transmitted?

# Clue: Where does it come from?

NEWSWEEK MAGAZINE

# Dogfights in Japan Are a Family Outing

BY TAYLOR WOFFORD ON 09/01/16 AT 10:33 AM EDT

# IACUC: Wouldn’t approve dogfight at NCSU…

# Geographic distribution of babesiosis among dogs in the United States and association with dog bites: 150 cases (2000–2003)

Adam J. Birkenheuer, DVM, PhD, DACVIM; Maria T. Correa, PhD; Michael G. Levy, PhD; Edward B. Breitschwerdt, DVM, DACVIM

| | *Babesia gibsoni* | *Babesia canis vogeli* | Other piroplasm |
|---|---|---|---|
| **Total** | **131** | **11** | **3** |
| **APBT*** | **121** | **0** | **1** |
| **Greyhound** | **0** | **8** | **0** |
| **Other Breed** | **10** | **3** | **2** |

* APBT: American Pit Bull Terrier

# Now what?

- We know what it is…
- We know who gets it…
- We “know” how it’s transmitted…
  - Tick-bite
  - Dog fight
  - Blood-transfusion
  - Transplacental
- We have no idea how to treat it!
- No treatments at that time could clear the infection

# Search for better treatments

- IMHO most science is not truly novel

The New England Journal of Medicine

## ATOVAQUONE AND AZITHROMYCIN FOR THE TREATMENT OF BABESIOSIS

PETER J. KRAUSE, M.D., TIMOTHY LEPORE, M.D., VIJAY K. SIKAND, M.D., JOSEPH GADBAW, JR., M.D., GEORGINE BURKE, PH.D., SAM R. TELFORD III, SC.D., PETER BRASSARD, M.D., DIANE PEARL, M.D., JABER AZLANZADEH, PH.D., DIANE CHRISTIANSON, R.N., DEBRA MCGRATH, R.N., AND ANDREW SPIELMAN, SC.D.

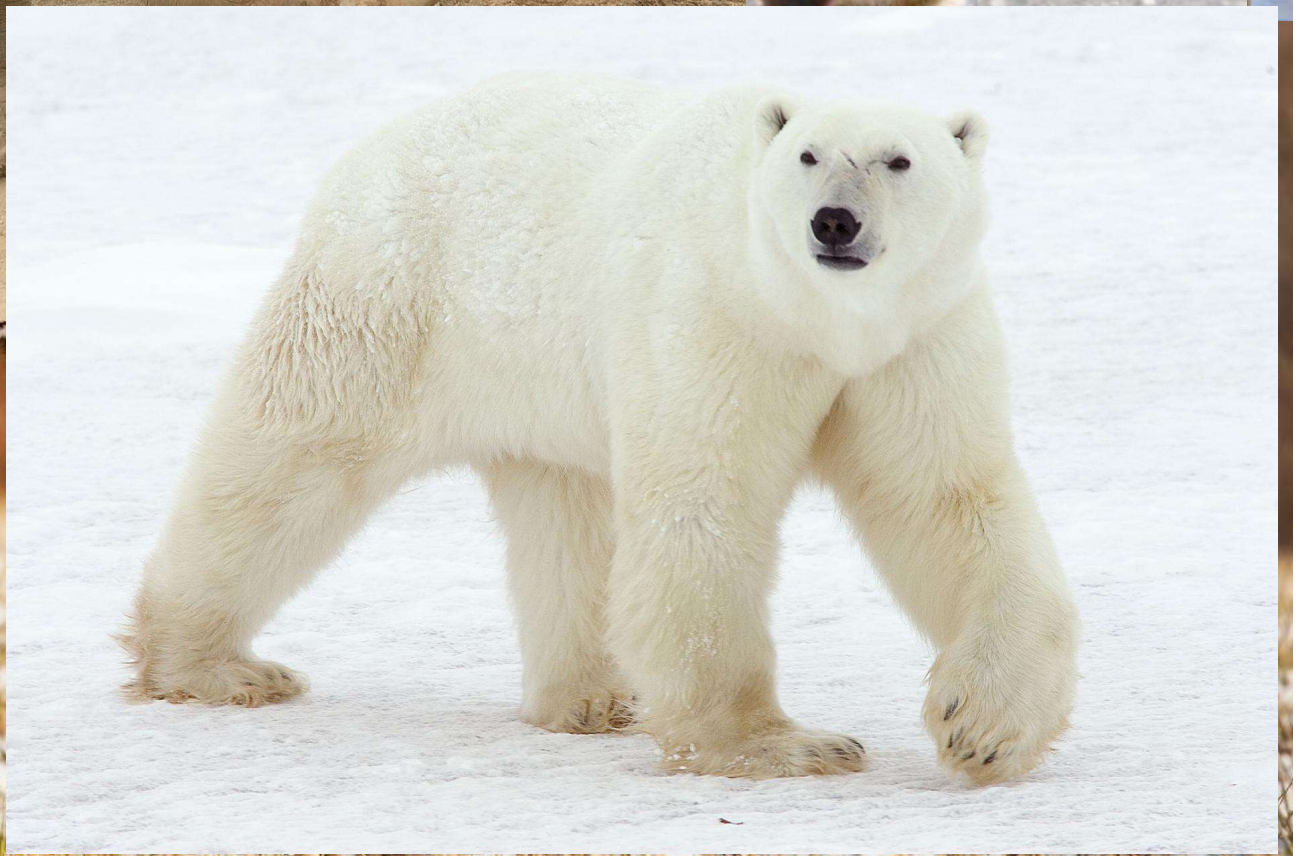
- Did a pilot study
- Then a randomized placebo-controlled trial
- About 80% effective at “clearing” *B. gibsoni*

*J Vet Intern Med* 2004;18:494–498

# Efficacy of Combined Atovaquone and Azithromycin for Therapy of Chronic *Babesia gibsoni* (Asian Genotype) Infections in Dogs

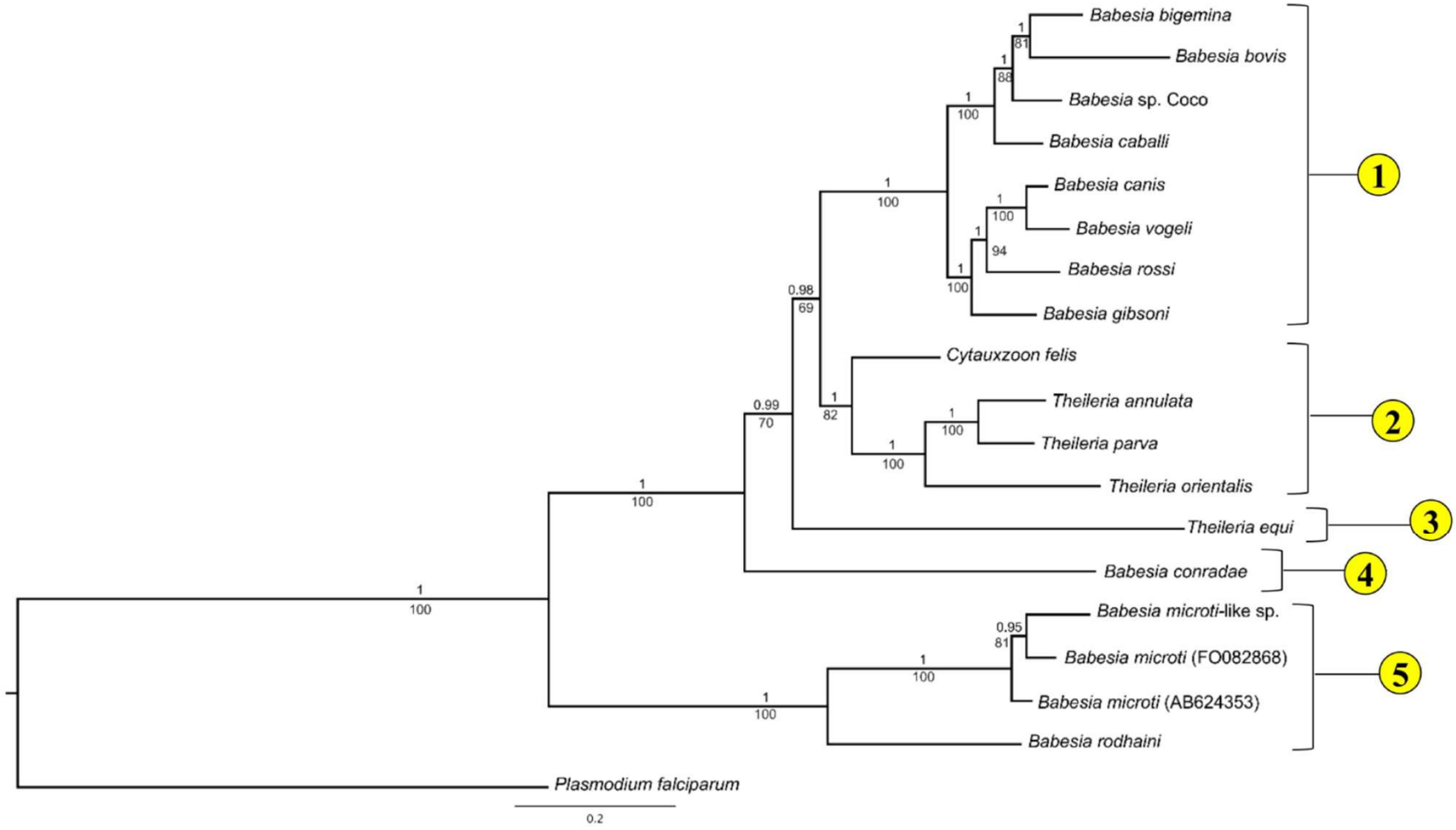
Adam J. Birkenheuer, Michael G. Levy, and Edward B. Breitschwerdt


WALLPAPERS
HDR



# We Started to Better Understand the “Who”

**A) Concatenated mitochondrial and 18S nucleotide sequence**



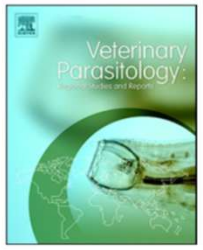
# Could we better understand the where?

Veterinary Parasitology: Regional Studies and Reports 22 (2020) 100471

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr




Original Article

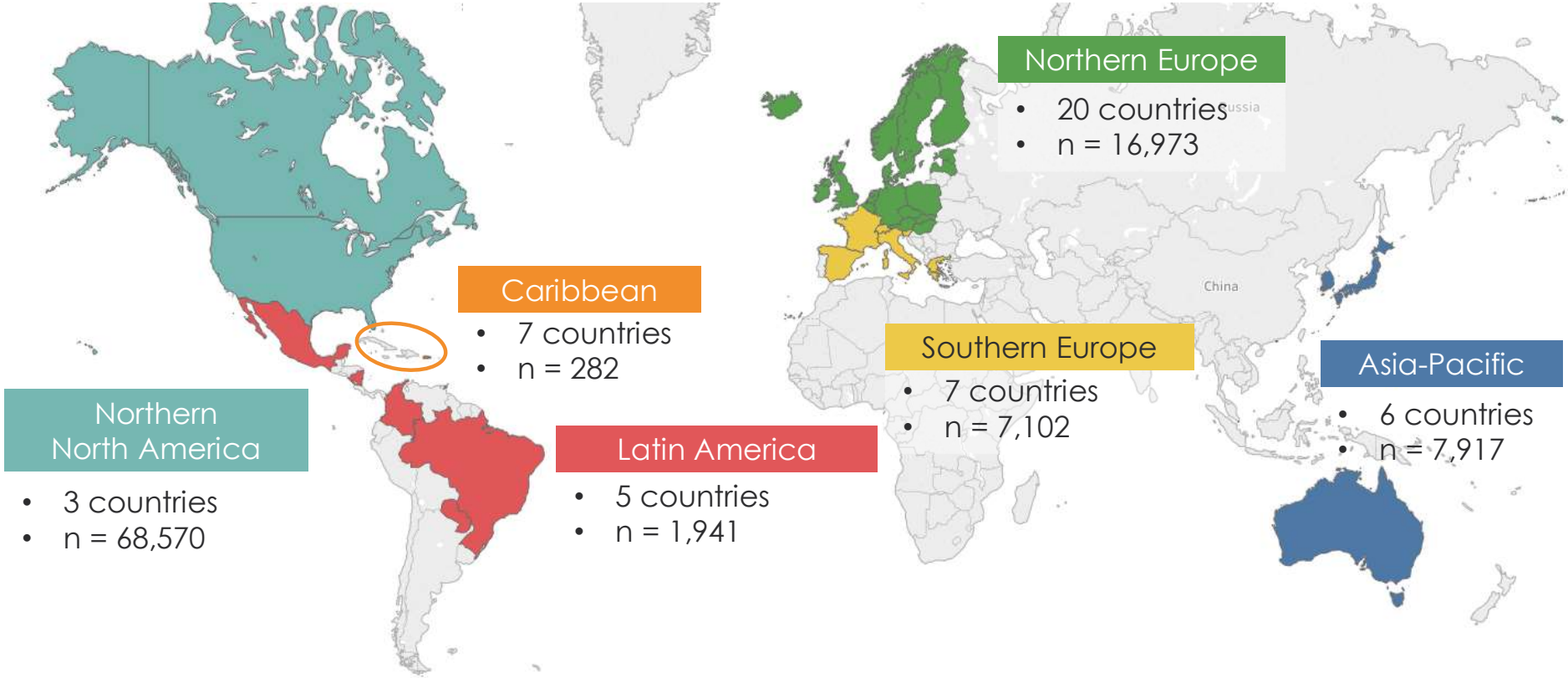
## Global distribution of canine *Babesia* species identified by a commercial diagnostic laboratory

Adam J. Birkenheuer[a,*], Jesse Buch[b], Melissa J. Beall[b], Jennifer Braff[b], Ramaswamy Chandrashekar[b]

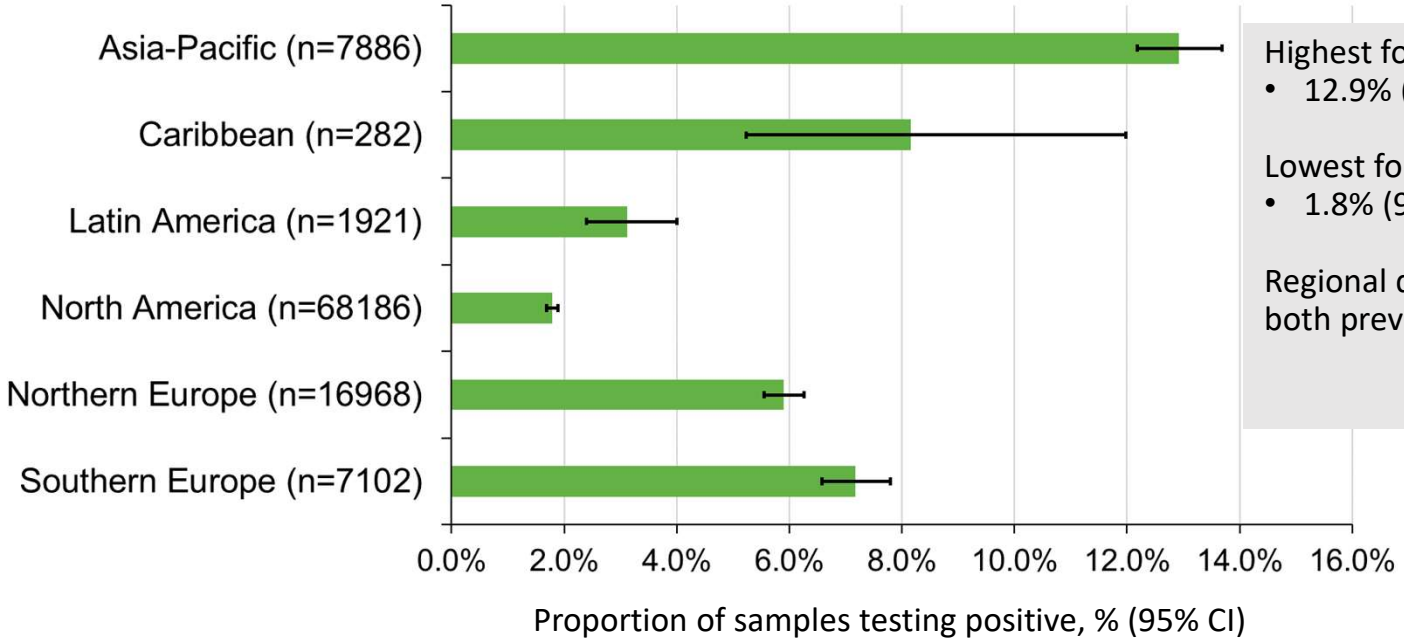
[a] North Carolina State University, College of Veterinary Medicine, 1061 William Moore Drive, Raleigh, NC 27607, USA
[b] IDEXX Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME, USA

# Geographic Description of the Data

102,345 results from 48 countries in 6 regions



# Proportion of *Babesia spp.* PCR Positives By Region



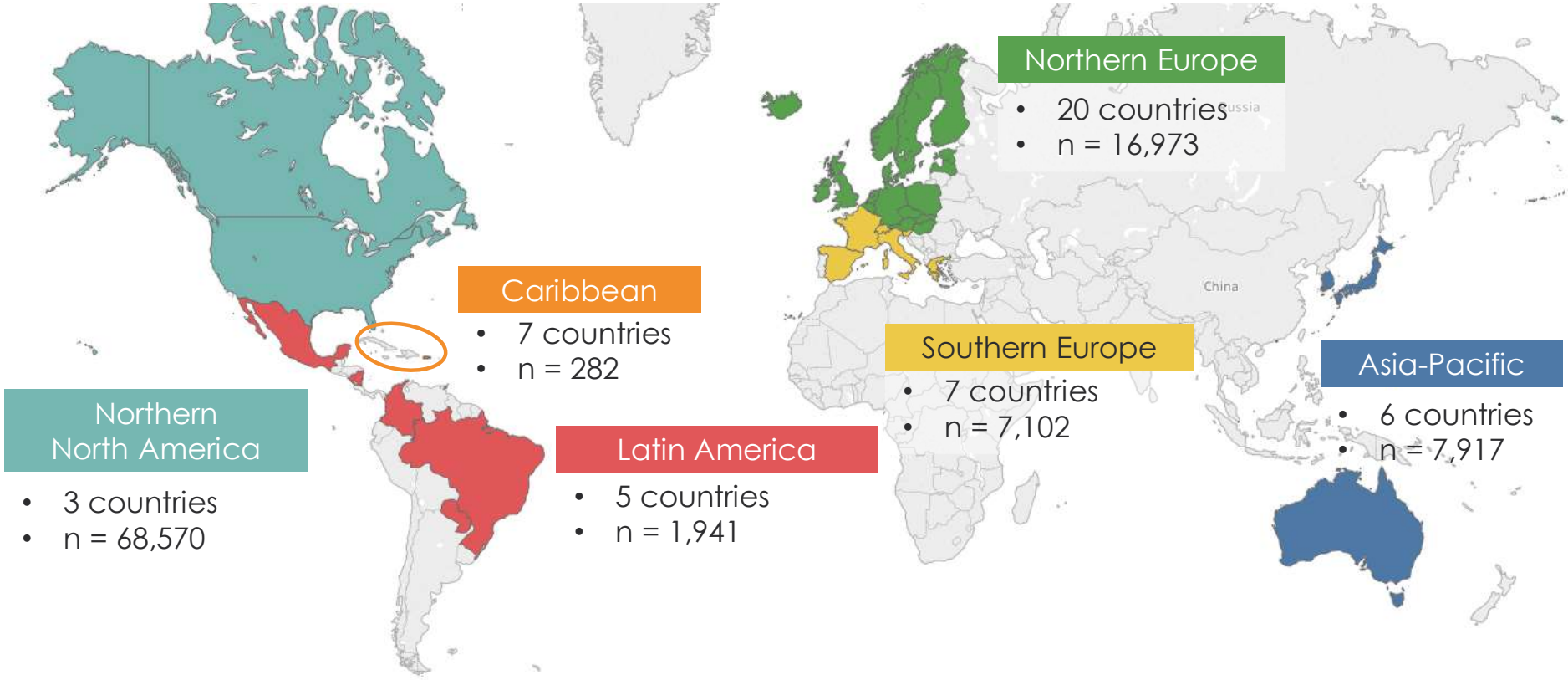

Highest for Asia-Pacific region
- 12.9% (95% CI 12.2% to 13.7%)

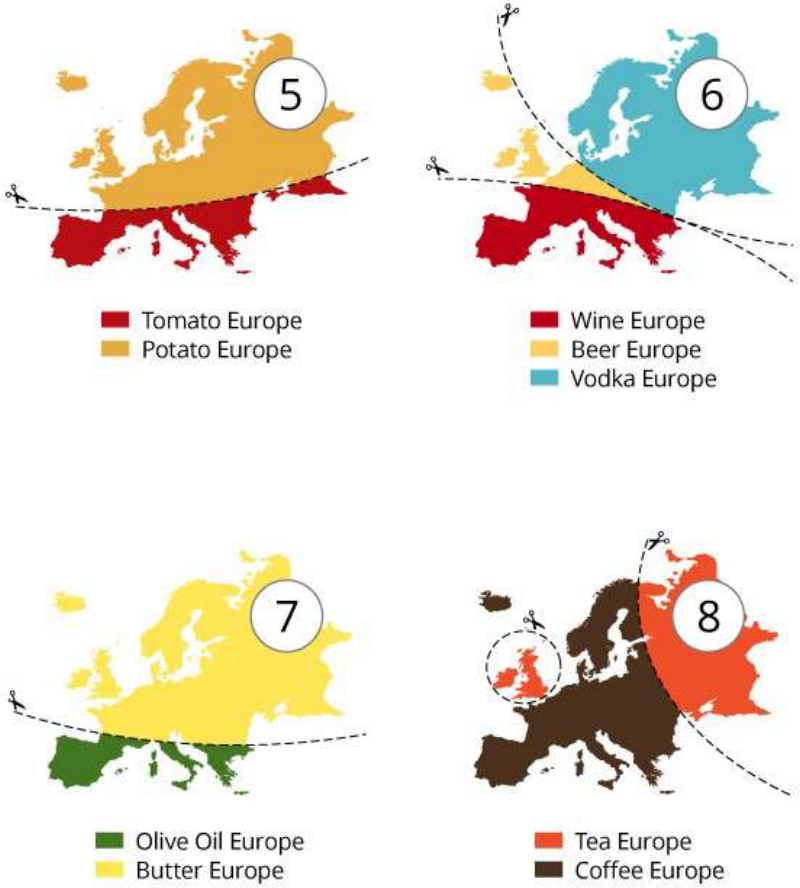
Lowest for Northern North America
- 1.8% (95% CI 1.7%-1.9%)

Regional differences likely driven by both prevalence and testing practices.

# What Are the Speciation Patterns for *Babesia spp.* PCR Positives by Region?

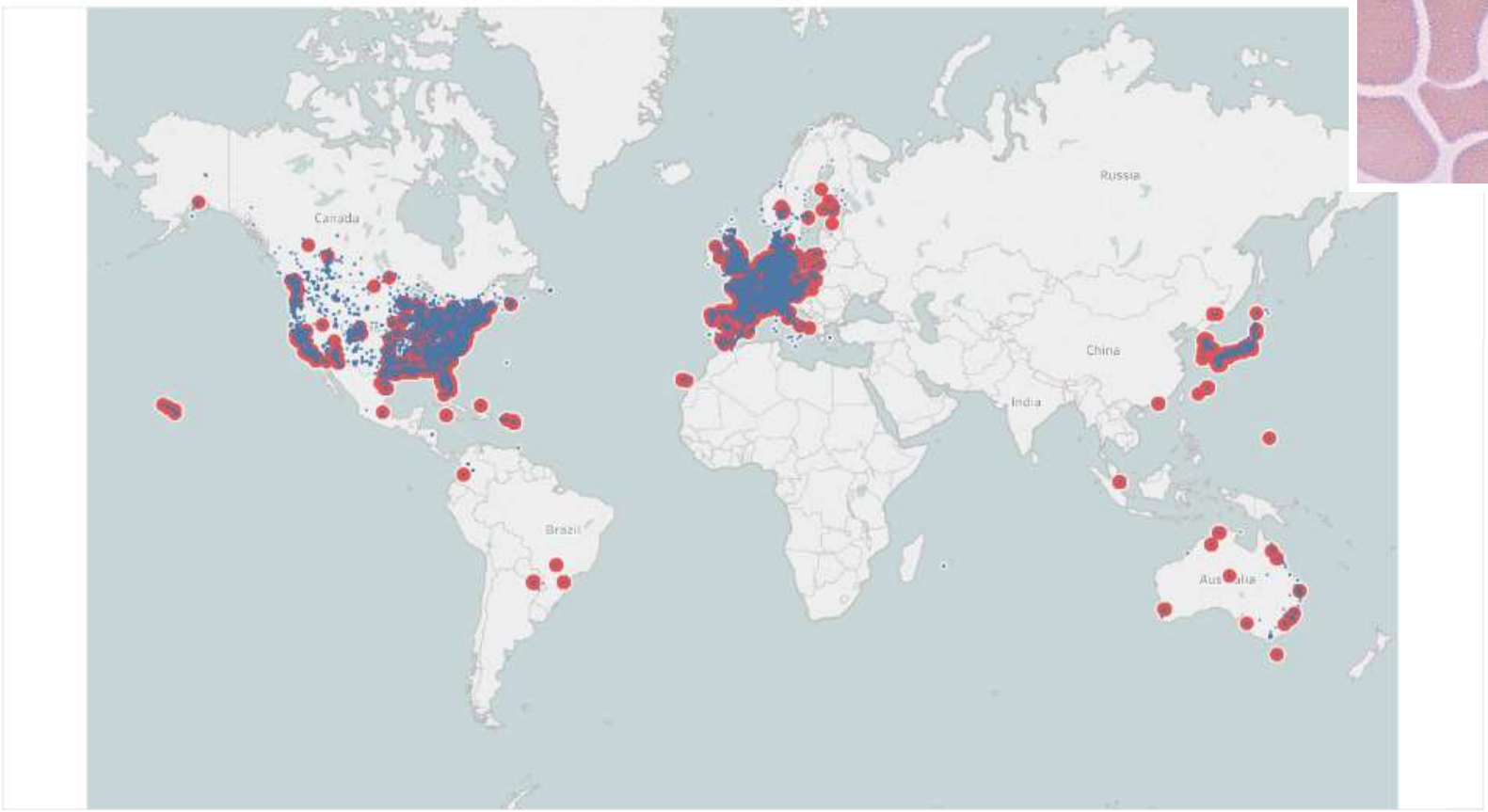
102,345 results from 48 countries in 6 regions

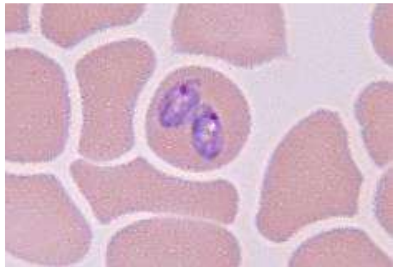



5
Tomato Europe
Potato Europe
6
Wine Europe
Beer Europe
Vodka Europe
7
Olive Oil Europe
Butter Europe
8
Tea Europe
Coffee Europe

Global Distribution of *Babesia* spp.

# PCR Positives



CK [2]1

## Global Distribution of *B. gibsoni*

# PCR Positives

Locations with postive *Babesia gibsoni* PCR test results (2013-2017).
Positives without valid speciation and samples positive for multiple *Babesia* species are excluded.

**Slide 38**

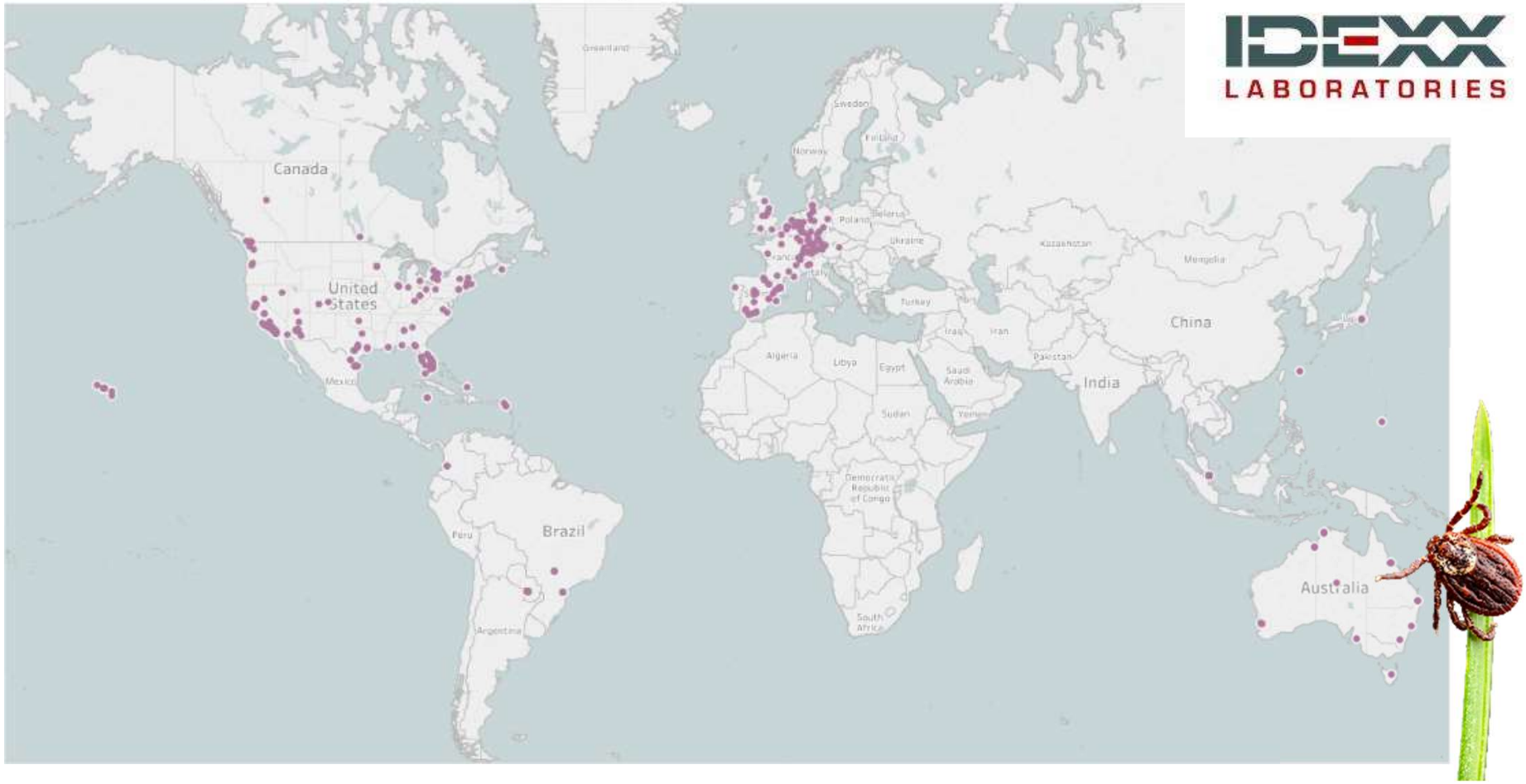
**CK [2]1** Is it okay that the 3 numbers above do not add up to the total listed at the bottom of the slide?
Cathleen Kurz, 03/01/2020

CK [2]2

## Global Distribution of *B. vogeli*

# PCR Positives

Locations with postive *Babesia vogeli* PCR test results (2013-2017).
Positives without valid speciation and samples positive for multiple *Babesia* species are excluded.



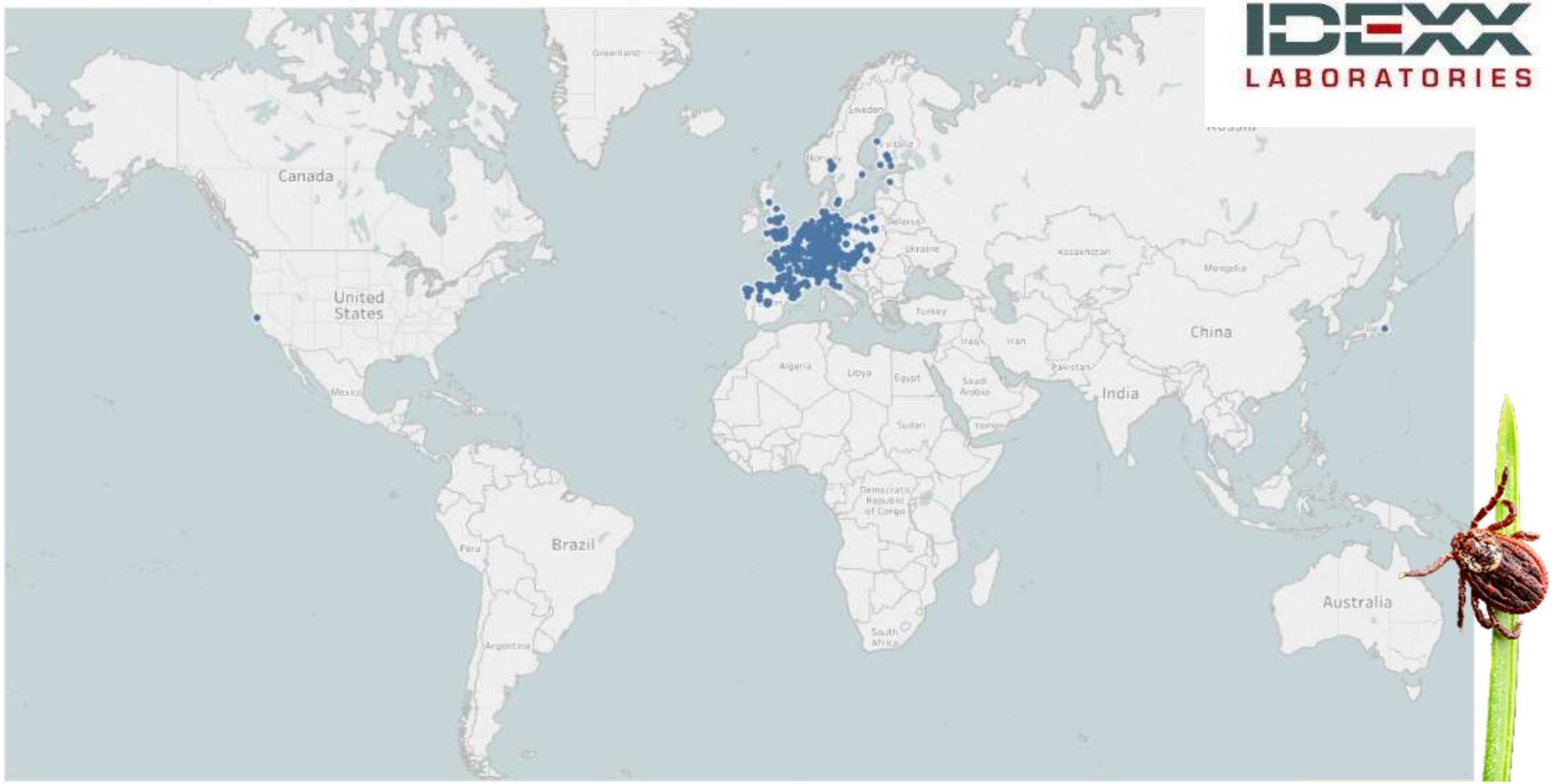
**Slide 39**

**CK [2]2** Is it okay that the 4 numbers above do not add up to the total listed at the bottom of the slide?
Cathleen Kurz, 03/01/2020

Global Distribution of *B. canis*

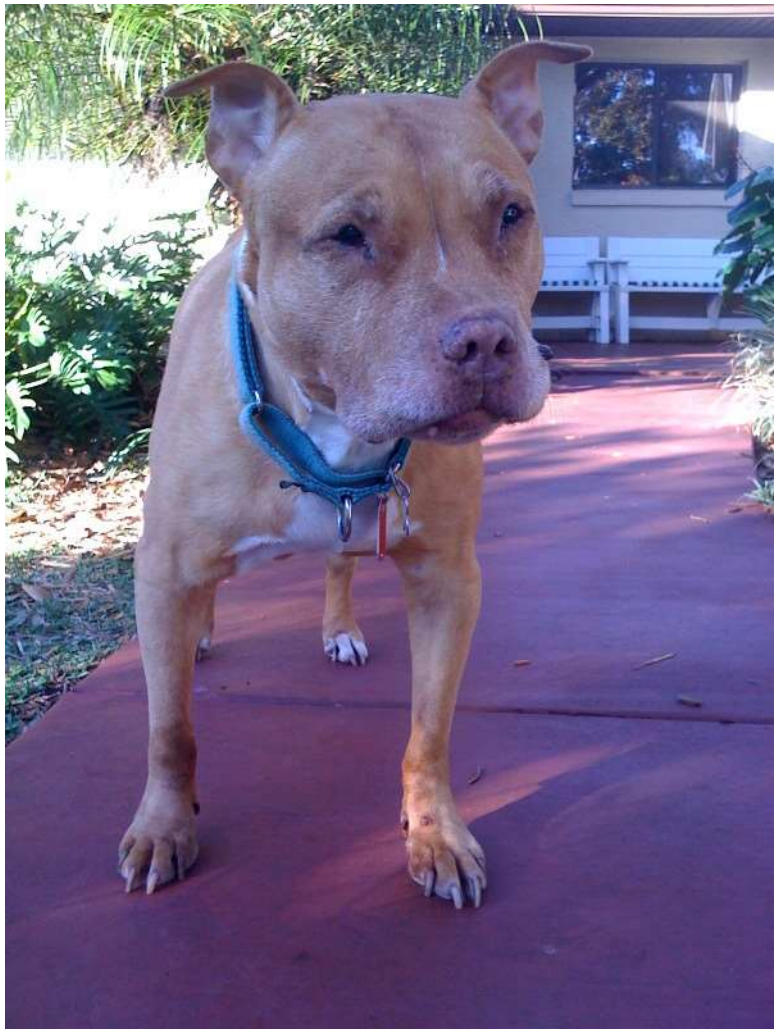
# PCR Positives

Locations with postive *Babesia canis* PCR test results (2013-2017).
Positives without valid speciation and samples positive for multiple *Babesia* species are excluded.



# Bonz

- 13 yr MC APBT
- Bonz's owners noticed dark colored urine on 4-15-09
- Diagnosed with a UTI and prescribed enrofloxacin
- On 4-16-09, Bonz was very weak and was diagnosed anemia
- Who would test for Babesia now?
- Good! So did the RDVM
- Found Babesia on a smear
- Why is Bonz “atypical”



# The rest of the story…

- Bonz was attacked by two other pitbulls Dec. 2008
- His surgery history includes wound surgery on the right hind limb (Jan. 2009)
- Bonz's PCV dropped dramatically after Sx. and an abdominal ultrasound revealed two nodules on the stomach and an enlarged spleen
- During endoscopy, biopsies were taken of the stomach nodules and they were benign.
- Splenectomy: Histopathology of the spleen was benign

# The rest of the story…

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- During endoscopy, biopsies were taken of the stomach nodules and they were benign.
- Splenectomy: Histopathology of the spleen was benign

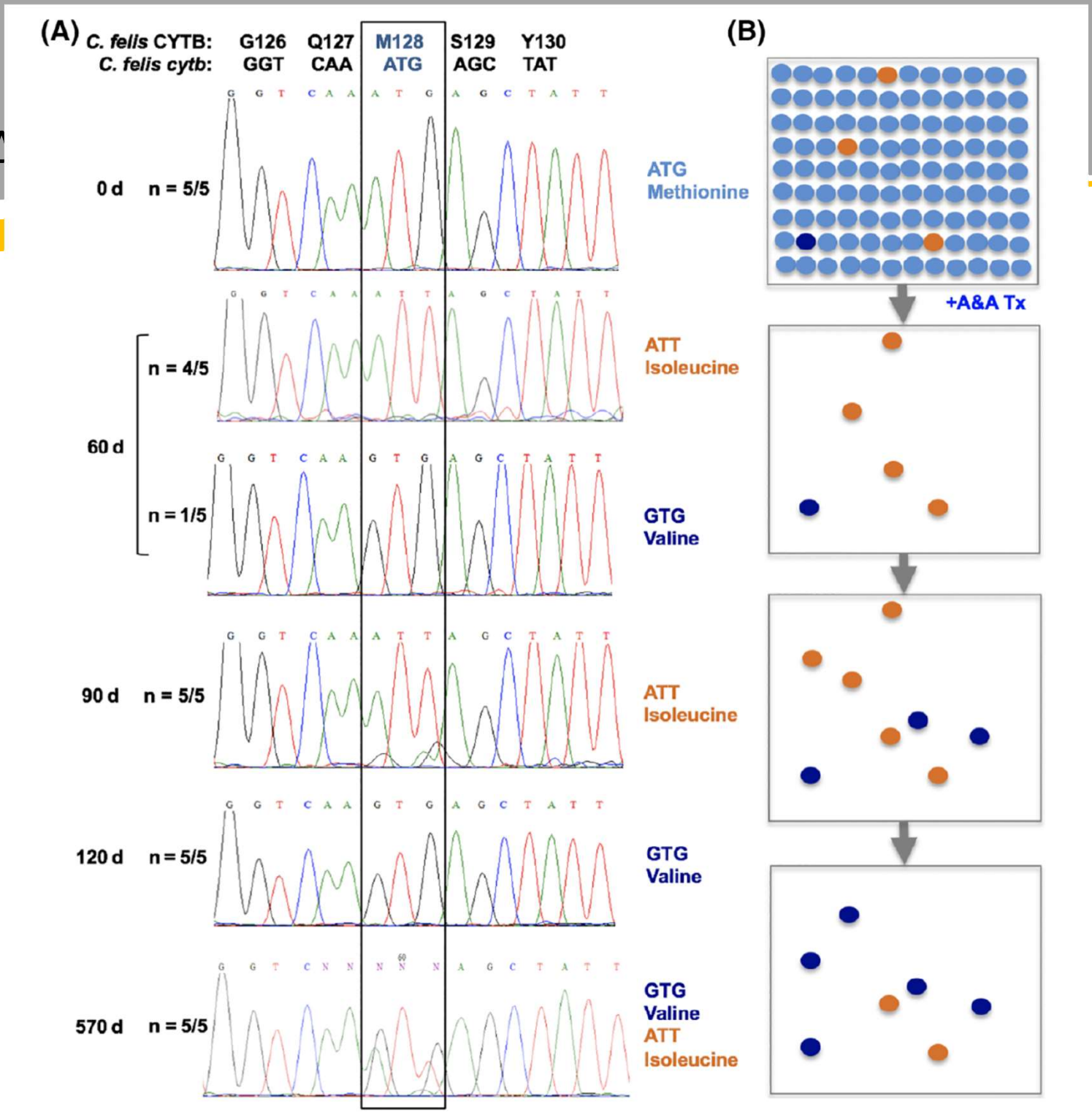
# What does this mean for Bonz?

- Dogs that have been splenectomized frequently fail to respond to treatment!!!
- Atovaquone and azithromycin combination therapy is NOT perfect!

# *Babesia gibsoni:* Treatment of Choice

- Atovaquone (13.5mg/kg PO TID with a fatty meal)
- Azithromycin (10mg/kg PO Q24)
- BOTH treatments for 10 days

# A&A


(A)
C. felis CYTB: G126 Q127 M128 S129 Y130
C. felis cytb: GGT CAA ATG AGC TAT
0 d n = 5/5
ATG Methionine
60 d
n = 4/5
ATT Isoleucine
n = 1/5
GTG Valine
90 d n = 5/5
ATT Isoleucine
120 d n = 5/5
GTG Valine
570 d n = 5/5
GTG Valine
ATT Isoleucine
(B)
+A&A Tx

# Bailey

**HEMATOLOGY - SMALL ANIMAL CBC**

| TEST | RESULT | | UNITS | REF RANGE |
|---|---|---|---|---|
| WBC | 5.64 | | X10^3/UL | 4.39 - 11.61 |
| RBC | 7.82 | | X10^6/UL | 5.7 - 8.01 |
| HEMOGLOBIN | 19.3 | | G/DL | 13.8 - 20.3 |
| HEMATOCRIT | 58.1 | H | % | 39.2 - 55.9 |
| MCV | 74.3 | | FL | 64 - 75.2 |
| MCH | 24.7 | | PG | 22.7 - 26.8 |
| MCHC | 33.3 | L | G/DL | 34.5 - 36.6 |
| RDW | 12.8 | | % | 11.3 - 13.5 |
| PLATELETS | 210 | | X10^3/UL | 190 - 468 |
| MPV | 12.3 | | FL | 7.9 - 13.8 |

| TEST | RESULT | | UNITS | REF RANGE |
|---|---|---|---|---|
| PACKED CELL VOLUME | 56 | | % | 39 - 58 |
| PLASMA PROTEIN | 9.5 | H | G/DL | 6.1 - 7.5 |

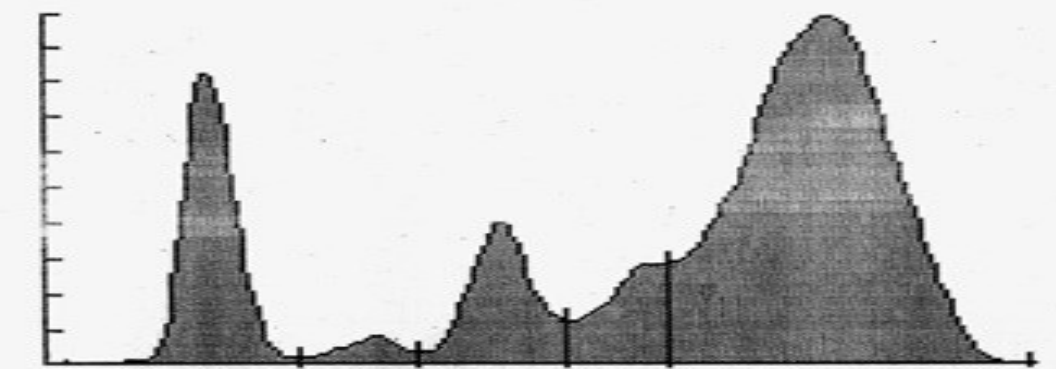
Who would test for Babesia now?

# Bailey: the rest of the story…

- Bailey actually lives with Bonz
- Surprise…Bailey and Bonz got into the fight Dec. 2008
- Globulin: 6.9 g/dl
- Albumin: 2.7 g/dl
- UPC: 0.41
- PCR positive for *Babesia gibsoni*

# What did we learn from Bailey?

- Dogs with *Babesia gibsoni* infections do NOT have to be anemic OR thrombocytopenic!
- May appear “perfectly healthy” to owners and veterinarians alike
- Hyperglobulinemia may be the sole abnormality detected



# Resolution of a Proteinuric Nephropathy Associated with *Babesia gibsoni* Infection in a Dog

Dennis J. Slade, DVM[*,†], George E. Lees, DVM, MS, DACVIM, Brian R. Berridge, DVM, PhD, DACVP, Fred J. Clubb, DVM, PhD, DACLAM, Leslie A. Kuczynski, VMD[§,**], Meryl P. Littman, VMD, DACVIM

# Azotemia and Proteinuria in Dogs Infected with *Babesia gibsoni*

Tarini Ullal, DVM*, Adam Birkenheuer, DVM, PhD, DACVIM, Shelly Vaden, DACVIM

# What’s Next?

# Canine Babesia Genome Sequencing Consortium (NCSU and LSU)

- Goal is to sequence genomes from canine Babesia species for comparative genomics
- Currently sequenced at NCSU
  - Babesia gibsoni
  - Babesia vogeli
- Currently sequenced by LSU
  - Babesia rossi
- Work in progress
  - Babesia canis
  - Babesia conradae
  - Babesia vulpes

# Take Home Messages

- There are a lot more Babesia species on earth than we know about
- The “tend” to infect one species or group…
- If you aren’t looking for them, you won’t find them
- Once you find them, they might be difficult to treat

# Thank you and Questions