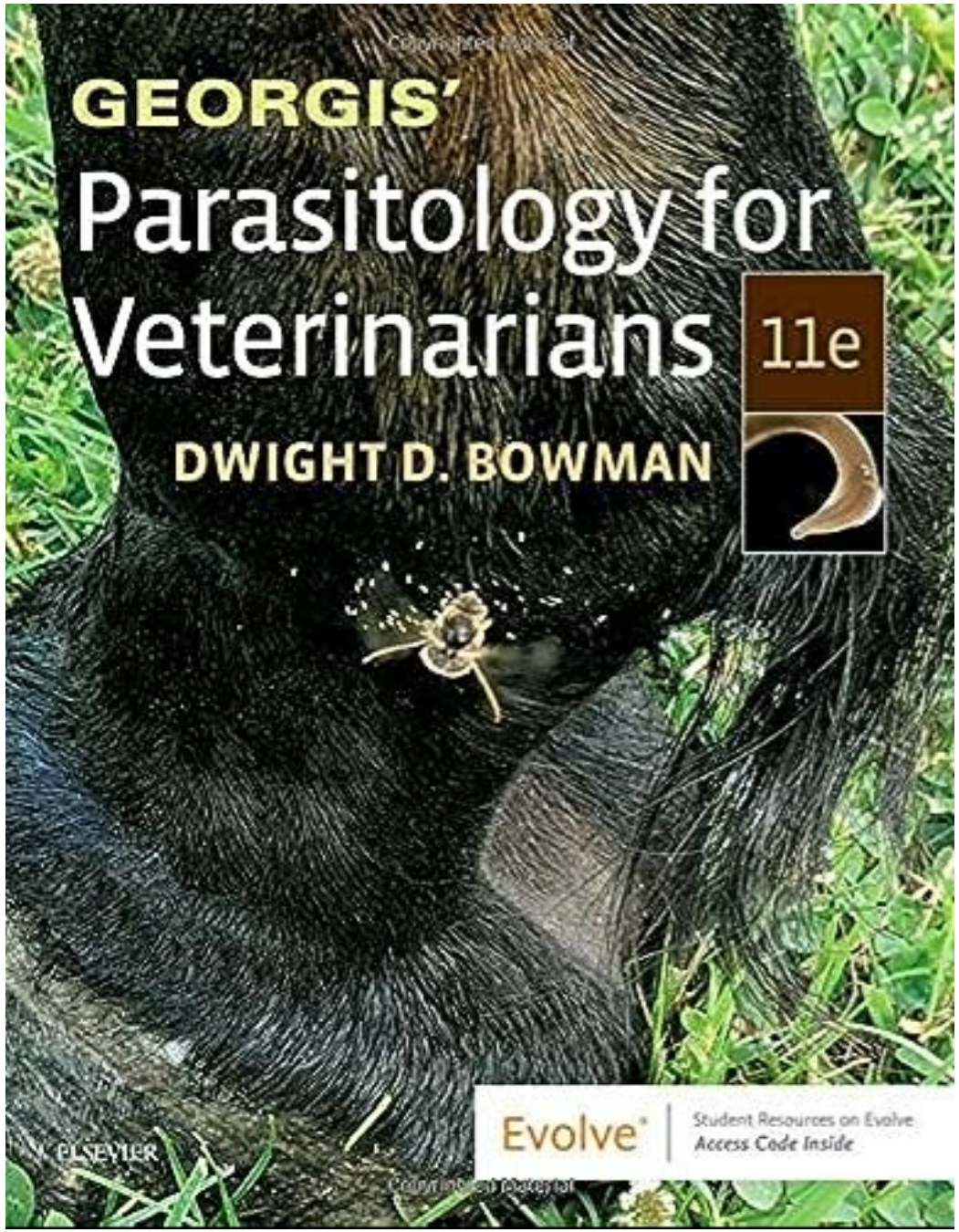
Introduction to Blood-borne Apicomplexans

Small Animal Apicomplexans:

Babesia spp. and *Cytauxzoon felis*



https://capcvet.org/

https://parasitology.cvm.ncsu.edu/

Veterinary Parasitology
North Carolina State University

VMP 930

VMP 930 Home Page
(Moodle Course Site)

Lecture

Laboratory

Lab Word Keys

Lab Image Keys

Lab Quizzes

Supplemental Course Materials

Parasitology Keys

Dichotomous Word Keys

Diagnostic Image Keys

Modules in Development

Life Cycles

Canine Diagnostic Keys

Links

Companion Animal Parasite Council

American Heartworm Society

Merck Veterinary Manual

American Association of Equine Practitioners

American Consortium for Small Ruminant Parasite Control

Tropical Council for Companion Animal Parasites

Veterinary Parasitology
VMP 930: Lecture

LECTURE SCHEDULE

(Print Schedule)

INTRODUCTION (Flowers)

08/12 **Definitions, life cycles, and classification**
PDF sPPT1 sPPT2

PROTOZOA (Qurollo)

08/14 **Introduction & Hemoflagellates** PDF PPT
08/15 **Mucoflagellates** (during lab period) [in B112] PDF PPT
08/19 **Coccidia 1** PDF PPT
08/21 **Coccidia 2** PDF PPT
08/22 **Coccidia 3** (during lab period) [in B112] PDF PPT
08/26 **Toxoplasma & Neospora** PDF PPT
08/28 **Sarcocystis & EPM** [in D239] PDF PPT
08/29 **Piroplasms** (during lab period) [in B112] PDF PPT

ARTHROPODS (Qurollo & Flowers)

09/04 **Ticks** PDF PPT
09/05 **Protozoa Moodle Quiz [5%]** (during lab period)
09/09 **Mites** PDF PPT questions answers
09/11 **Fleas & Lice** PDF PPT questions answers
09/12 **Arthropod Moodle Quiz [5%]** (during lab period)
09/16 **Select Flies** PDF PPT questions answers

EXAM #1 [ExamSoft] (Qurollo & Flowers)
Wednesday, 9/18 in B112, 8:00 to 8:50am
Protozoa & Arthropods [15%]

Past Exam: 2019 Exam 1; 2019 Exam 1 Key
Past Exam: 2018 Exam 1; 2018 Exam 1 Key
Past Exam: 2017 Exam 1; 2017 Exam 1 Key

NEMATODES (Flowers & Moorhead)

09/23 **Intro, Pasture-borne Nematodes**
PDF PPT questions answers
09/25 **Ostertagia & minor Trichostrongyles**
PDF PPT questions answers
09/30 **Strongyle & Oesophagostomum**
PDF PPT questions answers
10/02 **Strongyloides & Trichuris**
PDF PPT questions answers
10/03 **Nematode 1 Moodle Quiz [5%]** (during lab period)
10/07 **Ascarids & Physaloptera**
PDF PPT questions answers
10/09 **Haemonchus & Hookworms**
PDF PPT questions answers
FYI: Resistant Hookworms
10/14 **Respiratory Nematodes**
PDF PPT questions answers
10/16 **Oxyuris and Misc. Nematodes**
PDF PPT questions answers
10/28 ***Dirofilaria immitis* 1** PPT
American Heartworm Society Guidelines
10/30 ***Dirofilaria immitis* 2** PPT
10/31 **Nematode 2 Moodle Quiz [5%]** (during lab period)

EXAM #2 [ExamSoft] (Flowers & Moorhead)
Monday, 11/04 in B112, 8:00 to 8:50am
Nematodes [15%]

Past Exam: 2019 Exam 2; 2019 Exam 2 Key
Past Exam: 2018 Exam 2; 2018 Exam 2 Key
Past Exam: 2017 Exam 2; 2017 Exam 2 Key

PLATYHELMINTHES (Flowers)

11/06 **Trematodes 1** PDF PPT questions answers
11/11 **Trematodes 2** PDF PPT questions answers
11/13 **Cestodes 1** PDF PPT questions answers

Parasitic Protozoa we cover

Grouped by Infection Site and Motility

Apicomplexa (sg =Alveolates)	**Flagellates** (sg = Excavates)

Blood

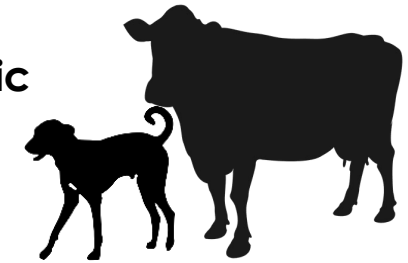
Blood apicomplexa (piroplasms)

Babesia spp.
Cytauxzoon felis
Theileria spp.

Hemoflagellates

Trypanosoma cruzi
Leishmania infantum

Systemic



Systemic apicomplexa

Toxoplasma gondii
Neospora caninum
Sarcocystis spp.
Hepatozoon americanum

Intestines/ urogenital

Intestinal apicomplexan (coccidia)

Cryptosporidium parvum
Eimeria spp.
Cystoisospora spp.

Mucoflagellates

Tritrichomonas foetus
Tritrichomonas blagburni
Giardia

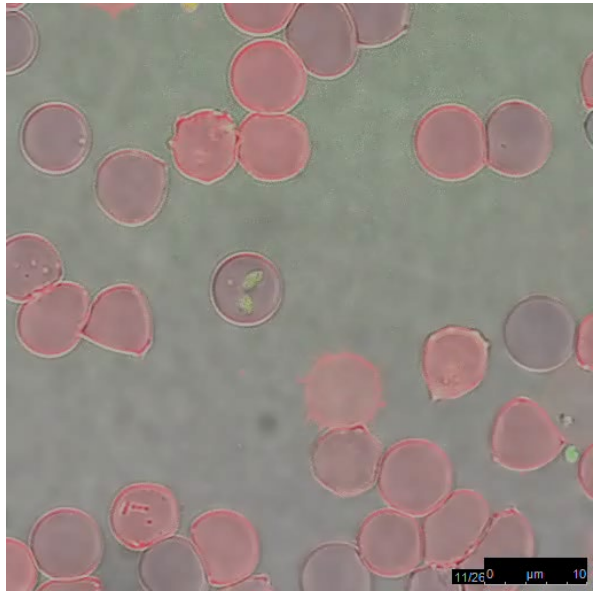
Learning Objectives: Blood Apicomplexans Introduction

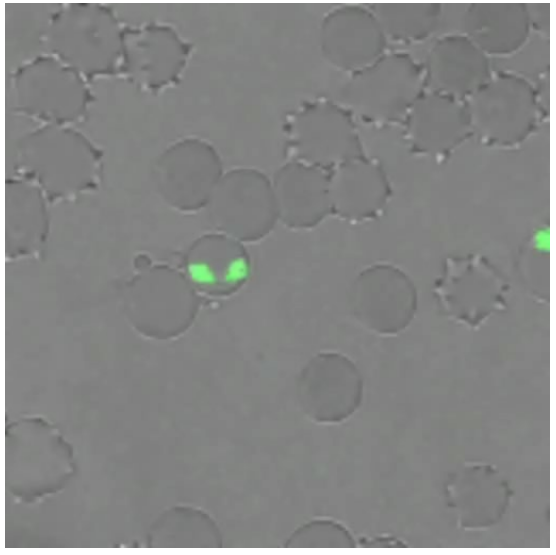
1. Know the select characteristics of apicomplexans
2. Know the select terms used to describe apicomplexan replication
3. Understand that piroplasms are blood-borne apicomplexans
4. Understand how piroplasms are transmitted and what life cycle they utilize
5. Know that *Babesia*, *Theileria* and *Cytauxzoon* spp. are medically important piroplasms

Apicomplexan: Select Characteristics

- Intracellular protozoa with **apical complex**

- Gliding motility



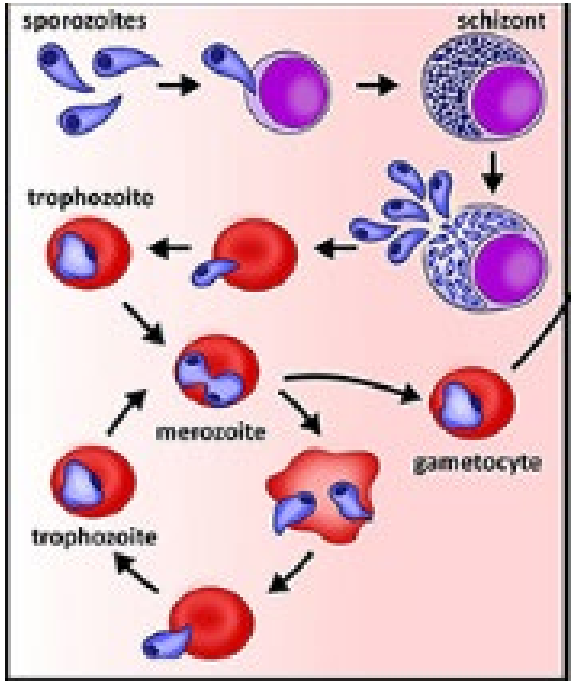


- Life cycle alternates b/w **sexual and asexual reproduction**

- Different stages, with terms like:

"-zoites"

"-onts"



Cytauxzoon example

What are Piroplasms?

1. Intracellular, apicomplexan protozoal parasites
2. **Tick-transmitted**

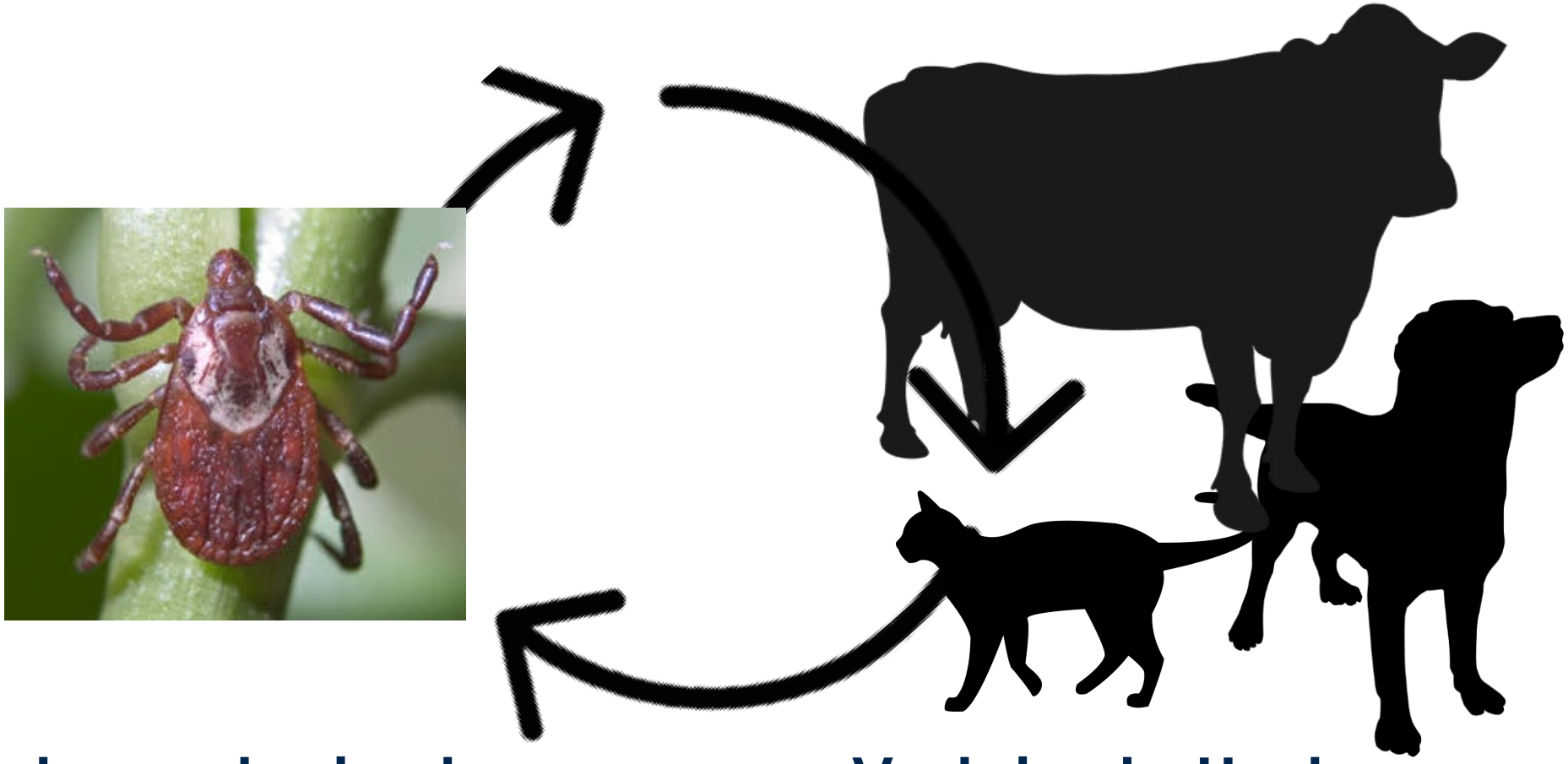
Dermacentor spp.

Rhipicephalus sanguineus

Haemaphysalis spp.

3. **Indirect life cycles** (tick and vertebrate hosts)
4. Infect **vertebrate blood cells**
5. Genera include ***Babesia***, ***Theileria***, and ***Cytauxzoon***

Piroplasms are transmitted by ticks and utilize an Indirect Life Cycle

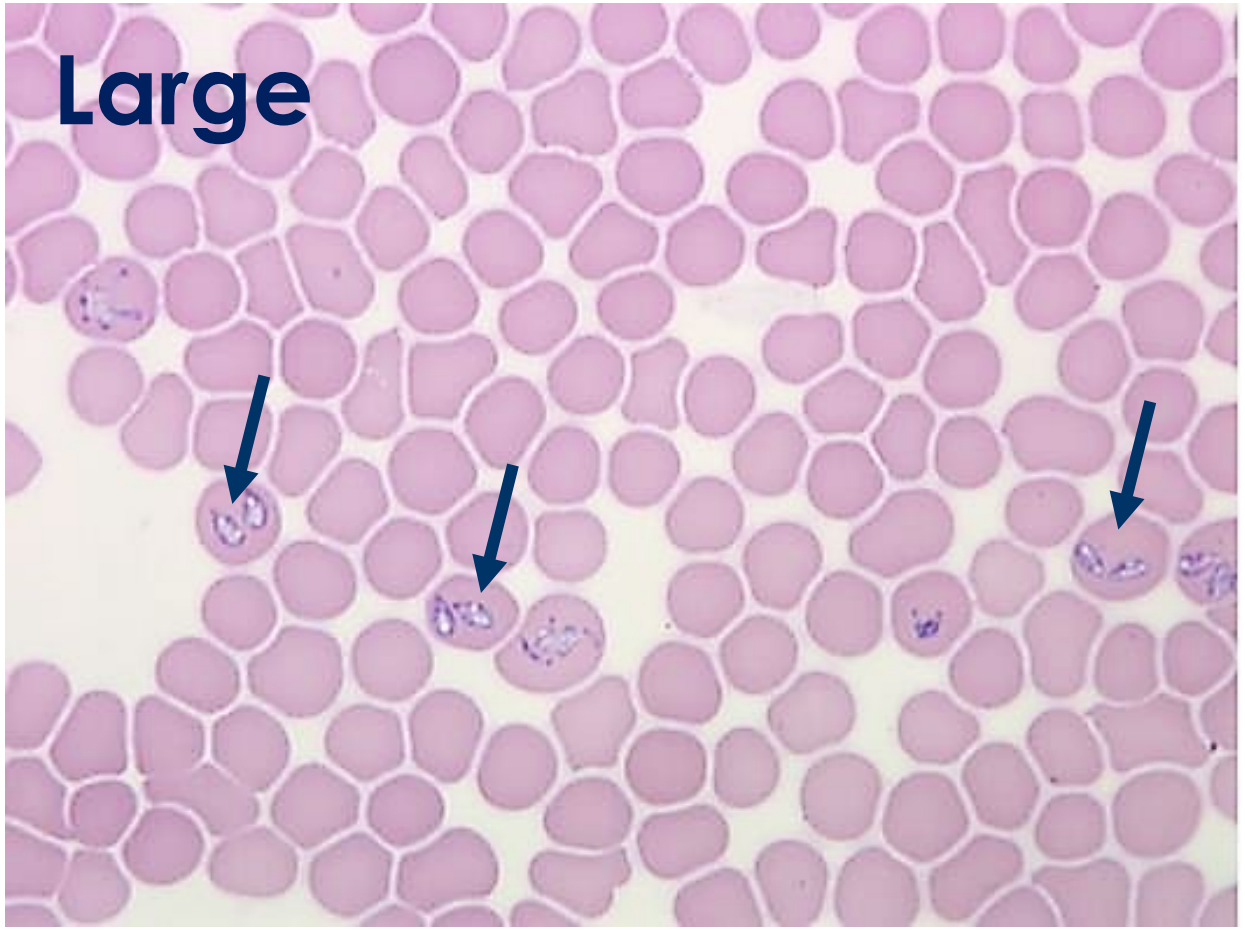


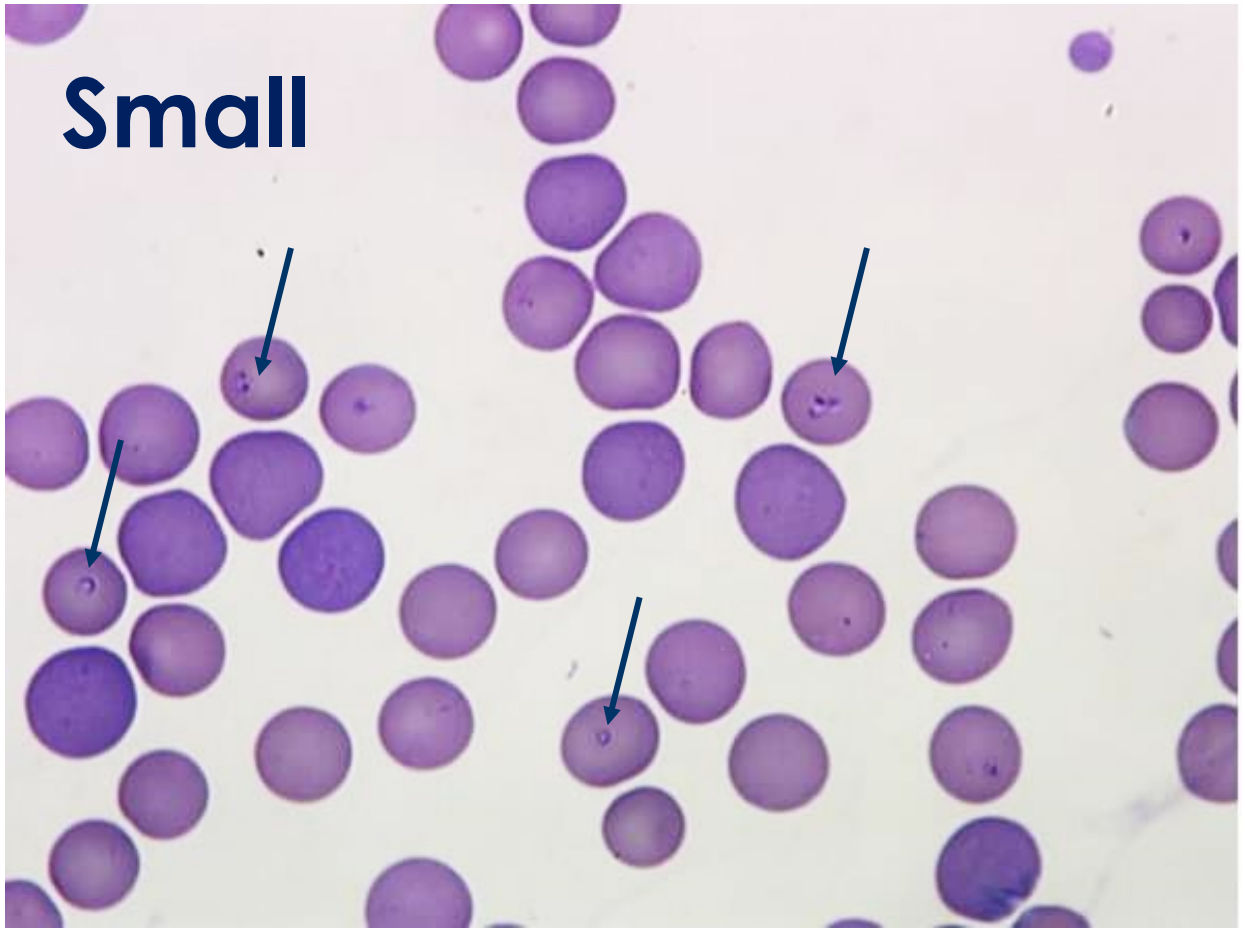
Invertebrate-vector hosts
(definitive host because sexual reproduction of the parasite occurs ticks)

Vertebrate Hosts
(intermediate hosts because only **A**sexual reproduction occurs in vertebrates)

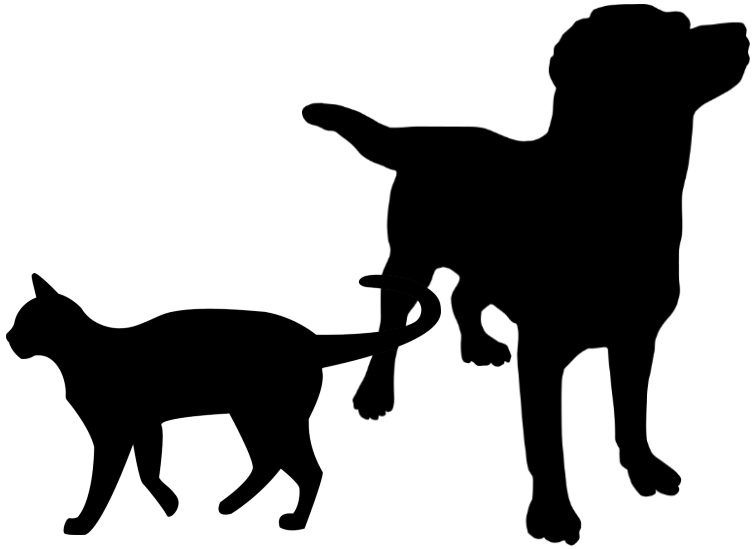
Morphology: Piroplasmids

- Piroplasms are "zoites" (trophozoites, merozoites) that infect erythrocytes and white blood cells depending on the species of pathogen
- When identified in erythrocytes, they are classified as…





Medically important Piroplasms in the U.S. (and their vertebrate hosts) we will discuss

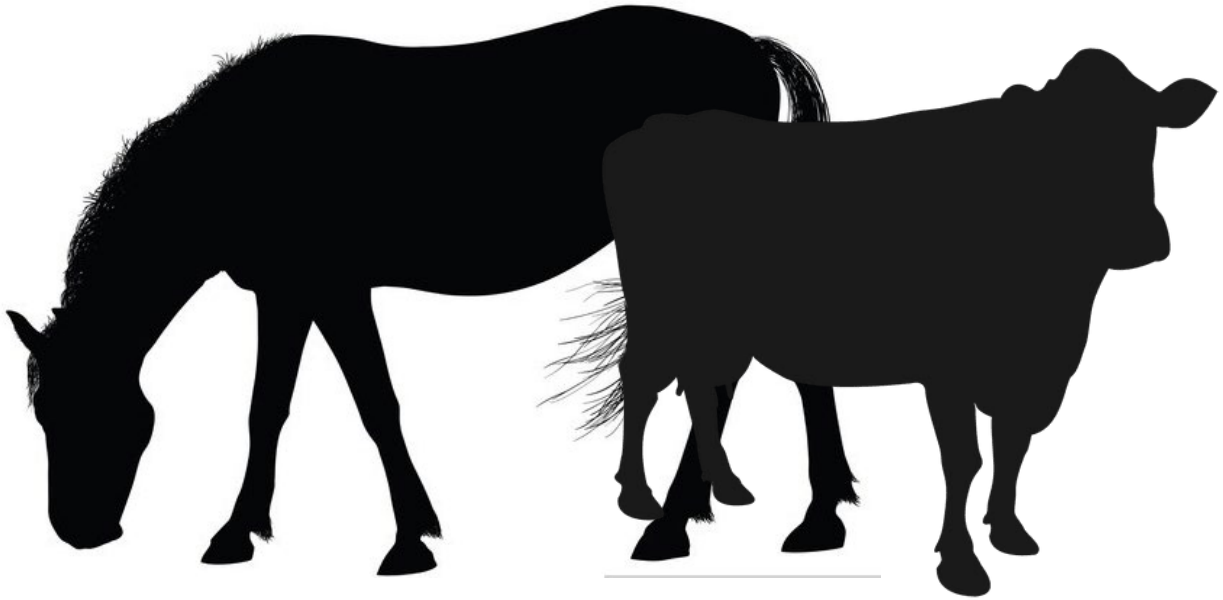


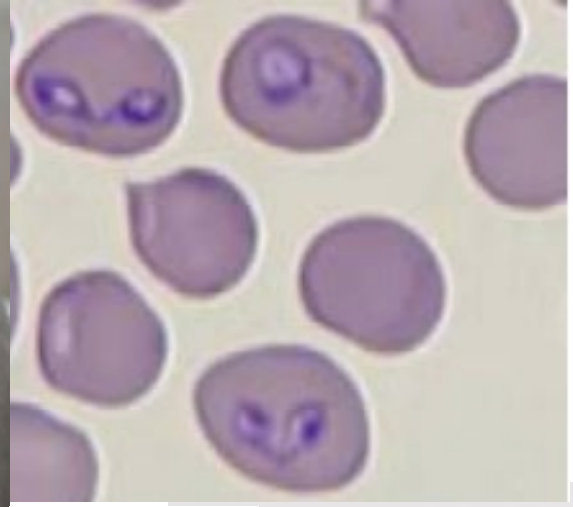
Small Animals

Babesia gibsoni (dogs)
Babesia vogeli (dogs)
Cytauxzoon felis (cats)

Large Animals

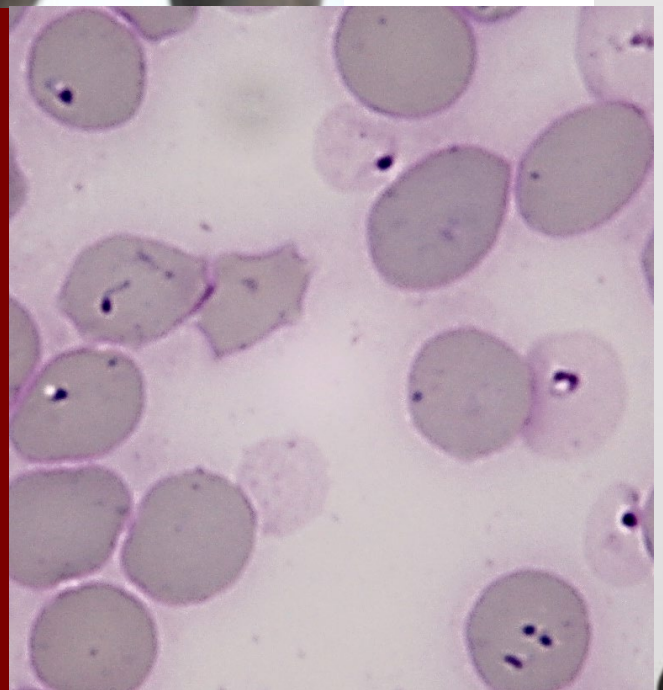
Theileria orientalis (cattle)
Theileria equi (horses)
Babesia caballi (horses)





Babesia spp.

- Canine piroplasm
- Anemia/Thrombocytopenia
- Indirect Life Cycles
  -Tick-borne disease



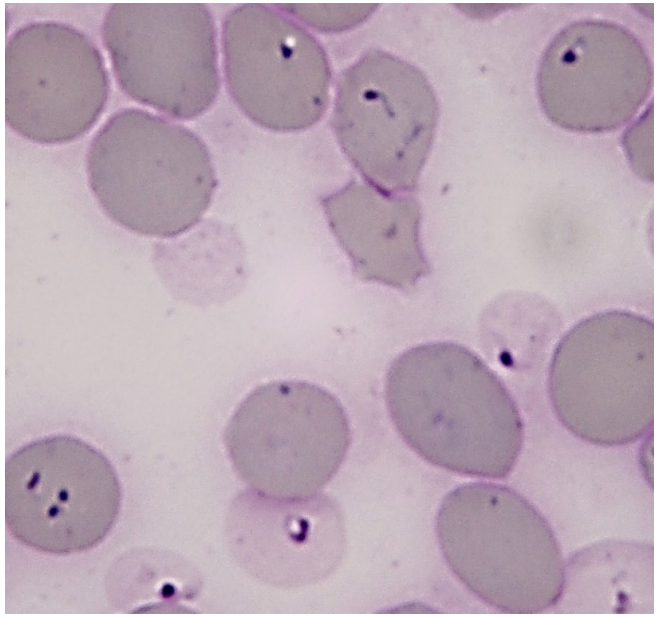
https://houndsavers.org/adopt-a-greyhound/

Learning Objectives: *Babesia* spp.

1. Life cycle: understand the indirect life cycle and know the highlited details.
2. Transmission: understand how *B. vogeli* and *B. gibsoni* are transmitted.
3. Pathogenesis: understand how *B. vogeli* and *B. gibsoni* cause disease
4. Clinical signs: understand the specified common clinicopathologic findings for canine babesiosis and the difference in disease severity between acute and chronic infections.
5. Diagnosis: understand the ways we can diagnose babesiosis, and of the 3 discussed, which is the most useful and why.
6. Treatment: understand that treatment is different for large *Babesia* vs. small *Babesia* in dogs.
7. Epidemiology: understand the risk factors for canine babesiosis and which breeds are more commonly infected with which *Babesia* spp.

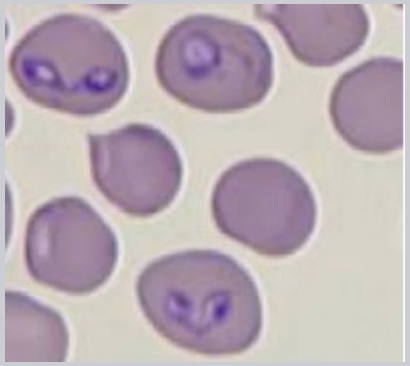
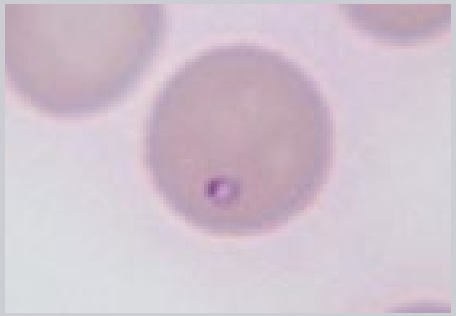
“FYI” = won’t be tested on

- Molly is a 4-year-old female spayed American Staffordshire terrier who presents to the NCSU VHC Small Animal Internal Medicine service for **decreased appetite and lethargy.**
- **Thrombocytopenia** and **anemia**.



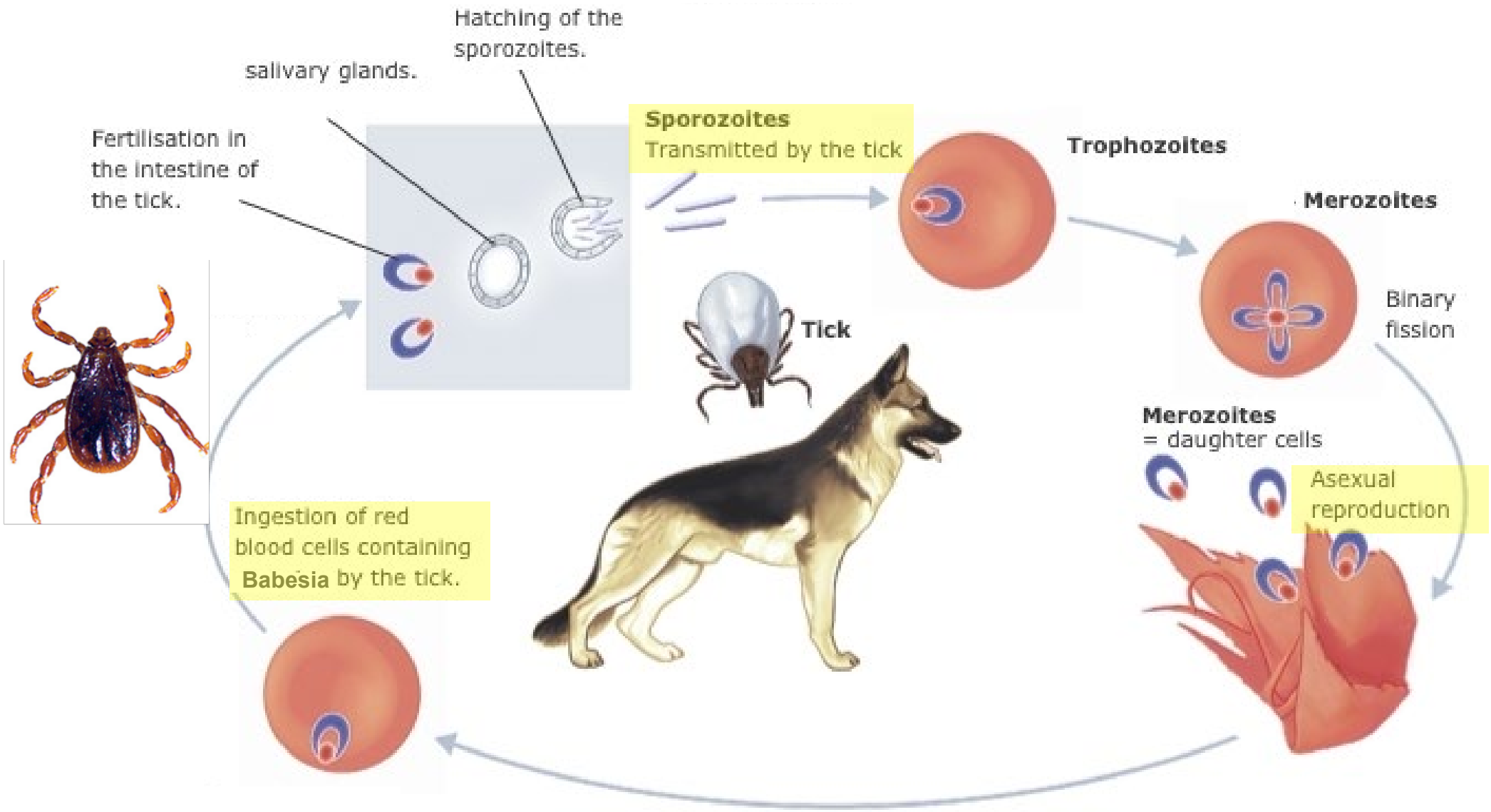
She has Canine Babesiosis

Babesia spp. that infect dogs

Babesia species	Size	Tick Vector	Reported Distribution
B. canis	**Large** 	*Dermacentor* spp.	Europe
B. vogeli ★ *(B. canis vogeli)*		*Rhipicephalus sanguineus*	Worldwide
B. rossi		*Haemaphysalis elliptica*	South Africa
B. coco		*Amblyomma americanum*	North America
B. gibsoni ★	**Small** 	*Haemaphysalis spp.*	Worldwide
B. conradae		?	California
B. vulpes *(B. microti-like)*		*Ixodes spp.* ?	Europe and North America

FYI this chart, except *B. vogeli* and *B. gibsoni*

Indirect Life Cycle: *Babesia*



Indirect Life Cycle: *Babesia*

Canine Vertebrate Host

Transmission

1. **Sporozoites injected by tick bite**
2. **Infected dog-to-dog (fighting)**
3. **Blood transfusion**
4. **Trans placentally (vertically)**

Invasion -- Sporozoites invade erythrocytes

Asexual reproduction

- Merogony → merozoites burst out of the erythrocytes and infect other erythrocytes

Transmission: *Babesia* in US

Babesia vogeli

(AKA B. canis or B. canis vogeli)

Rhipicephalus sanguineus

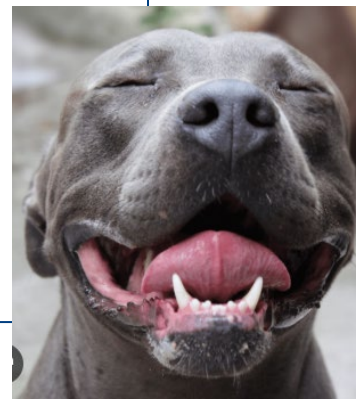
Babesia gibsoni

Haemaphysalis spp.

United States?

Dog bites / fighting

Blood transfusion



Babesiosis Pathogenesis

1. Direct destruction of erythrocytes during multiple asexual cycles

2. Autoimmune Reactions

- Autoantibodies directed against host erythrocytes and platelets

Babesiosis Clinical Signs

Acute or chronic

History

- Any age dog
- Lethargy
- Depression
- Pale mucous membranes (MM)
- Discolored urine
- Dog fight/bite
- Tick attachment
- Blood transfusion

Physical Exam Findings

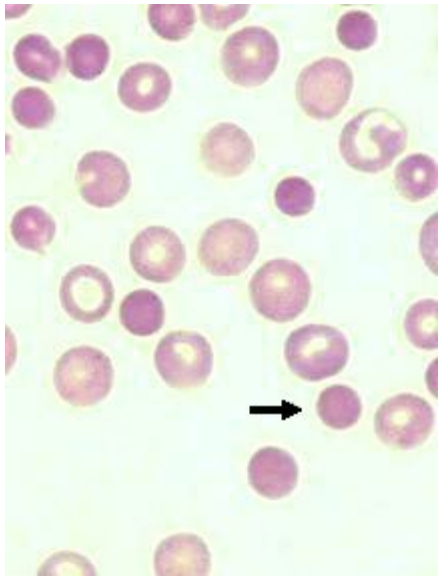
- Fever
- Splenomegaly
- Pale MM
- Lymphadenomegaly
- Jaundice
- Normal?

Babesiosis Common Hematological findings

- **Thrombocytopenia (more common)**
- **Anemia**

- If it looks like **IMHA or ITP** You'd better think about *Babesia*!

Spherocytes



Autoagglutination

Babesiosis Biochemical findings

- +/- Hyperglobulinemia
- +/- Hyperbilirubinemia
- +/- Increased liver enzymes (mild)
- +/- Mild azotemia
- +/- Metabolic acidosis
- No pathognomonic biochemical findings!

Babesiosis Diagnosis

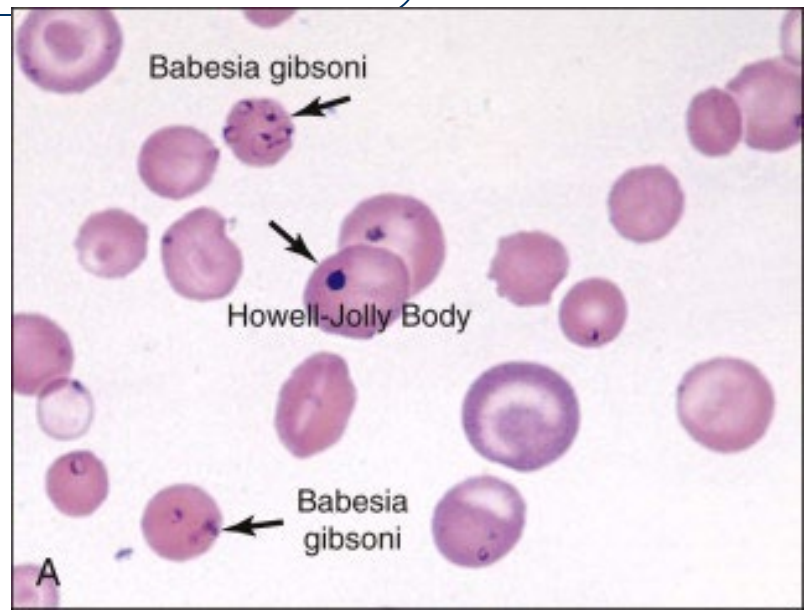
- **Parasite visualization**
- **Serology (testing for antibodies)**
- **PCR (will allow you to speciate the *Babesia* spp.)**

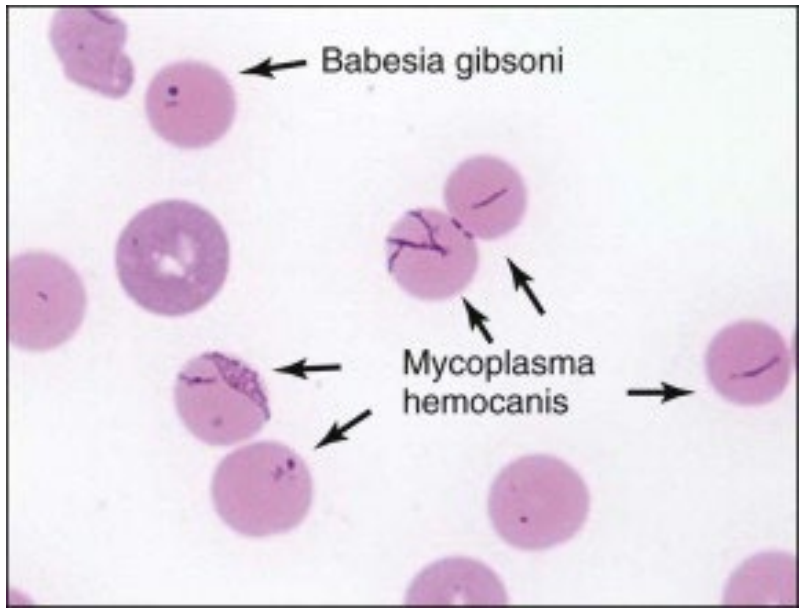
All 3 tests are useful, but PCR is probably the "best test" if you choose only 1

Babesia Parasite Visualization

- Diff-Quik stain with oil immersion
- Capillary blood = ear or toenail
- Look at the entire slide(s)
- False positives (artifacts etc)
- Not very sensitive (a negative slide exam does not rule out babesiosis)

water artifact

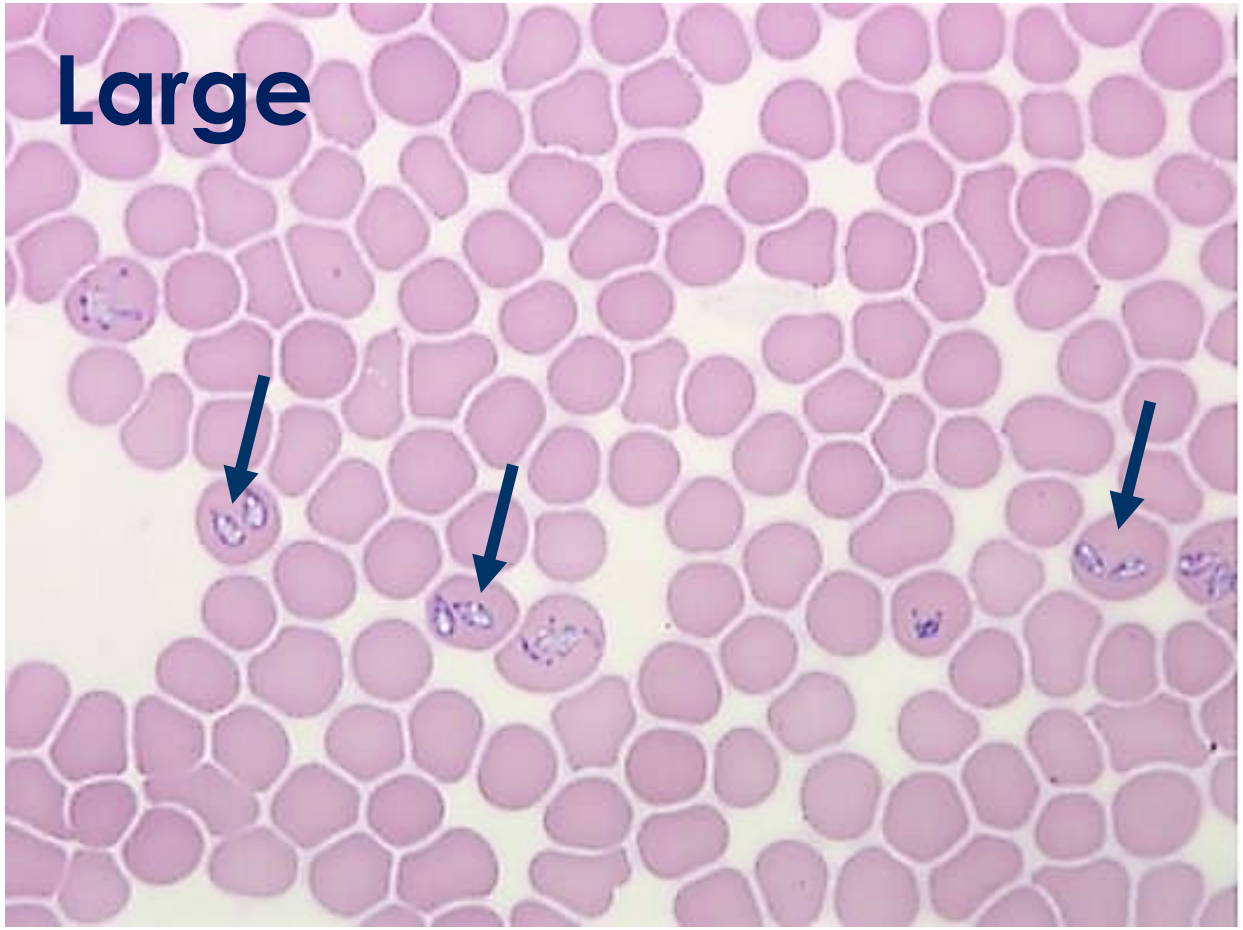


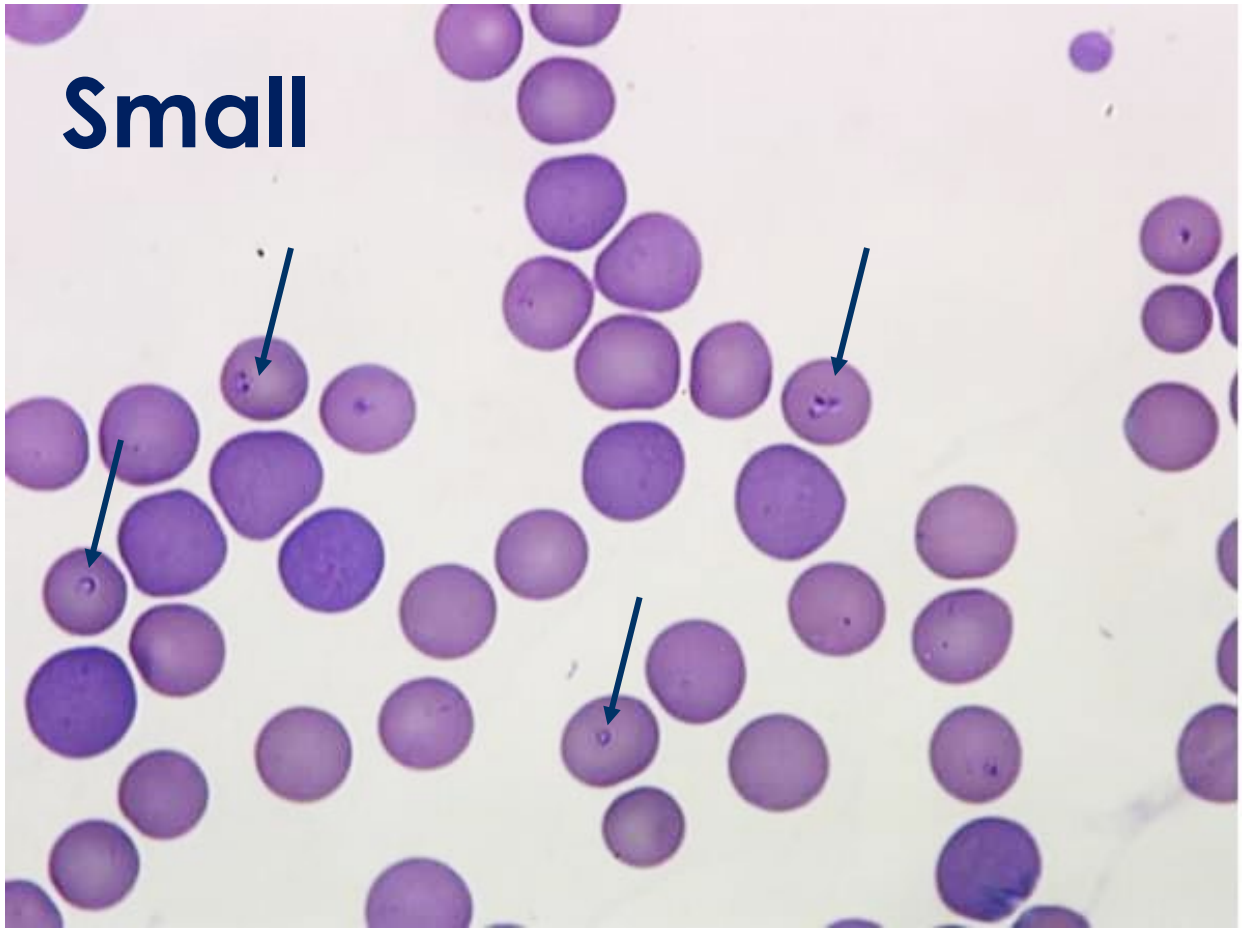


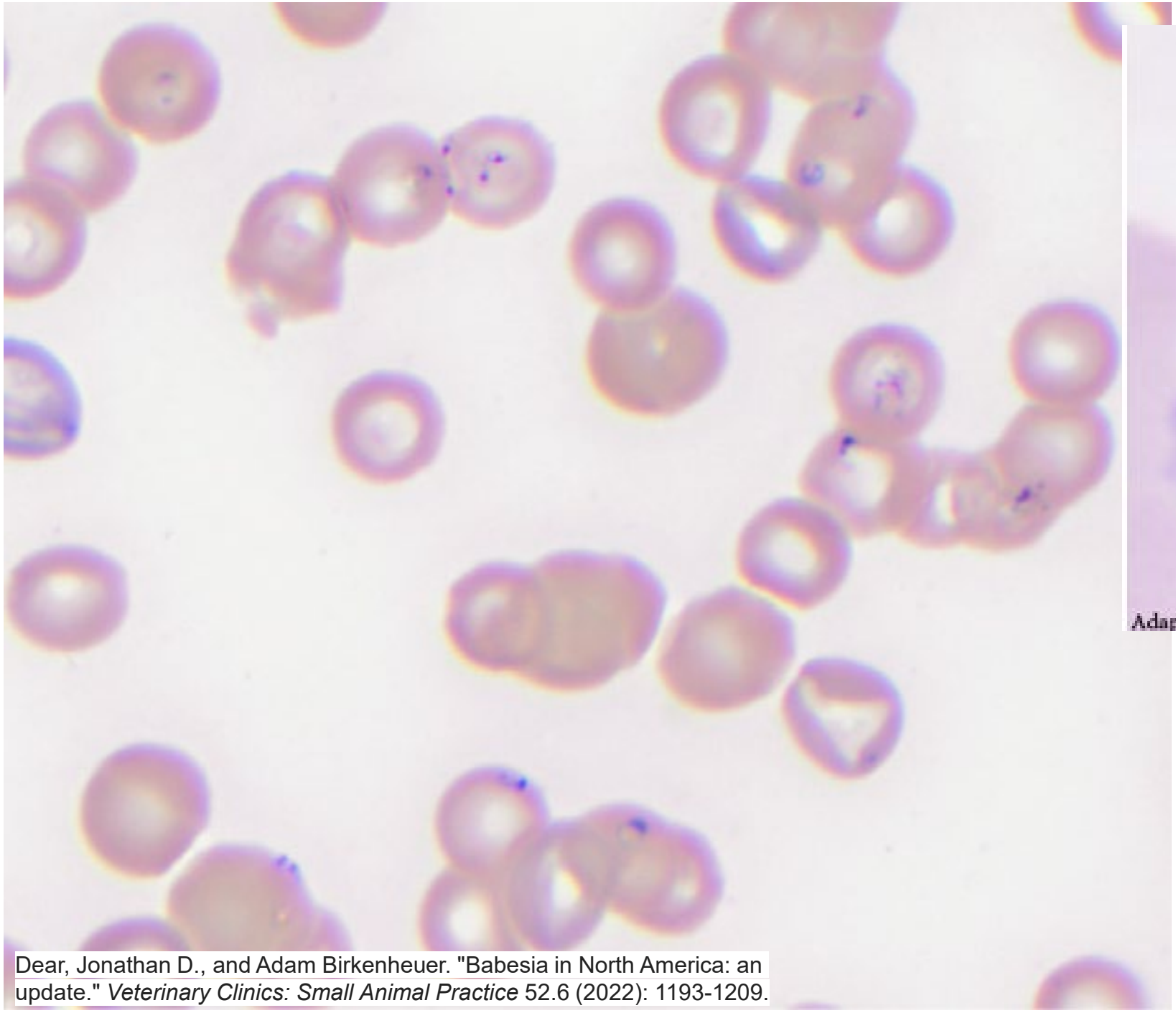
Babesia Parasite Visualization

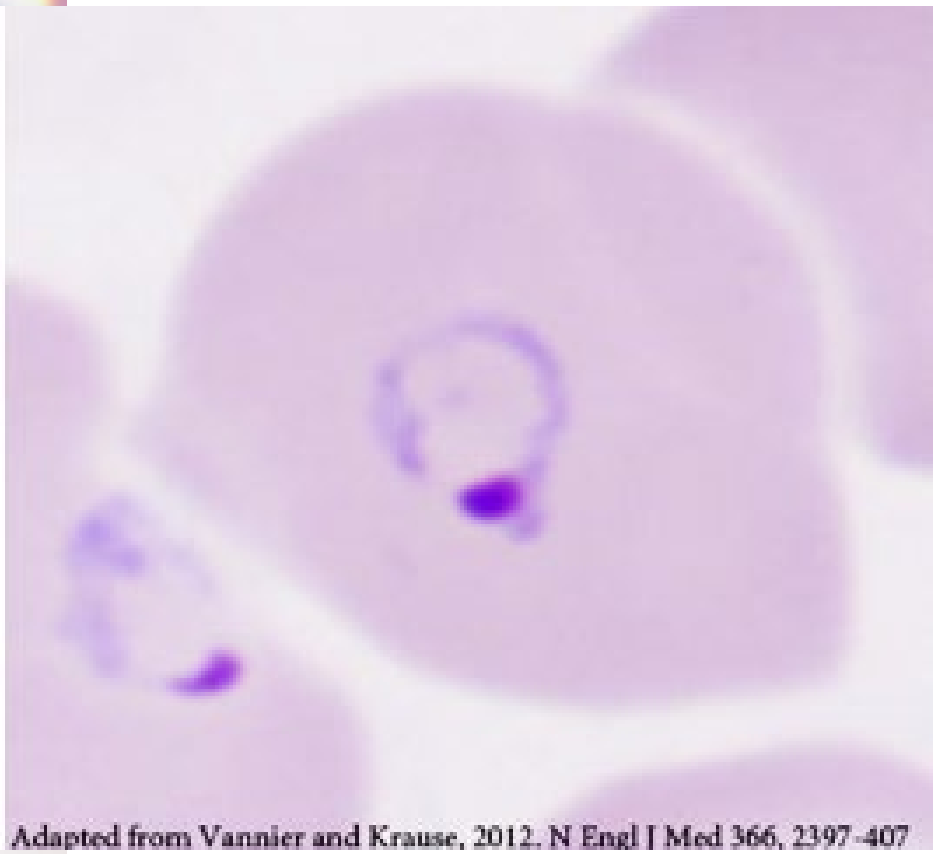
Babesia vogeli (large)

Babesia gibsoni (small)









Adapted from Vannier and Krause, 2012. N Engl J Med 366, 2397-407

Dear, Jonathan D., and Adam Birkenheuer. "Babesia in North America: an update." *Veterinary Clinics: Small Animal Practice* 52.6 (2022): 1193-1209.

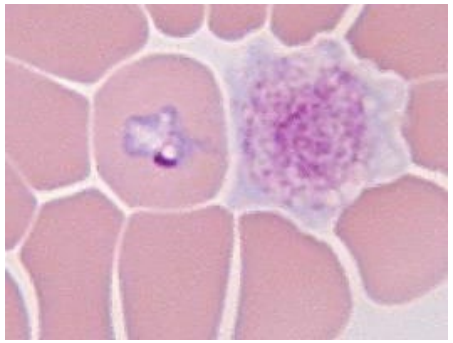
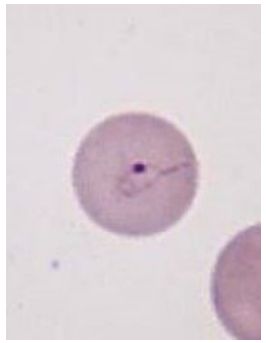
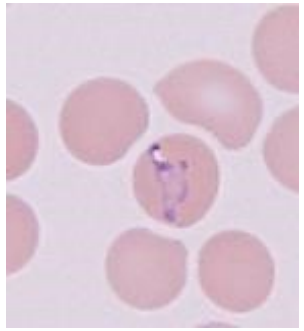
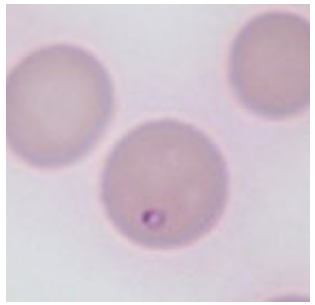
Babesia Serology

- **Serology (detection of antibodies) is not species-specific**
  - Antibodies against *B. vogeli* may cross-react with *B. gibsoni* test (vise versa)
  - You can't determine the *Babesia* species with serology
- There are not always antibodies present in an infected dog at time of testing
- Run acute and convalescent titers (~2 weeks apart) and look for an increase in antibodies

Babesia PCR Testing

- A positive PCR is consistent with infection
- DNA sequencing of PCR product can determine the infecting *Babesia* species
- A negative PCR = true negative **OR** the parasite load is too low to be detected (false negative)

Babesia Diagnostic Summary


- Microscopy and serology are NOT able to definitively identify the species of *Babesia*
- Molecular testing (PCR, DNA sequencing) is required to differentiate *Babesia*
- **It's better to do microscopy, PCR and serology for all species**
- **If you can only do one test, pick PCR**
- If trying to confirm negative a PCR, ideally obtain two consecutive negative tests

Why should you care so much about speciating *Babesia* infections?

Treatment: *Babesia* spp.

Babesia species	Piroplasm Size	Recommended Rx
B. canis	Large	Imidocarb dipropionate
B. vogeli (B. canis vogeli)		
B. rossi		
B. coco		
B. gibsoni	Small	Azithromycin + Atovaquone
B. conradae		
B. vulpes		

Optimal treatment is different for large vs. small *Babesia* spp.

Babesia Epidemiology

- Worldwide distribution (*B. gibsoni* and *B. vogeli*)
- Not Zoonotic
- Any dog breed can become infected

Risk Factors for Canine babesiosis

- Breed
  - *Babesia vogeli* (greyhounds)-*R. sanguineus* kennel ticks
  - *Babesia gibsoni* (Pitbull) –dog-to-dog transmission
- Dog bite (by a Pitbull)
- Exposure to ticks
- Transfusions
- Splenectomy

Babesia Epidemiology

Greyhounds and *Babesia conradae*

Journal of Veterinary Internal Medicine Open Access ACVIM

Standard Article | Open Access

Babesia conradae **infection in coyote hunting dogs infected with multiple blood-borne pathogens**

Jonathan D. Dear, Sean D. Owens, LeAnn L. Lindsay, Alex W. Biondo, Bruno B. Chomel, Mary Marcondes, Jane E. Sykes

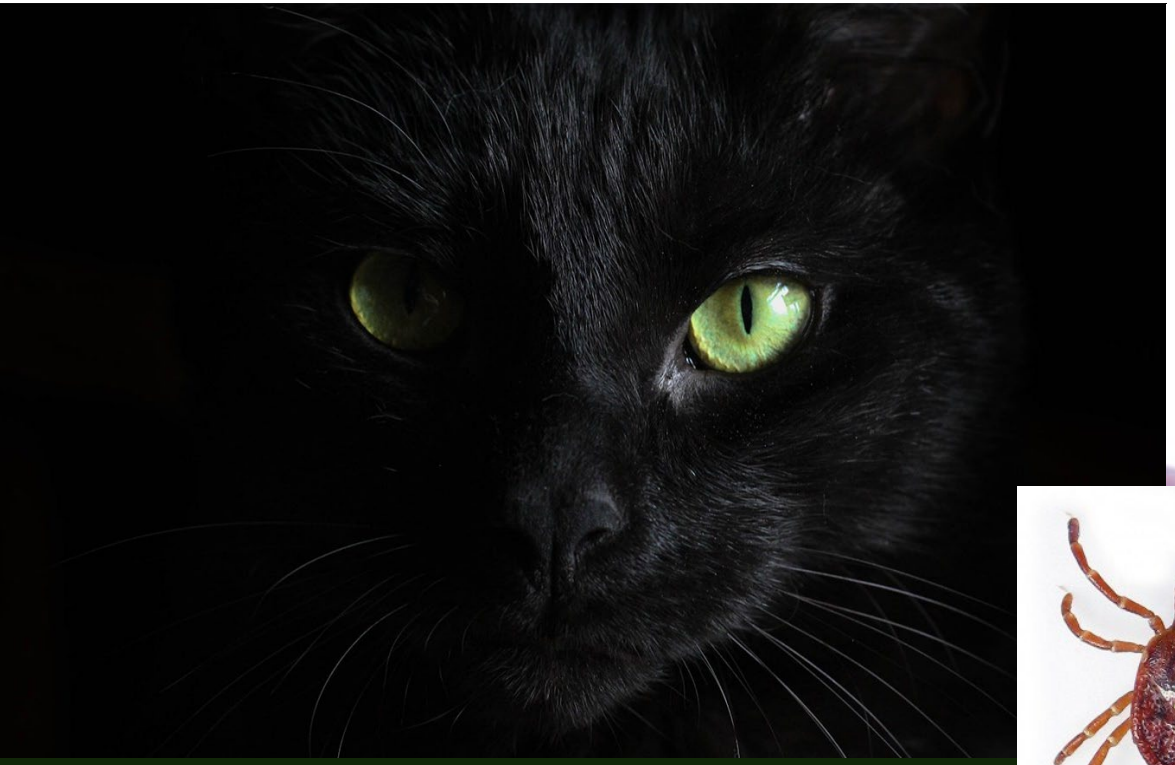
Aggressive interactions with other canids may play a role in *B. conradae* transmission.

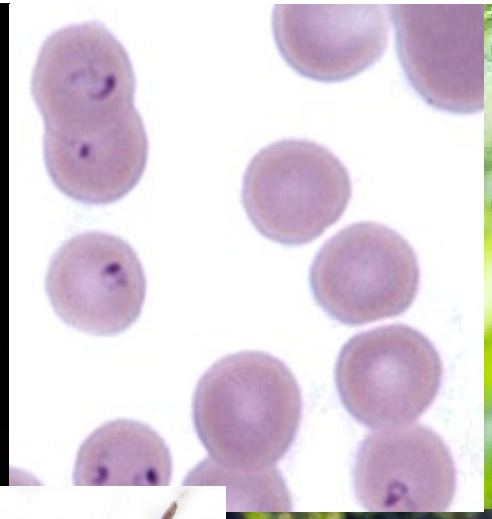
Dogs take a break from a Coyote hunt. (Scott Squire/Courtesy of University Press of Mississippi).
https://www.washingtonpost.com/news/in-sight/wp/2015/12/17/the-beauty-and-controversy-of-hunting-coyotes-with-hounds/

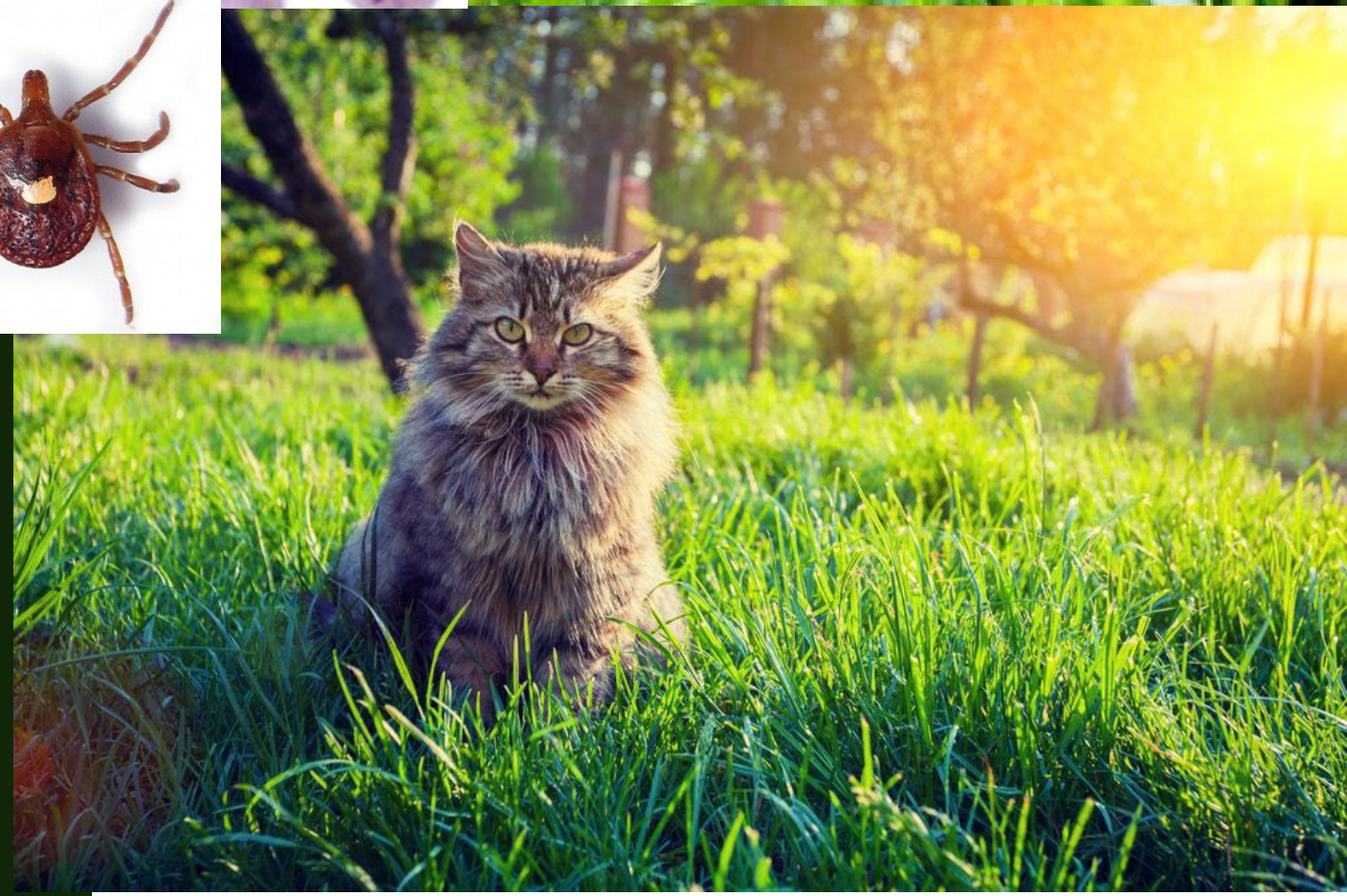
FYI

Babesiosis Take-Home Points

- This disease can be Acute or Chronic
- Thrombocytopenia more common than anemia
- Pit Bulls, Greyhounds, Ticks
- PCR best way to definitively diagnose
- Correct identification (by PCR) needed for best treatment







Cytauxzoon felis

- Feline piroplasm
- Severe Feline Disease
- Indirect Life Cycle
  -Tick-borne disease

Learning Objectives: *Cytauxzoon felis*

1. Life cycle: understand the indirect life cycle and the highlighted details.
2. Transmission: understand how it is transmitted and the 2 tick species mentioned.
3. Pathogenesis: understand the specified ways it can cause disease (including the role of schizont-laden macrophages).
4. Clinical signs: understand the specified clinical / laboratory findings
5. Diagnosis: understand how to diagnose cytauxzoonosis
6. Treatment: understand how to treat and what the survival rate is.
7. Epidemiology: understand where this disease is most prevalent and what the risk factors are.

"FYI" = won't be tested on

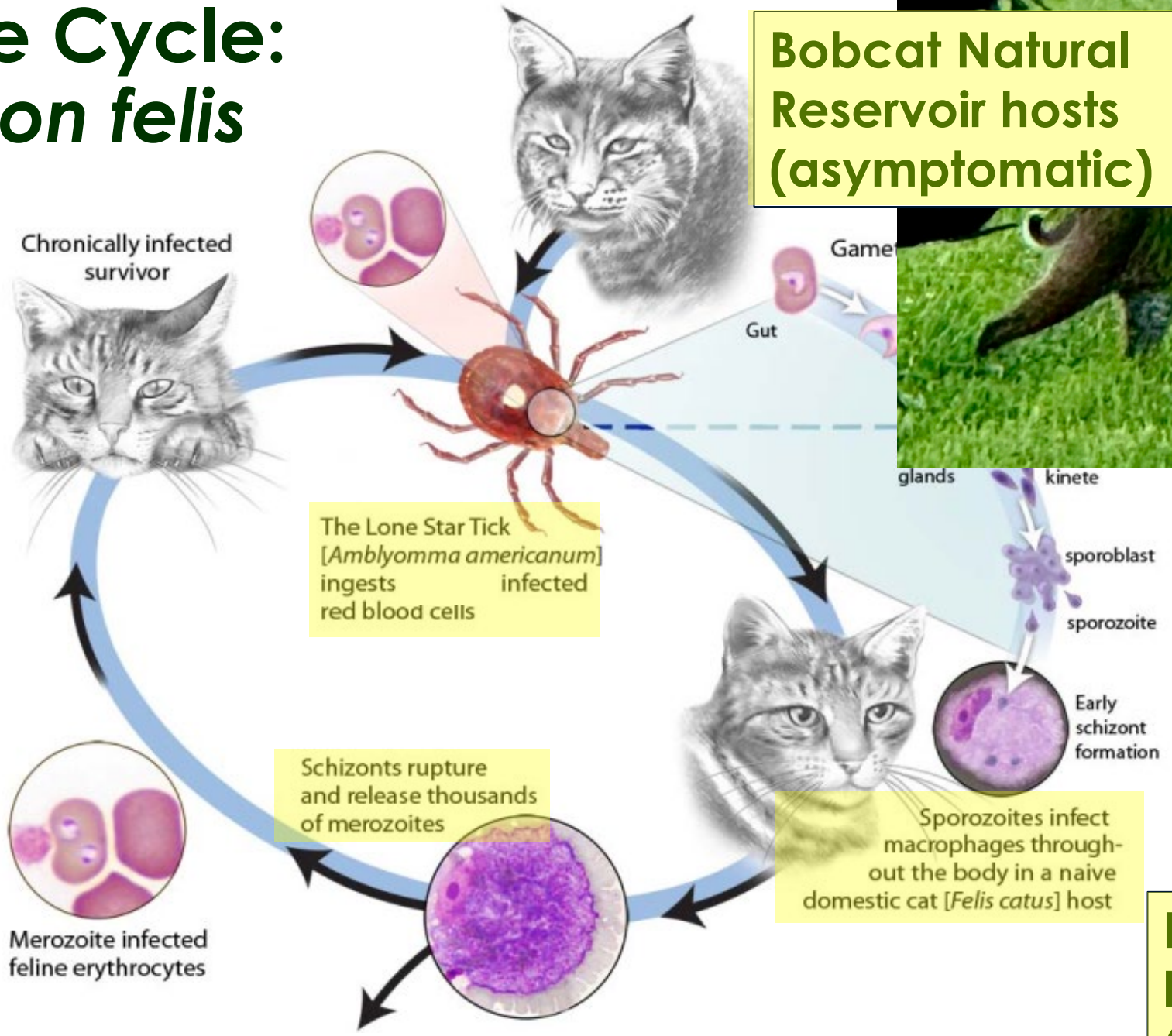
- Whirley is 14-month-old FS DSH cat who lives in NC, is primarily indoors but is allowed to go outside during the day.
- 7-day history of lethargy, fever and vomiting
- She presents to the NCSU VTH ER recumbent

- An engorged tick was attached
- CBC showed marked pancytopenia (anemia, neutropenia, thrombocytopenia)

Feline Cytauxzoonosis

Indirect Life Cycle: *Cytauxzoon felis*

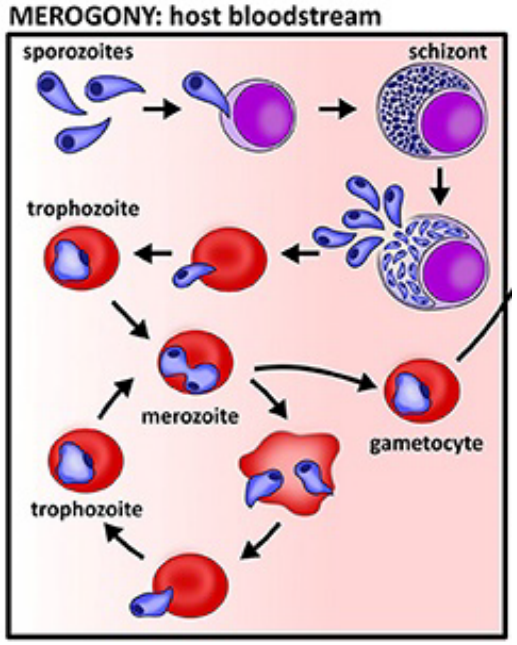
Know the highlighted information



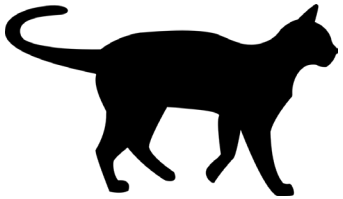
Bobcat Natural Reservoir hosts (asymptomatic)

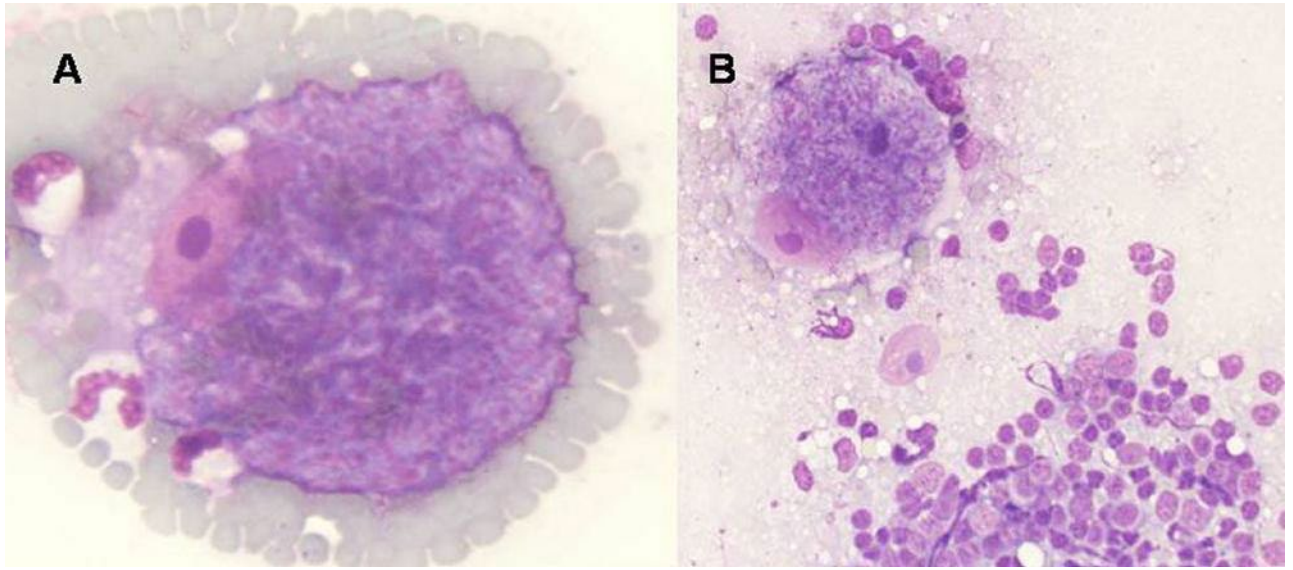
Domestic cat host (symptomatic)

Indirect Life Cycle: *Cytauxzoon felis*

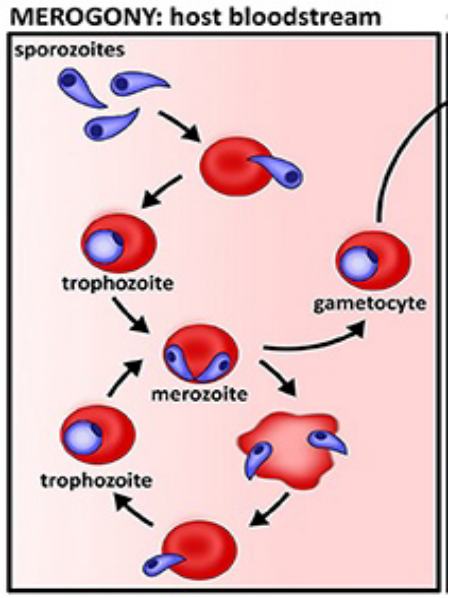


Cytauxzoon **in the cat** **<u>WBCs and RBCs</u>**





Schizonts inside monocytes at feathered edge of a blood smear



Babesia **only in the dog in RBCs**

Schizonts infect monocytes and macrophages

Transmission of *Cytauxzoon felis*

Amblyomma americanum

Dermacentor variabilis
(less common)

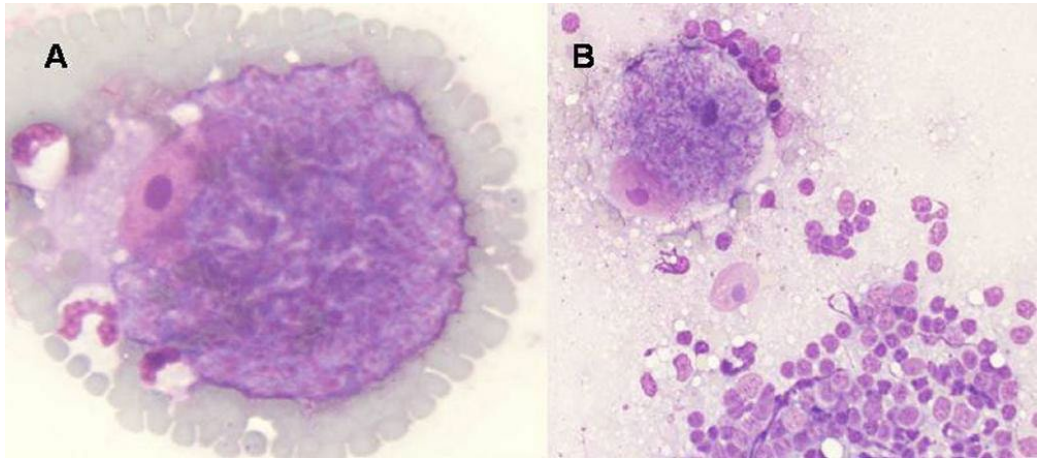
Cytauxzoon felis Pathogensis

1. Direct destruction of erythrocytes (minimal disease)

2. Widespread dissemination of schizont-laden macrophages → become lodged in vessels → multi-organ failure and death

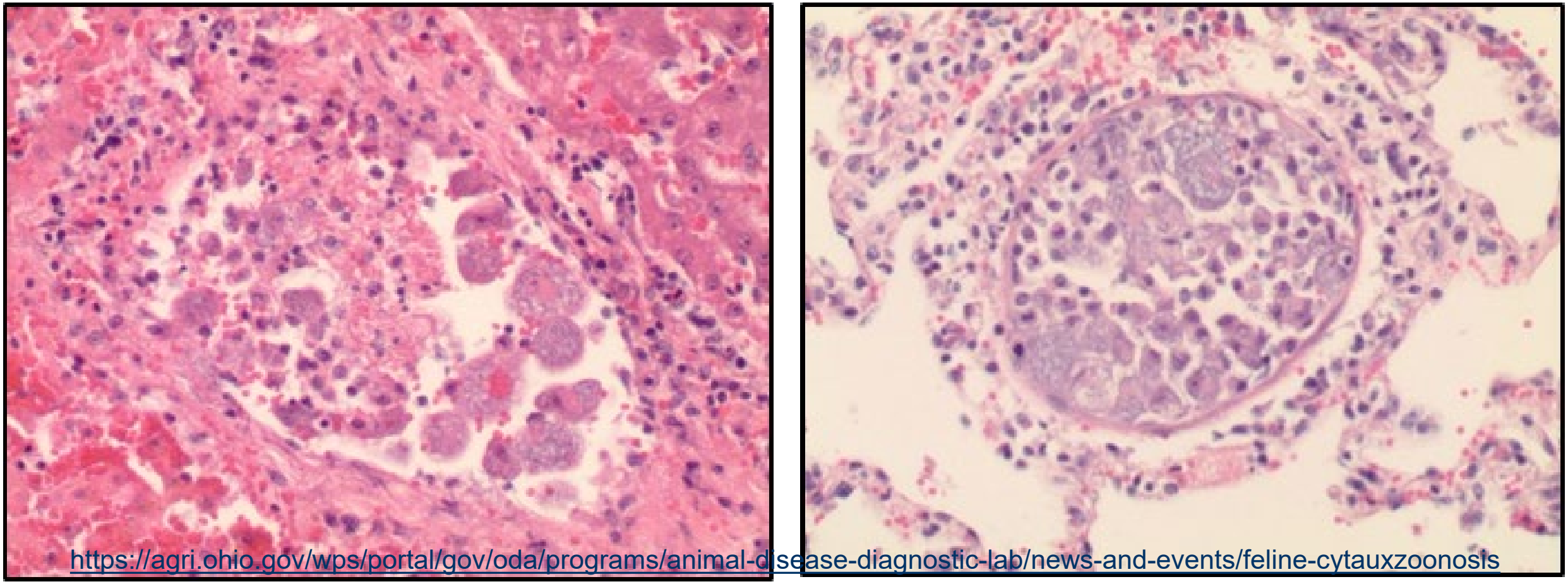
3. Inflammation → strong inflammatory response

- Most of the clinical signs and death are due to the schizogenous phase



- Infection of cats with merozoites stages in RBCs only results in minimal disease

Schizont-laden Macrophages in vessels



https://agri.ohio.gov/wps/portal/gov/oda/programs/animal-disease-diagnostic-lab/news-and-events/feline-cytauxzoonosis

Macrophages laden with *C. felis* schizonts in vessel lumens of a section of liver (left) and lung (right) from a 6-year-old DSH cat that died from this infection.

Cytauxzoonosis: Clinical Signs/History

- Acute febrile disease
- Access to outdoors
- History of ticks
- Lethargy
- Dyspnea
- Neurologic disease
- Jaundice / icteric

FYI:
Some evidence for subclinical infections of domestic cats

Whirley is icteric

Whirley's CBC

Anemia: PCV: 17% (32 – 48)

Leukopenia: WBC: $3.3x10^3$ (4.28 – 14.3)

Thrombocytopenia: Plt: $60x10^3$ (198 – 434)

Neutropenia: Segs: 660/ul (2.773 – 6.975)

pancytopenia

C. felis: Laboratory Findings

Pancytopenia

CBC

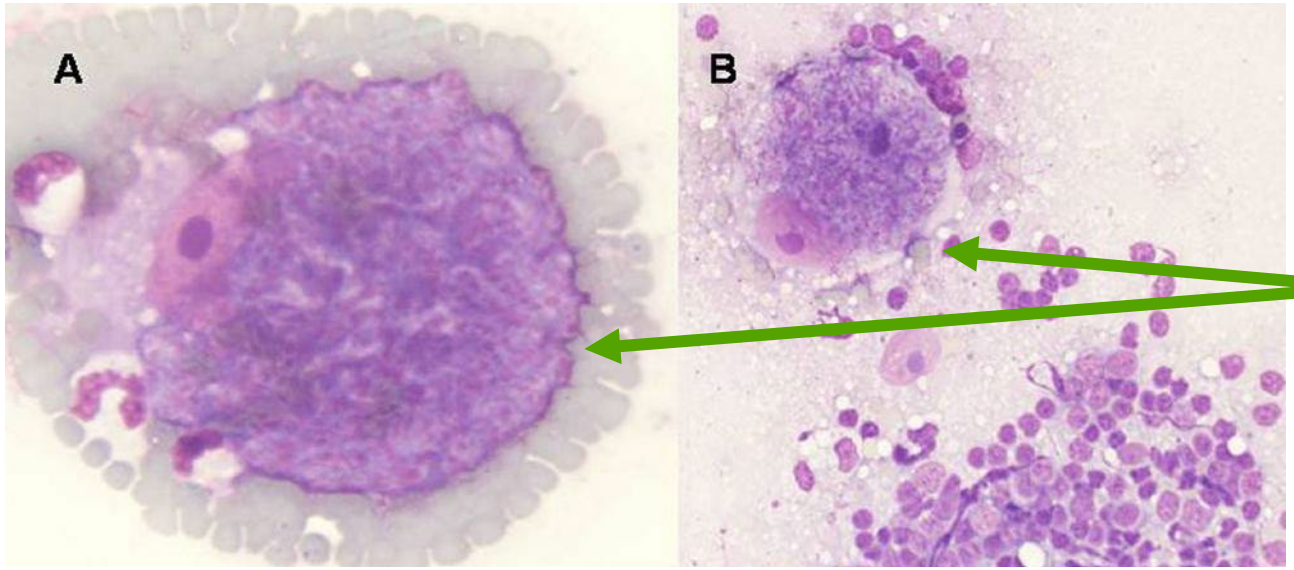
- Leukopenia
  - maybe leukocytosis
- Thrombocytopenia
- Anemia
- Organisms

Serum Biochemistry

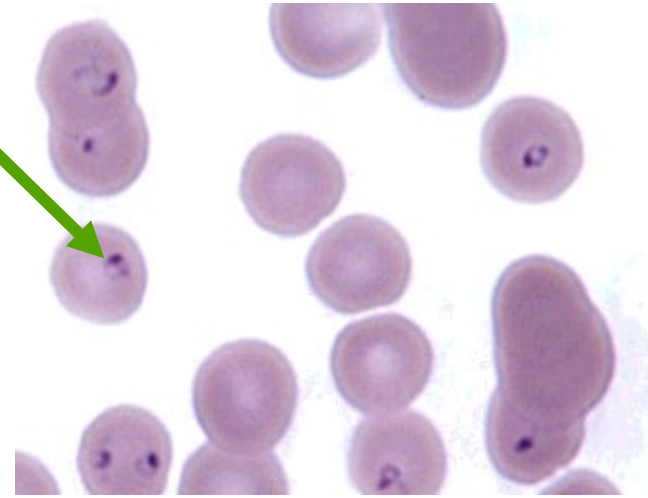
- Elevated liver enzymes
  - Not always severe
- **Hyperbilirubinemia**
- Hyperglycemia
- Hypoalbuminemia
- Azotemia (pre-renal)
- Electrolyte/acid-base disorders

C. felis: Diganostics

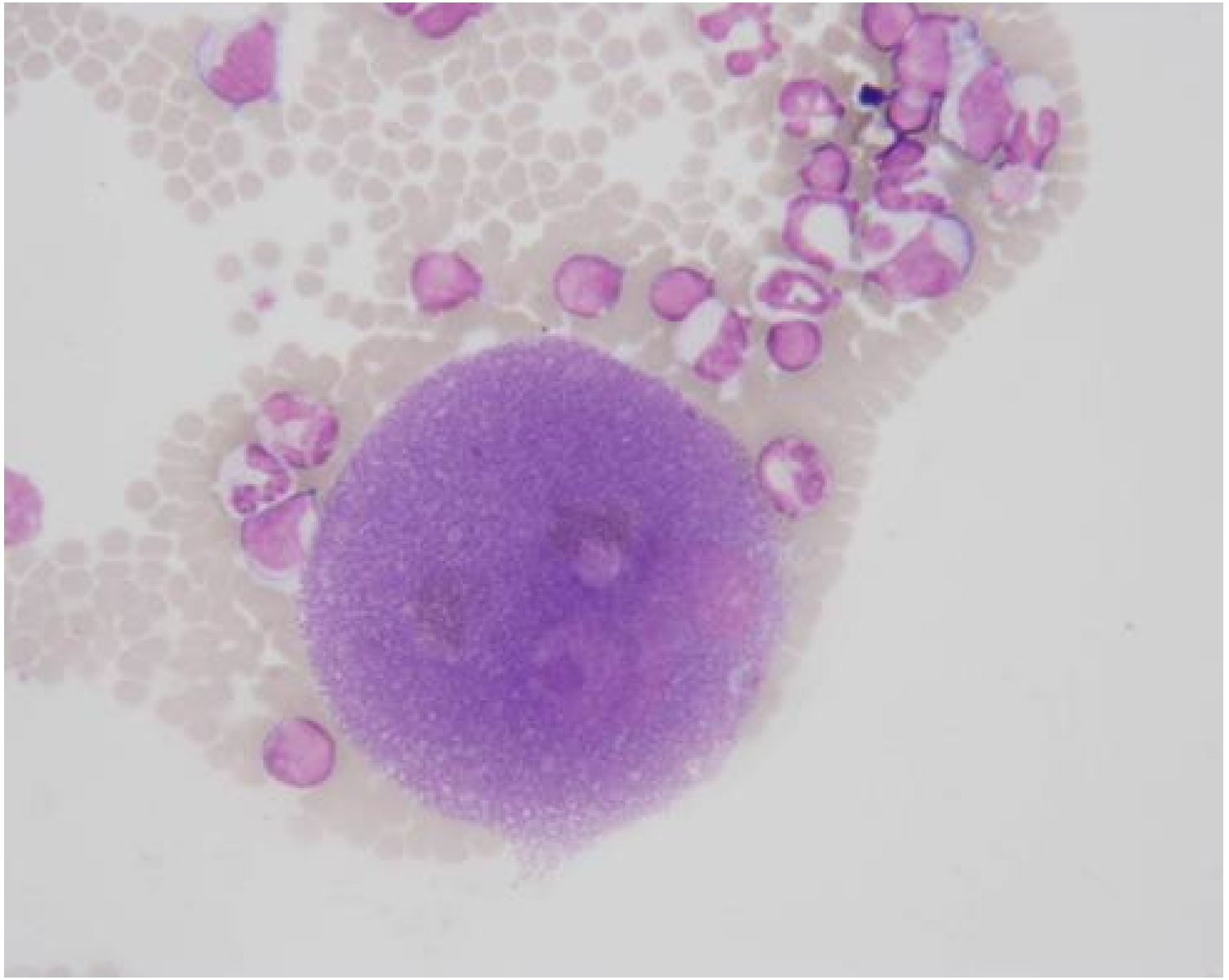
- **PCR (stat if available)**→ very sensitive
- NO serology tests
- **Blood smear** to look for organisms (not very sensitive but may get immediate diagnosis)



Piroplasms and/or Schizonts
(feathered edge of blood smear)

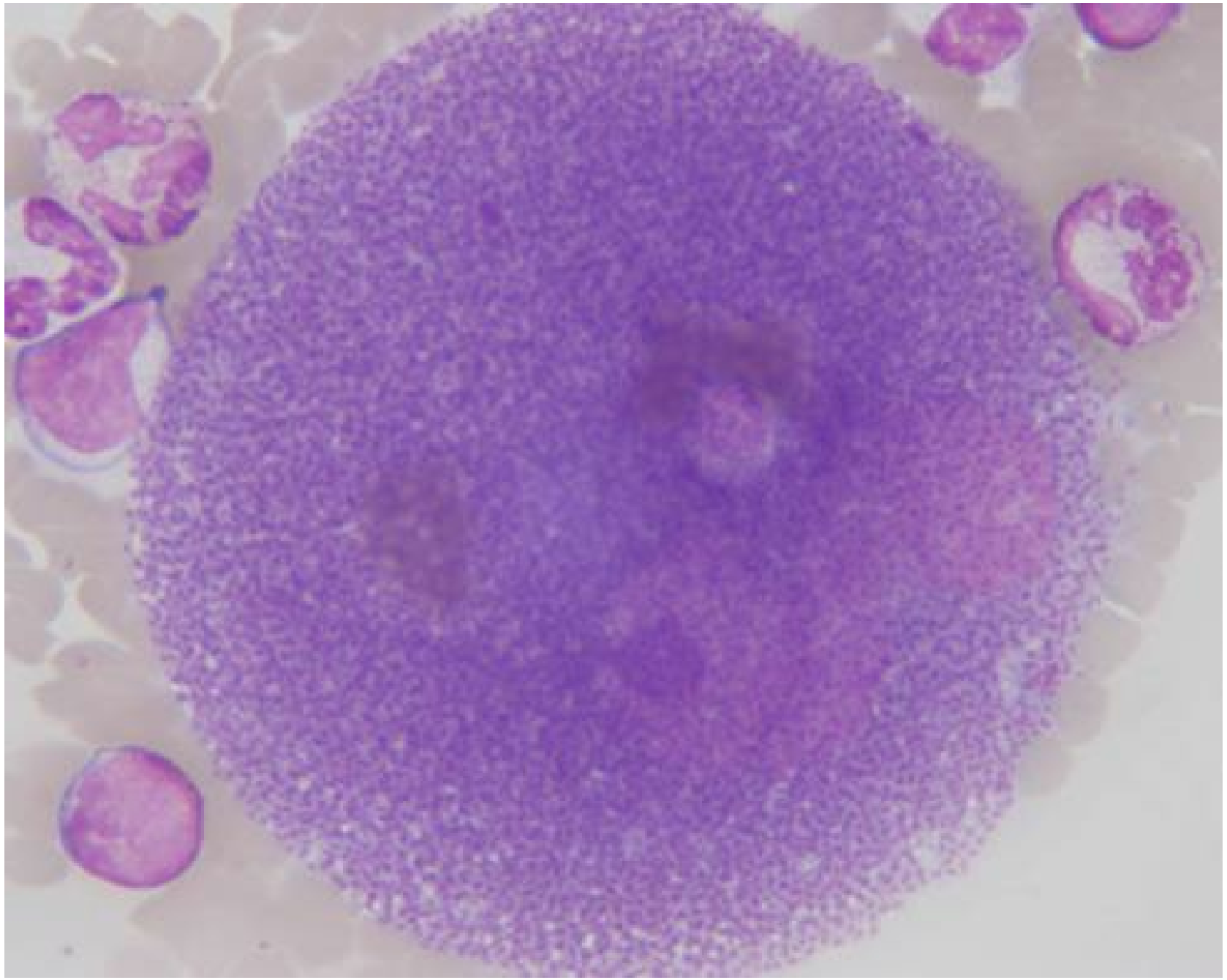


Are these clumped platelets on blood smear exam?



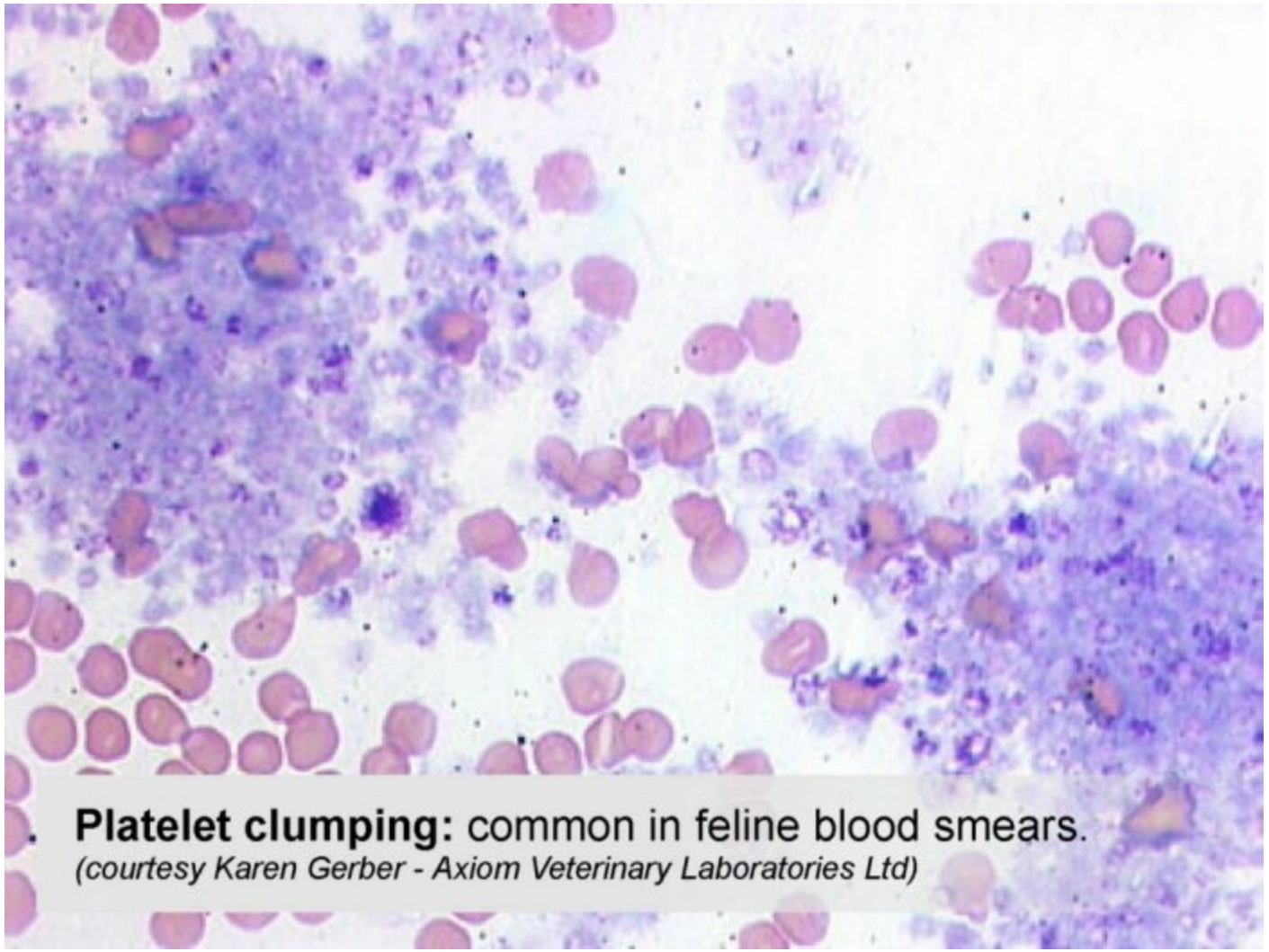
Closer examination…

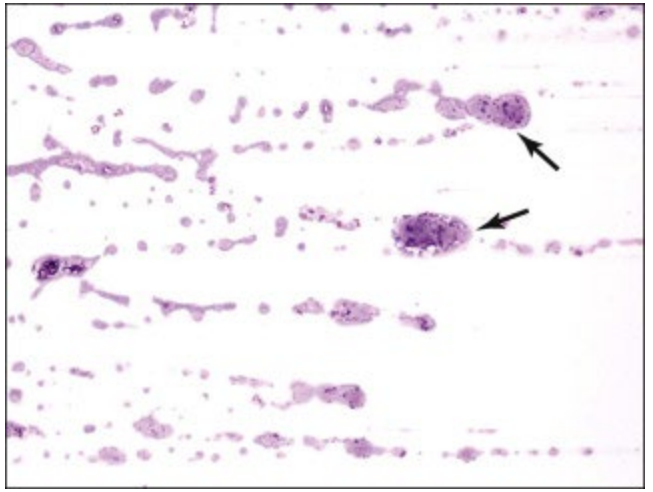
this is a schizont!

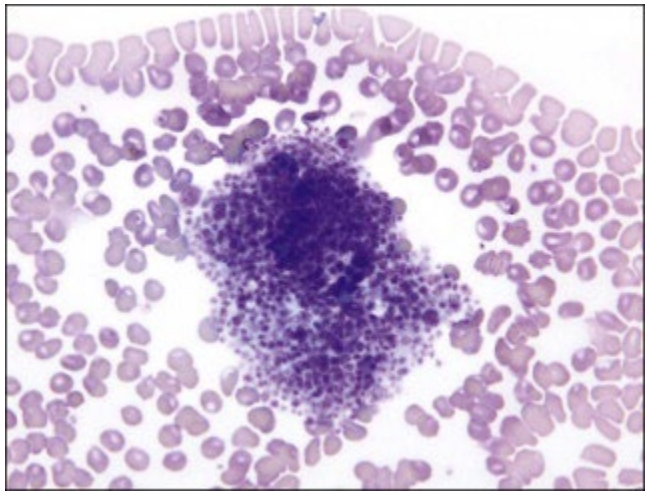


Schizonts
(cell full of merozoites

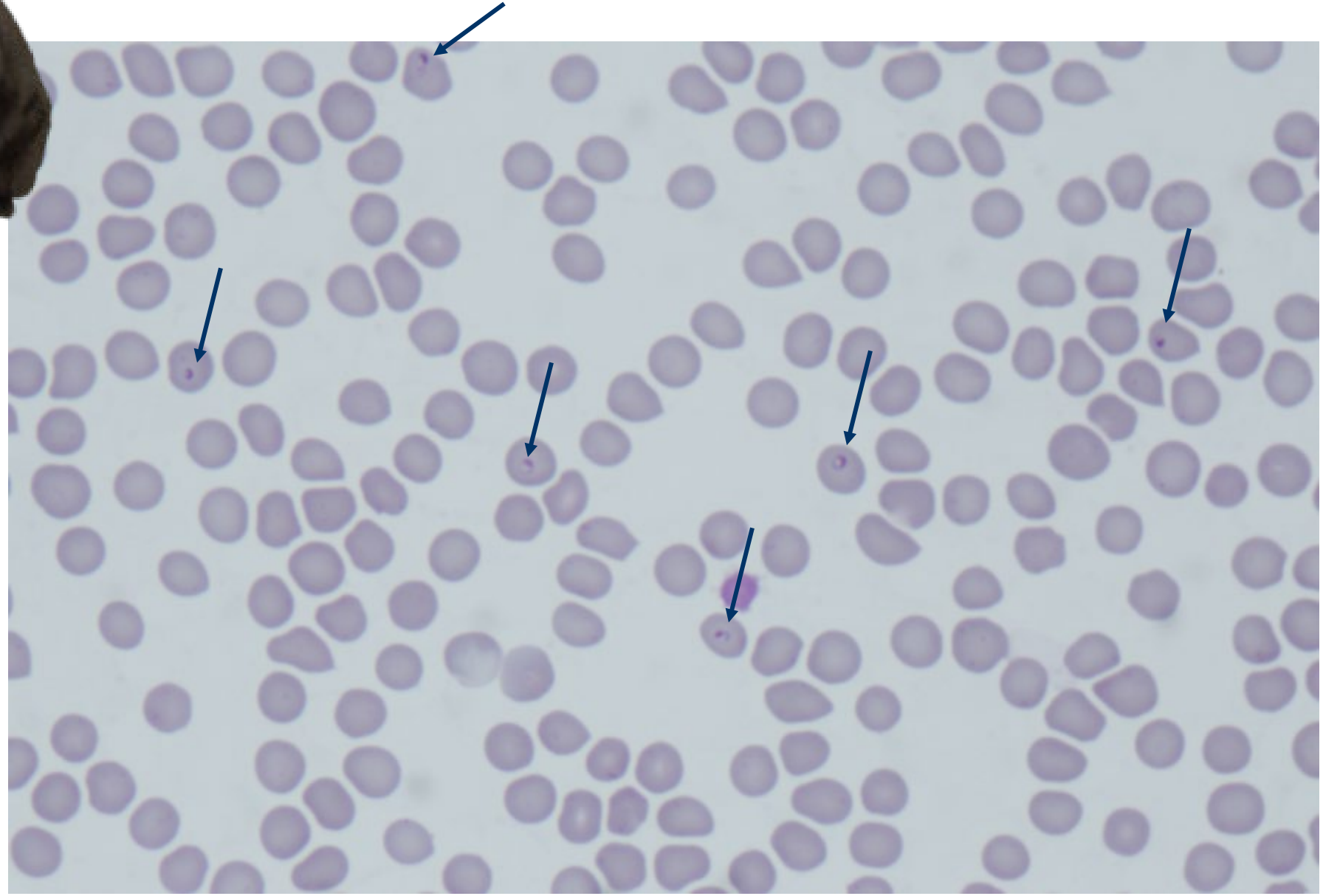
These are clumped platelets







You may also see piroplasms in RBCs



Course of events following *C. felis* infection of cats

Entire process is very quick

- Signs begin 12-15 days after infection
- Progress from ADR → coma in days
- Tissue schizonts accompany the illness
- Piroplasms may or may not be evident
- Death within 5 days of clinical signs is typical

Without treatment, almost all cats die from cytauxzoonosis.

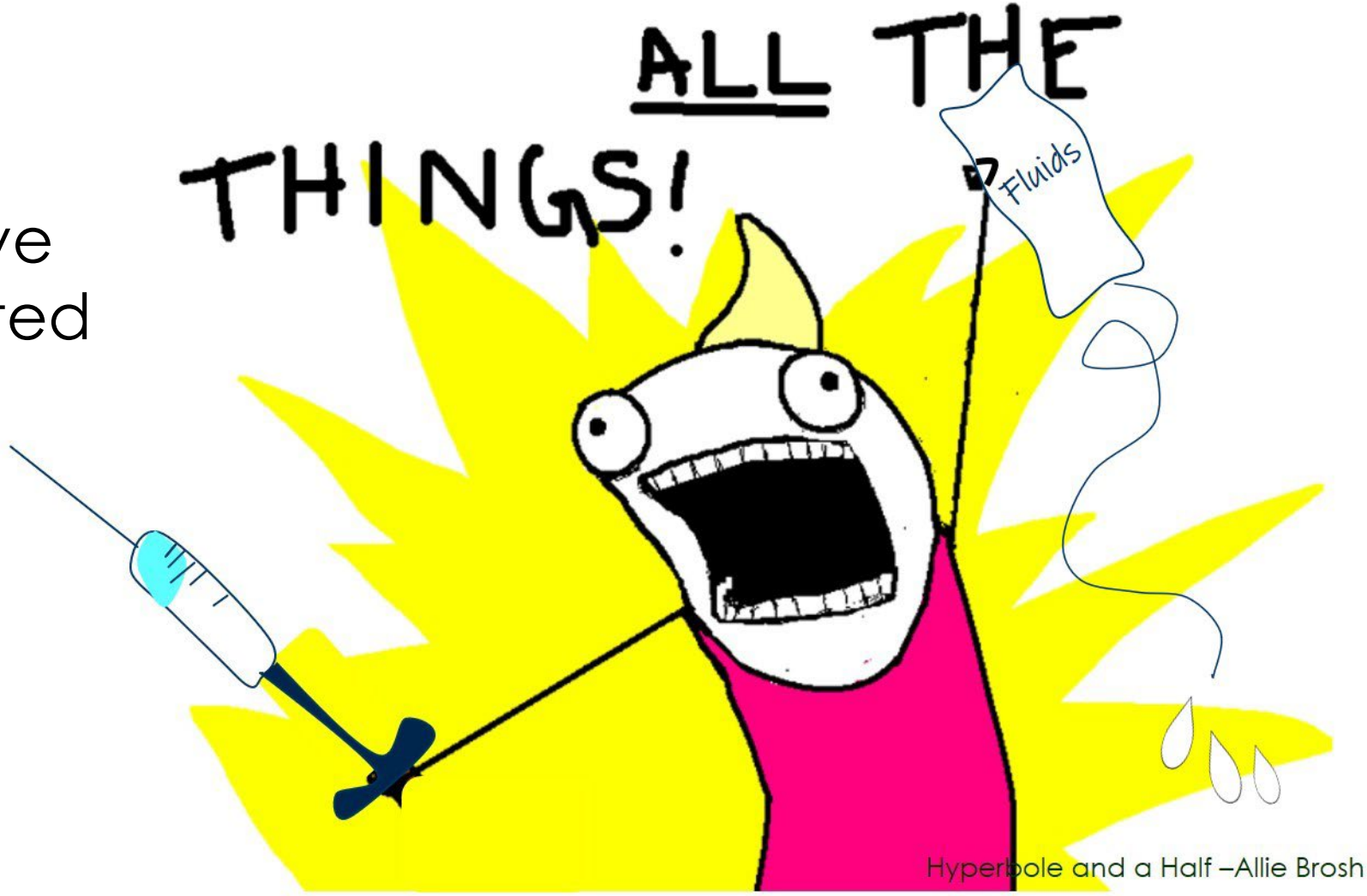
Cytauxzoonosis Treatment

Atovaquone and Azithromycin

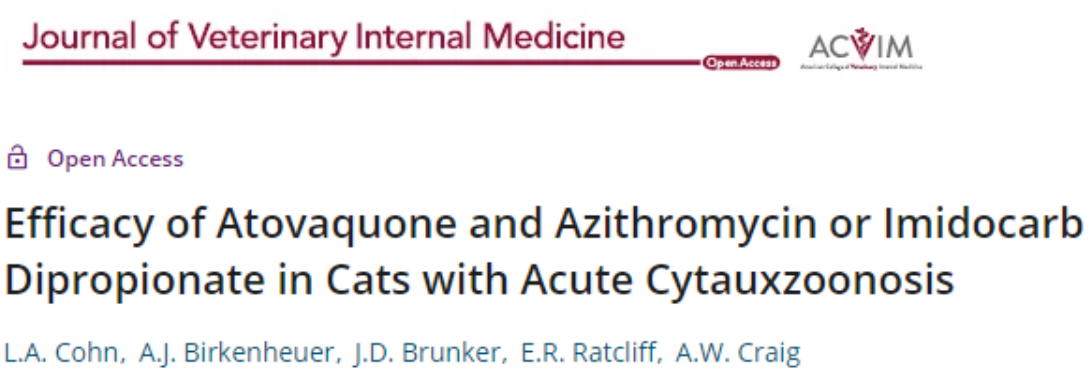
- **~60% survival**
- Demonstrated to have efficacy against related protozoan parasites

Avoid all of it with **TICK PREVENTION**

Supportive Care



Atovaquone /Azithromycin Full-scale Trial (FYI)



- An open-label, randomized prospective study compared survival in cats treated with atovaquone (15 mg/kg PO q 8 hrs) and azithromycin (10 mg/kg PO q 24 hrs) or imidocarb (3.5 mg/kg sc)

- 80 acutely ill cats with confirmed *Cytauxzoon felis*

Atovaquone /Azithromycin Full-scale Trial (FYI)

- 32 of 54 cats (**60.5%**) treated with A&A survived
- 7 of 27 cats (26%) treated with imidocarb survived

- Clinical data was not available for most cases
- NCSU cats were VERY VERY VERY SICK!!!
- ICU patients, needed oxygen therapy, treatment for shock, etc.
- **Clinical recoveries were NOT rapid**, patients slowly recovered after 4-7 days of intensive care
- Mild hemolytic anemia during second week

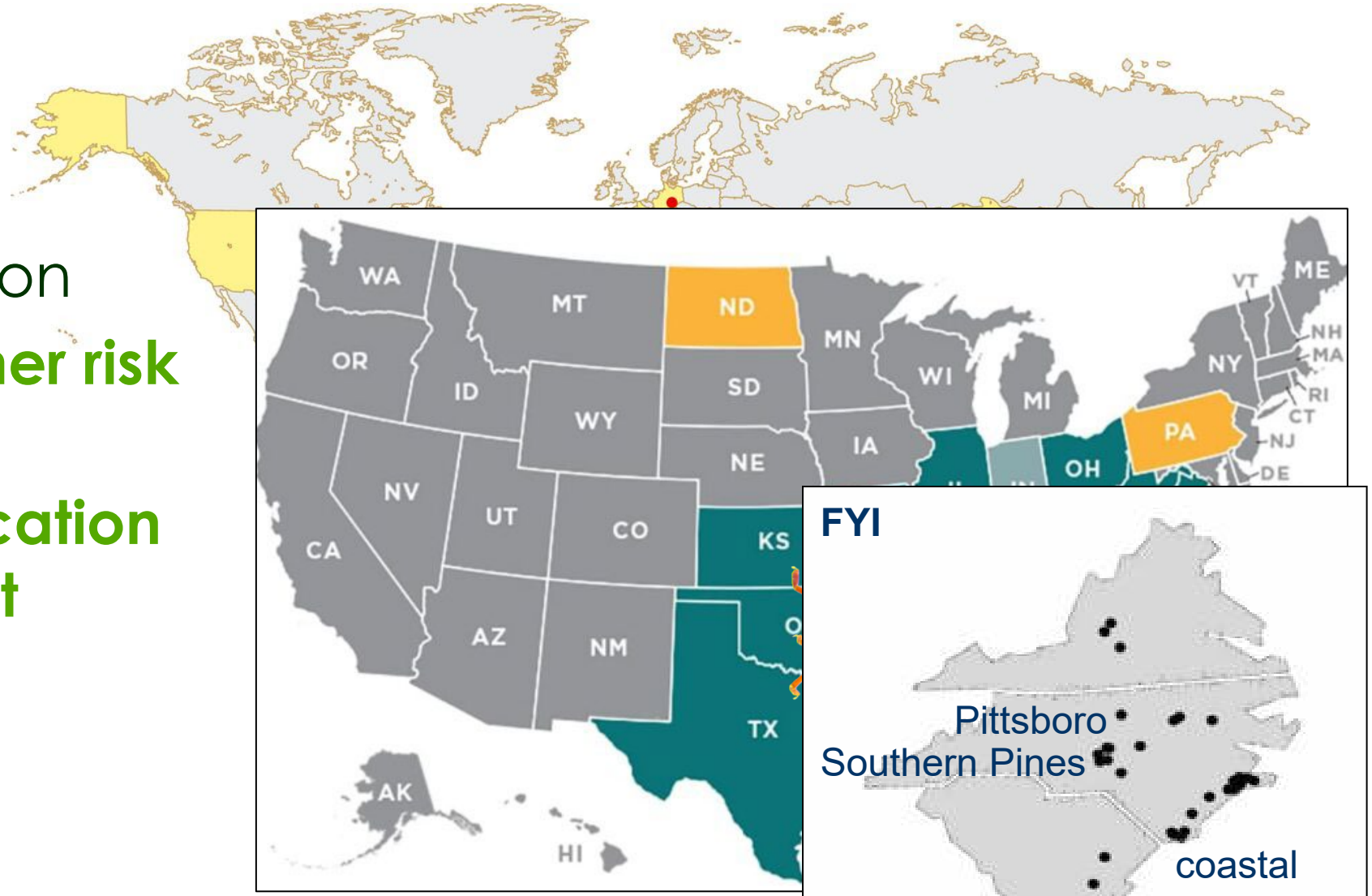
Atovaquone /Azithromycin Full-scale Trial (FYI)

But….

- By 10-14 days after discharge, cats COMPLETELY normal
- Complete resolution of all abnormalities during follow-ups
- Detection of parasites post-treatment was variable
- Survivors continued to do well 5 years post-treatment

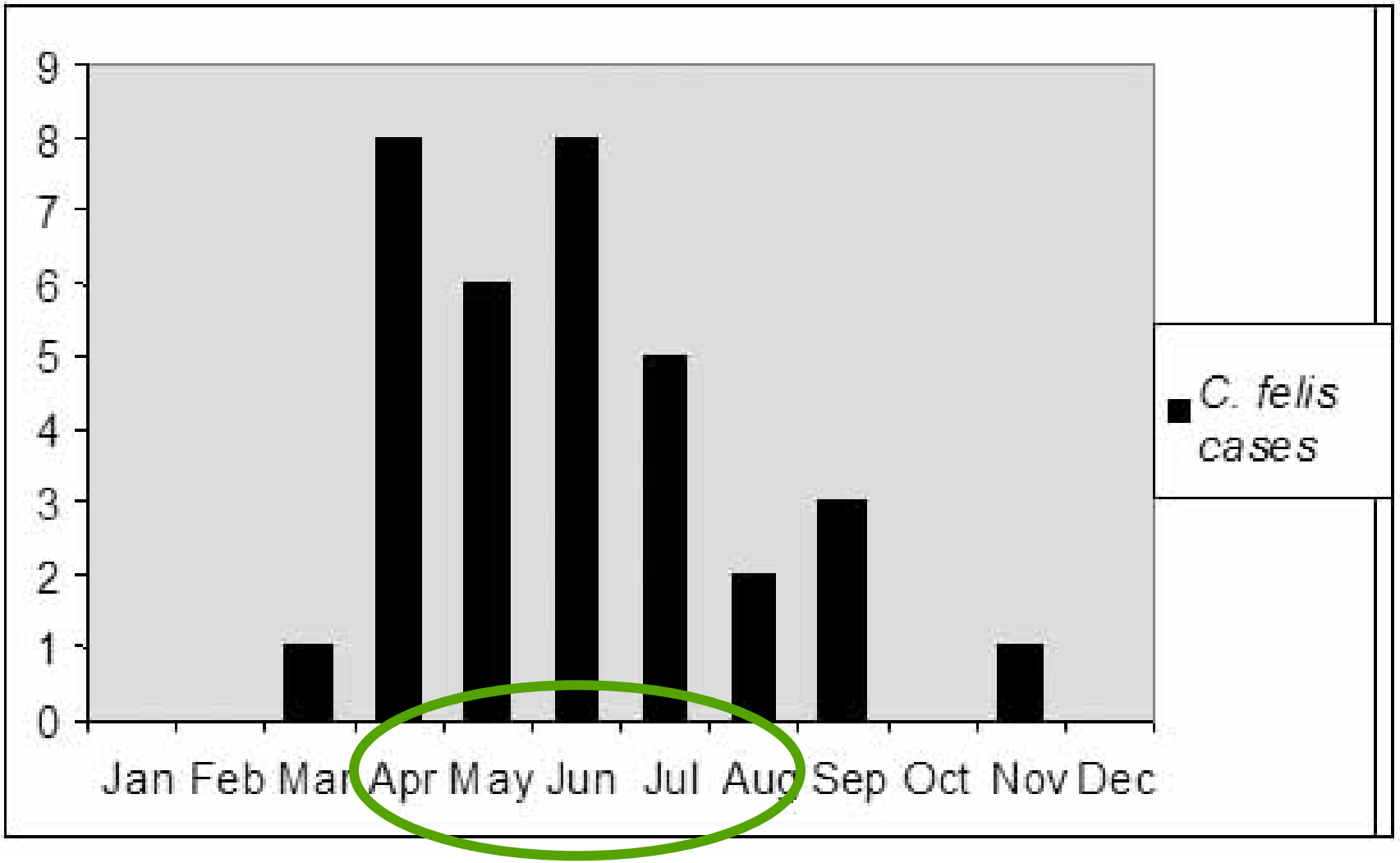
Cytauxzoon felis Epidemiology

- Not Zoonotic
- World-wide distribution
- **Outdoor cats at higher risk for disease**
- **US Geographical location = Southeast, Midwest**



Birkenheuer et al. *JAVMA* 2006;228(4)
Shock et al. *Vet Parasitol*, 2011;175(3)

Cytauxzoon felis Seasonal Distribution



Highest prevalence in spring and summer

Cytauxzoon felis Prevention

Acaricides (without permethrin)

Preventatives tested against *A. americanum*

Seresto (imidacloprid and flumethrin collar)

Revolution Plus (selamectin and sarolaner topical solution)

Other tick preventatives probably effective

Cytauxzoon Take Home Points

- Acute Febrile Illness
- Outdoor access
- Pancytopenia
- Hyperbilirubinemia
- Blood smear and stat PCR!
- Diagnose and treat quickly!
- 60+% Survival
- Tick-borne disease, Southeastern, Midwestern US

Discussion Questions

A dog (or cat) presents to you with a fever and multiple ticks attached.

What CBC abnormality is most common in tick-transmitted piroplasmids?

You can only run 1 tick-borne disease diagnostic test…which one would you choose?

Have Questions?

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