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South Florida
WILDLIFE CENTER

In partnership with The Humane Society of the United States

A Novel *Babesia* Species in the Virginia Opossum (*Didelphis virginiana)*

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Virginia Opossum

Common in N. American rehabilitation centers

- SFWC numbers – 2015 = 1,507 2016 = 1,757 2017 = 1,550

Prolific breeder – in S. Florida breed bi-annually

Altricial young born 13 days post-conception

On average 7-9 survive early post-natal period

Wean around 95 days post-partum, disperse over next 3 months

Virginia Opossum

Short life span – fewer than 10% survive first year

Life span = 2-4 years

Opportunistic omnivores

Adapt easily to urban and semi-urban environments

Lives in close proximity to humans and domestic animals

Encounters with humans are common

Virginia Opossum

- Reasons for presentation:
  - Trauma –
    - HBC
    - Animal bites
    - Traps
  - Parasites –
    - GI parasites – *Physaloptera (Turgida) turgida, Gnathastoma didelphis, Cruzia americana*
    - Pulmonary parasites – *Didelphostrongylus hayesi, Capillaria didelphis*
    - Protozoal parasites – *Sarcocystis* spp., *Besnoitia darlingi*

Virginia Opossum

- Reasons for presentation:
  - Orphaned
  - Bacterial infections/ sepsis
    - mastitis
    - Atypical mycobacteria
  - Opportunistic fungal infections
  - General debilitation – geriatric, other
  - Other infectious disease?

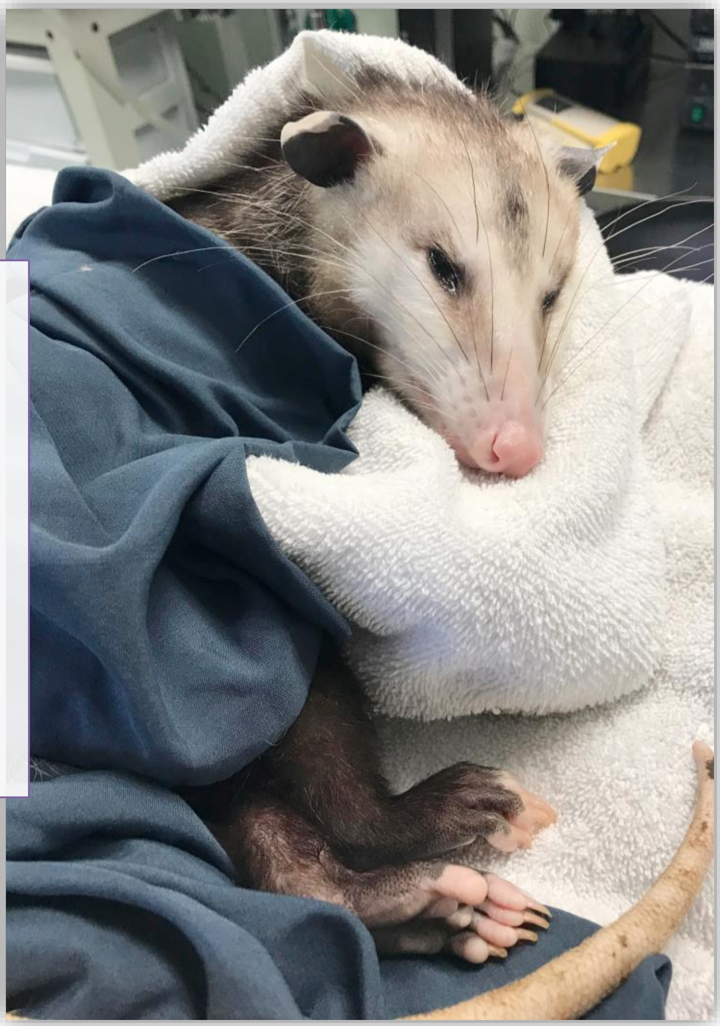
Case #1

- Female opossum, older; wounds on tail, hair loss
- Occasional head twitch and circling, emaciated
- Treated with antibiotics (TMS and Cipro), anti-inflammatory (Meloxicam) and topical wound care (Silver-Elta)
- Improved somewhat – but continued to be ataxic.

Case #1

Worsening anemia :

- 2/10 – PCV = 20% (chemistry nsf)
- 2/15- PCV = 16% - odd-looking blood smear w/ RBC agglutination
- 2/22 – HCT = 10%



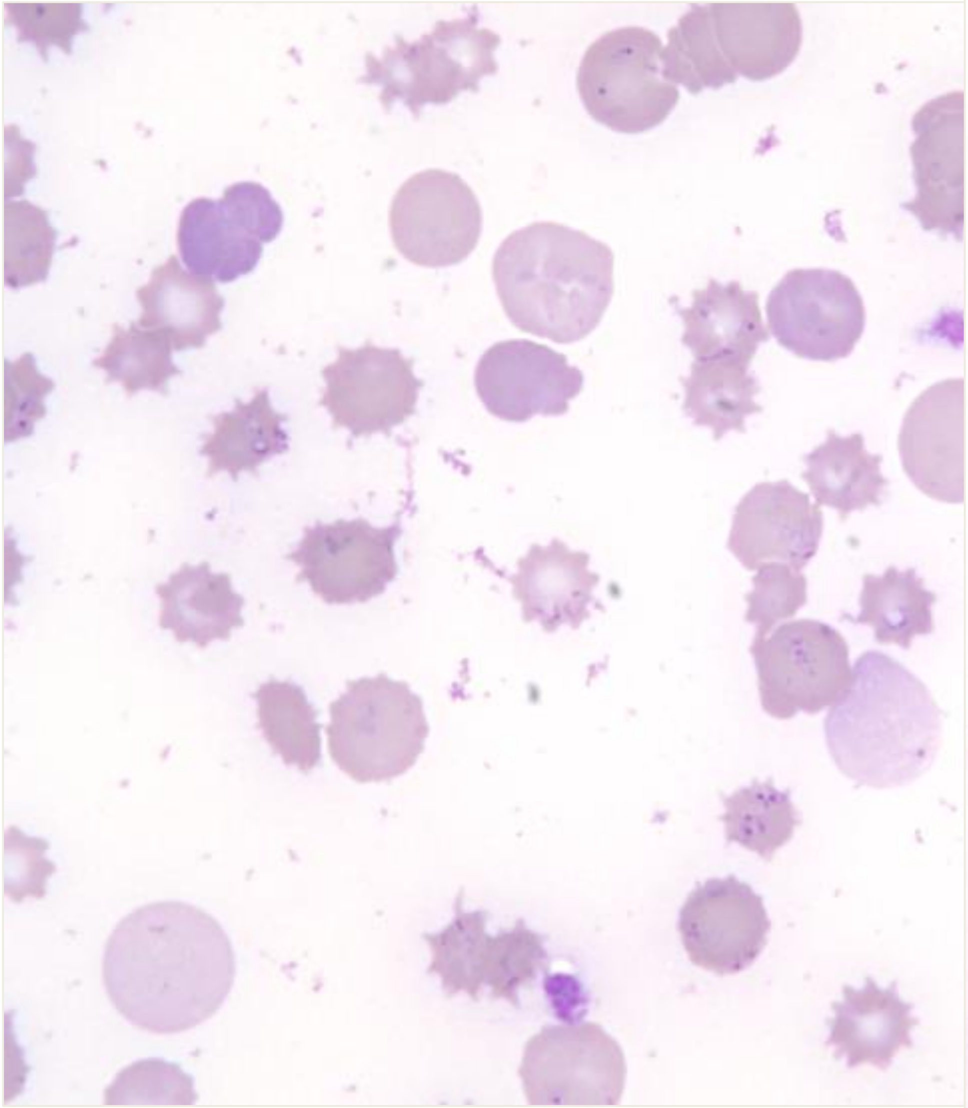
Case #1

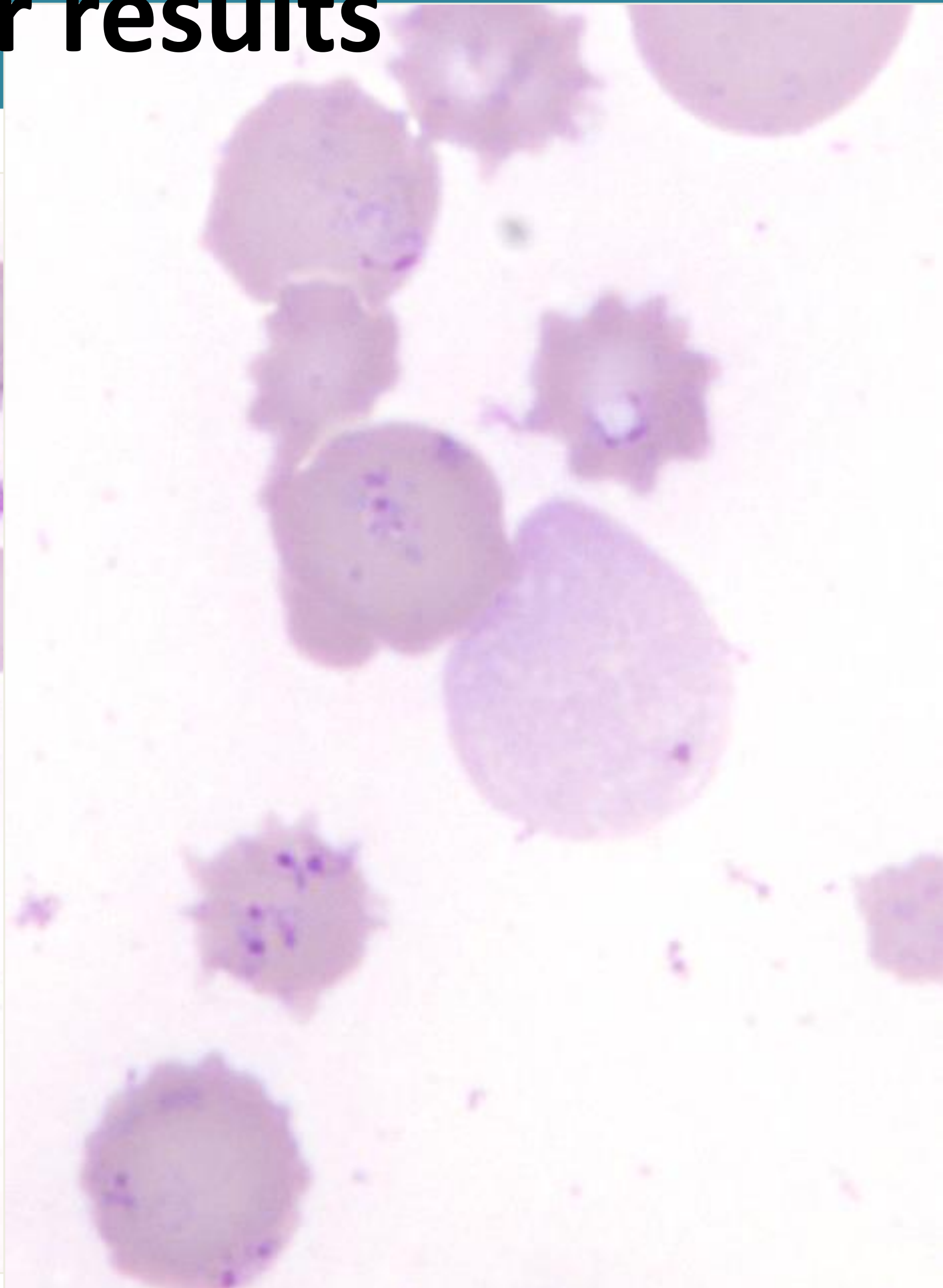
- Necropsy revealed Besnoitia lesions on liver, kidney and lungs; nodular lesions on lung; GI parasites
- Bone marrow aspirates collected right humerus and right illiac crest

CBC results

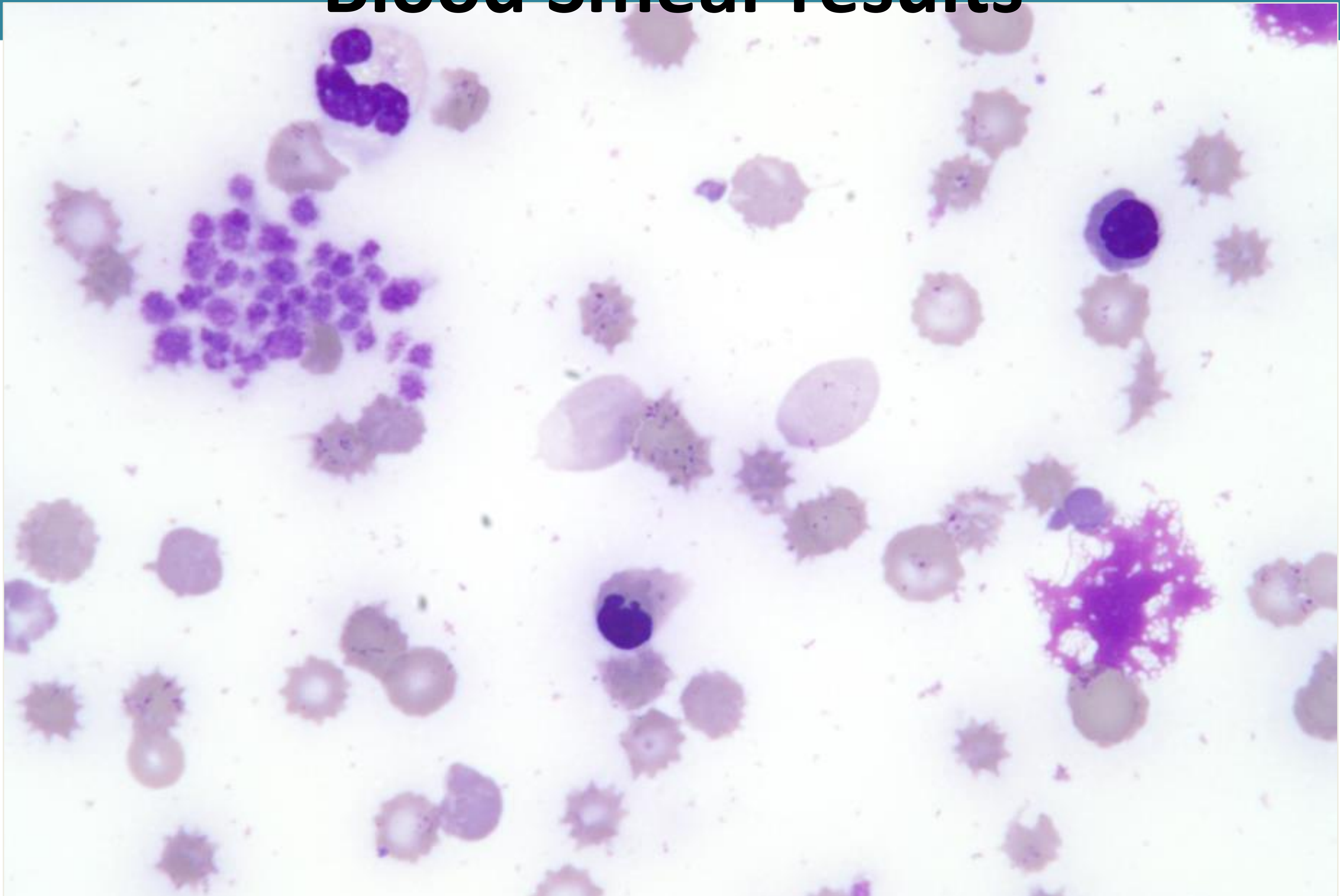
- Leukocytosis (WBC = 38,0000)
- Multiple red blood cell abnormalities
  - Anisocytosis, polychromasia, hypochromasia
  - Echinocytes, schistocytes, keratocytes, ghost cells
- Intra-erythrocytic *Babesia* seen

Blood Smear results

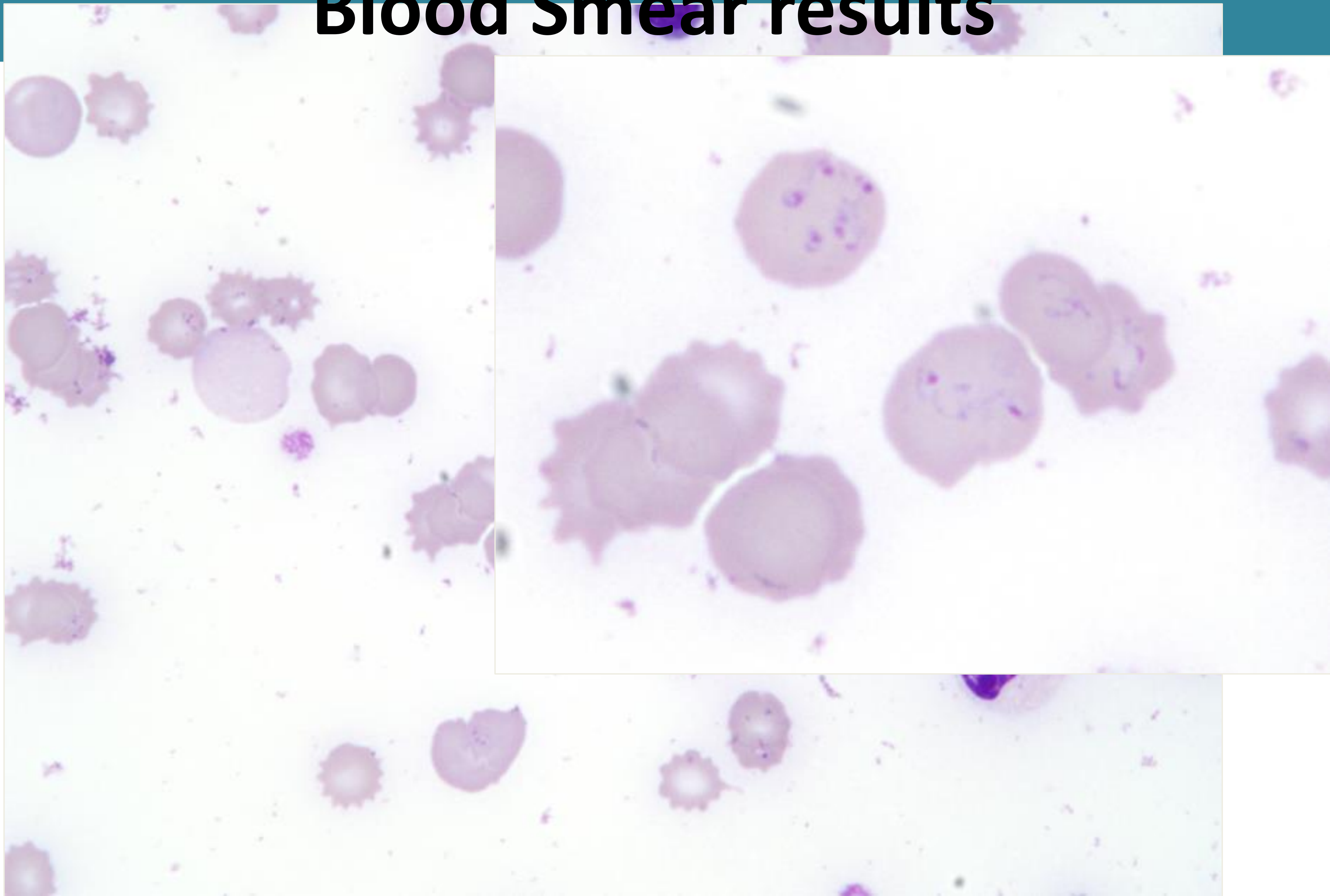




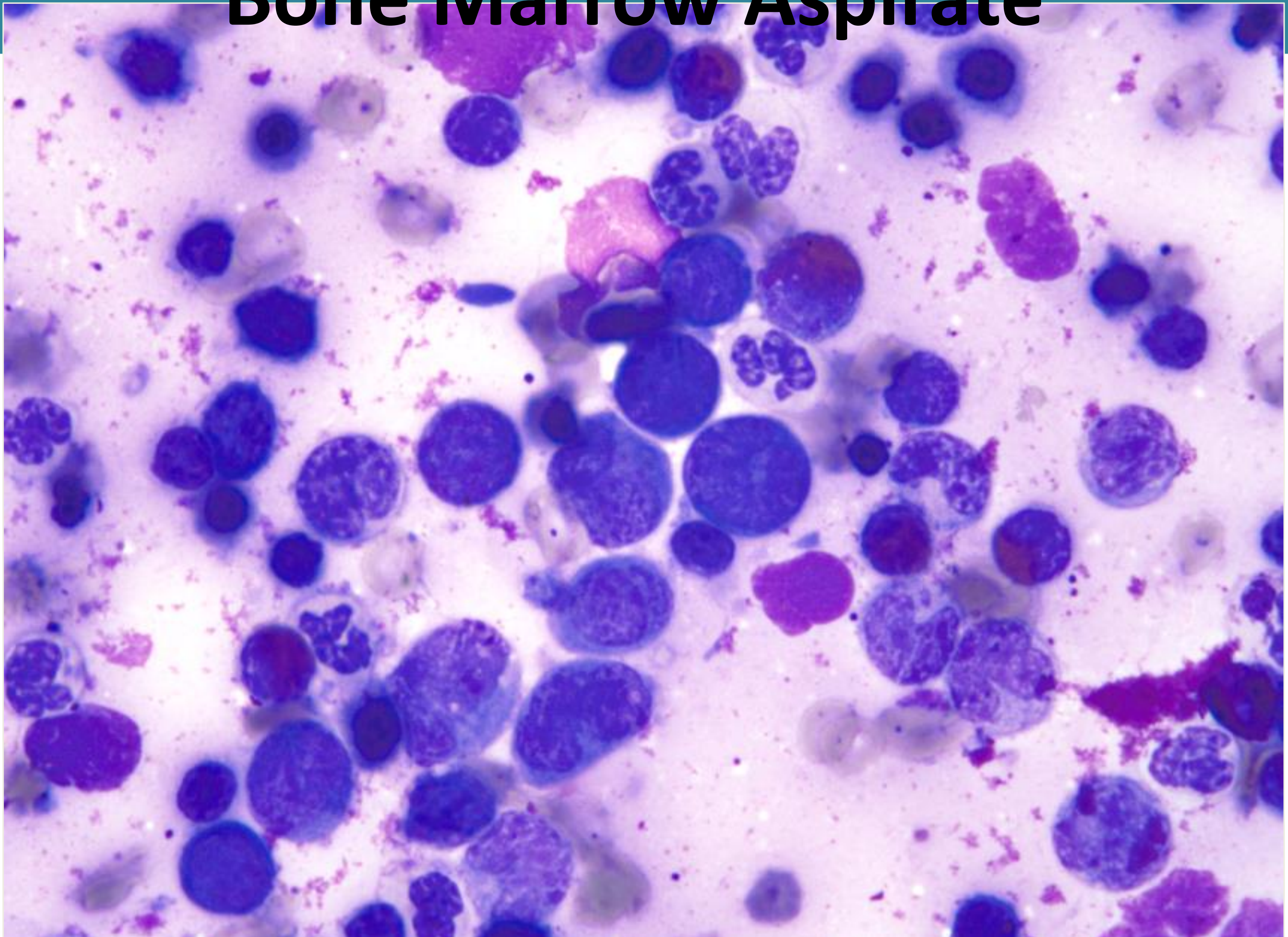
Blood Smear results



Blood Smear results



Bone Marrow Aspirate



Case #1

Due to RBC inclusions consensus PCR w/ sequencing was run

Babesia DNA found – Sequence analysis revealed novel *Babesia*

Collected samples from other patients with a variety of presentations

Case #2

- Adult (geriatric?) male admitted ~ 3 months later
- Alert but recumbent; pale, emaciated, severe flea infestation, superficial abrasions
- Supportive care, treatment for anemia and wounds initiated
- Condition worsened over next two days

Case #2

PCV/ TS = 15%/ 6.7 mcg/dl

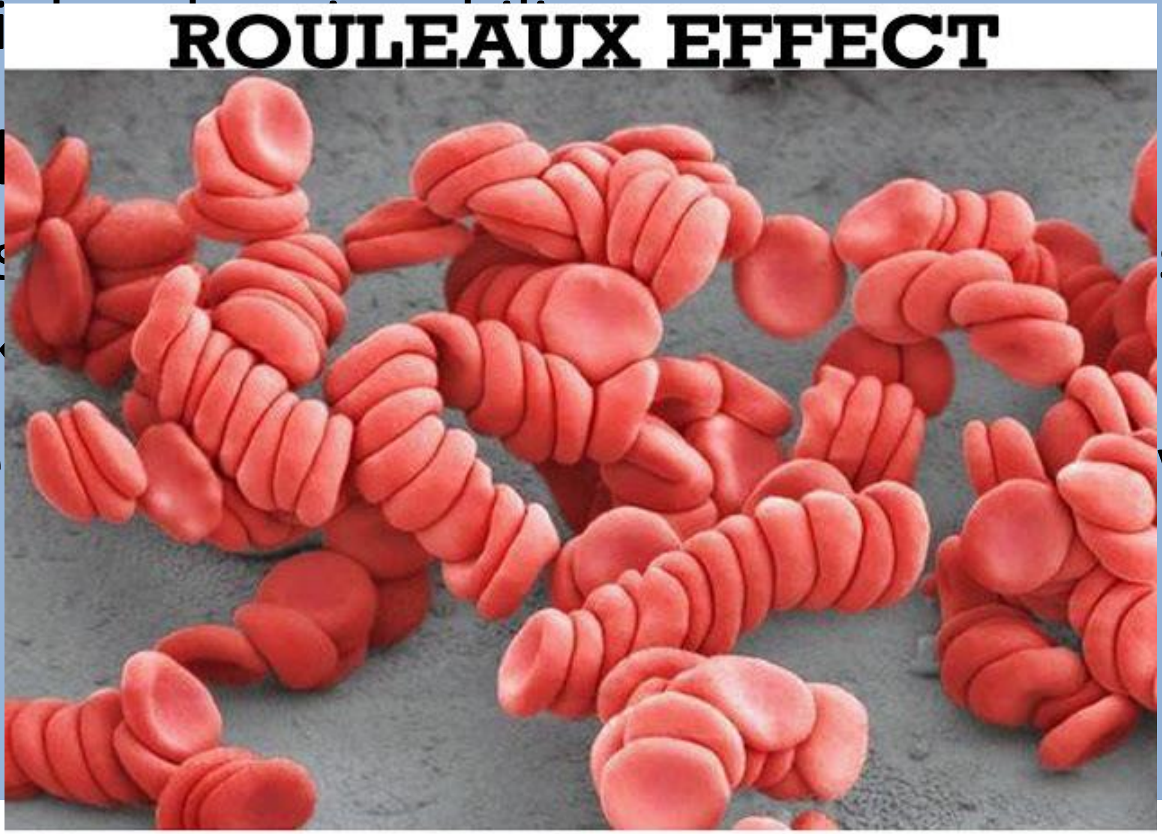
Bilateral eye issues noted, including *Besnoitia* cysts in anterior chamber

Multiple parasites found on fecal exam

- Euthanized due to poor prognosis
- Blood submitted for CBC, Babesia PCR

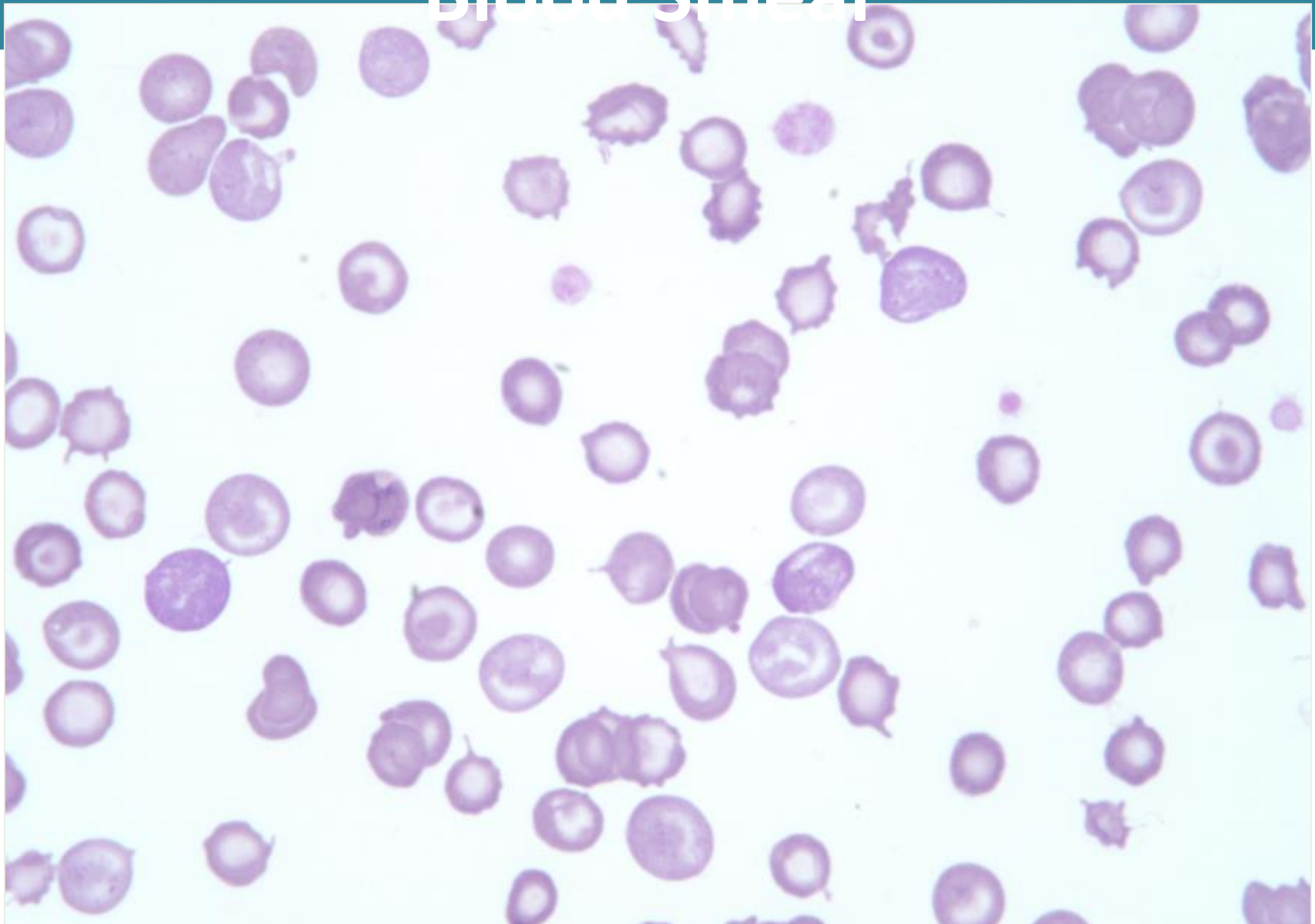
Case #2

- Leukocytosis – mild (WBC = 15,0000)
  - Peri
- Multi
  - Anis … sia,
    poik
- Roule … ved

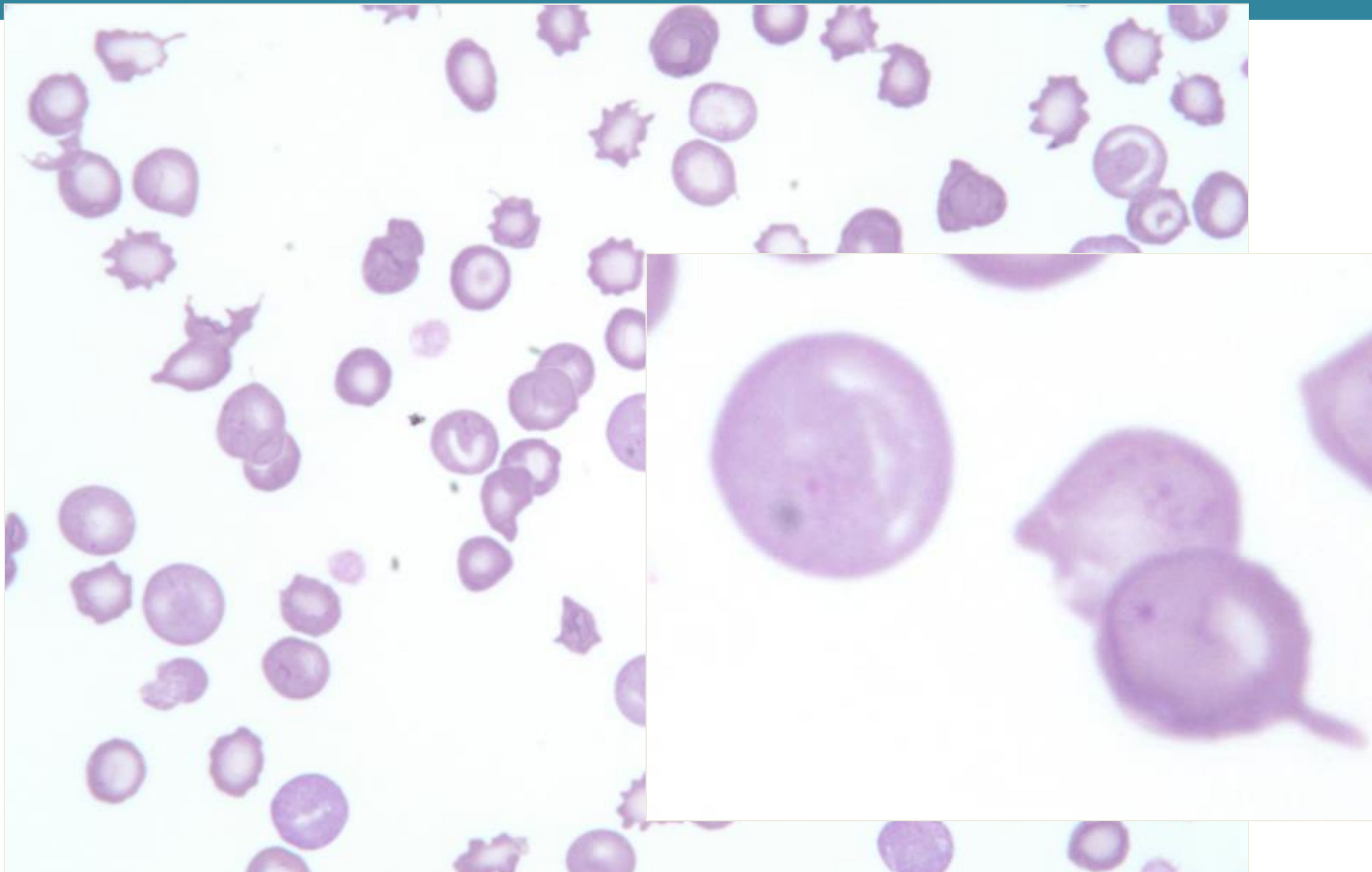


Stacking of Red Blood Cells

Blood Smear



Blood Smear



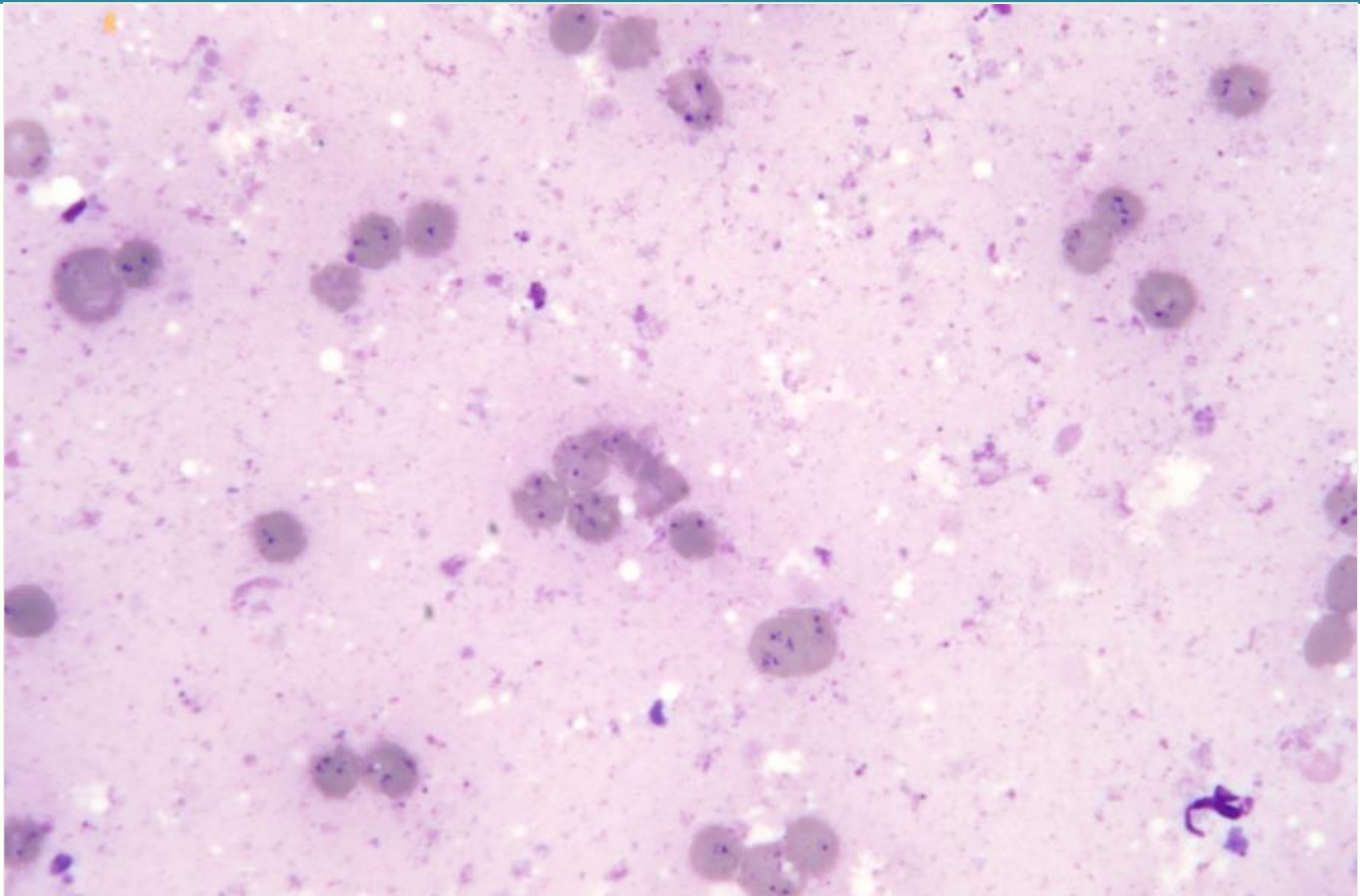
Case #3

- Adult opossum, slightly thin, icteric gums and skin
- Slightly thin w/ heavy flea infestation
- Given SQ fluids – died shortly after admission

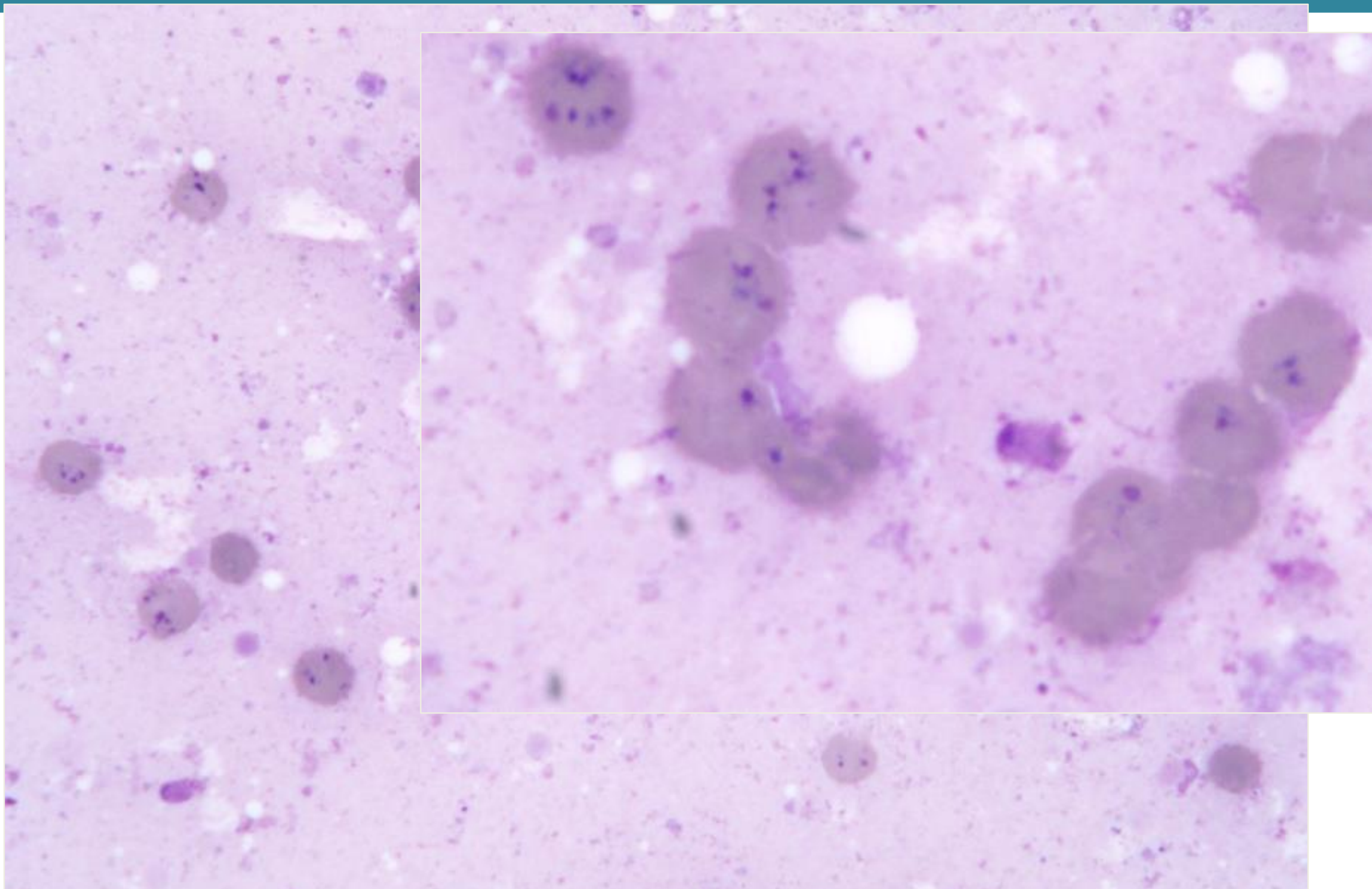
Case #3

- Enlarged spleen and liver, reddish urine noted on necropsy
- Liver and spleen tissue imprints taken, samples frozen
- Urine also submitted for leptospirosis PCR

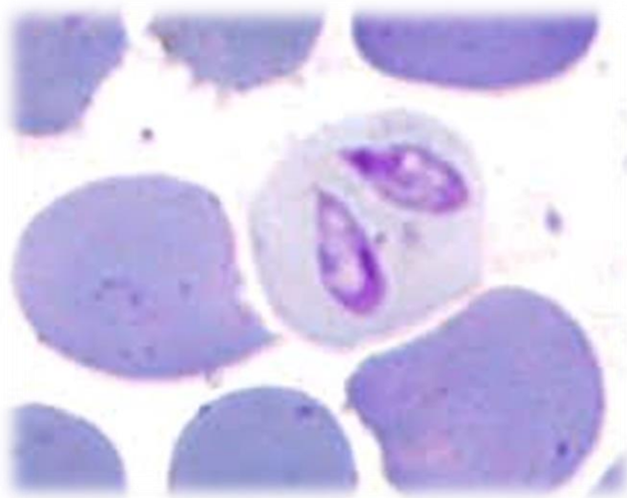
Liver Imprint



Liver Imprint



PCR results

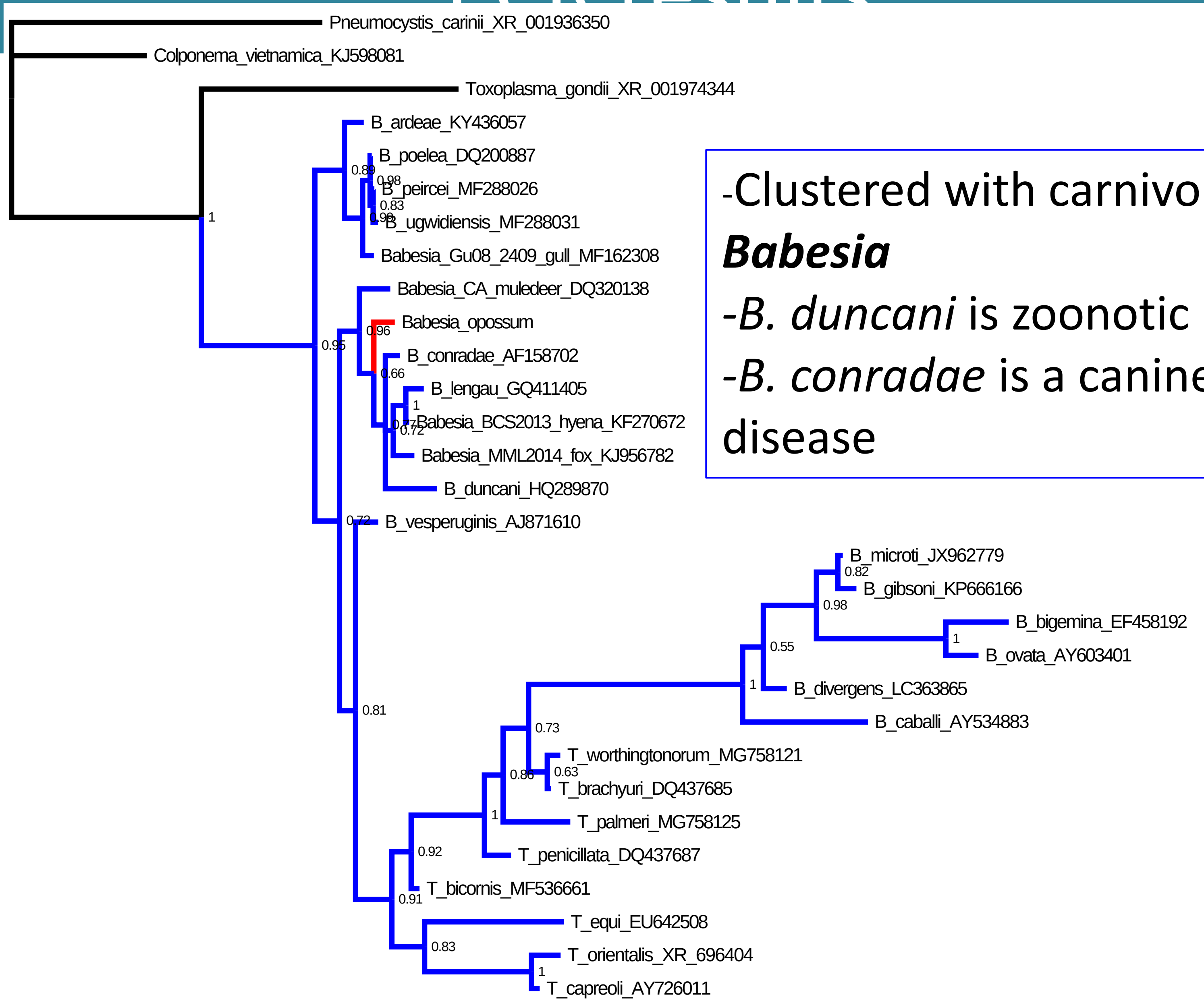


Case 2

Case 3

Same novel *Babesia*

PCR results



-Clustered with carnivore ***Babesia***

-*B. duncani* is zoonotic

-*B. conradae* is a canine disease

Babesiosis

Babesia are common hemoparasites of vertebrates

- Over 100 species described
- Vector-borne – Ixodid ticks most common

Wildlife infections considered common

- Can be host-specific, or infections found in closely related domestic species

Wildlife infections most commonly considered sub-clinical

Babesiosis

Babesia are RBC parasites from the phylum apicomplexa

In the piroplasm order with *Theilleria* and *Cytauxzoon*

Broadly classified as small- or large-form

Babesiosis

Babesia infections can be acute or chronic

- Acute stage varies on severity, depending on age, immunity, co-morbidities, etc.

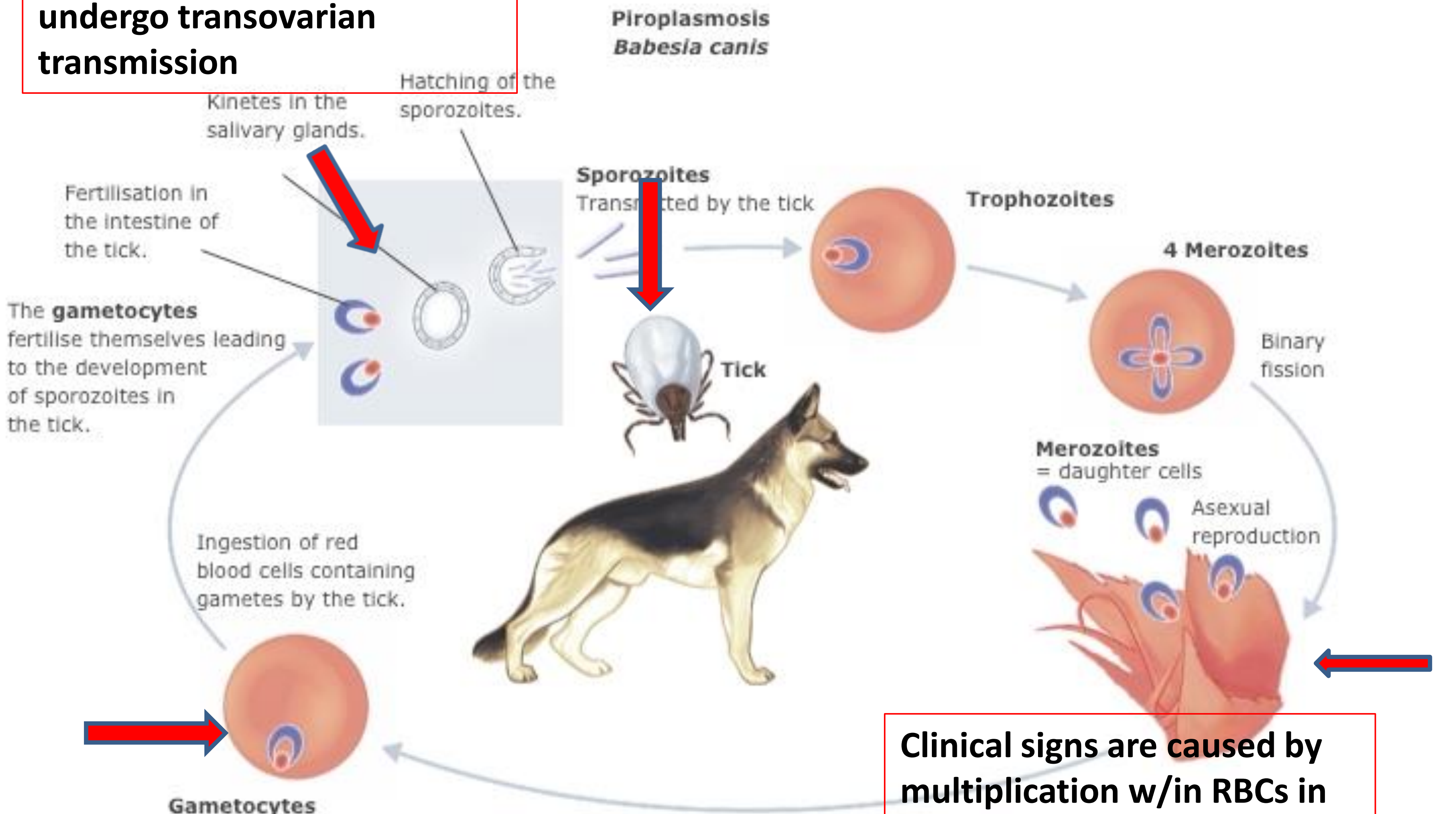
Acute symptoms:

- Fever, anemia
- Signs of hemolysis (jaundice, hematuria)
- Lethargy, anorexia

Chronic infections are usually subclinical – but stress can cause recrudescence

Babesia Life Cycle

Large-form *Babesia* can undergo transovarian transmission



Clinical signs are caused by multiplication w/in RBCs in host

Babesia diagnosis

- CBC – regenerative anemia, leukocytosis, hemolytic anemia
- Blood smear cytology – Visualization of parasite in RBCs
  - Fresh blood samples are ideal
- Serology – positive test can indicate exposure instead of active infection
- PCR – most sensitive and specific
  - Can diagnose and differentiate sub-species

Babesia in marsupials

- Australian marsupials:
  - Woylie, bandicoot, quoll
    - Incidental findings, clinically insignificant
  - Brown antechinus
    - Severely infected after under-going semelparity
  - E. gray kangaroo, agile wallaby
    - High morbidity and mortality

Babesiosis

- Babesia in South American marsupials:
  - Found in M. domestica, D. marsupialis
  - Other surveys found no piroplasms in S. American marsupials
- Only *B. microti* has been investigated in Va. Opossums
  - Found to have low reservoir competence
  - Prevalence also thought to be very low

Treatment

- Anti-protozoal drugs – many costly and difficult to find, various side effects
  - Imidocarb – injectable given 2 weeks apart, up to 4 doses
    - Often does not clear carrier state
    - SLUD side effects
  - Diminazene – single injection clears parasitemia
    - Not available in US
  - Atovaquone (combined w/ Azithromycin)
    - Oral 10 day course
    - Used in specific small-form *Babesia*
    - Parasitemia may recur

Treatment

- Anti-protozoal drugs – many costly and difficult to find, various side effects
  - Malarone (Atovaquone + Proguanil)
    - Vomiting and hyperphosphatemia side effects
    - High chance of relapse
  - Clindamycin and Metronidazole
    - Used for human *B. microti*
  - Carrier state may not be eliminated
  - Many combinations have been studied – varying results – but carrier state usually persists

Treatment

- Blood transfusions often necessary due to severe anemia
- Supportive care – fluid and electrolyte support, treatment of underlying issues
- Prevention – prevent ticks from feeding

Ixodes scapularis

Conclusions

Babesia should be considered as a differential for anemia in opossums

- Especially hemolytic anemia

Pre-existing conditions may play a part in severity

Opossum *Babesia* closely related to zoonotic species, domestic dog species

PCR most likely most sensitive diagnostic

Further Investigation

Epidemiology

- Prevalence
- Morbidity/ mortality
- Distribution
- Vector biology

Further Investigation

- Potential for zoonosis, spread to domestic carnivores
- Phylogenetic relationship to other hemoparasites

WILDLIFE CENTERS CAN HELP!

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