# Diagnosis of tick-borne diseases in dogs

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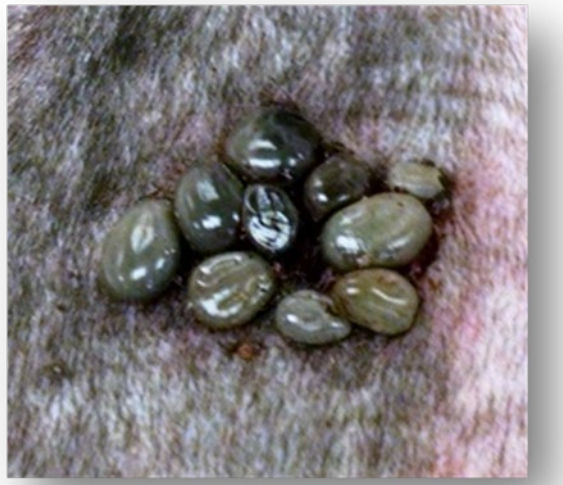
companionanimals.colostate.edu



Biogal Galed Labs.

# Conflict of Interest Disclosure:

## FINANCIAL DISCLOSURE:

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**This lecture only includes information on drugs based on their approved labels or if there is not a labeled drug for veterinary use, the information provided is backed up by peer reviewed references.**

Journal of Veterinary Diagnostic Investigation
1–4

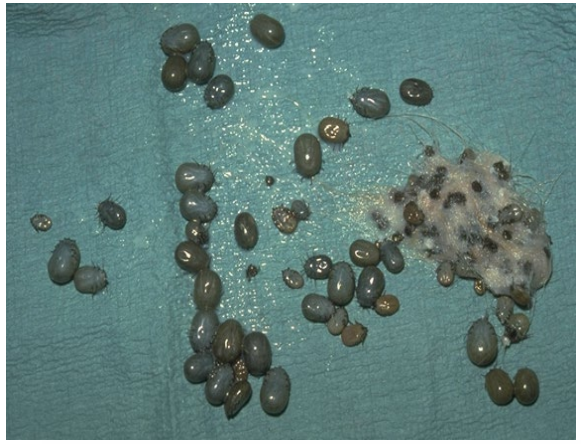
DOI: 10.1177/1040638717729187
jvdi.sagepub.com

# Amplification of *Mycoplasma haemofelis* DNA by a PCR for point-of-care use

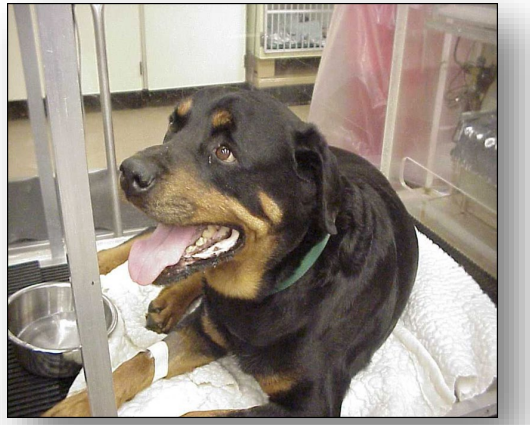
**Jennifer Hawley,[1] Tal Yaaran, Sarah Maurice, Michael R. Lappin**

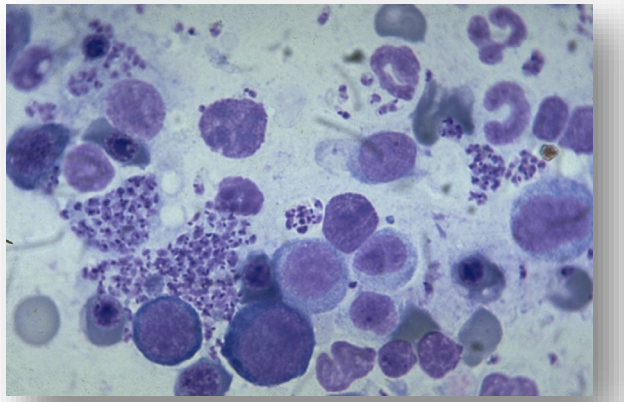
**Abstract.** We compared a qualitative in-clinic (IC)-PCR for the detection of *Mycoplasma haemofelis* DNA with the results of a commercial qualitative laboratory-based, conventional (c)PCR. In order to determine the specificity of both tests, *Bartonella* spp. samples were included. Forty-three previously tested blood samples with known PCR results for hemoplasmas and *Bartonella* spp. were selected. The samples were split between 2 laboratories. At the first laboratory, DNA was purified and run on 2 cPCR assays for the detection of hemoplasmas and *Bartonella* spp. At the second laboratory, DNA was purified using 2 purification protocols and both run in the IC-PCR assay. The cPCR results confirmed that 18 samples were positive for *M. haemofelis*, 5 for '*Candidatus M. haemominutum*', 8 for *Bartonella henselae*, 2 for *Bartonella clarridgeiae*, and 10 were negative for both genera. No mixed infections were observed. The IC-PCR assay for the detection of *M. haemofelis* had a sensitivity of 94.4% and specificity of 96%, when using the same DNA purification method as the first laboratory. Using the second purification method, the sensitivity of the IC-PCR assay was 77.8% and specificity was 96%. *Bartonella* species were not detected by the IC-PCR *M. haemofelis* assay. The IC-PCR assay decreased the amount of time to final result compared to a cPCR assay.

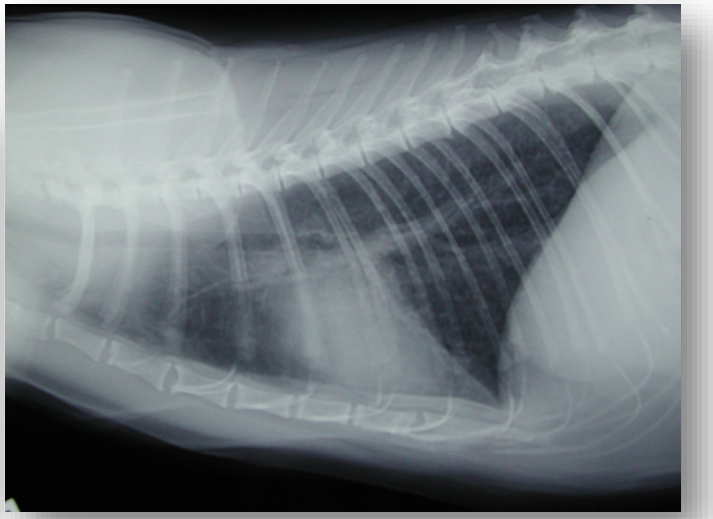
**Key words:** Anemia; cats; DNA; hemolytic; hemoplasmas; PCR; point-of-care systems.

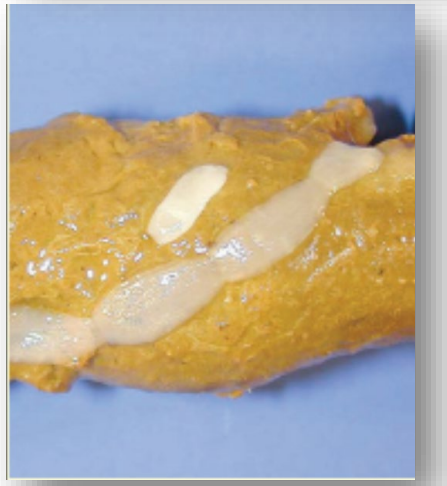


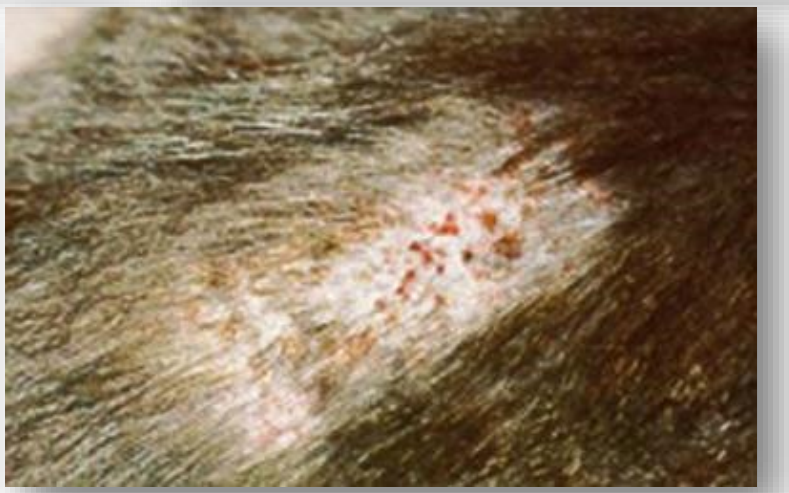
**There are a lot of vector borne disease agents!**











- **Flea**
  - *Babesia vogeli*
  - *Bartonella henselae*
  - *B. clarridgeaie*
  - *B. koehlerae*
  - *B. quintana?*
  - *B. vinsonii*
  - *Coxiella burnetii?*
  - *Hepatozoon felis*
  - *Hemoplasmas*
  - *Rickettsia felis*
  - *R. typhi*
  - *Yersinia pestis*
- **Tick**
  - *Anaplasma platys*
  - *A. phagocytophilum*
  - *Bartonella spp.?*
  - *Babesia spp.*
  - *Borrelia burgdorferi*
  - *Cytauxzoon felis*
  - *Ehrlichia canis*
  - *E. chaffeensis*
  - *E. ewingii*
  - *Hemoplasmas*
  - *Hepatozoon* spp.
  - *Rickettsia rickettsii (Americas)*
  - *Rickettsia spp. (other SFG)*
  - *Others*

**Classic Flea and Tick Agents**

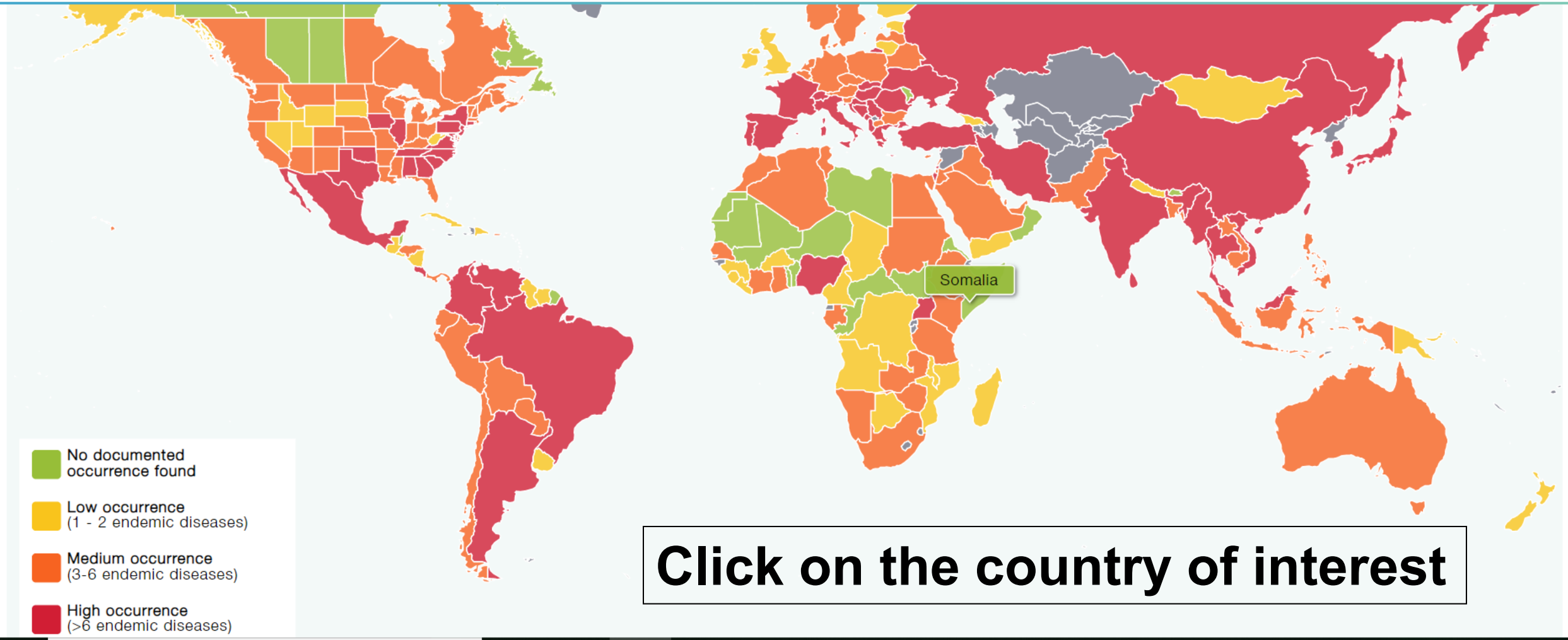
College of Veterinary Medicine ar | Your Employee Intranet | Fleas and Flea-Associated Barton | Occurrence Maps | CVBD

cvbd.elanco.com/cvbd-maps

Apps | Veterinary Continui... | eProtocol - CSU - P... | Employee Resources | Echo360 login | Home - College of... | My Profile - Zoom | SAIM Zoom Round... | CVMBS-Scope

CVBD Companion Vector-Borne Diseases

CVBD Maps | Diseases | Vectors | Resources | CVBD World Forum | Search




**Click on the country of interest**

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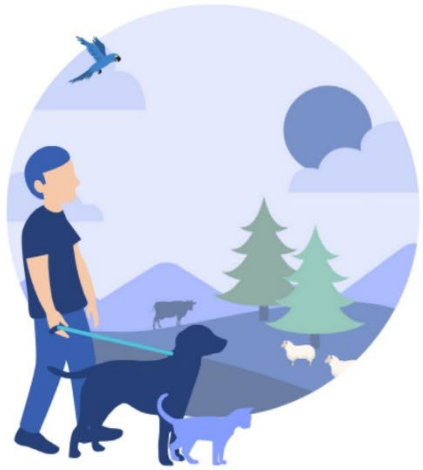
# Israel

| Disease | Vector | Comment |
|---|---|---|
| **Anaplasmosis** (*Anaplasma phagocytophilum*, *Anaplasma platys*) | Suspected to be hard ticks (*Ixodes* spp.) Brown Dog tick (*Rhipicephalus sanguineus*), *Rhipicephalus turanicus* | Seropositivity to *Anaplasma phagocytophilum* has also been detected in jackals and human patients. |
| **Babesiosis** (*Babesia canis*, *Babesia gibsoni*) | Ornate Cow or Marsh tick (*Dermacentor reticulatus*) vector to be determined | *Babesia vogeli* has also been detected in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks, but more data is needed on the occurrence in dogs |
| **Ehrlichiosis** (*Ehrlichia canis*) | Brown Dog tick (*Rhipicephalus sanguineus*) | Pathogen (*Ehrlichia canis*) has also been detected in ticks (*Rhipicephalus sanguineus*, *Rhipicephalus turanicus*), red foxes, golden jackals and in a human case report. Additionally *Ehrlichia chaffeensis* has been suspected in humans and seropositivity has been detected in jackals, but more data regarding cross-reactivity and regarding the occurrence in dogs is needed |
| **Hepatozoonosis** (*Hepatozoon canis*) | Brown Dog tick (*Rhipicephalus sanguineus*) | Seropositivity against *Hepatozoon canis* has also been detected in red foxes. |
| **Leishmaniosis** (*Leishmania infantum*) | Sand flies (*Phlebotomus* spp.) | Seropositivity against *Leishmania infantum* has also been detected in golden jackals, horses and presumably also in cats. *Leishmania major* and *Leishmania tropica* as pathogens of cutaneous leishmaniosis are reported in humans from different regions and in single canine cases. *Leishmania infantum* as agent of visceral leishmaniosis is reported in humans. |
| **Lyme Borreliosis** | | Seroreactivity against a *Borrelia* species has been detected in dogs, but species characterization was not possible. Questionable single autochthonous human cases have also been reported, but generally more data is needed |
| **Rickettsiosis** (*Rickettsia conorii* â€“ different strains, | *Rhipicephalus* spp. Cat flea | Seropositivity against SFG rickettsiae has also been detected in golden jackals. Mediterranean spotted fever (*Rickettsia conorii*) is also reported in human patients and seropositivity against *Rickettsia typhi* has been detected in rats. |

# Lecture Plan

- Tick borne diseases as a One Health Issue
- Tick borne diseases of importance to dogs (almost all infect cats!)
- Overview of tick-borne disease diagnostic options using *E. canis* as an example
  - Cytology
  - Serology
  - Nucleic acid amplification
- How to prove a tick borne “disease”



*Ensuring the prominence of the small companion animal-human interface in the global One Health agenda*

WSAVA
One Health Committee

**Main website**

**www.wsava.org**

**One Health Certificate Course**

**www.col.st/WSAVAOneHealth**

**Fleas and ticks on cats and dogs are a One Health issue!**

CENTER FOR COMPANION ANIMAL STUDIES
COLORADO STATE UNIVERSITY

*Journal of Feline Medicine and Surgery* (2020) **22**, 31–39

PREMIER REVIEW

# ROLE OF VECTOR-BORNE PATHOGENS IN THE DEVELOPMENT OF FEVER IN CATS
## 1. Flea-associated diseases

*Journal of Feline Medicine and Surgery* (2020) **22**, 41–48

PREMIER REVIEW

# ROLE OF VECTOR-BORNE PATHOGENS IN THE DEVELOPMENT OF FEVER IN CATS
## 2. Tick- and sandfly-associated diseases

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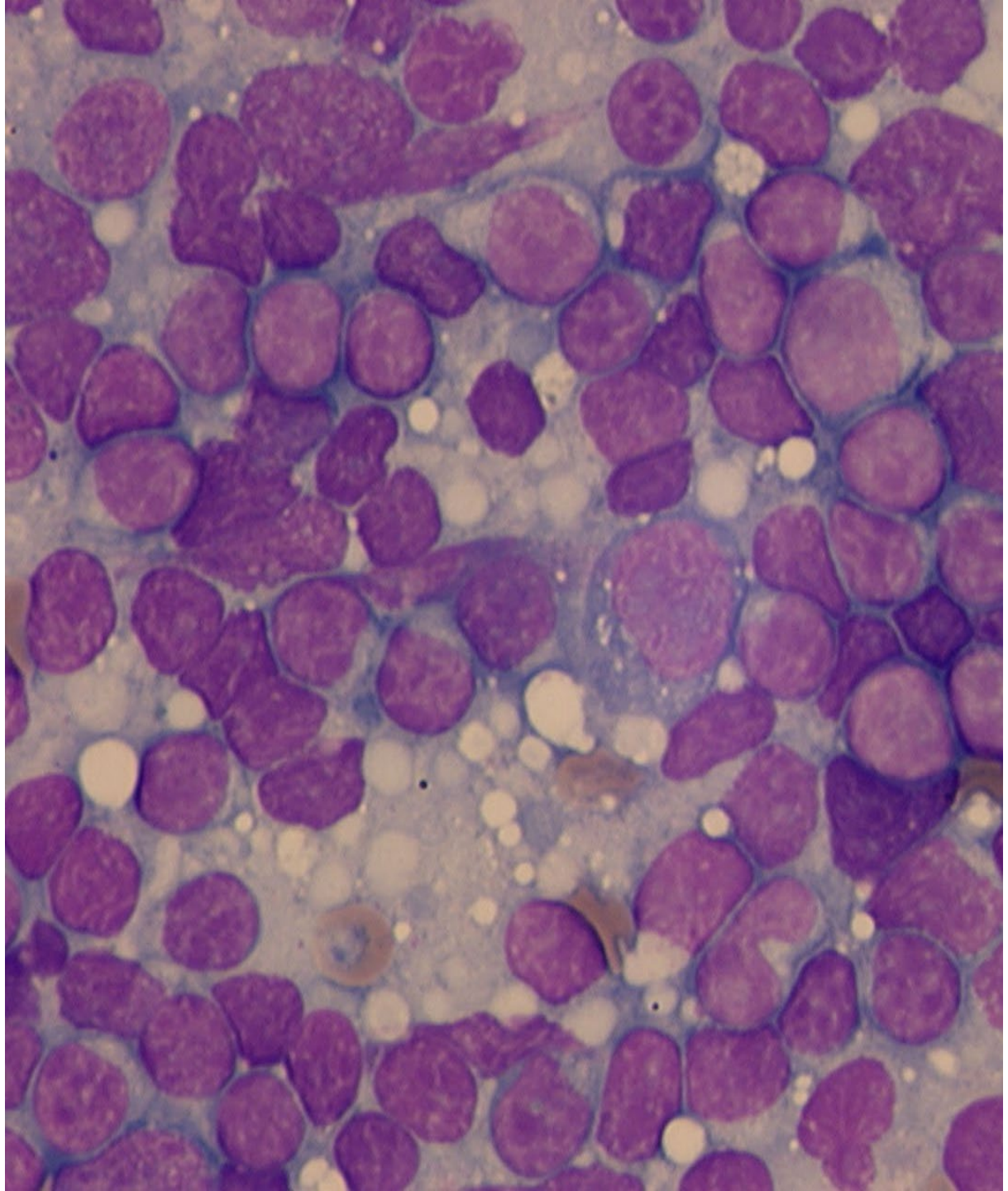
# “Norman”

- Lethargy - Fever
- Epistaxis
- 150,000 platelets
- 20,000 neutrophils
  - No band neutrophils
- Generalized lymphadenopathy
- No known ticks
- Never left Colorado
  - *Rhipicephalus* most common
- No tick control



- Lymph node hyperplasia

- Likely agent?


## If you have *Rhipicephalus*, you have 6 significant vector agents!

***Ehrlichia canis***
***Anaplasma platys***
***Hepatozoon canis***
***Babesia vogeli***
***Rickettsia rickettsii***
***Mycoplasma haemocanis***

# “Norman”

**Cytology and *Ehrlichia canis* antibody test**

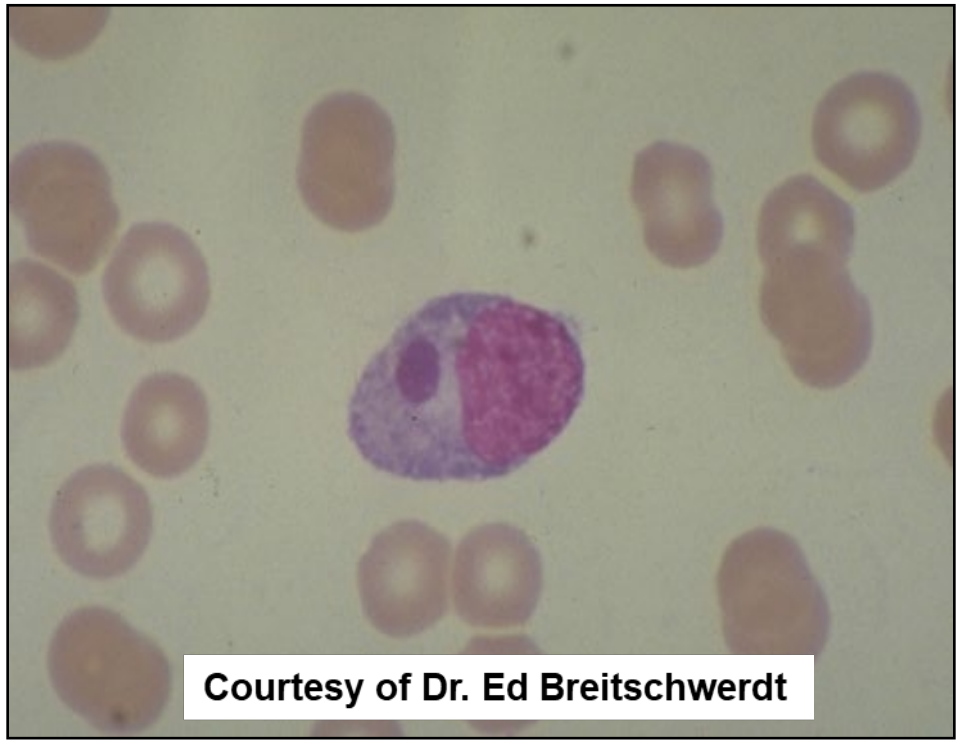
**Negative!**

**Could he still have a tick-borne agent?**

YES!

# “Norman”

## Finding *E. canis* morulae is rare




# “Norman”

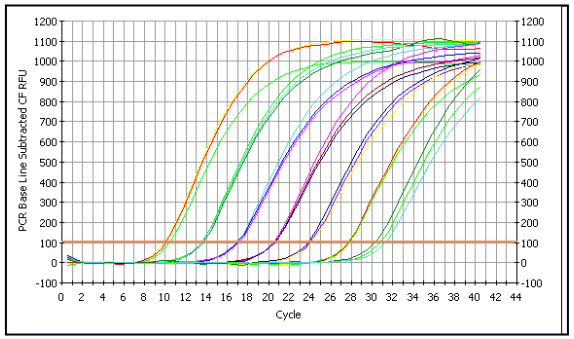
## Acute Ehrlichiosis

**What is a more sensitive test than cytology or antibodies?**

# Acute Ehrlichiosis

## What is a more sensitive test than cytology or antibodies?

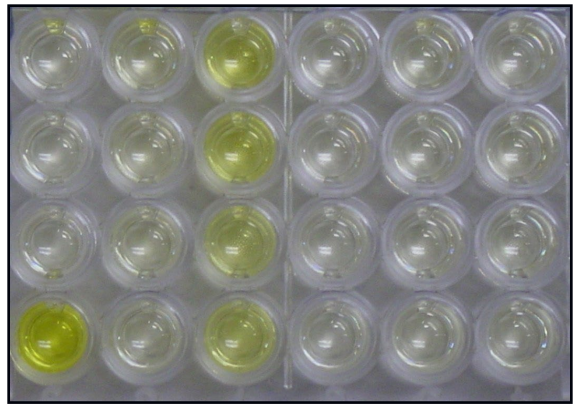
## PCR!

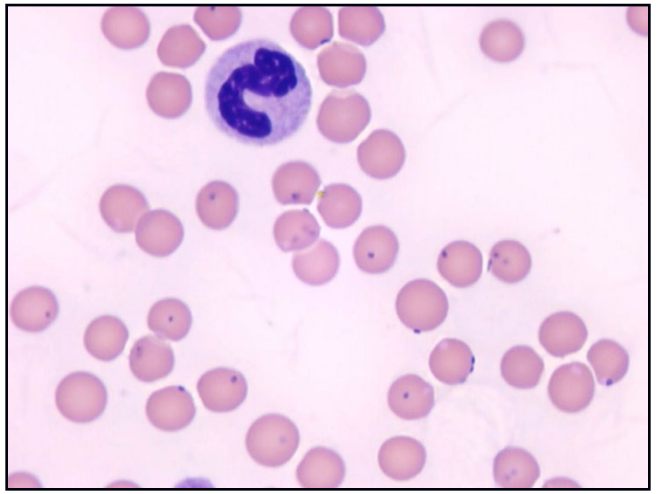



# "Norman"

# Diagnostic Tests

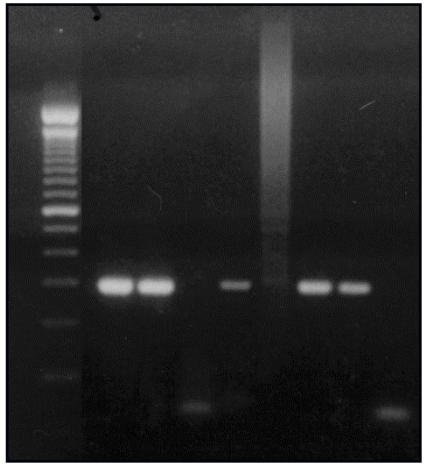
- **Organism demonstration**
  - Document infection
    - Cytology/immunocytochemistry
    - Histology/immunohistochemistry
    - Culture
    - Antigen tests
    - Polymerase chain reaction
- **Antibody tests**
  - Document exposure





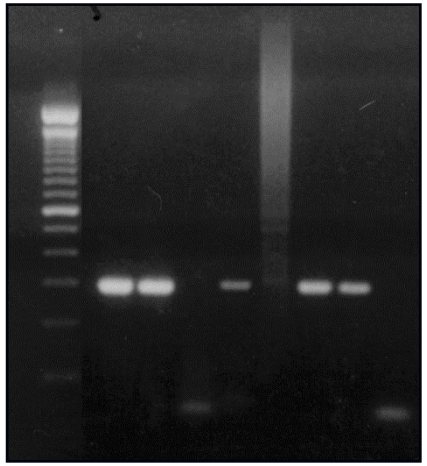
# PCR Assays

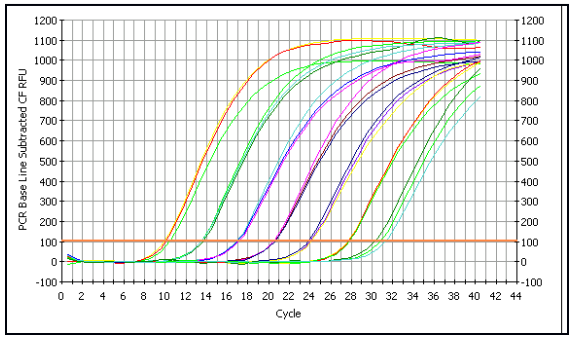
- Polymerase chain reaction (PCR)
  - DNA amplification
  - Examples
    - *Anaplasma, Babesia, Ehrlichia* spp.
- Reverse transcriptase PCR
  - Convert RNA to DNA
  - PCR reaction
  - RT-PCR
  - Examples
    - Coronaviruses, Caliciviruses



# PCR Assays

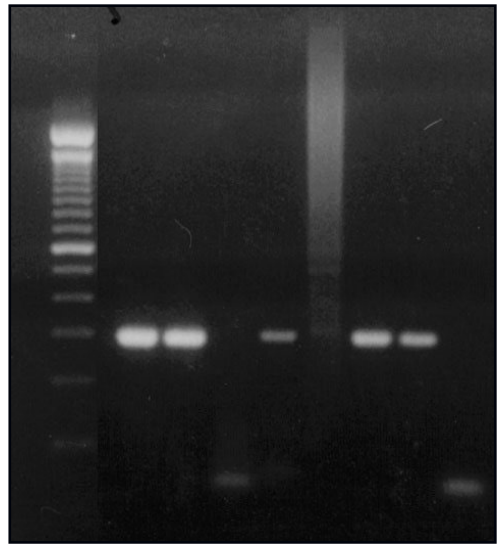
- Primers
  - Genus specific
  - Species specific
  - Multiplex
- Conventional (end point)
  - Positive or negative
- Fluorogenic (real time)
  - Positive or negative
  - Potential for quantitative





# PCR Assays-General Comments

- Rapid analysis
- Proves current infection
- Analytical sensitivity high
- Analytical specificity high
- Positive predictive values
  - Vary by the syndrome
- Negative predictive values
  - Vary by the syndrome
- Not all labs are the same!



# Monocytotropic Ehrlichiosis

- Diagnosis
  - Morulae detection
  - Polymerase chain reaction (best in acute histories)
  - Antibody detection (best in chronic histories)


*Brief Research Report*

Journal of Veterinary Diagnostic Investigation
2014, Vol. 26(4) 558–562

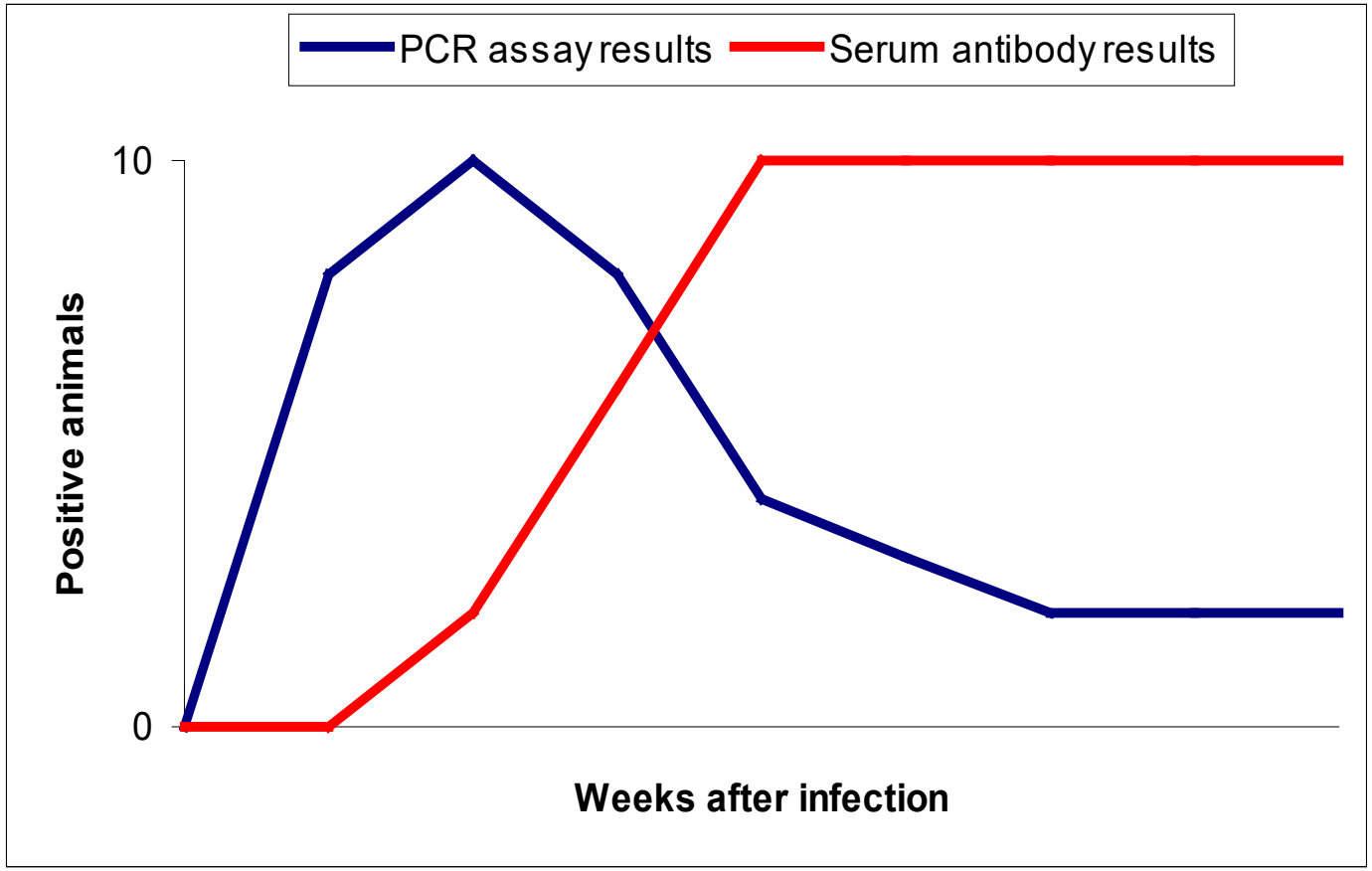
DOI: 10.1177/1040638714534849
jvdi.sagepub.com

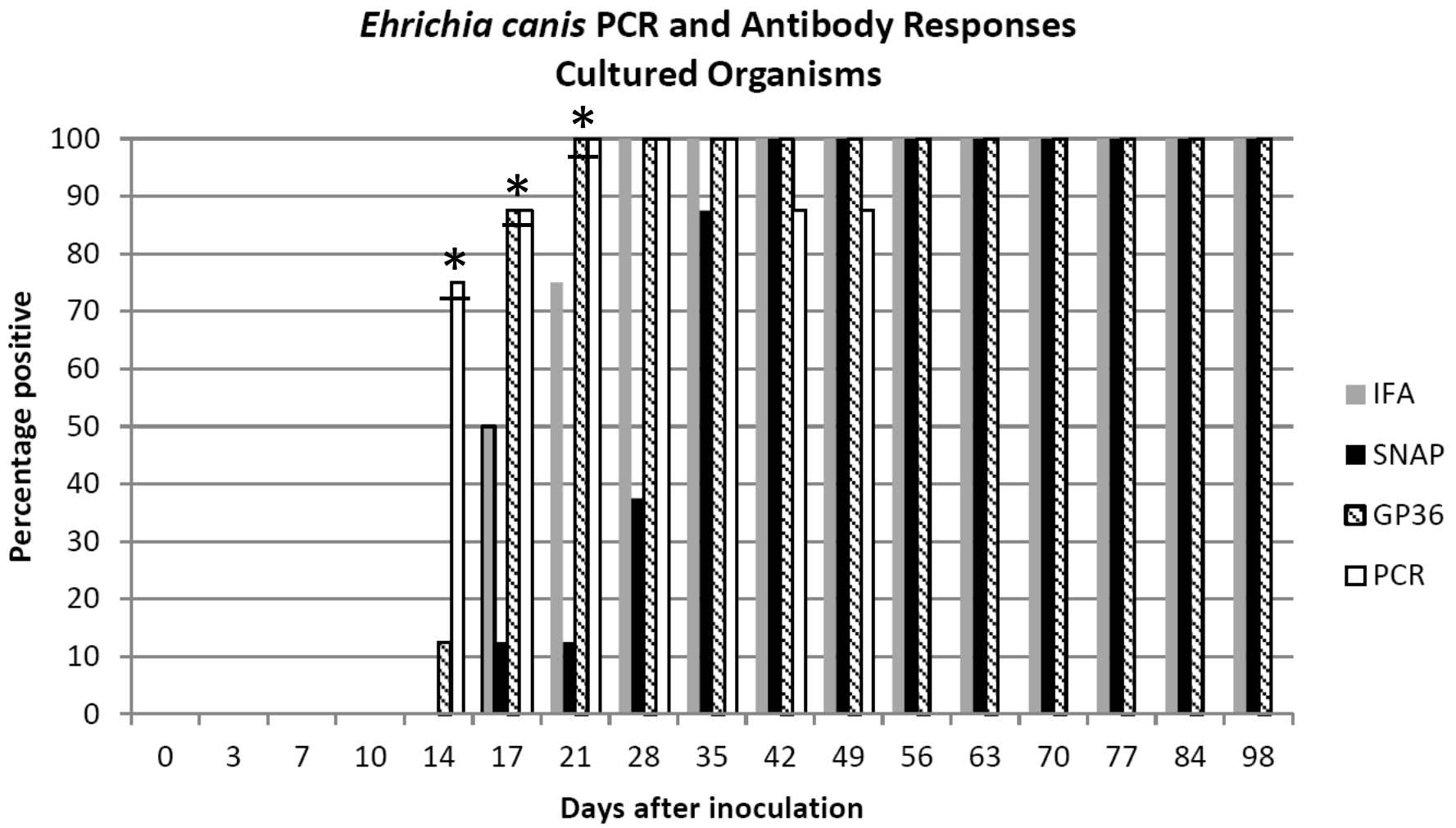
**Use of an automated system for detection of canine serum antibodies against *Ehrlichia canis* glycoprotein 36**

**Scott Moroff, Irene Sokolchik, Todd Woodring, Colby Woodruff, Brett Atkinson, and Michael R. Lappin[1]**

# PCR Positive Before Serology

- ***Anaplasma* spp.**
- ***Babesia* spp.**
- ***Bartonella* spp.**
- ***Ehrlichia* spp.**
- ***Rickettsia* spp.**






**8 dogs inoculated IV with cultured *E. canis* – Oklahoma State**
**Doxycycline starting on Day 56**

***PCR statistically faster than any serology on Day 14 (PCR not available Day 56)**
***PCR and GP36 faster than SNAP on Days 17, 21, and 28**

# “Norman”

## Hot topic

**If a tick-borne disease is suspected and both tests are available, assay for antibodies AND DNA**

The Veterinary Journal 187 (2011) 292–296

Contents lists available at ScienceDirect

The Veterinary Journal

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

Review

# Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): An overview

Shimon Harrus [a,*], Trevor Waner [b]

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[b] *Israel Institute for Biological Research, P.O. Box 19, Ness Ziona 74100, Israel*

# Can I do both types of assays in my clinic?


# How do in clinic PCR assays compare?

RESEARCH Open Access

## A new TaqMan method for the reliable diagnosis of *Ehrlichia* spp. in canine whole blood

Kirsty Thomson[1*], Tal Yaaran[2], Alex Belshaw[1], Lucia Curson[1], Laurence Tisi[1], Sarah Maurice[2] and Guy Kiddle[1]

**Abstract**

**Background:** Ehrlichiosis is an important emerging infectious disease of the canid family and humans worldwide. To date, no extensive evaluation or validation of a molecular diagnostic test for ehrlichiosis has been published. Here, we present data for a newly designed TaqMan assay and compare its performance to a commercial technology (PCRun®). Both of these real-time methods of analysis were evaluated using a comprehensive number of prospective and retrospective samples collected from dogs exhibiting symptoms of ehrlichiosis.

**Results:** Whole blood samples collected from dogs, retrospectively in the United Kingdom and prospectively in Israel, were analysed for the presence of *Ehrlichia canis* and *Ehrlichia minasensis* DNA using the TaqMan PCR, developed specifically for this study. The results were compared to those of a real time commercial isothermal amplification method (PCRun® system developed by Biogal Galed Labs ACS, Galed, Israel). The sensitivity and specificity (CI: 95%) of the TaqMan PCR and PCRun® were both determined to be 100% and absolute, for all of the samples tested. Interestingly, both tests were demonstrated to be highly comparable, irrespective of differences in amplification chemistry or sequences targeted. Host differences, incidence of disease and geographical location of the isolates had little impact on the positivity recorded by each of the diagnostic methods.

**Conclusions:** It was evident that both amplification methods were equally suited for diagnosing canine ehrlichiosis and while the PCRun® clearly amplified all clinically relevant *Ehrlichia* species known to infect dogs and humans, the TaqMan method was more specific for *E. canis* and *E. minasensis*. This work demonstrates that despite good analytical sensitivities and specificities for *Ehrlichia* spp. neither method could fully account for the clinical diagnosis of thrombocytopenia.

**Keywords:** PCR, PCRun®, Clinical validation, Emerging, Tick-borne

## *Molecular detection of Babesia canis vogeli in dogs in the city of São Luís - MA, Brazil*

**ABSTRACT.** Babesiosis is an emerging, worldwide disease caused by protozoa of the genus Babesia, which infects domestic animals and wildlife, as well as humans. The objective of this study was to determine the occurrence of Babesia canis vogeli in dogs in the city of São Luís - MA, Brazil. Blood samples were collected from 65 animals for direct examination, through blood smear and molecular diagnosis, through PCRun. Among the 65 animals analyzed by direct diagnosis, none were positive for Babesia spp. while PCRun showed that 5 (7.69%) were infected by B. canis vogeli, demonstrating the presence of this species in dogs from an urban environment in the city of São Luís. It was concluded that PCR is a more sensitive and specific for this diagnosis, and can be used to define more precisely the infection of dogs by B. canis vogeli. This study confirmed the presence of B. C. vogeli in dogs in the city of.

**Keywords**: B. canis vogeli, dogs, direct diagnosis, PCR

pubvet
MEDICINA VETERINÁRIA E ZOOTECNIA
ISSN 1982-1263

https://doi.org/10.22256/pubvet.v12n6a108.1-4

**Detecção molecular de *Babesia canis vogeli* em cães da cidade de São Luís – MA, Brasil**

Journal of Veterinary Internal Medicine

ACVIM
American College of Veterinary Internal Medicine

Open Access

*Standard Article*

*J Vet Intern Med* 2017;31:1081–1090

# Prevalence of Vector-Borne Pathogens in Southern California Dogs With Clinical and Laboratory Abnormalities Consistent With Immune-Mediated Disease

L. Kidd, B. Qurollo, M. Lappin, K. Richter, J.R. Hart, S. Hill, C. Osmond, and E.B. Breitschwerdt

**Background:** Studies investigating the prevalence of vector-borne pathogens in southern California dogs are limited. Occult infections might be misdiagnosed as idiopathic immune-mediated disease.

**Hypothesis/Objectives:** (1) To determine the prevalence of vector-borne pathogens in southern California dogs with compatible clinical findings using PCR and serologic panels and (2) to determine whether testing convalescent samples and repeating PCR on acute samples using the same and different gene targets enhance detection.

**Animals:** Forty-two client-owned dogs with clinical signs of vector-borne disease presenting to specialty practices in San Diego County.

**Methods:** Combined prospective and retrospective observational study. Forty-two acute and 27 convalescent samples were collected. Acute samples were prospectively tested for antibodies to *Rickettsia, Ehrlichia, Bartonella, Babesia, Borrelia, and Anaplasma species*. PCR targeting *Ehrlichia*, *Babesia*, *Anaplasma*, hemotropic *Mycoplasma*, and *Bartonella* species was also performed. Retrospectively, convalescent samples were tested for the same organisms using serology, and for *Ehrlichia*, *Babesia*, *Anaplasma*, and *Bartonella* species using PCR. Acute samples were retested using PCR targeting *Ehrlichia* and *Babesia* species.

**Results:** Evidence of exposure to or infection with a vector-borne pathogen was detected in 33% (14/42) of dogs. *Ehrlichia* and *Babesia* species were most common; each was identified in 5 dogs. Convalescent serologic testing, repeating PCR, and using novel PCR gene targets increased detection by 30%.

**Conclusions and Clinical Importance:** Repeated testing using serology and PCR enhances detection of infection by vector-borne pathogens in dogs with clinical signs of immune-mediated disease. Larger prevalence studies of emerging vector-borne pathogens in southern California dogs are warranted.

**Key words:** Anaplasmosis; Babesiosis; Ehrlichiosis; Flea; Immune-mediated; Rickettsioses; Tick.

**Will doing a PCR diagnose all infections?**

# Can measuring *E. canis* antibodies help me in chronic phase illness?

## Can measuring *E. canis* antibodies help me in chronic phase illness?

**YES!**

## Most dogs in subclinical and chronic phases have high *E. canis* titers and so a negative antibody test helps RULE OUT the infection

**(high negative predicative value)**

# Do positive test results (antibodies or DNA) for tick borne disease agents prove illness?


**These dogs were all considered healthy by their owners!**

Table 2. Clinical Abnormalities Associated with *Ehrlichia canis* Infection in Dogs

| STAGE OF INFECTION | ABNORMALITIES |
|---|---|
| Acute | Fever |
| | Serous or purulent oculonasal discharge |
| | Anorexia |
| | Weight loss |
| | Dyspnea |
| | Lymphadenopathy |
| | Tick infestation often evident |
| Subclinical | No clinical abnormalities |
| | Ticks often not present |
| Chronic | Ticks often not present |
| | Depression |
| | Weight loss |
| | Pale mucous membranes |
| | Abdominal pain |
| | Evidence of hemorrhage: epistaxis, retinal hemorrhage, etc. |
| | Lymphadenopathy |
| | Splenomegaly |
| | Dyspnea, increased lung sounds, interstitial or alveolar lung infiltrates |
| | Ocular: perivascular retinitis, hyphema, retinal detachments, anterior uveitis, corneal edema |
| | Central nervous system: meningeal pain, paresis, cranial nerve deficits, seizures |
| | Hepatomegaly |
| | Arrhythmias and pulse deficits |
| | Polyuria and polydipsia |
| | Stiffness and swollen, painful joints |

***Ehrlichia canis*** **can cause EVERYTHING since it causes vasculitis**

**You have blood vessels in almost all tissues!**

Table 3 Clinicopathologic Abnormalities Associated with *Ehrlichia canis* Infection in Dogs

| STAGE OF INFECTION | ABNORMALITIES |
|---|---|
| Acute | Thrombocytopenia |
| | Leukopenia followed by neutrophilic leukocytosis and monocytosis |
| | Morulae |
| | Low-grade, nonregenerative anemia unless hemorrhage has occurred |
| | Variable *Ehrlichia* titer |
| | PCR positive |
| Subclinical | Hyperglobulinemia |
| | Thrombocytopenia |
| | Neutropenia |
| | Lymphocytosis |
| | Monocytosis |
| | Positive *Ehrlichia* titer |
| | PCR positive |
| Chronic | Monocytosis |
| | Lymphocytosis |
| | Thrombocytopenia |
| | Nonregenerative anemia |
| | Hyperglobulinemia |
| | Hypocellular bone marrow |
| | Bone marrow/spleen plasmacytosis |
| | Hypoalbuminemia |
| | Proteinuria |
| | Polyclonal or immunoglobulin G monoclonal gammopathy |
| | Cerebrospinal fluid mononuclear cell pleocytosis |
| | Nonseptic, suppurative polyarthritis |
| | Rare azotemia |
| | Increased alanine aminotransferase and alkaline phosphatase activities |
| | Positive *Ehrlichia* titer |
| | PCR positive |

*PCR,* Polymerase chain reaction.

***Ehrlichia canis*** **can cause EVERYTHING since it causes vasculitis**

**You have blood vessels in almost all tissues!**

# *Ehrlichia canis* "Classics"

- **Fever** (lethargy and inappetance)
- **Epistaxis** (from vasculitis or anti-platelet AB acutely)
  - Surgical incisions "seeping" blood
- **Mild thrombocytopenia in acute stage**
  - Usually 75,000 to 200,000/microliter
- **Severe thrombocytopenia is in the chronic phase**
  - Usually combined with anemia and neutropenia
  - "Tropical pancytopenia"

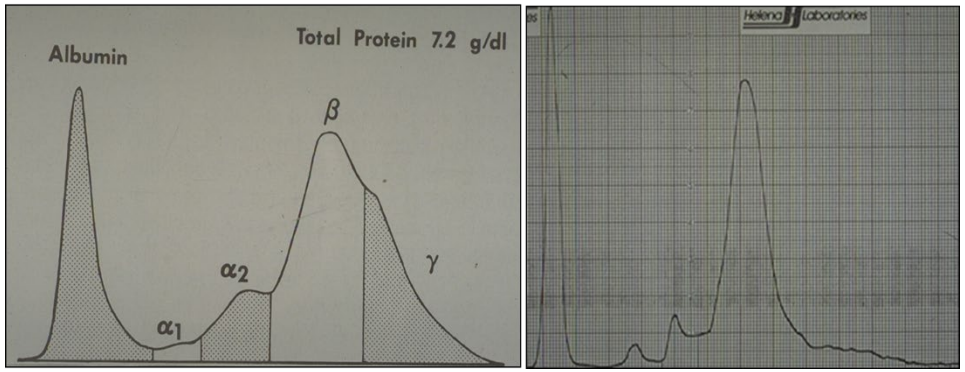
# *Ehrlichia canis* "Classics"

- **Lymphadenopathy (hyperplastic on cytology)**
- **Proteinuria (immune complexes in kidneys)**
- **Most likely tick borne to give:**
  - **Pancytopenia (bone marrow wipeout)**
  - **Monoclonal gammopathy**
- **Only 25% of owners know their dog had ticks!**

# *Ehrlichia canis*

- **Clinical pathology-chronic**
  - **Monoclonal or polyclonal gammopathy**
  - **Mononuclear CSF**
  - **Proteinuria**
  - **Coomb's positive frequently**
  - **ANA positive frequently**

**Polyclonal** **Monoclonal**



# Ehrlichiosis
# (and other tick-borne agents)

- **Presumptive diagnosis**
  - Seropositive/PCR positive
  - Appropriate clinical signs
  - Exclusion of other causes
  - Response to anti-rickettsial drugs

**“Possibly the infection you think it is!”**

# Ehrlichiosis

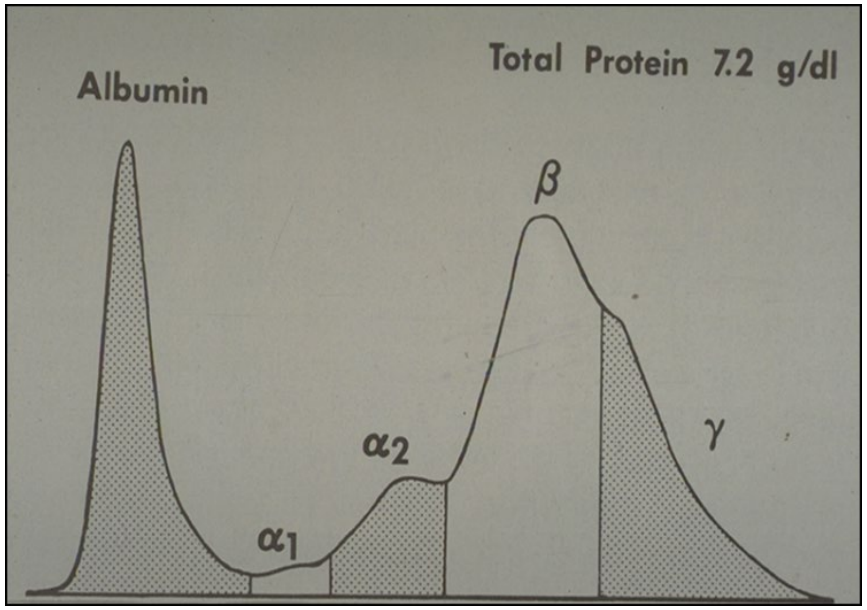
- Treatment
  - Doxycycline
    - 5 mg/kg, PO, q12hr for 28 days OR
    - 10 mg/kg, PO, q24hr for 28 days
  - Imidocarb diproprionate
    - 5.5 mg/kg, IM, q 14 days, twice
    - Combine with doxycycline if co-infection with *Babesia vogeli*
  - Chloramphenicol
  - Quinolones ineffective!!

# Ehrlichiosis

## How long should I treat?

- Minimum treatment duration is 28 days

## What should I monitor?




- Clinical abnormalities
- Laboratory abnormalities
  - Platelet count
  - Anemia
  - Hyperglobulinemia
- Treat until these are normal or stop improving

# Ehrlichiosis

**Following antibody titers or PCR assay results in not beneficial for *E. canis* dogs**

**- Titers usually do not go to negative**

**- Blood PCR assay results can go negative, but *E. canis* is usually hiding in the spleen.**

# Ehrlichiosis

- **Should I treat all seropositive dogs?**

- **Probably not**
  - **Cost**
  - **Side effects**
  - **Failure to clear the infections**
  - **Development of resistant strains**

- **Minimal diagnostic plan**
  - **Complete blood cell count**
  - **Globulin**
  - **Urinalysis**
- **All normal**
  - **Recheck in 2 months**
  - **Still normal, recheck yearly**
- **Any abnormal**
  - **Treat**
  - **Recheck 28 days**
    - **Normal, recheck yearly**
    - **Abnormal, continue RX and rechecks**

# *Ehrlichia* Seropositive Healthy

- **Flea**
  - *Babesia vogeli*
  - *Bartonella henselae*
  - *B. clarridgeaie*
  - *B. koehlerae*
  - *B. quintana?*
  - *B. vinsonii*
  - *Coxiella burnetii?*
  - *Hepatozoon felis*
  - *Hemoplasmas*
  - *Rickettsia felis*
  - *R. typhi*
  - *Yersinia pestis*
- **Tick**
  - *Anaplasma platys*
  - *A. phagocytophilum*
  - *Bartonella spp.?*
  - *Babesia spp.*
  - *Borrelia burgdorferi*
  - *Cytauxzoon felis*
  - *Ehrlichia canis*
  - *E. chaffeensis*
  - *E. ewingii*
  - *Hemoplasmas*
  - *Hepatozoon* spp.
  - *Rickettsia rickettsii (Americas)*
  - *Rickettsia spp. (other SFG)*
  - *Others*

**Classic Flea and Tick Agents**

# Diagnosis of tick-borne diseases in dogs

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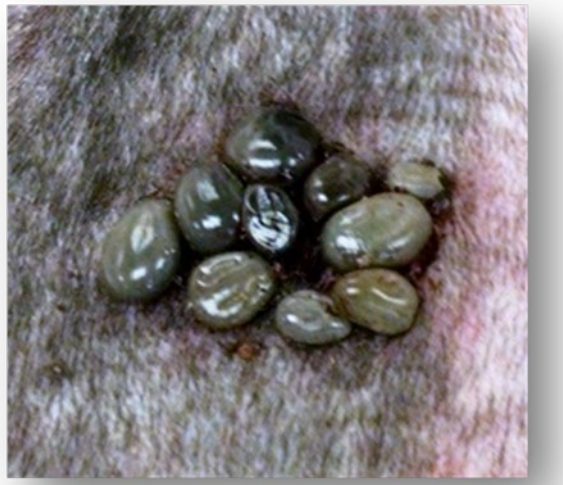

Biogal Galed Labs.