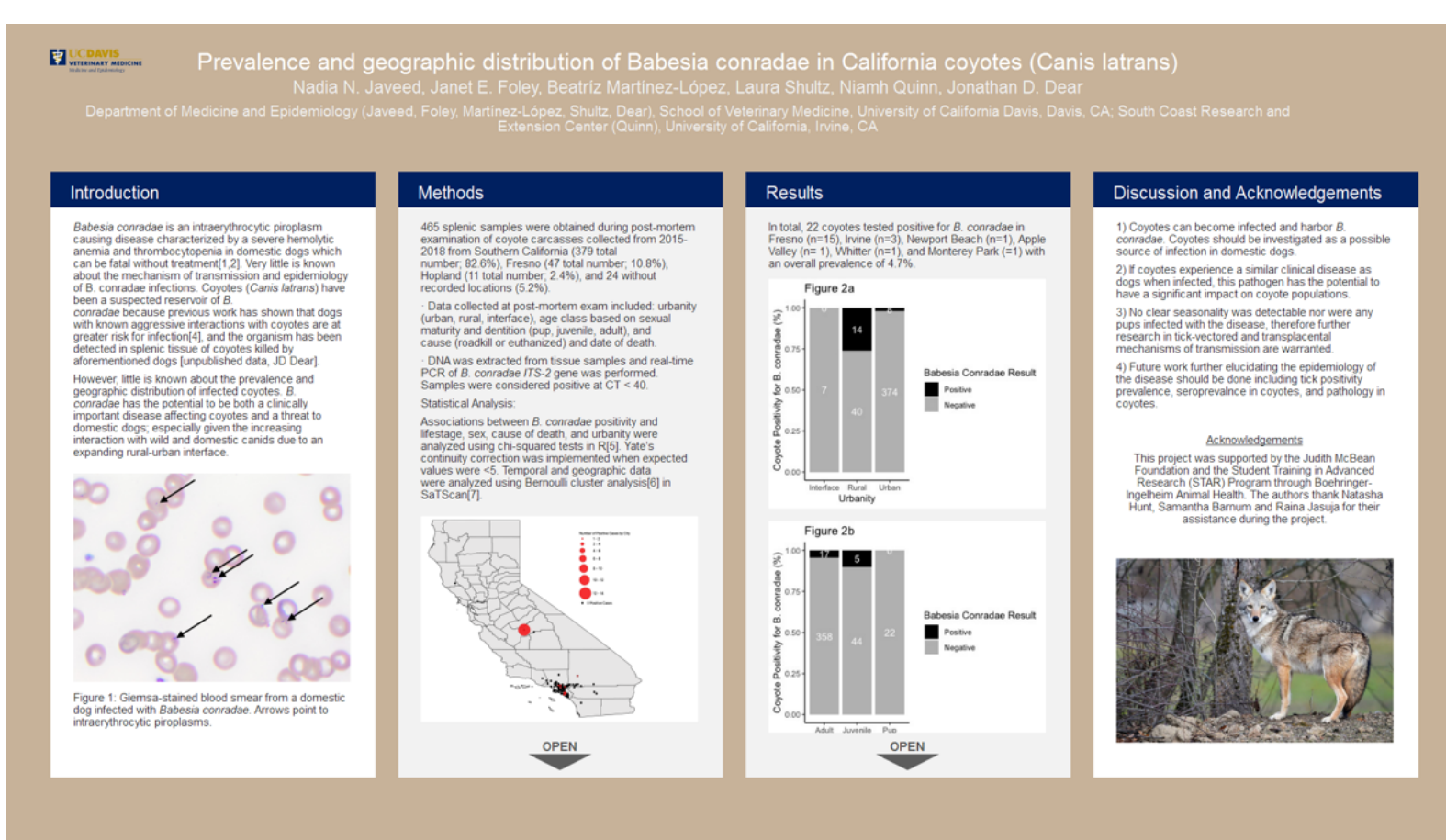


Prevalence and geographic distribution of *Babesia conradae* in California coyotes (*Canis latrans*)



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INTRODUCTION

Babesia conradae is an intraerythrocytic protozoan causing disease characterized by a severe hemolytic anemia and thrombocytopenia in domestic dogs which can be fatal without treatment [1,2]. Very little is known about the mechanism of transmission and epidemiology of *B. conradae* infections. Coyotes (*Canis latrans*) have been a suspected reservoir of *B. conradae* because previous work has shown that dogs with known aggressive interactions with coyotes are at greater risk for infection [4], and the organism has been detected in splenic tissue of coyotes killed by aforementioned dogs [unpublished data, JD Dear].

However, little is known about the prevalence and geographic distribution of infected coyotes. *B. conradae* has the potential to be both a clinically important disease affecting coyotes and a threat to domestic dogs; especially given the increasing interaction with wild and domestic canids due to an expanding rural-urban interface.

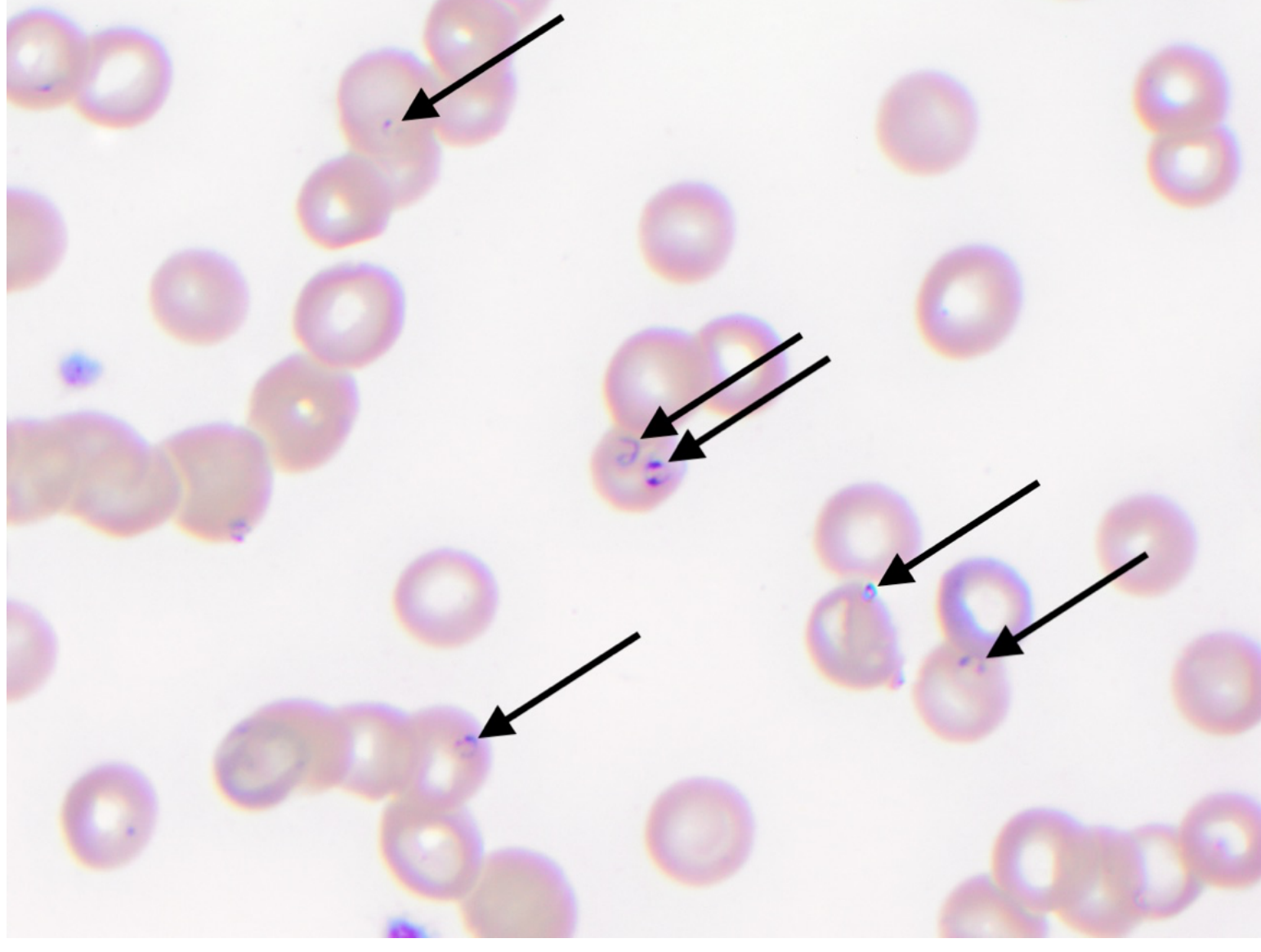


Figure 1: Giemsa-stained blood smear from a domestic dog infected with *Babesia conradae*. Arrows point to intraerythrocytic proplasms.

METHODS

465 splenic samples were obtained during post-mortem examination of coyote carcasses collected from 2015-2018 from Southern California (379 total number; 82.6%), Fresno (47 total number; 10.8%), Hopland (11 total number; 2.4%), and 24 without recorded locations (5.2%).

Data collected at post-mortem exam included: urbanity (urban, rural, interface), age class based on sexual maturity and dentition (pup, juvenile, adult), and cause (roadkill or euthanized) and date of death.

DNA was extracted from tissue samples and real-time PCR of *B. conradae* ITS-2 gene was performed. Samples were considered positive at CT < 40.

Statistical Analysis:

Associations between *B. conradae* positivity and lifestage, sex, cause of death, and urbanity were analyzed using chi-squared tests in R [5]. Yate's continuity correction was implemented when expected values were <5. Temporal and geographic data were analyzed using Bernoulli cluster analysis [6] in SaTScan [7].

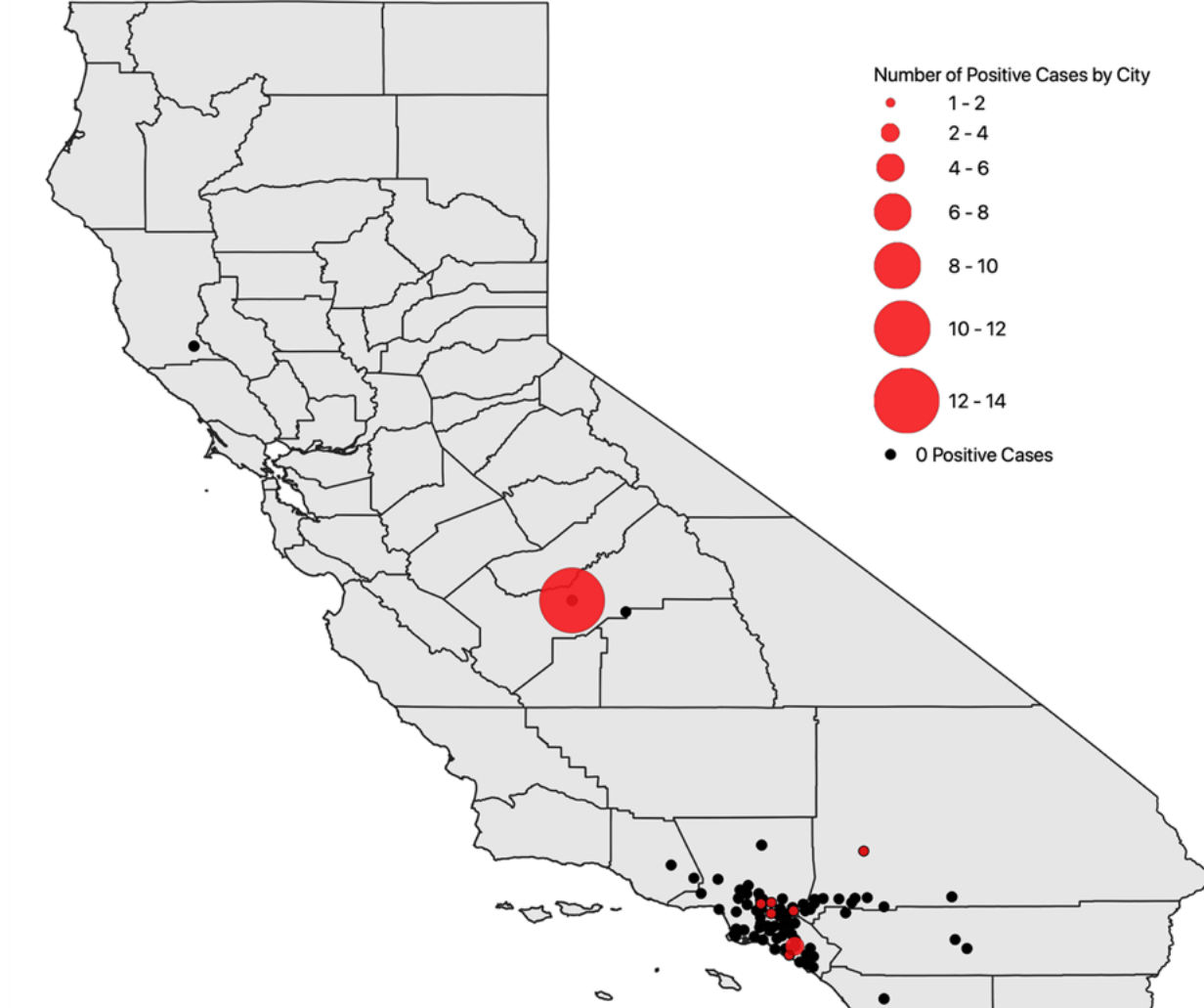


Figure 2: Distribution of coyotes (*Canis latrans*) that tested positive for *Babesia conradae* by city in California where black points denote cities sampled without positive coyotes, and red points indicate cities with positive coyotes. The size of the point is proportional to the number of positive coyotes found in that city. Fresno was found to have a significant space-time cluster of *B. conradae* positive coyotes ($P < 0.0001$).

RESULTS

In total, 22 coyotes tested positive for *B. conradae* in Fresno (n=15), Irvine (n=3), Newport Beach (n=1), Apple Valley (n=1), Whittier (n=1), and Monterey Park (n=1) with an overall prevalence of 4.7%.

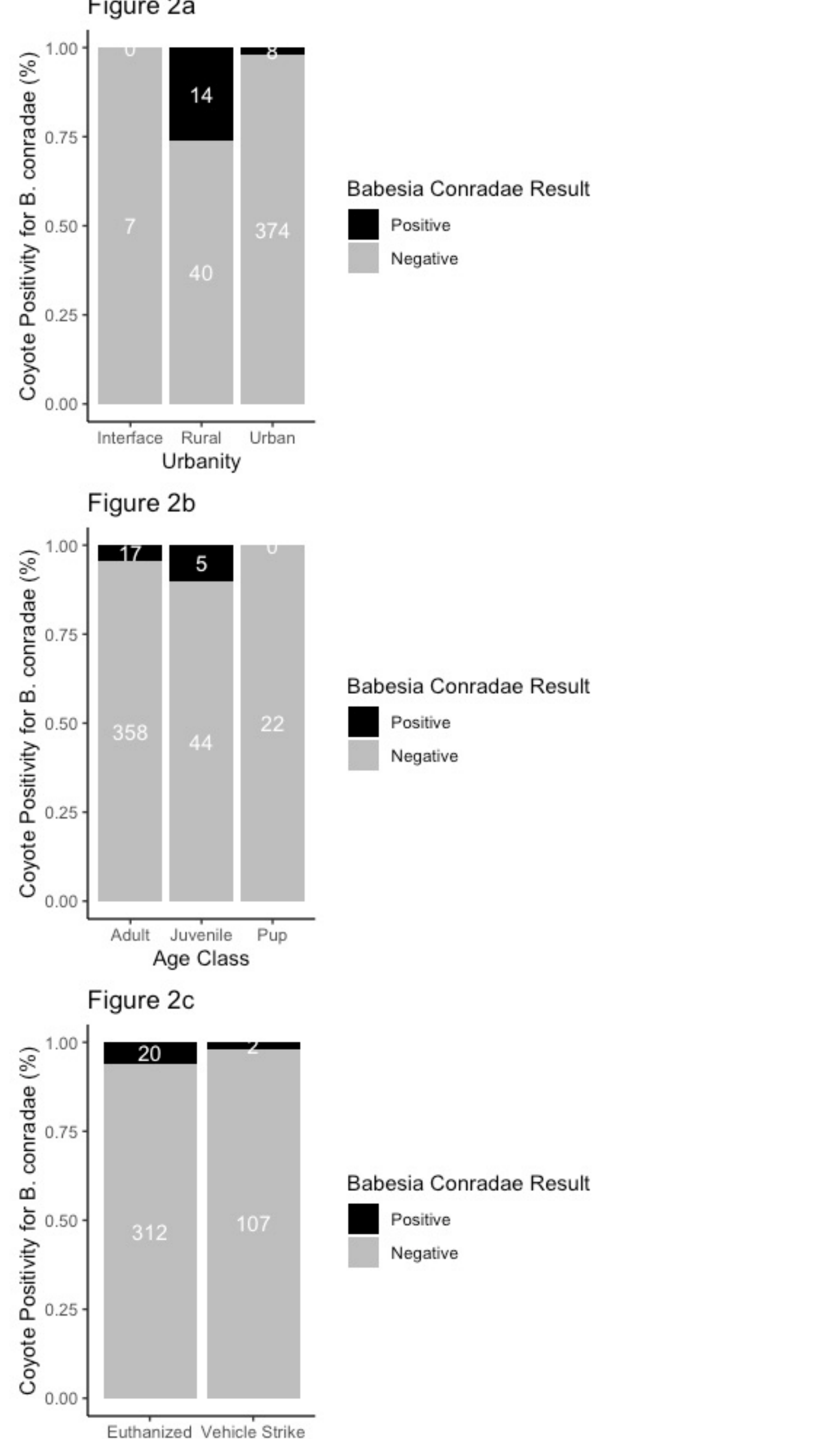


Figure 3: Comparisons of the proportion of *B. conradae* positivity coyote (*Canis latrans*) populations in California using urbanity, age class, and cause of death.

a) Proportion of *B. conradae* positivity between urban, rural, and interface coyote populations. Absolute number of coyotes are labeled on the bar plot. The proportion of coyotes infected was significantly different between urbanity classifications ($P < 0.0001$) and a greater proportion of rural coyotes were positive than urban or interface coyotes.

b) Proportion of *B. conradae* positivity between pup, juvenile, and adult coyotes. Age class was determined by sexual maturity and dentition. Absolute number of coyotes are labeled on the bar plot. Age class was not a predictor of infection with *B. conradae* ($P = .13$).

c) Proportion of *B. conradae* positivity between coyotes that were euthanized and those who are suspected to have died from vehicular strike. Absolute number of coyotes are labeled on the bar plot. Cause of death was not a predictor of infection with *B. conradae* ($P = .14$).

DISCUSSION AND ACKNOWLEDGEMENTS

- 1) Coyotes can become infected and harbor *B. conradae*. Coyotes should be investigated as a possible source of infection in domestic dogs.
- 2) If coyotes experience a similar clinical disease as dogs when infected, this pathogen has the potential to have a significant impact on coyote populations.
- 3) No clear seasonality was detectable nor were any pups infected with the disease, therefore further research in tick-vectored and transplacental mechanisms of transmission are warranted.
- 4) Future work further elucidating the epidemiology of the disease should be done including tick positivity prevalence, seroprevalence in coyotes, and pathology in coyotes.

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ABSTRACT

Babesia conradae is a re-emerging intraerythrocytic protozoan causing disease characterized by severe hemolytic anemia and thrombocytopenia in domestic dogs and can be fatal without treatment. Although the mechanism of transmission is not known, coyotes (*Canis latrans*) have been a proposed reservoir of disease, and previous work has shown that dogs with known aggressive interactions with coyotes are at greater risk for infection than those without these interactions. This study aimed to determine the prevalence of *B. conradae* in wild coyote populations in California in order to assess the viability of coyotes as a potential source of infection for domestic dogs. 465 splenic samples were obtained during post-mortem examination of coyote carcasses collected as part of a convenience sample or from previous epidemiological studies from Southern California, Fresno, and Hopland. DNA was extracted from samples and amplified using real-time PCR with primers specific for the *B. conradae* ITS-2 gene. In total, 22 coyotes tested positive in Fresno (n=15), Orange (n=4), San Bernardino (n=1), and Los Angeles counties (n=1) with an overall prevalence of 4.7%. Coyotes from Fresno ($p < 0.00001$) and rural coyotes ($p < 0.0001$) were significantly more likely to be infected with *B. conradae*. This study demonstrates that coyotes can become infected and harbor *B. conradae* and should be investigated as a possible source of infection in domestic dogs. Further avenues for research include investigating antibody seroprevalence and mechanism of transmission in coyotes.

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