

### Introduction

- Ehrlichiosis is a risk for humans and dogs across the country, but Missouri and Arkansas have shown the largest increasing trends for disease and highest risk of infection. It is commonly vectored by the Lone Star tick, the American dog tick, and the brown dog tick.
- Ehrlichiosis can manifest variably, from an asymptomatic infection to a fatal multi-organ monocytic ehrlichiosis, with the causative agent being *E. chaffeensis*, *E. ewingii*, or *E. canis*.
- Dated prior research has found *E. ewingii* to be the predominant species in Missouri; however, with changing climatic and ecological patterns, there is a need for an updated evaluation of local *Ehrlichia* spp. and their individual incidences of infection and clinical disease.
- The SNAP® 4Dx® Plus Test tests dog blood for four vector-borne pathogens—two *Ehrlichia* spp., two *Anaplasma* spp., *Borrelia burgdorferi*, and *Dirofilaria immitis*—and is a valuable and inexpensive screening tool. Despite its value clinically, the test does not differentiate to a species level, so to further elucidate pathogenic *Ehrlichia* presence in an endemic area, the tick vectors themselves can be screened with more discerning methods including nucleic acid sequencing.

**The principal objective of this study is to characterize an initial epidemiological picture of canine *Ehrlichia* infection and disease risk in Boone County, Missouri, utilizing retrospective data to build context and establishing long-term active surveillance.**

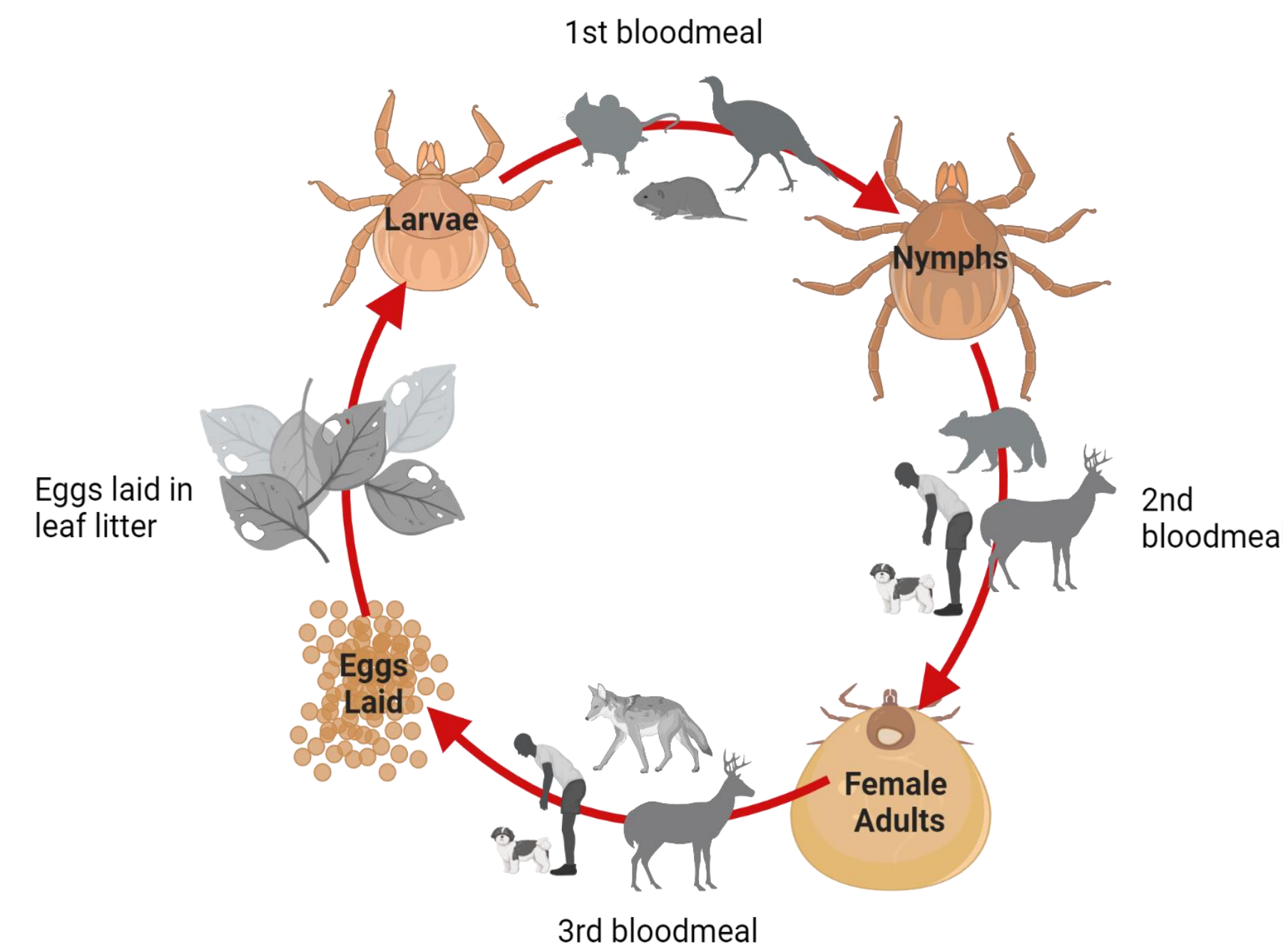


Figure 1: Transmission cycle of the Lone Star tick, *Amblyomma americanum*.

### Discussion

- Although *Ehrlichia* positivity rates from the SNAP® test results far outshadow those of the screened *Anaplasma* spp., *Borrelia burgdorferi*, and *Dirofilaria immitis*, the vast majority of *Ehrlichia*-positive dogs present no apparent clinical signs (Fig. 3, Tab. 1).
- Tick-borne illness was suspected as the cause of severe clinical signs in just 1 case of a positive *Ehrlichia* result, where the patient presented with polyarthritides and fever. Out of the 74 nascent positive SNAP® test results, only 10 received a documented diagnosis of canine ehrlichiosis, and of those, 5 were in the VHC for a wellness visit. Within this setting, clinical signs (on top of a positive test result) did not necessarily precede diagnosis, and diagnosis did not precede a prescription of doxycycline, the broad antimicrobial treatment for tick-borne illness. Diagnoses may not be a reliable tool for characterizing *Ehrlichia* infection and disease risk within this dataset (Tab. 2).
- In July 2022, only 1 tick species, *Amblyomma americanum*, was identified from the ticks collected on popular hiking trails and dog parks in Boone County. The other two vectors appear to be less consequential in Boone County this season for Ehrlichia transmission (Fig. 4).

### Methods

#### Retrospective clinical analysis:

- SNAP® 4Dx® Plus test data pulled from the University of Missouri Veterinary Health Center's VetView database is organized and analyzed for the years 2012-2022.



#### Active surveillance:

- Sites are selected for public health relevance, optimal habitat, and tick density.
- Ticks are collected by cloth drags for 750 square meters per site, and stored live in vials. Ticks collected from shelters and rescues are stored directly in RNALater solution.
- Adult and nymphal ticks are identified using dichotomous keys for species, sex, and life stage.
- Using a sterile blade, ticks are sliced in half with internal tissues collected and exoskeleton discarded.
- Nucleic acid is extracted with Qiagen AllPrep kit and stored at -80 °C.
- Using the V4 region of the highly conserved 16S rRNA gene, samples will be sequenced for three *Ehrlichia* species.

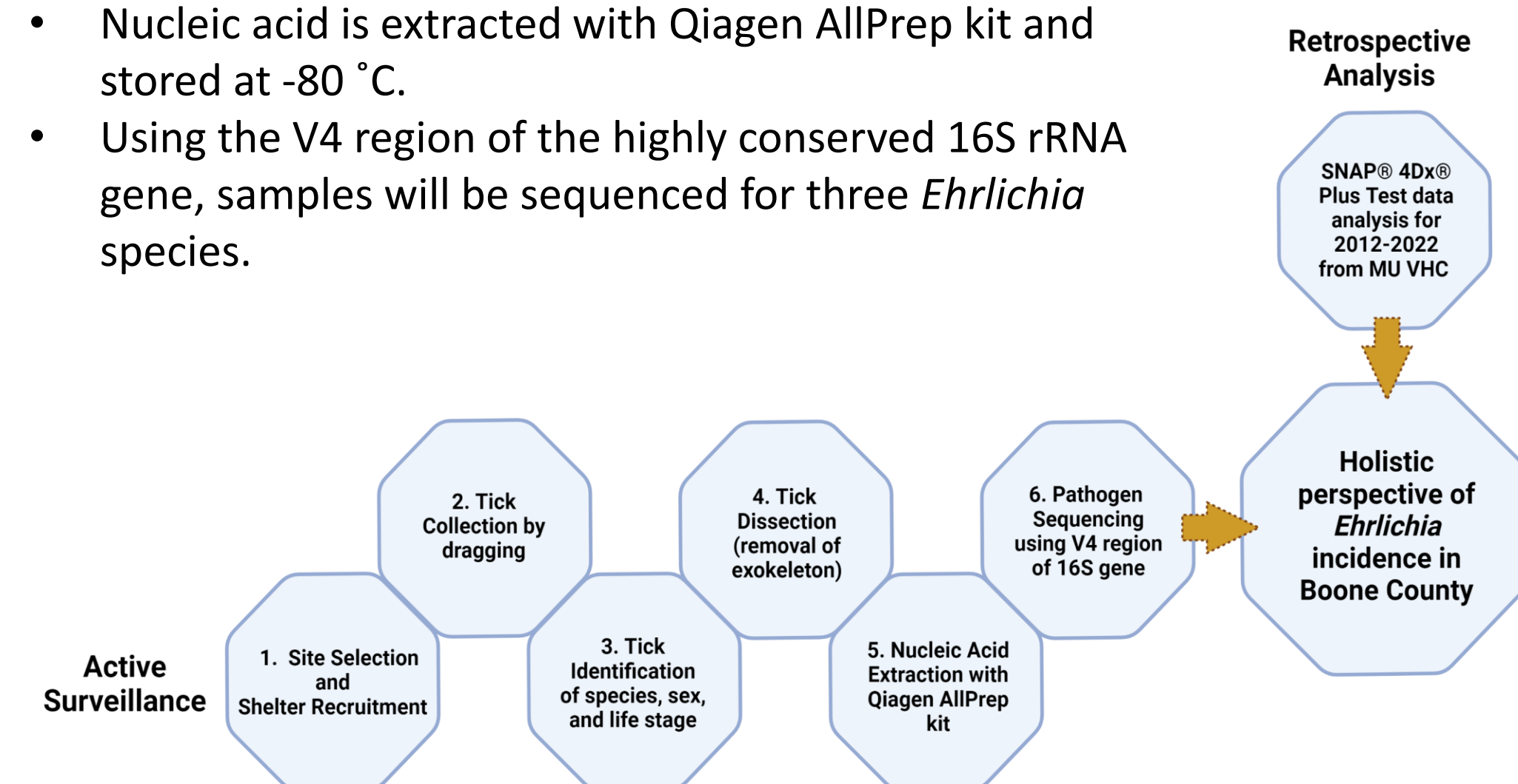


Figure 2: Schematic of experimental methodology.

### Results

Figure 3: Preliminary data from January 2021 to June 2022 of positive SNAP® 4Dx® Plus Test results from the University of Missouri Veterinary Health Center.

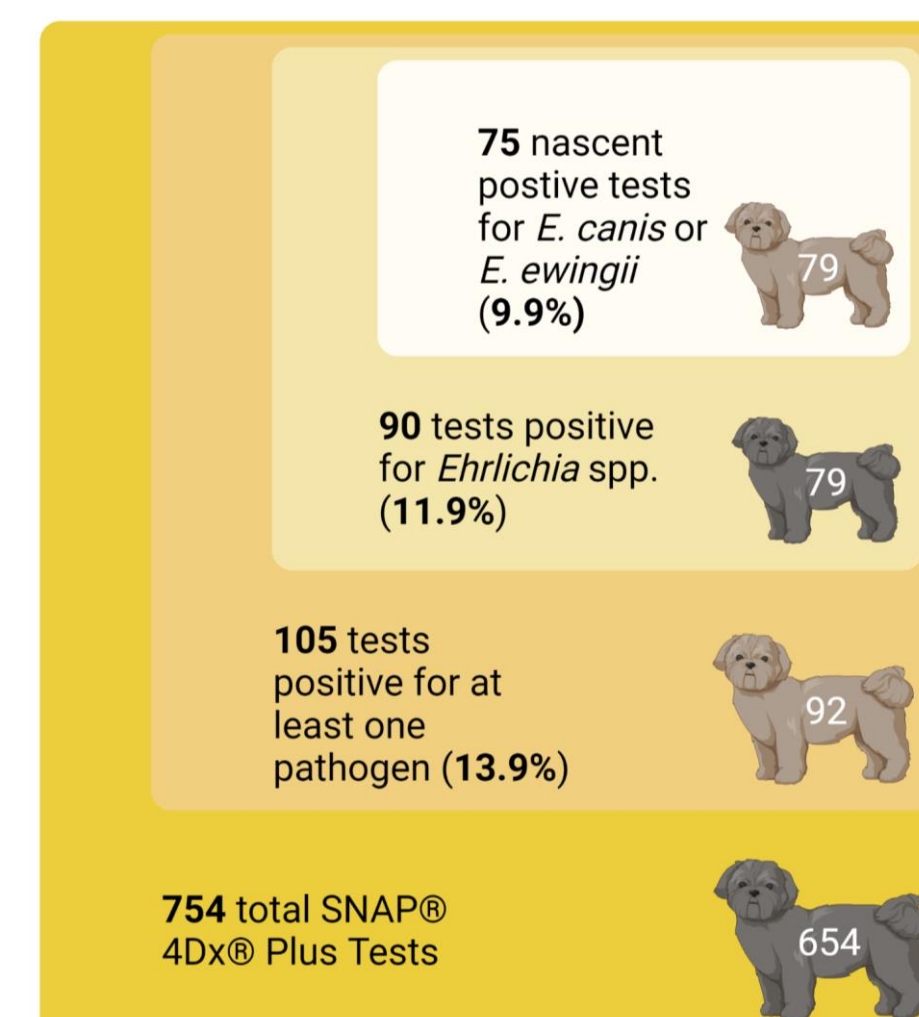


Figure 4: Tick drag sampling in July 2022. 6 sites were sampled for 750 square meters each on wooded trails with suitable habitat. Ticks were then identified based on species, life stage, and sex. A total of 24 ticks was collected this month.

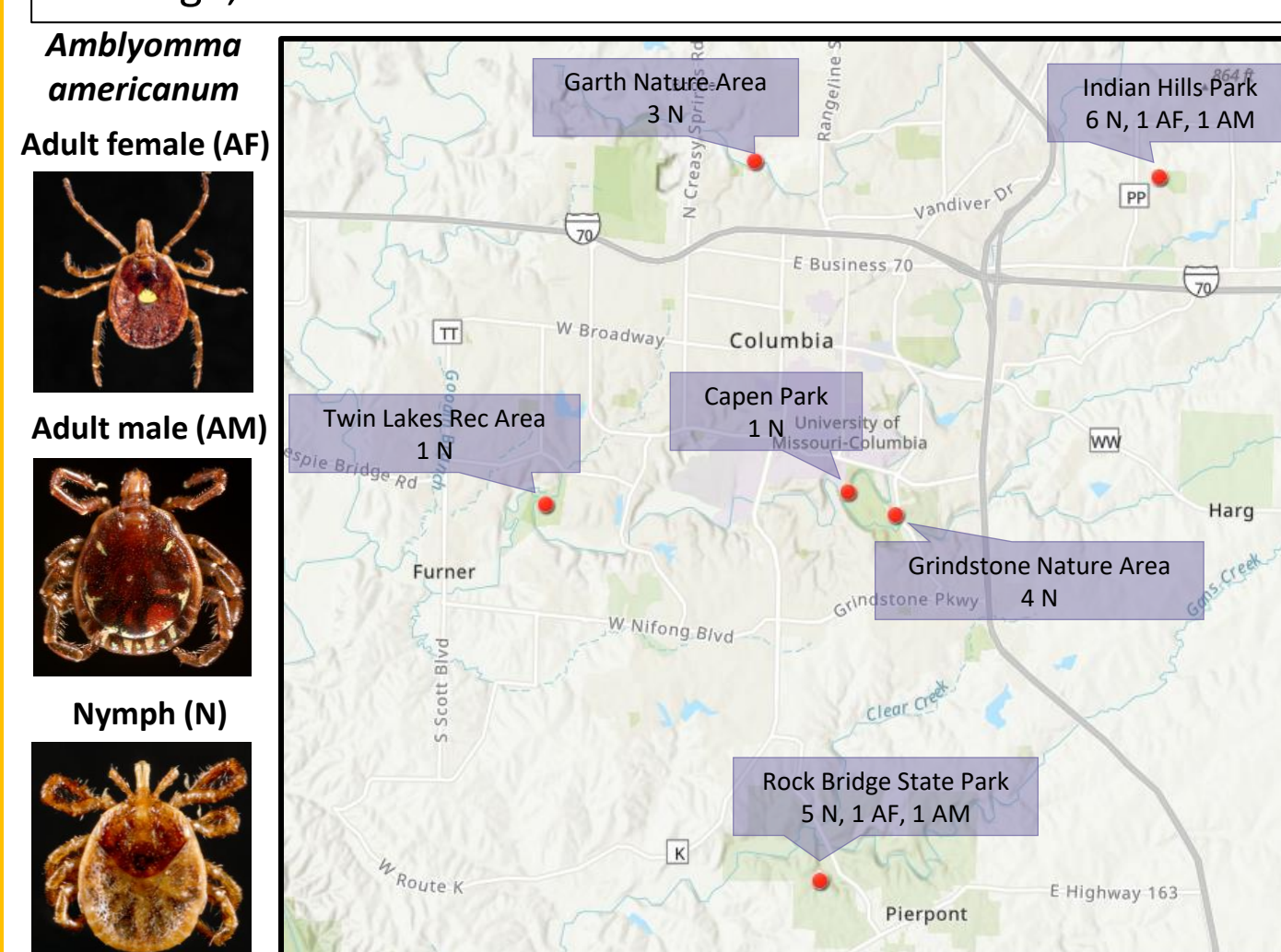


Table 1: Nascent infections from January 2021—June 2022 based on SNAP® test results. The test does not discriminate between new and old infections; prior infections were noted by clinician.

Vector-borne pathogen	Positive case count	Positive case percentage
<i>Ehrlichia ewingii</i> and <i>Ehrlichia canis</i>	74	70.50%
<i>Anaplasma phagocytophilum</i> and <i>Anaplasma platys</i>	1	0.95%
<i>Borrelia burgdorferi</i>	6	5.70%
<i>Dirofilaria immitis</i>	7	6.70%

Table 2: Confirmed canine ehrlichiosis diagnoses from January 2021—June 2022. 10 cases of the 75 nascent *Ehrlichia* infections received a recorded diagnosis of ehrlichiosis. For half the patients, diagnosis was assigned with a positive SNAP® test result and a lack of clinical signs.

Patient	Clinical Signs
1	none
2	none
3	vomiting and possible dysautonomia
4	checkup for limping
5	possible tick borne illness with polyarthropathy
6	none
7	none
8	none
9	enlarged abdomen
10	checkup for infiltrative lipoma

### Moving Forward

- SNAP test data from 2012-2020 will be analyzed for positivity rates, clinical signs, diagnoses, treatments, and outcomes.
- Local sites will be selected for frequent active surveillance and will be sampled on a weekly basis.
- We will attempt different protocols to discern optimal storing methods to reduce degradation of nucleic acids in vectors, including storage in RNALater solution at room temperature.
- We will explore variable methods for tissue collection and preparation for extraction of ticks, optimizing for low contamination and high concentrations of DNA, optimally 50 nanograms per microliter.
- Utilizing primers for the V4 region of the highly conserved 16S gene, we will design an assay to detect the individual pathogenic species of *Ehrlichia* including *E. chaffeensis*, *E. ewingii*, or *E. canis*. This will allow more inclusive and accurate species-level screening and help us discern the dominant species in the county.
- Given a successful pathogen assay is developed for *Ehrlichia*, we are interested in building the assay further to detect other tick-borne pathogens posing health threats to both pets and their owners.

### Acknowledgments

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### References

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