

Active surveillance for the tick-vectored hemoparasite *Theileria orientalis* in three Missouri beef herds

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Background

- The newly emerging hemoparasite, *Theileria orientalis*, is a pathogenic protozoa that causes *Theileria*-associated bovine anemia (TABA) in cattle.
- Clinical disease resembles bovine anaplasmosis. Unlike bovine anaplasmosis, there is no treatment for TABA.
- Theileria orientalis* is transmitted by the invasive longhorned tick (*Haemaphysalis longicornis*) and could result in severe economic losses for the U.S. cattle industry.
- The longhorned tick, native to eastern Asia, was first identified in the U.S. in 2017 and has since spread to 20 states and the District of Columbia.
- Epidemiologic studies in Australia and New Zealand have shown that *T. orientalis* spreads quickly in areas where longhorned tick populations are established.
- Longhorned ticks are currently known to be established in 3 Missouri counties.
- Sporadic cases of *T. orientalis* have been identified in MO, but data on the extent of its spread is limited.

Objective

Our objective is to surveil for the presence of *Theileria orientalis* in 3 Missouri cow-calf herds grazing premises where *H. longicornis* ticks are known to be established.

Methods

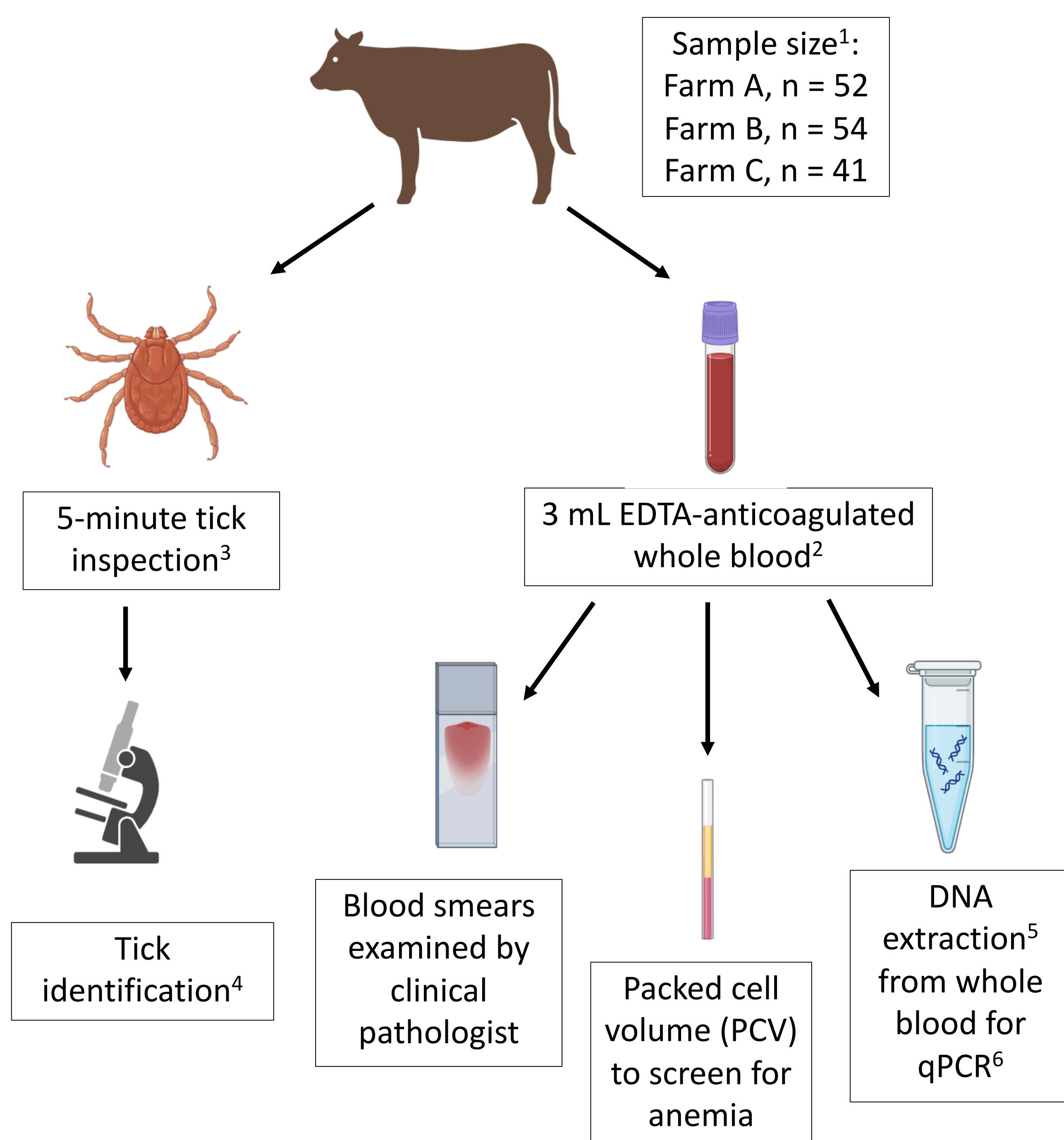


Figure 1: Summary of methods. ¹ Sample size from each herd calculated to detect infected cattle with 95% confidence ($\alpha = 0.05$) at $\geq 5\%$ prevalence. ² Collected from the coccygeal vein with an 18-gauge needle. ³ Ticks collected into 100% ethanol. ⁴ Ticks taxonomically identified by species and life stage using morphologic keys (Keirans, 1989; Egizi, 2019). ⁵ DNA extracted from whole blood using the Thermo Scientific GeneJET Genomic DNA Purification Kit according to the manufacturer's instructions. ⁶ Quantitative PCR performed in duplicate with primers designed to amplify the major piroplasm surface protein (MPSP) gene of all *T. orientalis* genotypes, as previously described (Oakes, 2022). Created with BioRender.

Study Herd Locations

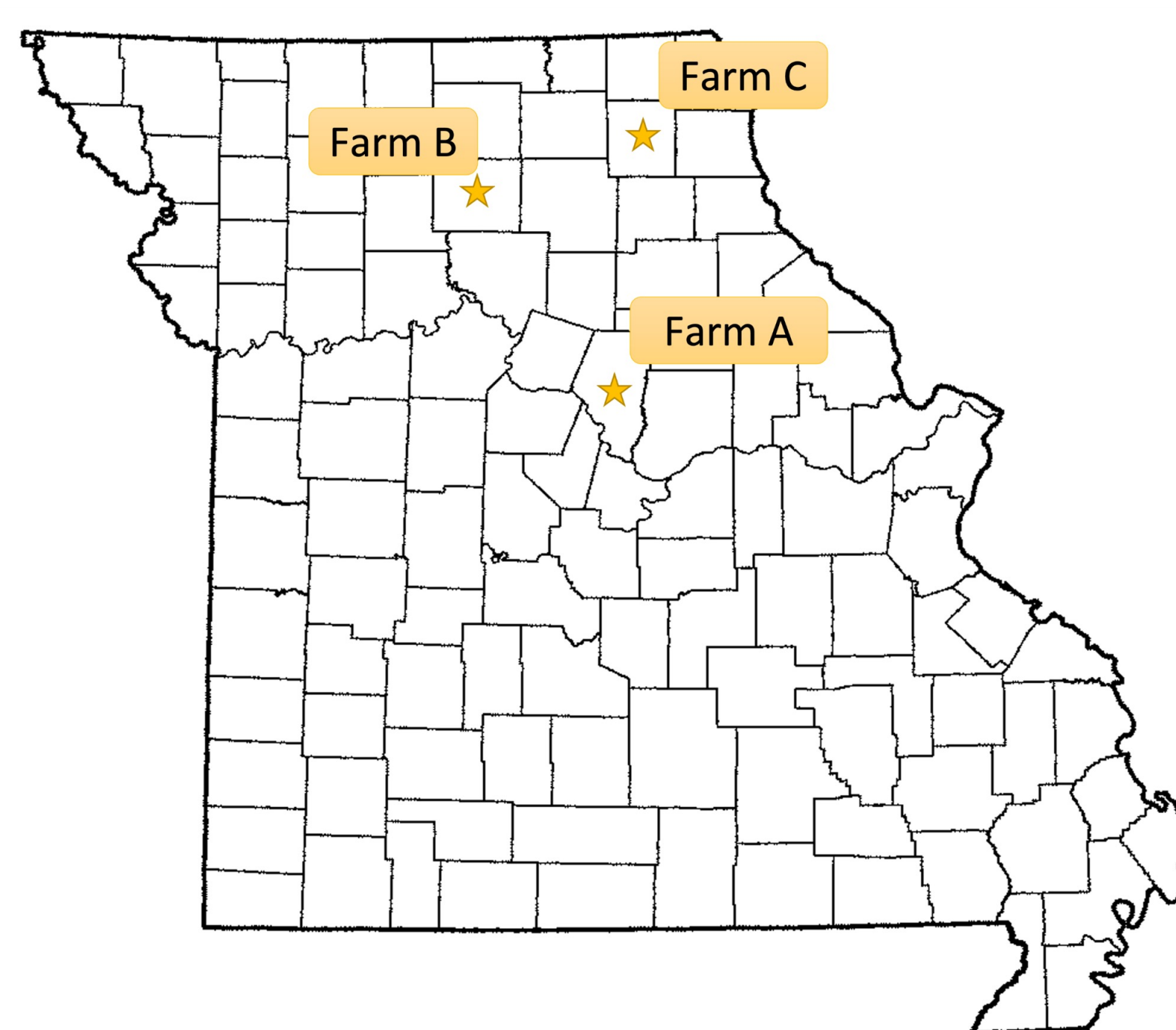


Figure 2: County map of Missouri showing approximate locations of study herds (A, Boone County; B, Linn County; C, Knox County). Longhorned ticks are currently established in these 3 counties.

qPCR Results for *T. orientalis*

Herd	Sample ID	C _T (Mean)
Farm A (n = 52)	7	28.7
	24	31.2
	25	32.6
	30	29.2
	31	29.6
	44	27.8
Farm B (n = 54)	45	26.7
	9	33.1
	51	30.3
Farm C (n = 41)	53	29.8
	10	28.9

Tick Identification Results

	Farm A	Farm B	Farm C	Total
<i>Amblyomma americanum</i> (lone star tick)	131	110	65	306
<i>Dermacentor variabilis</i> (American dog tick)	0	10	191	201
<i>Haemaphysalis longicornis</i> (longhorned tick)	2	4	12	18
Total	134* (2.6/cow)	125** (2.3/cow)	268 (6.5/cow)	527 (3.6/cow)

*1 tick from Farm A was an adult *Ixodes* but was too damaged to identify at the species level.

**1 tick from Farm B was too damaged to be identified.



Eighteen *H. longicornis* ticks, including 9 adult females and 9 nymphs, were collected from 16 cows (Farm A, 2 cows; Farm B, 4 cows; Farm C, 10 cows).

Figure 3: CDC Image of a nymph (left) and an adult female (right) of *Haemaphysalis longicornis*.

PCV and Blood Smear Results

- PCVs of all cows except for one were within normal limits (Farm A, mean = 32.2%; Farm B, mean = 33.5%; Farm C, mean = 35.8%)
- One cow on Farm B was anemic with a PCV of 18% and died several days after sample collection. *Theileria orientalis* was not detected by blood smear or qPCR. *Anaplasma marginale* was detected by qPCR (C_T 27.9) but was not detected by blood smear. No necropsy was performed.
- Theileria* organisms were detected on blood smears in 6 cows from Farm A and 1 cow from Farm B.

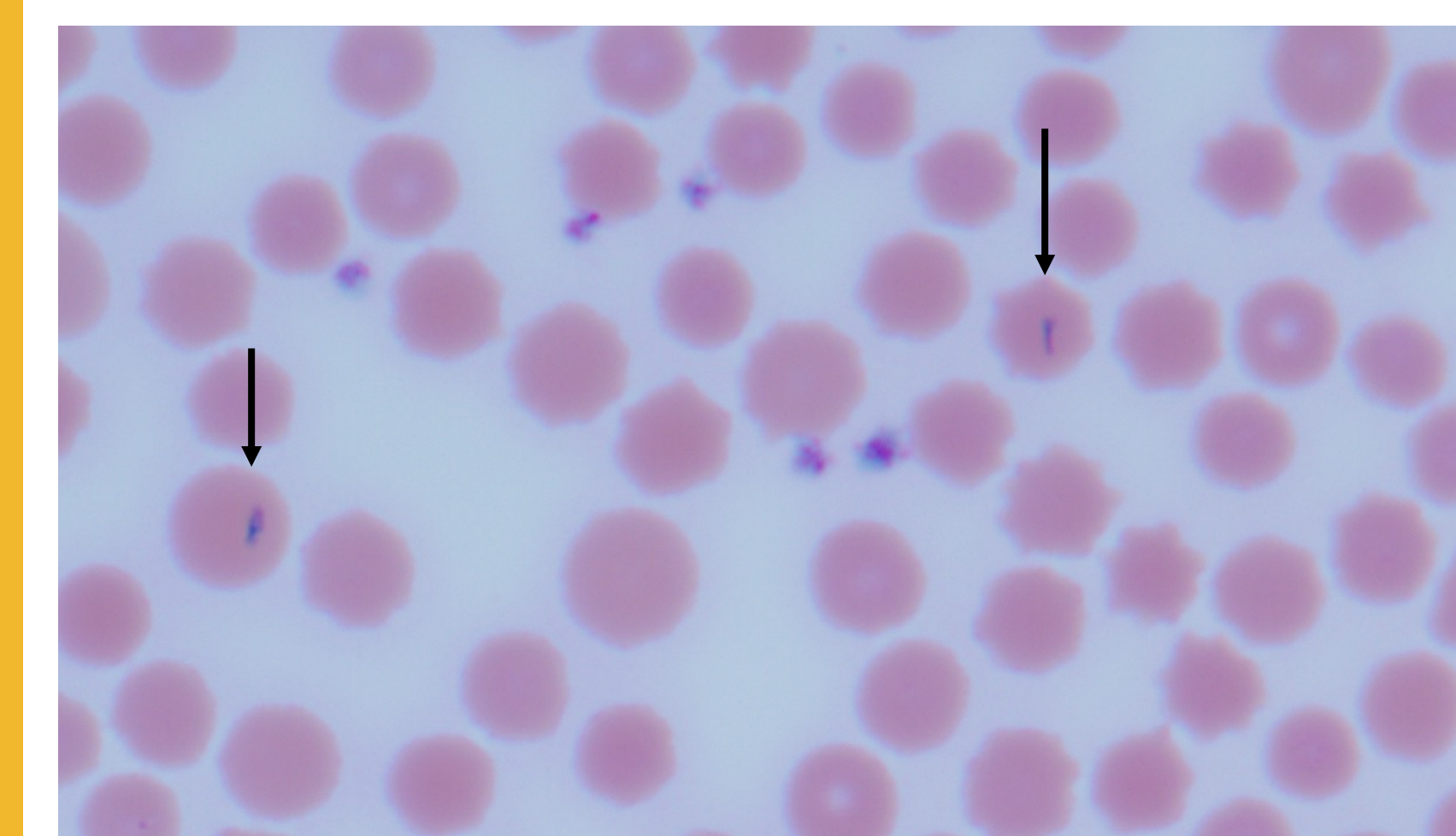


Figure 4: Blood smear from a cow infected with *T. orientalis*. Rare erythrocytes contain slender and elongate protozoa, approximately 2-4 μm long, with basophilic cytoplasm and a pink-purple internal region. Photo credit: Dr. Jacqueline Nunnolley, DVM.

Discussion

- Longhorned ticks were first identified on pastures at these 3 farms in August 2022 (Farm B), April 2023 (Farm A), and August 2023 (Farm C). Collection of multiple life stages on all premises met criteria for establishment.
- The longhorned tick is a successful invasive species because females are parthenogenetic (capable of asexual reproduction) and they feed on a variety of domestic and wild hosts.
- Our results show no obvious relationship between the number of *H. longicornis* collected from cows and the number of *T. orientalis*-infected cows within a herd; however, we do not know when these cows were initially infected with *T. orientalis*, and tick numbers can vary widely depending on the time of year.
- Our next steps include a genotype-specific qPCR to distinguish which of the 3 most common *T. orientalis* genotypes (Ikeda, Chitose, and Buffeli) are present in the study herds.
- In conclusion, our findings show that *Theileria orientalis* is currently prevalent among cattle in the 3 herds tested, and given the concurrent presence of the tick vector, there is likely local transmission of *T. orientalis* in these herds.

References

- Egizi, A. M., et al. (2019). A pictorial key to differentiate the recently detected exotic *Haemaphysalis longicornis* Neumann, 1901 (Acari, Ixodidae) from native congeners in North America. *ZooKeys*(818), 117-128.
- Keirans, J. E., et al. (1989). Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. *J Med Entomol*, 26(5), 435-448.
- Oakes, V. J., et al. (2022). Coinfection of cattle in Virginia with *Theileria orientalis* Ikeda genotype and *Anaplasma marginale*. *J Vet Diagn Invest*, 34(1), 36-41.

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