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Monitoring for arboviruses in mid-Missouri by local tick sampling and presence of viremia in domestic and wild animals.

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Background

Tick-borne diseases have been on the rise in Missouri for several years. Indeed fatal human cases of infection with novel arboviruses have recently emerged and continue to increase in incidence. Heartland virus (HRTV), a Phlebovirus in the family Bunyaviridae was first reported in 2009, and was associated with severe fever, leukopenia, and thrombocytopenia in affected individuals. Bourbon virus, a Thogotovirus in the family Orthomyxoviridae, was first detected in in 2014. Surveillance studies have identified these viruses in ticks collected around the geographical locations of cases in the states of Missouri and Kansas.

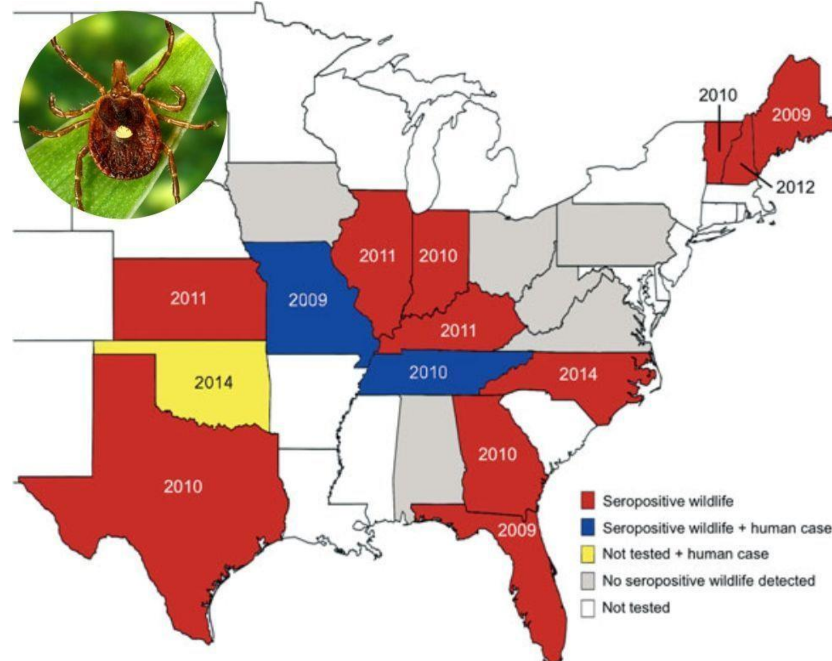


Figure 1: Map of seropositive results for HRTV in the U.S.

Limited serological survey demonstrating exposure of wild and domestic animals to these viruses has suggested that viremic vertebrate hosts may play a role in the transmission cycle. However, there is paucity of information on active infection of wild and domestic animals in the state of Missouri and whether these viruses are present in ticks found in local peri-urban parks. This information is vital because newly emerging viruses can pose a serious threat to human and animal health – especially with the growing popularity of outdoor recreation in this area.

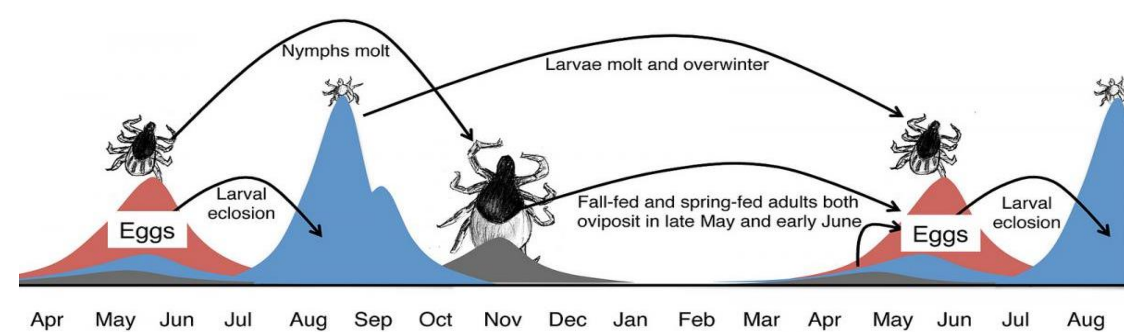


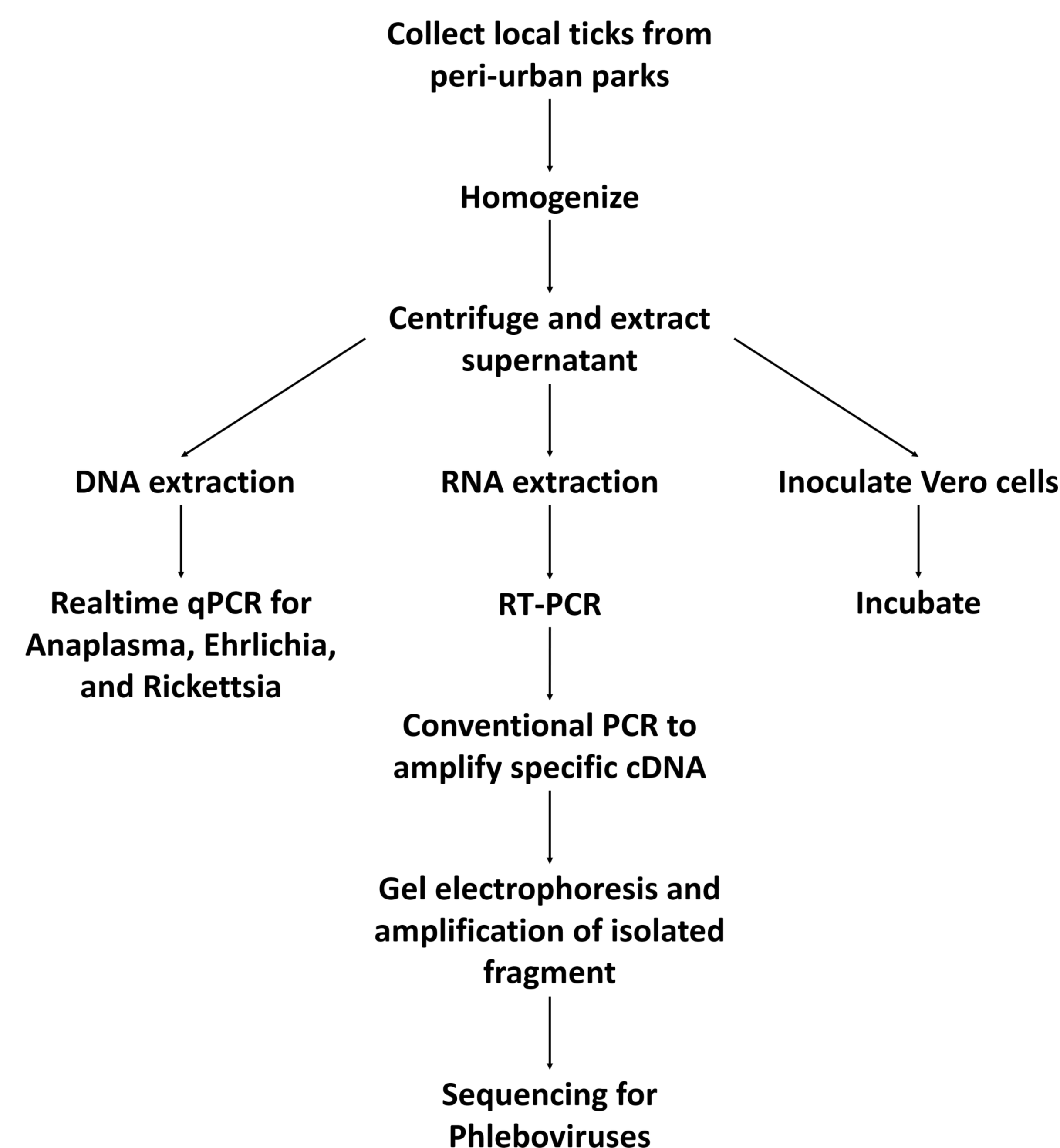
Figure 2: General life cycle of a tick throughout the year.

Specific Aims

(1) To determine the incidence of Phleboviruses and Thogotoviruses in ticks collected from peri-urban parks in mid-Missouri.

(2) To analyze diagnostic samples submitted to the VMDL in Columbia, MO from domestic animals and wildlife.

Methods



1. **Tick Analysis**: Ticks will be collected, with a drag technique, from peri-urban parks and recreational areas around the city of Columbia, MO during the summer of 2018.
2. Family, genus and species specific real-time qPCR will be used to probe DNA isolated from tick homogenates for common tick-borne pathogens (e.g. Anaplasma, Ehrlichia, and Rickettsia).
3. RT-PCR will be used to convert all RNA isolated from tick homogenates to cDNA, with random primers.
4. Conventional PCR will be used to amplify family, genus, and species specific cDNA rom tick homogenates with primers for Phlebovirus and Thogotovirus.
5. Gel electrophoresis will be used to isolate possible Phlebovirus and Thogotovirus RNA (100-300bp) following RT-PCR and sequencing will be used to verify the identity of the fragments.
6. Supernatant from tick homogenates will also be inoculated into Vero cell culture and incubated with regular observation to observe effects.
7. **Retrospective Analysis**: Stored RNA extracted from domestic animal and wildlife samples submitted to the VMDL from 2013-2017 will be randomly selected and tested in the same manner.

Preliminary Results

Local tick collection:
Adult and nymph ticks were successfully collected, using self-made drags, from peri-urban parks around Columbia, MO. Areas where they were found abundantly included Hinkson Woods Conservation Area (72) and Grindstone Nature Center (25). Species found included Lone star ticks and American dog ticks.

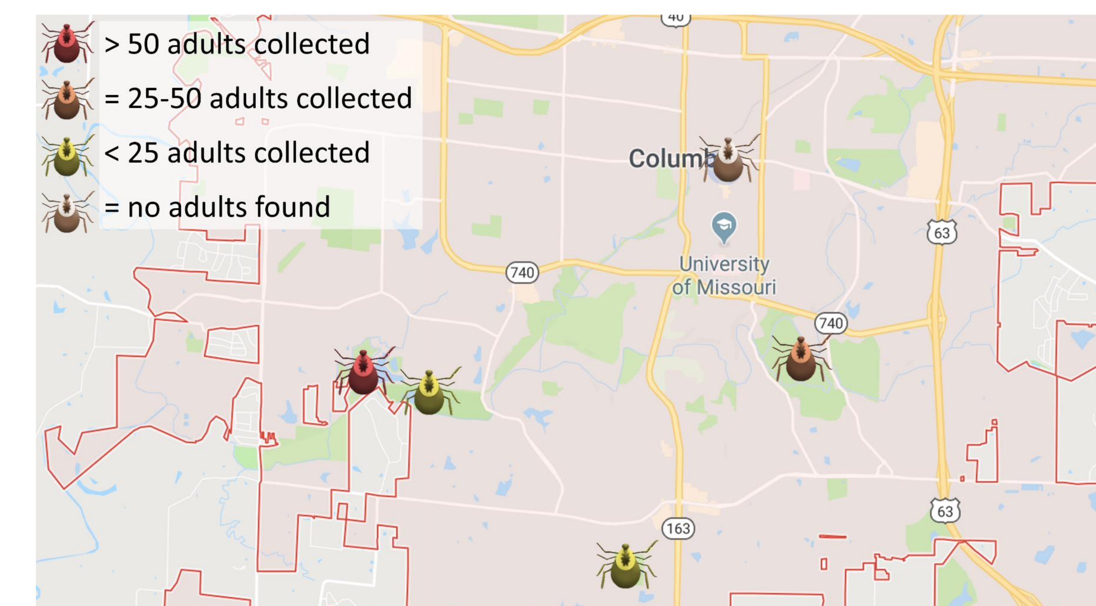


Figure 3: Geographical map of tick collection in Columbia, MO.

Identification of carried pathogens:
Of the 72 ticks collected from Hinkson Woods Conservation Area, 54 tested positive for Rickettsia by real-time qPCR. All ticks collected were negative for Anaplasma and Ehrlichia.

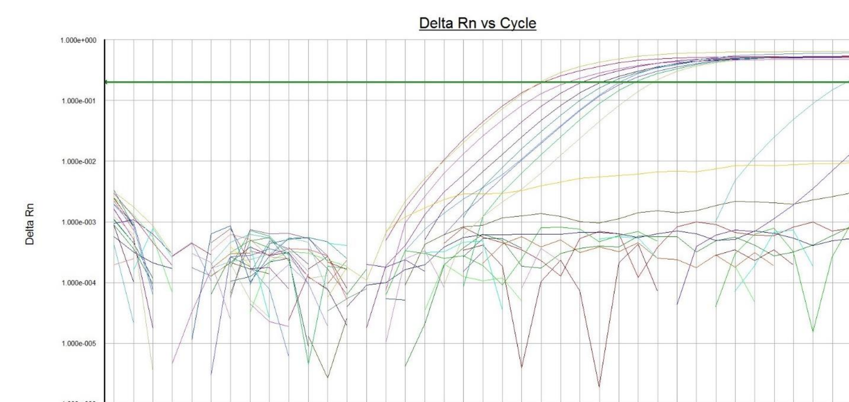


Figure 4: Amplification plot of real-time qPCR for Anaplasma, Ehrlichia, and Rickettsia (+).

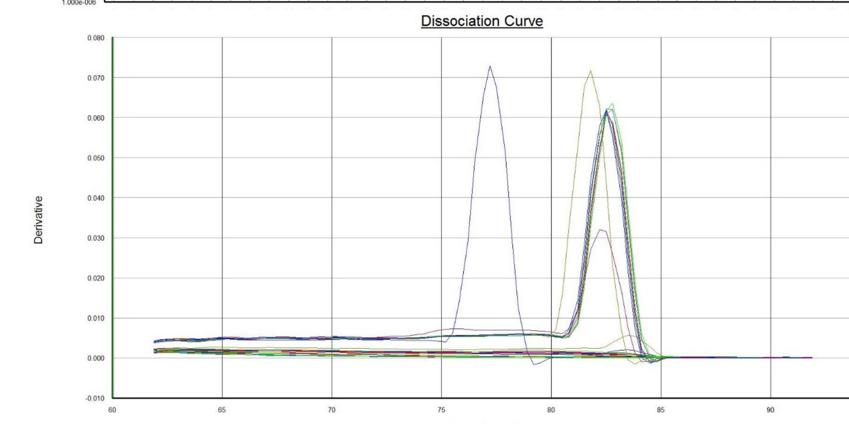


Figure 5: Melt curve of real-time qPCR for Anaplasma, Ehrlichia, and Rickettsia (+).

Identification of Possible Phlebovirus:
All Lone star adult and nymph ticks collected from Grindstone Nature Center displayed a band around 380bp on gel electrophoresis, following RT-PCR and then conventional PCR on resulting cDNA with Phlebovirus primers.

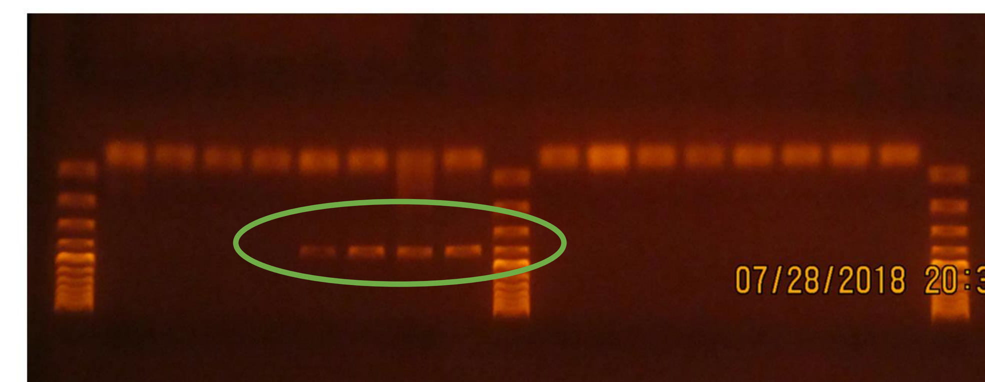


Figure 6: Gel electrophoresis of tick sample cDNA with band present at ~380bp.

Unexpected challenges

Figuring out the best way to homogenize ticks:
Ticks posses a very tough cuticular exoskeleton that resists pressure. This prevents researchers from easily accessing their genetic material and any pathogens they may be carrying. Initially, a TissueLyser II system was used – sample (ticks), media and a steel ball bearing were added to a lock-top Eppendorf tube and shaken vigorously by a machine. However:

(1) One steel bearing was not enough to adequately homogenize the samples. So, a second was added, which successfully broke up the samples, such that nucleic acids could be extracted.

(2) Using multiple steel bearings would cause small cracks to appear in the lids of some lock-top Eppendorf tubes and samples were lost. Another type of tube with a thicker screw-top was used for the next round of samples and the lids successfully held up to the bearing impact.

(3) Small cracks began to appear in the bottom of some screw-top tubes when shaken in the machine for longer than 15 minutes and samples were lost. Wrapping the bottom of these tubes with parafilm, to prevent fluid loss, is proposed for the next run of samples.

(4) Alternatively, using multiple smaller sized beads has been proposed to help reduce impact to the tube, but still completely homogenize the sample.

Figure 6: TissueLyser II machine, tubes and steel beads used to homogenize tick samples.

Future Aims

1. To sequence PCR products obtained from local tick samples that show bands around 380bp after amplification with Phlebovirus specific primers.
2. RT-PCR of tick samples with Thogotovirus specific primers.
3. RT-PCR of samples obtained through the VMDL from domestic and wild animals from 2013-2014 with Phlebovirus and Thogotovirus specific primers.
4. Deep sequencing of RNA obtained from local tick samples, following RT-PCR, to detect novel viruses.