Testing for immune system adaptivity: hemocyte response to bacterial infection in ticks

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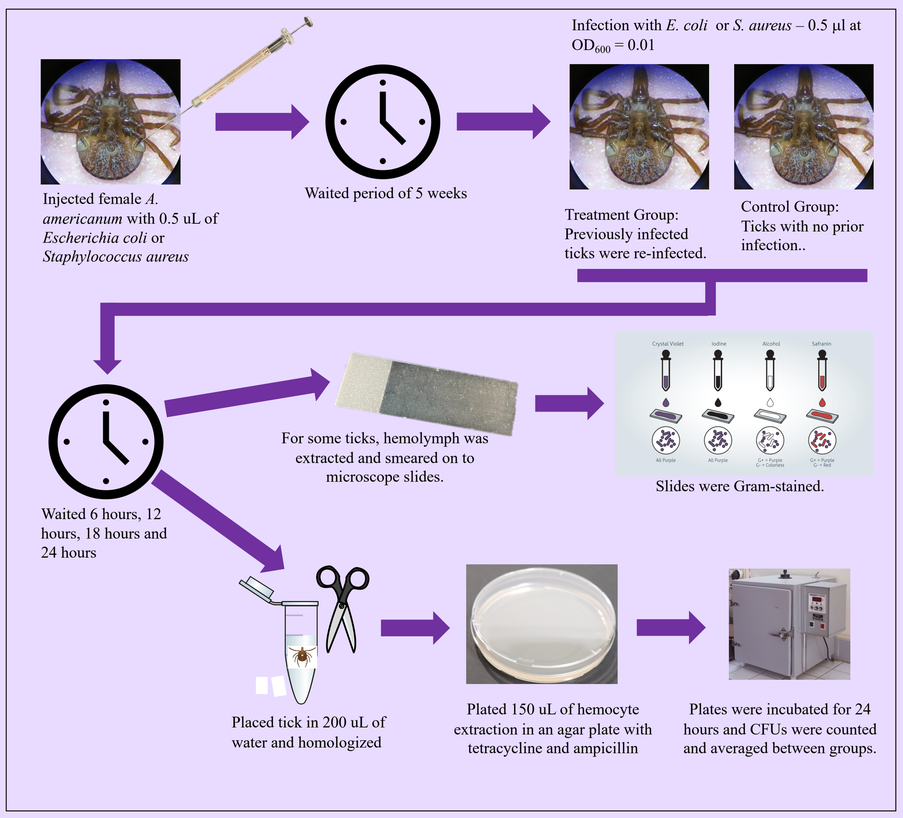
Background

A prevailing and hotly debated uncertainty in immunology is whether invertebrates, including arthropods such as ticks, possess an immune system with adaptive abilities or if, as it has long been assumed, they are merely capable of generating an innate immune response. Innate immunity is characterized by a lack of memory of prior infections, no specificity in response to a particular pathogen, and no change in intensity of response between first and subsequent infections with the same pathogen. Adaptive immunity, however, is oppositely characterized and a growing list of studies have contributed evidence to suggest that invertebrate immune adaptability does indeed exist. To date, zero studies have been published investigating the possibility of any aspect of adaptive immune potential in ticks. This study aims to assess whether the tick immune response can increase in intensity or decrease time taken to respond to a repeated infection with the same bacterial species.

Materials and Methods



Results

Repeated gram-negative infection stimulates faster peak hemocyte response time increases from 24 h to 12 h

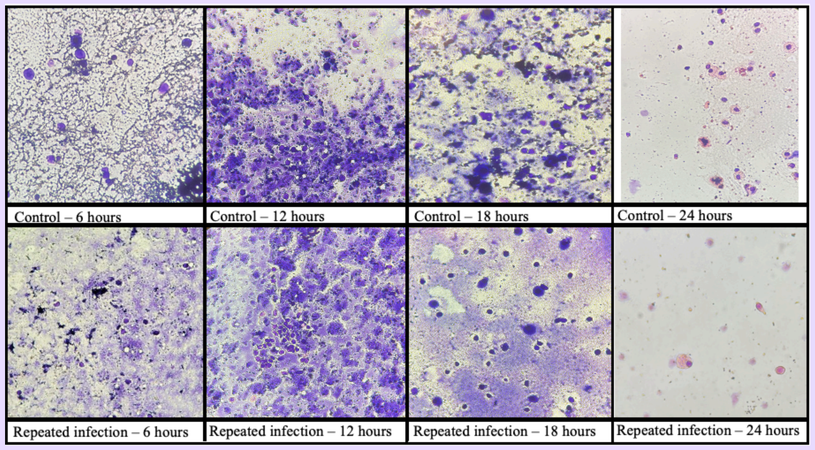


Fig. 1. Gram-stained hemolymph from gram-negative (*E. coli*) initial-infected (control) or repeat-infected ticks.

Repeated gram-positive infection does not stimulate an adaptive response

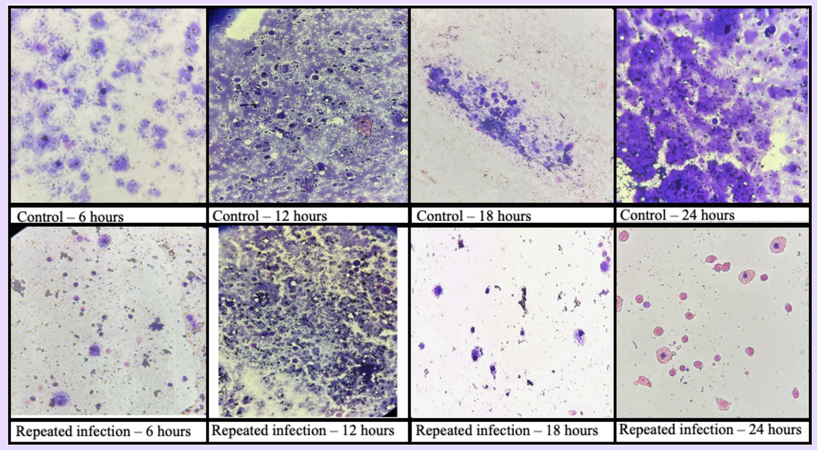


Fig. 2. Gram-stained hemolymph from gram-negative (*S. aureus*) initial-infected (control) or repeat-infected ticks.

Bacterial load in ticks may increase in second infection

Time points	Average Control	Average reinfection	P-Value
6-hour	3	5.5	———
12-hour	57	70.5	0.33929
18-hour	23	118.5	0.11924
24-hour	14.5	29.75	———

Table 1. The table shows the average colony forming units (CFUs) growing on agar plates smeared with hemolymph extracted at 6-, 12-, 18-, and 24 h post-initial (control) or post-repeated infection. Sample sizes for 6- and 24 h were too small for statistical analysis.

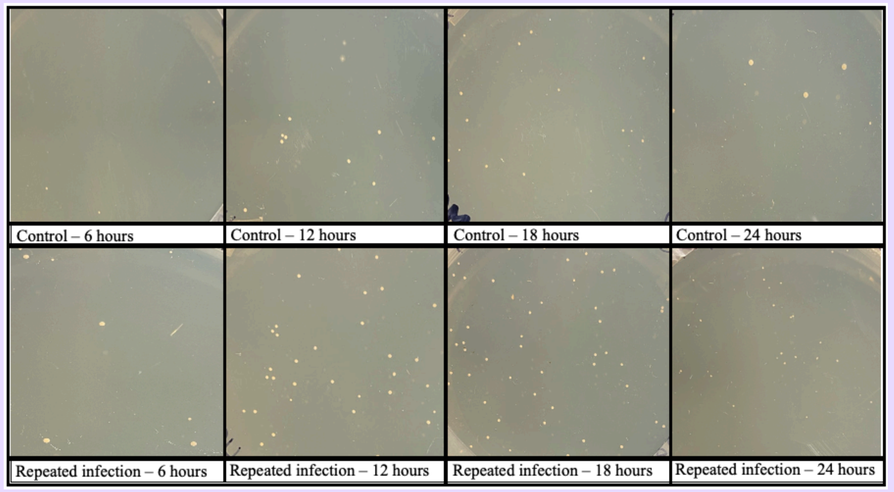


Fig. 3. Comparison of bacterial (*E. coli*) growth on agar plates smeared with hemolymph extracted at 6-, 12-, 18-, and 24 h post-initial (A) or post-repeated (B) infection.

Discussion

Adaptive potential of the tick immune response was assessed in two ways in this study. First, we smeared hemolymph containing hemocytes on microscope slides at 6-, 12-, 18-, and 24 h post-initial (control) or post-repeat (treatment) infection. Qualitative analysis suggests that repeated gram-negative infections induce a faster hemocyte response peak that was not seen for gram-positive infections and differences in abundance were not obvious. Second, bacterial infection clearance from hemolymph was assessed. Differences in bacterial colony counts between initial and repeated tick infection, while not supported statistically, did trend towards significance. Notably, however, results were contradictory to our hypothesis that less bacteria would be present in ticks during a repeated infection.

Conclusion

There is some evidence that for repeated gram-negative infections, ticks respond more swiftly by producing more hemocytes, however overall bacterial loads do not appear to be well-controlled. There is little evidence in this study of a similar increase in response to repeated gram-positive infection, however overall bacterial load could not be assessed in this study and warrants further investigation. One possible explanation is that bacteria from the initial infection still remained in the ticks. Future studies should investigate bacterial persistence in infected ticks.

Acknowledgments

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