

Tick mischief: what can be detected in juvenile ticks *Dermacentor reticulatus* collected from rodents?

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INTRODUCTION. Juvenile *Dermacentor reticulatus* ticks inhabit nests and burrows of their rodent hosts and cannot be collected from vegetation. To detect vertical transmission of *Babesia canis* in *D. reticulatus*, we aimed to study larvae and nymphs collected from rodents. However, the molecular techniques used for the detection of pathogen DNA are sensitive enough to detect not only pathogens vectored by ticks but also those taken up with current or previous blood meals ('meal contamination') or just present in the environment and on the tick or host surface ('environmental contaminations'). Thus, an additional aim of our study was to evaluate the extent of such contamination while studying feeding ticks collected from rodents.

METHODS. Juvenile *D. reticulatus* were collected from 140 rodents: 91 bank voles trapped in two forest sites in the Mazury Lake District and 49 rodents (*Apodemus* and *Microtus* spp). from an open habitat near the town of Białobrzegi in Central Poland. Altogether 504 *D. reticulatus* ticks, comprising 266 individually evaluated nymphs and 238 larvae assigned to 50 larval pools, were studied for the presence of *Babesia*, *Bartonella* and *Rickettsia* spp. DNA. Statistical analyses were conducted to: 1) evaluate the effect of rodent host factors (host species, sex and age) on prevalence of infection in ticks; 2) to compare the frequency of positive samples between groups of pathogen-positive and pathogen-negative rodent hosts. To complete the last aim, blood samples obtained from 49 rodents from Białobrzegi were studied for the presence of *Babesia* and *Bartonella* DNA.

RESULTS. Infestation of rodent hosts with juvenile ticks ranged between 46–78%, with a mean abundance was of 3.6 ticks/rodent for *D. reticulatus* and 4.8 ticks/rodent for *I. ricinus*. The highest prevalence of PCR-positive *D. reticulatus* samples was obtained for *Rickettsia* spp. (28%) and *R. raoultii* was identified in 22 sequenced PCR products. *Babesia* DNA was detected in 20 (7.5%), *D. reticulatus* nymphs, including B. microti in 18 (18/266=6.8%) and B. canis in two (2/266=0.8%) of 266 D. reticulatus nymphs that were analyzed. Babesia microti DNA was also detected in four pools of D. reticulatus larvae (4/50 pools=8%). The detection success of *B. microti* in *D. reticulatus* ticks was associated with the species of the rodent hosts of the ticks species (much higher for typical *B. microti*-host-species such as *Microtus* spp. than for *Apodemus* spp.) and host age (3× higher in ticks collected from adult hosts in comparison to juvenile ones). Moreover, the DNA of *B. microti* was detected in 68% of *D. reticulatus* nymphs collected from *B. microti*-positive rodents in comparison to only 1.6% of nymphs collected from *B. microti*-negative rodents.

Bartonella DNA was detected in 18% of *D. reticulatus* tick samples (38% of larvale pools, 14% of nymphs). Again, host factors played important roles for 'tick positivity' – the highest prevalence

of positive ticks was collected from *Apodemus* spp., which are regarded as *Bartonella* reservoirs. The DNA of *Bartonella* was detected in 42% of nymphs and 57% of larval pools collected from *Bartonella*-positive rodents in comparison to 28% of nymphs and 11% of larvae collected from *Bartonella*-negative rodents.

CONCLUSIONS. Vertical transmission of *B. canis* in *D. reticulatus* ticks was confirmed in the field. Additionally, we demonstrated that 'meal contamination' generates a confounding signal in molecular detection of pathogen DNA extracted from ticks collected from infected hosts and must be taken into account in evaluating the suitability of tick species as vectors.

ACKNOWLEDGEMENTS. The study was funded by National Science Centre (NCN) Sonata Bis grant no. 2014/14/E/NZ7/00153.