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Dehydration and starvation yield energetic consequences that affect survival of the American dog tick

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ABSTRACT

Ticks are obligate hematophagous arthropods, but may have to endure extended time (1–2 years) between feedings. During these off-host periods, ticks must contend with a multitude of environmental stresses including prolonged or repeated exposure to desiccating conditions. In this study, we measured the energetic consequences of single and repeated bouts of dehydration of American dog ticks, *Dermacentor variabilis*, and examined the impact of energy reserves on tick survival during dehydration. Recently molted ticks exposed to a single period at 0% relative humidity (RH) for 5 d lost ~26% of their body water and showed 1.3- and 1.7-fold reductions in protein and lipid, respectively. These reduced energy reserves coincided with increased O_2 consumption in dehydrated ticks. Exposure to repeated cycles of dehydration (0% RH, 48 h) and rehydration (100% RH, 24 h) also reduced energy reserves; however, ticks were able to fully recover their body water after 12 cycles of dehydration/rehydration and endured > 20 cycles. Starvation of ticks, in the absence of dehydration, for 18 or 36 weeks resulted in the loss of ~20–40% of protein and 60% of lipid reserves. When ticks were exposed to continuous dehydration at 0% RH, their survival after 18 weeks of starvation was only minimally impacted; however, individuals starved for 36 weeks succumbed to dehydration much more rapidly than recently fed ticks. Both single and repeated dehydration exposures resulted in substantial energetic costs and ticks with limited energy reserves were more susceptible to dehydration-induced mortality, indicating that adequate energy reserves are critical for tolerance to dehydration stress and long-term success of ticks.

1. Introduction

The American dog tick, *Dermacentor variabilis*, is a hard tick (Ixodidae) that is widely distributed in the United States and acts as an important vector for diseases such as Rocky Mountain spotted fever (de la Fuente et al., 2008). As with other ixodid ticks, *D. variabilis* has a multi-stage life cycle (larva, nymph, adult) with each stage requiring a blood meal for development and/or reproduction (Sonenshine, 1991). Although ticks are obligate hematophagous arthropods, they spend the majority of their lives off host where they must contend with environmental stressors while relying on energy reserves acquired from blood feeding by the previous stadia (reviewed in Needham and Teel, 1991). The ability to survive these stresses while off host significantly impacts the specific environments where ticks and their pathogens can establish (Goethert and Telford, 2009; Klompen et al., 1996).

During off-host periods, ticks are at risk for dehydration due to their small body size and consequent high surface-area-to-volume ratio (Benoit and Denlinger, 2010), and maintaining water balance is critical for tick survival (Needham and Teel, 1991). Ticks can prevent dehydration through physical and physiological adaptations to conserve water, behavioral mechanisms, and acquisition of exogenous water (Benoit and Denlinger, 2010; Needham and Teel, 1986; Sonenshine, 1991). However, some of these preventative measures come at an energetic cost. Increased movement between humid microhabitats and questing locations, which occurs under desiccating conditions (Crooks and Randolph, 2006; Short et al., 1989), and the active, solute-driven process of water vapor absorption to replenish water content (Gaëde and Knülle, 1997) can reduce lipid reserves (discussed in Randolph, 2008).

When conditions drop below the critical equilibrium humidity (CEH; the relative humidity at which ticks can absorb water vapor), typically 75–95% relative humidity (RH) depending on tick developmental stage and species, ticks begin to dehydrate as water loss exceeds their ability for water vapor uptake (Needham and Teel, 1991; Yoder et al., 2012). To increase survival times at low RH, some ticks rely on energy reserves during dehydration as a source of metabolic water

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(Dautel, 1999). Additional energetic costs would be incurred during rehydration as dehydration-induced damage is repaired and water balance is restored through active water vapor absorption. When dehydrated ticks are moved to hydrating conditions, there is an increase in observed VCO_2 (Fielden and Lighton, 1996), likely as ticks metabolize resources to facilitate water vapor uptake. Another potential source of energy use when arthropods are dehydrated is the variety of molecular mechanisms employed to limit dehydration-induced damage including, expression of proteins such as heat shock proteins, antioxidant enzymes, and aquaporins (Benoit and Denlinger, 2010). *D. variabilis* upregulates similar pathways in addition to accumulating molecules such as glycerol, which act to prevent damage to cellular components, in various insects and ticks (Rosendale et al., 2016; Yoder et al., 2006). These physiological adjustments that improve tick resistance to dehydration are likely energetically costly.

In addition to the risk of dehydration-induced mortality, ticks must contend with limited nutrient availability during extended off-host periods. Although ticks show a remarkable ability to withstand prolonged starvation, in part through low metabolic rates (Lighton and Fielden, 1995), exhaustion of energy reserves is a major cause of mortality under field conditions (Nieto et al., 2010). In addition to starvation-induced mortality, limited energy reserves can also reduce tolerance of environmental stress. Ticks with high fat reserves survive exposure to low temperatures better than starved ticks with low lipid levels (Herrmann and Gern, 2013). Additionally, older ticks with limited energy stores show a greater susceptability to water loss at low RH as compared to more recently fed ticks (Williams et al., 1986). In several dipterans, dehydration exposure reduces lipid, carbohydrate, and/or protein levels (Benoit et al., 2010; Teets et al., 2012), leading to effects on long-term fitness such as reductions in fecundity (Benoit et al., 2010). These desiccating conditions may be particularly costly for ticks that have been fasting for extended periods and cannot replenish energy reserves until finding a host; however, energetic expenditure during dehydration has received little attention in ticks.

Water balance and starvation are arguably two of the main factors determining the ability of ticks to survive off host (Needham and Teel, 1991); however, little is known about the interaction between these two stresses. Additionally, most studies on tick dehydration have focused on a single dehydration event, although ticks often experience multiple bouts as they move between humid microhabitats and questing locations with less favorable hydric conditions. Therefore, the objectives of this study were to measure the effects of dehydration on energy balance, compare the energetic impacts of a single dehydration event and repeated dehydration/rehydration cycles, and examine the interaction between starvation status and dehydration resistance.

2. Materials and methods

2.1. Ticks

Engorged *Dermacentor variabilis* nymphs were obtained from laboratory colonies at the Oklahoma State University Tick Rearing Facility (Stillwater, OK, USA) where they were fed on sheep (*Ovis aries*). These colonies are maintained under 14:10 h, light:dark (L:D), 97% relative humidity (RH), and 25 ± 1 °C. Upon arrival, groups of 10 ticks were transferred to 15 cm^3 mesh-covered vials and placed in closed chambers containing a supersaturated solution of potassium nitrate, providing 93% RH (Winston and Bates, 1960). After nymphs developed and emerged as adults (ecdysis), a process that occurred ~27 days after completing their blood meal and dropping from the host, ticks were transferred to fresh tubes and kept in these chambers at 26 ± 1 °C, 15:9 h L:D, and 93% RH until used in experiments. The effects of single and repeated bouts of dehydration were examined on male ticks that were 2 weeks post-ecdysis. The interaction between starvation and dehydration was explored by comparing ticks that were 2, 18, and 36 weeks post-ecdysis.

2.2. Experimental conditions

Ticks were randomly chosen from multiple rearing batches and individually transferred to 1.5 cm^3 mesh-covered vials for treatments. Control ticks were kept at 93% RH under rearing conditions as described above. Desiccating conditions of 0% RH were maintained in a 5 L glass desiccator containing fresh anhydrous calcium sulfate with a cobalt chloride indicator (Drierite, Xenia, OH, USA). All treatments occurred at 26 ± 1 °C and 15:9 h L:D. Ticks were weighed (to 0.01 mg) using an electrobalance (CAHN 25; Ventron Co., Cerritos, CA, USA) at the beginning of the trial and measured daily until the treatment was complete. Featherweight forceps were used to handle the ticks and the weighing process required 30 s or less per individual. For survival studies, ticks were left under dehydrating conditions until succumbing to water loss, whereas ticks used in energetic studies were dehydrated until they lost ~25% of their initial body water. For repeated dehydration treatments, ticks were kept at 0% RH for 48 h, followed by rehydration for 24 h at 100% RH, which was maintained by placing distilled H_2O in sealed glass containers. Ticks were weighed twice per cycle, on the second day of each dehydration period (after 48 h at 0% RH) and on each day of rehydration (after 24 h at 100% RH). These cycles were repeated until mortality occurred in the survival study or after 3, 6, or 12 cycles of dehydration/rehydration for energetic studies.

2.3. Water and organic content and survival

A subset of ticks (N = 7–10) from each control or experimental group was used to determine body water content. Following treatment, each tick was weighed as described above, frozen at −80 °C, and then placed in a 75 °C oven. Ticks were weighed daily until they reached a constant mass, and water content was determined by subtracting dry mass (DM) from the fresh mass and expressed as mg H_2O mg^{-1} DM. These dried ticks were then transferred individually to crucibles and burned at 550 °C for 18 h in a muffle furnace (NEY model 2-525 SII). The organic content was calculated as the difference between the DM and mass of the remaining ash residue.

Ticks were assessed daily for survival. Ecological death was reached when ticks were unable to right themselves and/or crawl, displayed uncoordinated movements, and curled their legs. This point has been used as a measure of dehydration-induced mortality in *D. variabilis* as ticks do not recover after reaching this point (Yoder et al., 2012). However, we observed that ticks lacking coordinated movement were still capable of absorbing water when returned to 100% RH, albeit not to levels of water content observed at the beginning of the treatment. To prevent confounding results in our repeated dehydration studies, we opted to use physiological death as the measure of mortality for all our treatments. Under physiological death, ticks display no body movement and legs are tightly curled. To confirm physiological death, ticks were placed at 0% RH for 24 h to observe a body mass reduction (through evaporative water loss) that is much more rapid than occurs in live ticks under identical conditions. For each treatment, proportion of survival was based on 5–6 replicates of 5 ticks each.

2.4. Energy reserve assays

The effect of dehydration and starvation on energy stores was assessed with assays for total lipid, glycogen, and soluble protein. Following treatment or removal from control conditions, ticks were flash frozen and stored at −80 °C until used in assays. Individual ticks (N = 9–10) were weighed then homogenized in 500 µl of STE buffer (250 mM NaCl, 10 mM Tris, 5 mM EDTA, pH 8.3) with 2% Na_2SO_4 using a BeadBlaster 24 microtube homogenizer (Benchmark Scientific, Edison, NJ, USA). The resulting homogenate was divided into 3 aliquots and used for protein, lipid, and glycogen determination. Soluble protein was measured with 25–50 µl of homogenate using the Bradford method (BioRad; Hercules, CA, USA) with bovine serum albumin as the