Ticks and Tick-borne Diseases 9 (2018) 25–30

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Low and high thermal tolerance characteristics for unfed larvae of the winter tick *Dermacentor albipictus* (Acari: Ixodidae) with special reference to moose

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ARTICLE INFO

Keywords:
Range expansion
Moose
Alces alces
New hampshire
Alaska
United States

ABSTRACT

We report that larvae of the winter tick *Dermacentor albipictus*, the only stage that will quest for a host, can tolerate short-term cold shock down to −25 °C and short-term heat shock as high as 46 °C. Unlike a three host-tick, larvae of *D. albipictus* have no preconditioning response to low or high temperature exposure by rapid cold hardening (RCH) or heat hardening, and poor ability to acclimate to low and high temperature extremes. Thermal tolerance limits were not improved as the result of larval clustering, and there was only a minimal effect due to changes in photoperiod. These larvae are freeze intolerant and die at higher temperatures (−5 to −10 °C) from contact with ice by inoculative freezing. In absence of cold-associated resistance mechanisms, winter survival requires that larvae procure a host before the first snow cover. Their low and high temperature tolerance, however, is a key survival element that adapts them for off-host periods during summer, which in the arctic could allow for northern expansion.

1. Introduction

Development to adulthood by the one-host winter tick, *Dermacentor albipictus*, depends on larval survival during the questing period that occurs during short days and low temperatures in autumn (Addison and McLaughlin, 1988; Lindquist et al., 2016). Larvae are inactive when in the resting stage (dormancy) during summer, remaining grouped in sheltered reprieves in soil and leaf litter near where the egg mass was laid, to facilitate water conservation and survival during summer (Yoder et al., 2016, 2017a). In early fall, larvae cease resting periods and become active, climb vegetation, and aggregate in clumps of 100s at the tips of vegetation where they quest for a host (McPherson et al., 2000). Due to their one-host nature, development to adulthood (i.e., feeding, molting, and subsequent mating) occurs on the same host animal. Many wild mammals that serve as a host, including deer (*Odocoileus* spp.), elk (*Cervus elaphus*), and feral hogs (*Sus scrofa*) (Teel et al., 1990; McPherson et al., 2000; Liebisch et al., 2010; Musante et al., 2014), are habitual groomers that successfully remove a large number of winter ticks. Most notably, however, winter ticks can be a serious problem for moose (*Alces alces*) that are stimulus groomers and fail to remove larvae with any efficiency (Samuel, 2004). Problems arise for moose when tick infestation levels are high (> 35,000 ticks per moose), causing epizootic conditions when > 50% mortality can occur in calves in March–April (Musante et al., 2014; Jones, 2016). These 10–11-month-old calves experience negative energy balance in March–April while consuming a protein-deficient diet prior to spring green-up. They die from acute anemia and weight loss associated with the extreme and concentrated blood loss (3–4 weeks) from feeding by adult female winter ticks (Samuel, 2004; Musante et al., 2007, 2010; Jones, 2016).

The initiation of the larval questing period is such that the summer dormant period ends as days become shorter and moose enter their fall rut or breeding season. The increased activity and movement of moose during the breeding season, as well as increased feeding associated with seasonal adiposity, increases their relative exposure to questing larvae. An extended questing period from delayed winter conditions (i.e., snow cover, extended cold) is associated with epizootic years (Jones, 2016; Ball, 2017). Determining the temperature stress characteristics of larvae could reveal physiological strategies and thresholds related to off-host survival, and might provide for better prediction of epizootics and possible moose/tick management programs.

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http://dx.doi.org/10.1016/j.ttbdis.2017.10.013
Received 6 June 2017; Received in revised form 5 September 2017; Accepted 19 October 2017
Available online 31 October 2017
1877-959X/

Because extreme low and high temperatures can reduce tick populations (Apanaskevich and Oliver, 1991), knowledge of the temperature tolerance of winter ticks is important to understand their distribution and activity patterns in a given area. The majority of ticks studied to date are freeze-intolerant and cannot survive direct exposure to freezing (Burks et al., 1996; Dautel and Knülle, 1997, 1998; Rosendale et al., 2016; Yu et al., 2014), but ticks behaviorally combat exposure to low temperatures by crawling into sheltered, protected reprieves (Burks et al., 1996; Rosendale et al., 2016). Quiescence or behavioral diapause is also a survival mechanism against low temperature (Belozerov, 2009), and it is believed that cryoprotectants are produced for maximum cold hardiness (Neelakanta et al., 2010; Yu et al., 2014). Conversely, resting periods in winter ticks may occur during summer as a strategy to promote water balance (Yoder et al., 2016), and when questing in fall, larvae are exposed to the elements as they typically do not descend vegetation (Drew and Samuel, 1985; McPherson et al., 2000).

Studies examining cold tolerance indicate that larvae are hardier than post-larval stages due to their lower, lower lethal temperature (LLT; Rosendale et al., 2016). However, larvae appear to be vulnerable and die by inoculative freezing upon contact with ice (Burks et al., 1996; Rosendale et al., 2016). Long-term thermal acclimation and short-term preconditioning by rapid cold hardening (RCH) at sublethal temperatures can increase tolerance to cold (e.g. long-term acclimation and RCH has been observed in *Dermacentor variabilis*, but not *Ixodes scapularis*; Vandyk et al., 1996; Rosendale et al., 2016). No temperature tolerance data exist for *D. albipictus*; either cold temperature thresholds that inhibit larval activity/questing in autumn, or hot temperature thresholds affecting larval survival during summer. We tested the hypothesis that photoperiod (short day vs. long day), cold acclimation, or pre-dehydration may modify cold tolerance and heat tolerance of *D. albipictus* larvae. We also sought to examine whether larval clumping (i.e., group effect) influences survival of cold and heat stress.

2. Materials and methods

2.1. Study area

Winter ticks were collected from the Androscoggin River watershed (elevation: 300–1200 m) in New Hampshire. Mountains, lowlands, rivers, bodies of water, and diversified forestation are characteristics of the terrain. The watershed is mostly nestled on privately owned, commercially harvested land with off-road vehicle and logging trails. Prominent tree species include a variety of northern hardwoods, American beech (*Fagus grandifolia*), balsam fir (*Abies balsamea*), red spruce (*Picea rubens*), sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*), and white pine (*Pinus strobus*) (Musante et al., 2010; Yoder et al., 2017b). A decade (07/2007-07/2017) of average temperature, monthly precipitation, maximum snow depth, and extreme minimum and maximum temperatures were collected from the National Climatic Data Center for Berlin, NH (latitude/longitude: 44.4535°, −71.1854°; station: #270690; elevation: 280.4 m). Average monthly ambient temperature ranged from −15.6 to 21.0 °C, monthly precipitation ranged from 15.3 to 219.7 mm, maximum snow depth reached 1080.0 mm, the extreme minimum temperature plunged to −32.2 °C, and the extreme maximum temperature peaked at 33.3 °C.

2.2. Ticks

Ticks were collected from 5, 11-month-old calf moose that had been captured and radio-collared the previous January (P.J. Pekins holds collection permits). These animals succumbed in early April from heavy infestation of winter ticks and were field necropsied within 36 h of death (Jones, 2016); at least 70 engorged female ticks were collected from each calf. Identification as *D. albipictus* was based on keys (Clifford et al., 1961; Brinton et al., 1965) from observations of 10 slide-mounted, unfed larvae that were selected randomly from each egg mass. For subsequent oviposition and hatching, each was put into a mesh-covered 50 ml polypropylene centrifuge tube (Fisher Scientific, Pittsburgh, PA) placed in a glass desiccator within an incubator (Percival, Perry, IA) kept at 93% RH (SD ± 0.5% RH; sat. KNO_3; Winston and Bates, 1960) and 20 ± 0.5 °C. Fed females spent most of their crawling phase (pre-oviposition) and oviposited under short day (SD; 10 h:14 h L:D) or long day (LD; 14 h:10 h L:D) conditions. Eggs and resulting larvae remained under these SD and LD conditions until larvae were used in the experiment 3–6 weeks post-hatching (larvae compared within an experiment were of similar age). Following treatments, survival checks were administered after returning larvae to rearing conditions for 48 h, lethal temperatures were defined by 0% survival at 2 h exposure, and LT_{50} were calculated at 50% survival. Live ticks were characterized by the ability to spontaneously move several body lengths. Deceased ticks were identified by lack of movement in response to gentle agitation, and by curled appendages.

2.3. Cold-shock exposure

Tolerance of low temperatures by larvae was determined via 2 h exposures at variable temperature (−10 to −25 °C), 6 h exposures (−18, −20, and −22 °C), and 12 h exposures (−18 °C); experimental temperatures were based upon studies of cold tolerance in *D. variabilis* (Rosendale et al., 2016). Temperature equilibration was established in a 10-min period at the beginning of sample suspension (i.e., a total time of 2 h and 10 min occurred during the 2 h exposure group). Groups (n = 10) consisted of 10 larvae held in individual 1.5 cm^3 tubes placed in foam-plugged 50 ml tubes suspended in an ethylene-glycol (60:40) solution. Programmable baths (± 0.1 °C; Thermo Scientific, Pittsburgh, PA) were used to regulate temperature.

2.4. Heat-shock exposure

Tolerance of high temperature by larvae was determined via 2 h exposures at varying temperatures (35–46 °C) and 6 h exposures (39, 41, and 43 °C). Temperature equilibration, group size, tube arrangement, suspension solution, and temperature regulation were as described for the cold-shock experiments.

2.5. Pre-treatment effects on cold-shock survival

A discriminating temperature (~15% survival) was chosen to investigate the improvement in survival when ticks received a pre-treatment prior to cold shock. Pre-treatments of rapid cold hardening (RCH) (2 h at −5, 0, or 5 °C), dehydration conditions (1, 3, or 6 days at 75% RH), and extended acclimation (14 days at 0 °C) were utilized prior to the 2 h exposure at −23 °C (additional tests at −24 and −25 °C were conducted for RCH). Pre-treatments were performed immediately prior to testing; i.e., after 2 h and 10 min at 0 °C, the 50 ml tube was transferred to a −23 °C bath for 2 h and 10 min. Pre-treatment temperatures were adapted from the methods in Rosendale et al. (2016) and Drew and Samuel (1985).

2.6. Inoculative freezing

To investigate freeze tolerance in larvae, groups (n = 10) of 10 larvae were exposed for 2 h at either −5, −10, or −15 °C while in the presence of ice. The entire group was submerged in chilled (0 °C) water at the bottom of a 1.5 cm^3 tube that was cotton-plugged with a piece of ice placed on top of the plug. These tubes were transferred to 50 ml tubes and suspended in the cold bath. After testing, larvae were moved to a dry 1.5 cm^3 tube and placed back at the rearing condition.