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Original article

Cold hardiness and influences of hibernaculum conditions on overwintering survival of American dog tick larvae

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ABSTRACT

Understanding how ticks survive the multitude of stresses experienced during winter is integral to predicting population dynamics and transmission of tick-borne pathogens. The American dog tick (*Dermacentor variabilis*), a predominant vector of Rocky Mountain spotted fever, overwinters in any of its post-egg life stages. In this study, we characterized the cold tolerance of larval *D. variabilis* and examined the effect of various acclimatory conditions on cold hardiness. Cold-shock survival during 2 h exposure to various subzero temperatures was assessed and a lower lethal temperature of −20 °C and a 50% mortality temperature near −16 °C was established. Larvae exposed to −5 °C showed high survival (~70%) after 14 d and near 50% for up to 56 d at −5 °C. Larvae cycled between supra- and subzero temperatures showed better long-term survival than at constant −5 °C. The temperature of crystallization (T_c) was ~−23 °C and no larvae survived freezing after reaching their T_c. Larvae exposed to inoculative freezing survived brief, mild treatments (70% survival of −5 °C for 2 h) but survival was reduced compared to larvae cooled in the absence of ice. Reduced photophase, rapid cold hardening, dehydration, and long-term thermal acclimation all improved larval cold hardiness to varying degrees. Survival data were compared to measurements of hibernacula conditions, and we conclude that larvae face little threat from cold-induced mortality but inoculative freezing does pose a risk, and the geographic distribution of *D. variabilis* is only minimally influenced by the ability of larvae to survive low temperature exposure.

1. Introduction

Ticks require blood meals for developmental progression and reproduction, but most ixodid ticks spend the vast majority (~98%) of their lives off-host (Needham and Teel, 1991; Norval, 1977; Sonenshine, 1993). When these extended off-host intervals occur during winter, ticks must contend with a multitude of stresses including fluctuating temperatures and low relative humidity (Brunner et al., 2012; Nieto et al., 2010; Sonenshine, 1993). Like many overwintering arthropods in temperate regions, ticks are susceptible to mortality during exposure to low temperatures. In fact, it has been suggested that low winter temperatures are one factor that may limit the latitudinal and altitudinal distribution of various tick species (Daniel et al., 2003; Dantas-Torres and Otranto, 2011; Dergousoff et al., 2013; Lindgren et al., 2000). To promote overwintering survival, ticks undergo behavioral and physiological adjustments including seeking out sheltered hibernacula (Burks et al., 1996a,b), accumulating cryoprotectants such as glycerol and antifreeze proteins (Neelakanta et al., 2010; Yu et al., 2014), and entering a low activity state for improved water conservation (Yoder et al., 2016). These physiological changes presumably promote survival through a freeze-avoidance strategy, as most tick species have been classified as freeze-intolerant (Burks et al., 1996b; Dautel and Knülle, 1996; Needham et al., 1996; Yu et al., 2014). However, the influence of environmental conditions on tick cold hardiness is unclear as there are conflicting reports on the ability of ticks to acclimate to reduced temperatures or respond to photoperiod shifts (Burks et al., 1996b; Vandyk et al., 1996; Yu et al., 2014).

The American dog tick, *Dermacentor variabilis*, can overwinter in any of its nonfed stages, particularly at the northern extent of its range where summer is brief (Belozerov, 2009; Burg, 2001; Smith and Cole, 1941; Sonenshine, 1993), and may survive multiple winters in a single stage (Yunik et al., 2015). Throughout its range, which encompasses most of the United States except for regions of the Rocky Mountains, *D. variabilis* is a prolific vector of spotted

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fever rickettsioses and tularemia (de la Fuente et al., 2008; Goethert and Telford, 2009). The spring emergence of ticks coincides with increased host activity and plays an important epidemiological role in the spread of tick-borne diseases (Sonenshine, 1991). Therefore, discerning how *D. variabilis* survives potentially lethal cold exposure during winter is integral to our understanding of population dynamics and the capacity of these ticks to act as disease vectors.

D. variabilis overwinter beneath the leaf litter on or within the soil surface where thermal insulation occurs during extreme temperatures (Burks et al., 1996b; Sonenshine, 1993). Nymph and adult *D. variabilis* can tolerate brief exposure to temperatures below freezing, but survival is reduced when chilled in contact with ice (Burks et al., 1996a, 1996b). Ticks experience a reduced photophase within their hibernacula, which causes reduced activity and suppressed water loss rates in *D. variabilis* (Smith and Cole, 1941; Yoder et al., 2016). However, photoperiod does not appear to alter cold hardiness in *D. variabilis* nymphs (Burks et al., 1996a, 1996b). Although survival of immature ticks, including larvae, is important in determining vectorial capacities for various tick-borne diseases (LoGiudice et al., 2003), limits of cold hardiness and factors affecting overwintering survival have not been examined in larval *D. variabilis*. Here we characterize several aspects of cold hardiness in larvae of *D. variabilis*. Additionally, we attempt to clarify the role of microhabitat conditions on tick cold hardiness by examining the effects of photoperiod, short- and long-term thermal acclimation, and dehydration on survival following exposure to low temperatures.

2. Materials and methods

2.1. Ticks

Engorged, female *Dermacentor variabilis* were obtained from laboratory colonies at the Oklahoma State University (OSU) Tick Rearing Facility (Stillwater, OK, USA). Ticks at OSU are kept under 97% relative humidity (RH), 25 ± 1 °C, and 15:9 h, light:dark (L:D). Fed females were obtained within several days of dropping off the host and still in the crawling phase. Ticks were placed in 50 cm^3 mesh-covered vials upon arrival and held in closed chambers containing a supersaturated solution of potassium nitrate, providing 93% RH (Winston and Bates, 1960). Temperature (18 ± 0.5 °C) and photoperiod were controlled using programmable incubators (Percival, Perry, IA). Fed females spent the majority of their crawling phase (pre-oviposition period), and oviposited under short day (12:12 h L:D) or long day (15:9 h L:D) conditions. Eggs and the resulting hatched larvae remained in these short-day (hereafter SD) or long-day (hereafter LD) conditions until individuals were used in experiments 3–6 weeks post hatching. A summary of all experimental treatments is shown in Table 1.

2.2. Environmental temperature and relative humidity

Temperature and relative humidity were measured using data loggers (HOBO Pro, version 2; Onset, Bourne, MA, USA) that recorded data every 4 h from late November to late March. Data loggers were deployed at the University of Cincinnati's Center for Field Studies (39°17′07″N, 84°44′29″W) and one was positioned to record air conditions while another was positioned in a microhabitat approximately 7–9 cm beneath the leaf litter at the ecotone of an old-growth deciduous forest and natural meadow. This was an area where adult *D. variabilis* have been observed to overwinter, suggesting it is also a likely habitat for larvae. Data obtained from this microhabitat was used to ensure that our treatments were ecologically relevant and allowed us to predict how our laboratory findings may influence natural populations.

2.3. Cold-shock exposure

The lower lethal temperature for cold-shock survival was determined using a 2 h exposure to subzero temperatures. Groups of 10 larvae (N = 10 groups) were placed in 1.5 cm^3 mesh-covered tubes and these tubes were placed in 50 ml tubes, which were suspended

Table 1
Summary of experimental treatments and results of group comparisons.

Groups[a]	Pre-treatment[b]	Exposure temperature	Exposure duration	Result
Cold-shock survival				
LD	LD	−5 to	2 h	Survival higher under
SD	SD	−20 °C		SD
Long-term acclimation				
Acclimated	21 d at 0 °C	−19 °C	2 h	Survival higher after
Control	LD or SD			acclimation
Rapid cold hardening				
RCH	2 h at 0 °C	−18, −19,	2 h	Survival higher after
Control	LD or SD	−20 °C		RCH
Dehydration				
Dehydrated	1–6 d at 0% RH	−19 °C	2 h	Survival higher with 1
Control	LD or SD			or 3 day dehydration
Long-term survival				
Constant	LD or SD	−5 °C	7–56 d	No difference between
Cycle		Cycle from 3 to −5 °C		LD & SD
				Survival higher in cycle
T_c				
LD	LD	0 to −24	N/A	T_c under SD marginally
SD	SD			lower
Freezing – cold shock				
LD	LD	−5, −8, −10 °C with ice	2 h	No difference between LD & SD
SD	SD			
Freezing – duration				
LD	LD	−5 °C with ice	2 to 168 h	No difference between
SD	SD			LD & SD

[a] Groups compared within an experimental treatment. Long day (LD), short day (SD), rapid cold hardening (RCH), temperature of crystallization (T_c).
[b] Conditions experienced by larvae prior to exposure to discriminating temperature. LD and SD were larvae pulled directly from rearing conditions described in methods.