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Effect of Ambient Temperature on Competence of Deer Ticks as Hosts for Lyme Disease Spirochetes

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We determined whether the temperature of extrinsic incubation affects the competence of vector ticks as hosts for Lyme disease spirochetes (*Borrelia burgdorferi*). Larval *Ixodes dammini* ticks that had engorged on spirochete-infected C3H mice were incubated continuously at various temperatures, and the gut contents of the resulting nymphs were examined for spirochetes by direct immunofluorescence microscopy. Spirochetes were present in virtually all nymphs kept at 27°C or less for 6 months, in only 10% of those kept at 33°C, and in none kept at 37°C. Spirochetes became undetectable within 8 weeks when nymphs were warmed from 27 to 33°C beginning at the time of molting. Nymphs became virtually noninfective for mice after incubation at temperatures higher than 27°C for 2 weeks or longer. We conclude that ambient temperatures in excess of 27°C are not permissive for transmission of the agent of Lyme disease.

Ambient temperature crucially determines the course of infection of a variety of vector-borne pathogens (13). For mosquito-borne arboviruses, for example, ambient temperature correlates directly with the duration of extrinsic incubation (4, 6, 9, 12) and inversely with vector competence (14–17, 20, 27, 34). The course of infection may even be modified by the temperature at which the vector has been reared (14–16). Changes in ambient temperature affect tick-pathogen relationships in various ways, including release of developmental diapause in the pathogen (8, 19, 35), protein synthesis in pathogens (7), and stimulation of heat shock proteins in the vector (22). In addition, Lyme disease spirochetes (*Borrelia burgdorferi*) did not grow when cultured at temperatures in excess of 37°C (2). The effect of ambient temperature on the susceptibility of deer ticks to infection by Lyme disease spirochetes, as well as on their subsequent infectivity for vertebrate hosts, however, remains to be defined.

It may be that ambient temperature limits spirochetal vector competence of nymphal deer ticks by destroying Lyme disease spirochetes within the vector ticks. To explore this possibility, we determined the effect of rearing temperature on the susceptibility to spirochetal infection by nymphal deer ticks derived from larvae that had engorged on spirochete-infected vertebrate hosts. In particular, we defined the temperature limit that permits spirochete survival in infected larval ticks and affects the efficiency of spirochete transmission by derived nymphs.

MATERIALS AND METHODS

Ticks. Larval deer ticks used in this study were the second-generation progeny of adult deer ticks originally swept from the Great Island (Yarmouth, Mass.). This colony has been reared and maintained free of inherited spirochetal infection, as described previously (29). All ticks were stored in 3-dram (1 fluidram = 3.697 ml) mesh-covered, plaster-bottom plastic vials at 22°C at 95% relative humidity with 16 h of light per day. Engorged larvae were kept at 50 ticks per vial.

Animals. Groups of 3- to 4-week-old C3H/Hej mice, regardless of sex, reared from a stock originally obtained from the Jackson Laboratory (Bar Harbor, Maine) were used in these experiments. Spirochete-infected mice were subjected to feeding by clean larvae 2 months after they had been bitten by three to five infected nymphal ticks as previously described (30). All mice were ear tagged, and each caged group was derived from the same batch at an ambient temperature of 22°C with 16 h of light per day.

Spirochete strain. The spirochetal isolate (JD1) used in this study was originally derived from naturally infected deer ticks swept from vegetation at the Crane Wildlife Reservation (Ipswich, Mass.) as described previously (26). This isolate reacts with monoclonal antibody H5332 (3) and *B. burgdorferi*-specific DNA probes (28). It is highly infectious to rodent hosts (10, 18, 23, 25, 31) and is maintained in a system of alternate passage between deer ticks and laboratory mice (31).

Infection of ticks. To ensure successful attachment, groups of spirochete-infected C3H mice were anesthetized by injection with 0.2 ml of a pentobarbital solution (0.1%) and restrained in a small wire cage. Batches of noninfected larval ticks were randomly placed on these mice until fully engorged, and each mouse was infested with 200 to 300 voracious larvae. Replete larval ticks were collected and subsequently stored in separate mesh-covered, plaster-bottom plastic vials and then kept in environmentally controlled incubators maintained at various incubation temperatures until the ticks were molted to nymphs. The temperature was controlled precisely with an accuracy of ±0.5°C. In one experiment, spirochete-infected nymphs derived from engorged larvae that had been reared at a temperature of 21 or 27°C were placed in separate mesh-covered vials and maintained at temperatures higher than the initial incubation temperature.

Detection of infection of ticks. Ten to twenty derived nymphs were dissected at various intervals after molting and examined for the presence of spirochetes by direct immunofluorescence microscopy. Briefly, the gut tissues of these nymphs were dissected into a drop of phosphate-buffered saline (pH 7.2) on a slide, squashed with a coverslip, and allowed to air dry after removal of the coverslip. The prepared slides were subsequently fixed in acetone for 10 min and then incubated for 1 h at 37°C with fluorescein isothiocyanate-conjugated hyperimmune rabbit serum against *B. burgdorferi* JD1 (29). Slides were washed with phosphate-buffered saline, allowed to dry, and coverslipped after mounting in buffered glycerol. For all slides, 50 random fields were examined at a magnification of ×400 by epifluorescence microscopy.

Spirochetal transmission and infectivity assay. To determine Lyme disease spirochete transmissibility, three to five infective nymphs that had been kept at various temperatures for various periods of time after molting were permitted to feed and engorge on noninfected mice until repletion. Mice exposed to the bites of these nymphal ticks were examined for spirochetal infection at 1 month after being bitten.

Detection of infection of mice. A xenodiagnostic procedure was used to determine whether mice sustained persistent infection. Thus, at least 50 laboratory-reared, noninfected larval ticks were permitted to feed and engorge on each mouse at 4 weeks after it had been bitten by the infecting nymphal ticks. After xenodiagnosis, 10 engorged larvae and 5 to 10 derived nymphs were dissected and examined for the presence of spirochetes by direct immunofluorescent-antibody assay and dark-field microscopy, respectively.

Serologic tests. Sera collected from mice exposed to the bites of infecting nymphs were serially diluted from 1:10 to 1:1,000 and applied in duplicate to the wells of *B. burgdorferi*-coated immunofluorescent-antibody assay slides for detection of antibodies to the Lyme disease spirochete. This test was done at 2 and 4 weeks after the infected ticks became replete, and results were recorded as the greatest dilution of a test serum that reacted with the conjugate. Reactivity of serum at a dilution of 1:100 was used as a conservative criterion of infection (29).

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TABLE 1. Presence of Lyme disease spirochetes in nymphal deer ticks that fed as larvae on spirochete-infected hosts and were incubated at various temperatures continuously thereafter

Temp of incubation (°C)	Time (wk) after molting	% of ticks with spirochetes
15	2	90
	4	100
	6	100
	8	90
	24	90
21	2	100
	4	100
	6	90
	8	100
	24	90
27	2	90
	4	100
	6	100
	8	90
	24	90
33	2	10
	4–8	0
37[a]	0	0

[a] Engorged larvae fail to molt after incubation at 37°C.

TABLE 2. Persistence of Lyme disease spirochetes in nymphal deer ticks initially kept in a cool environment and then transferred to a warmer environment[a]

Temp of incubation (°C)		Time (wk) of final incubation	% of ticks with spirochetes
Initial	Final		
21	27	2	90
		4	90
		8	90
	33	2	30
		4	10
		8	0
	37	2	20
		4	0
		8	0
27	33	2	40
		4	10
		8	0
	37	2	20
		4	0
		8	0

[a] Spirochetal infection of nymphal deer ticks was 90 to 100% prior to transfer to a warmer environment.

RESULTS

First, we examined the prevalence of spirochetal infection in nymphal ticks that had continuously been exposed to particular temperature regimens. Thus, engorged larval ticks that had fed on spirochete-infected hosts were stored in separate mesh-covered vials (50 ticks per vial) and kept at various temperatures until molting. Derived nymphs were dissected and examined for the presence of spirochetes at various intervals after molting. Virtually all nymphs that had been kept at 27°C or less were infected by the agent of Lyme disease and retained the infection for as long as 6 months (Table 1). In contrast, no spirochetal infection was detected in any engorged larvae that had been incubated at 37°C for 2 weeks after repletion. Spirochetes generally failed to survive in derived nymphs that had been kept at temperatures higher than 27°C. Higher temperatures rapidly proved lethal to the spirochetes. These experiments demonstrate that Lyme disease spirochetes do not survive in vector ticks when the environment is relatively warm.

We then determined whether spirochetes were sensitive to elevated temperatures even after the larval-nymphal molt of the infected tick. Thus, spirochete-infected nymphal deer ticks that had first been kept at permissive temperatures (21 or 27°C) were incubated at various elevated temperatures. Even 2 weeks in an elevated environment proved lethal to spirochetes (Table 2). Spirochetal infection of nymphal deer ticks was undetectable at 8 weeks after incubation at elevated temperatures. Spirochetes fail to persist in nymphal deer ticks at temperatures that exceed 27°C. We concluded that vector-borne spirochetes are sensitive to elevated temperatures even in resting nymphal ticks.

Finally, we assessed whether a warm environment destroys the infectivity of vector-borne Lyme disease spirochetes. Thus, nymphal deer ticks that had been kept at various temperatures for various periods of time were permitted to feed and engorge on groups of noninfected C3H mice until repletion. All mice bitten by infective nymphs that had been kept at 27°C or less became infected by the agent of Lyme disease, regardless of the duration of incubation (Table 3). In contrast, only one mouse became infected after being bitten by infective nymphal ticks kept at 33 or 37°C for 2 weeks and none became infected after receiving the bites of infective nymphal ticks that had been kept for 8 weeks at temperatures higher than 27°C. This experiment confirmed that Lyme disease spirochetes fail to survive at temperature higher than 27°C within vector ticks.

DISCUSSION

The effect of the ambient temperature of extrinsic incubation on the susceptibility of vector ticks to Lyme disease spirochetes has never been reported. Our observations indicate that the ability of nymphal deer ticks to serve as hosts for Lyme disease spirochetes depends upon the ambient temperature and that temperatures in excess of 27°C limit the survival of spirochetes within vector ticks. Temperatures that approach the surface temperature of a rodent host are lethal within 2 weeks after the infectious feeding. Thus, the present study provides the first evidence of an inverse relationship between

TABLE 3. Transmission of Lyme disease spirochetes by nymphal deer ticks infected as larvae and kept at various temperatures for various periods of time after molting

Incubation temp (°C)	Duration of incubation (wk)	Spirochetal infection of mice[a]		
		No. of mice tested	% with spirochetes	% with antibody
15	2	3	100	100
	8	3	100	100
	24	5	100	100
21	2	3	100	100
	8	3	100	100
	24	5	100	100
27	2	3	100	100
	8	3	100	100
	24	5	100	100
33	2	5	20	20
	8	5	0	0
37[b]	2	5	20	20
	8	5	0	0

[a] All mice received three to five infecting nymphal tick bites.
[b] Ticks incubated at 37°C were derived from a transfer experiment as shown in Table 2.

the temperature of extrinsic incubation and the vector competence of deer ticks for the agent of Lyme disease.

The maintenance of spirochetal infection in nymphal deer ticks seems to be modulated by elevated temperature and duration of extrinsic incubation. Our results demonstrate that the persistence of spirochetal infection in nymphal deer ticks is reduced by incubation at temperatures higher than 27°C for longer than 2 weeks, and such nymphs clear the infection at 4 weeks thereafter. In general, the ability of nymphal deer ticks to maintain a spirochetal infection was limited by increased temperature and extended incubation. Thus, the differences in spirochetal infection of deer ticks in response to various extrinsic incubation temperatures may induce variations in infection rates in nature (1, 5, 11, 24, 32, 33).

The mechanism responsible for modulating spirochetal infection of deer ticks remains unclear. The effect of an elevated rearing temperature on the ability of vector mosquitoes to modulate their susceptibility to arboviral infection has been attributed to an intrinsic high-threshold phenomenon (17, 21). In the present study, we observed an increased ability of deer ticks kept at temperatures in excess of 27°C to modulate their competence for and maintenance of spirochetal infection after infectious feeding. Thus, we suggest that a maximal threshold temperature of extrinsic incubation may exist to regulate the susceptibility of deer ticks to infection and the development of Lyme disease spirochetes.

It is plausible that the higher temperature itself is directly detrimental to the development of spirochetes within vector ticks. In the natural life cycle, however, spirochetes must be able to adapt to at least two major temperature environments that are dramatically different, the various developmental stages of ticks and their mammalian hosts. In fact, temperature-modulated protein synthesis (7, 22) may be associated with maturation of spirochetes in ticks and provides the initial signal for dissemination of tick-associated pathogens (8, 19, 35, 36). Similarly, the effect of heat-induced proteins by such ticks on survival of spirochetes within vector ticks may not be excluded, especially in ticks kept at elevated temperatures for long incubation periods. Thus, additional studies intended to identify and characterize the potential factor induced by ticks at higher extrinsic incubation temperatures are needed to analyze the role of heat-induced proteins in the elimination of spirochetal infection within vector ticks.

In conclusion, we have demonstrated how an elevated extrinsic incubation temperature affects the vector competence of deer ticks as hosts for the agent of Lyme disease and may modulate spirochetal infection in vector ticks. In particular, we have defined a temperature threshold that may be responsible for the ability of deer ticks to maintain and efficiently transmit Lyme disease spirochetes.

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