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Short Communication

Effect of Temperature on Reproductive Fitness of the Engorged Tick, *Haemaphysalis longicornis* (Acari: Ixodidae)

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ZY, HW and JL designed the study. QJ, TW and ND conducted the experiments. SR and XY collected samples and analyzed the data. ZY and QJ wrote the article.

Key words
***Haemaphysalis longicornis*, Temperature stress, Oviposition, Fecundity.**

ABSTRACT

The tick *Haemaphysalis longicornis* is of great medical and veterinary importance, and can transmit a great diversity of pathogens. The current study investigated the effects of temperature on reproductive fitness of the engorged *H. longicornis,* results indicated that the engorged *H. longicornis* could survive all treatments at 0°C and 4°C, and pre-ovipositon periods were prolonged after 0°C, 4°C and 40°C treatment, whereas it was shortened after 37°C stress ($P<0.05$). The oviposition periods were varied among different temperature treatments, and the 0°C and 4°C treatments showed no influences, whereas the prolonged exposure to high temperatures (37°C and 40°C) led to a shorter oviposition periods which subsequently resulted in a low REI. Most incubation periods of eggs were shortened after temperature treatments on the engorged *H. longicornis*, and 37°C stress for more than 10 days resulted in no hatchment. Stress at 0°C on engorged ticks caused no effects on egg viability, whereas 4°C and prolonged 37°C and 40°C treatments on engorged *H. longicornis* decreased the hatchment rate of their eggs. These findings suggest that the tick *H. longicornis* could adapt a wide range of climate conditions and showed high tolerance to cold and low tolerance to heat.

As obligate hematophagous ectoparasites, ticks are recognized as notorious arthropod vectors which transmit the most diversity of pathogens and rank second only to mosquitoes causing life-threatening zoonotic diseases (Sonenshine and Roe, 2014). To date, approximately 900 species of ticks have been described and they have a worldwide distribution from arctic to tropical regions (Dantas-Torres *et al*., 2012). Climate is one of the main limiting factors which influences the geographical distribution of a tick species (Estrada-Peña, 1999; Despins, 1992), and the questing activities, offhost inter-stadial periods and development of ticks are largely depending on the ambient temperatures (Randolph, 2004). However, survival abilities under extreme environmental conditions are varied among species (Dantas-Torres *et al*., 2012; Dantas-Torres and Otranto, 2011), and probably playing vital roles in population maintenance of ticks (Randolph, 2004). Hence, information on the effects of temperature on life cycle and biological characteristics of ticks is important for future control measures making (Adejinmi and Akinboade, 2011; Khalid *et al*., 2017).

The tick *Haemaphysalis longicornis* is widely distributed in New Zealand, Australia (Tenquisf and Charleston, 2001) and Eastern Asia (Teng and Jiang, 1991), and can transmit a wide variety of pathogens including spotted fever group *Rickettsia*, *Borrelia*, *Bartonella*, *Anaplasma*, *Ehrlichia*, *Theileria and Babesia* (Yu *et al*., 2015). Recently, it has been proved to serve as reservoir and vector of severe fever with thrombocytopenia syndrome virus, which has caused many death in China, Japan and Korea (Luo *et al*., 2015). The whole life cycle of *H. longicornis* requires a mean duration of 135.8 days under laboratory conditions (Liu and Jiang, 1998), whereas

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under field conditions, the development periods vary dramatically in different months caused by fluctuating environmental conditions (Zheng *et al*., 2011). Therefore, it remains need to be determined how the temperature stress affects the biological characteristics of the tick *H. longicornis*. In the current study, the engorged female ticks were stressed at different temperatures, and their survival and reproductive fitness were analyzed, which would provide more information on future forecast of tick dispersion under global climate changes, and advance our knowledge for the control of tick-borne diseases.

Materials and methods

All ticks used in this study were the second generation ticks originated from the adult *H. longicornis* collected from vegetation by flag-dragging in Xiaowutai National Natural Reserve Area (39°50′to 40°07′N, 114°47′ to 115°30′E) in Hebei Province, north China. They were allowed to feed on the ear of rabbits, and the non-parasitize stages were maintained in colony incubator under laboratory conditions (23±1°C, 75±5% RH with a light: dark regime of 6: 18 h). The engorged females were collected and used on the day detached freely.

After detachment, the engorged females were rinsed with water, dried with tissue paper, and weighed individually. Females that within the normal range of the engorged body weights (174.3–317.7 mg) were placed into individual glass tubes. A group of 3 females was maintained in colony incubator and served as control. The remaining groups (three females each) were exposed to 0°C and 40°C for 12 h, 24 h, 48 h and 72 h, and at 4°C and 37°C for 5 d, 10 d, 15 d and 20 d, respectively. Each single female was observed daily, and after above exposure they were transferred back to the colony incubator.

After stressed at different temperatures, the mortality of the engorged female *H. longicornis* was determined. Biological characteristics of the survived females including pre-ovipositon period (number of days from detachment to the beginning of oviposition), oviposition period (number of days from the beginning to the end of oviposition), egg mass laid and the incubation period of the eggs (number of days from the beginning of oviposition to the hatching of the first larva) were recorded, and then the reproductive efficiency index (REI) (number of eggs/weight of the engorged female) was calculated (Dantas-Torres and Otranto, 2011). Analysis of variance was employed to test for the statistical significance of group differences using Statistica V6.0 (StatSoft, USA).

Results

The effects of temperature stresses on reproductive fitness of the engorged tick *H. longicornis* were listed in Table I. The engorged *H. longicornis* could survive all the time treatments at 0°C and 4°C, and both the temperature stress increased the pre-oviposition periods ($P<0.05$), except the group stressed at 4°C for 20 days failed to oviposit. When stressed at 37°C and 40°C, the prolonged exposure time (48 h and 72 h at 40°C, 15 days and 20 days at 37°C) resulted in 67% mortality of engorged *H. longicornis*. When stressed at 37°C, the 5 days and 10 days exposure groups showed shorter preoviposition periods ($P<0.05$). When stressed at 40°C, the 12 h and 24 h exposure prolonged the preoviposition periods ($P<0.05$) (Table I).

For the oviposition periods of *H. longicornis*, most temperature stresses showed no effects, except the groups exposed at 37°C for 5 days and 10 days, and 40°C for 24 h ($P<0.05$). As for REI of the engorged *H. longicornis*, most groups stressed at 0°C were elevated, and when compared to the control group, no differences were observed among the groups at 4°C and 37°C for 5 days, and 40°C for 12 h ($P>0.05$). With prolongation of time exposure at 4°C, 37°C and 40°C, the REI of *H. longicornis* declined progressively. The incubation periods were shortened when stressed at 37°C for 5 days and 40°C for 12 h, and at 0°C regardless of the exposure time ($P<0.05$). No egg hatchment was observed at 37°C for 10 days or longer, as well as at 40°C for 72 h and 4°C for 20 days. The hatachment rate of *H. longicornis* showed no differences among groups at 40°C for 12 h and 0°C regardless of time exposure ($P>0.05$), whereas other treatment groups all resulted in low hatchments (Table I).

Discussion

The present study investigated the effects of temperature stress on fecundity of the engorged *H. longicornis*. Results indicated that the engorged *H. longicornis* showed high tolerance to cold and low tolerance to heat, as exposure at 37°C for 15 days and 20 days, and at 40°C for 48 h and 72 h resulted in 67% mortality, whereas no mortality was observed when stressed at 0°C and 4°C, though these stressed at 4°C for 20 days failed to oviposition. The high temperature stress (~40°C) resulting in death of *H. longicornis* has been described previously (Heath, 2016). The tick *H. longicornis* is found freeze susceptible, and the low temperature stress can enhance the cold hardiness of unfed ticks (Yu *et al*., 2014). In China, the unfed nymphal and adult *H. longicornis* are able to survive overwinter in the field, and the adults are active from April when the lowest teperature around 5°C to September with lowest temperature around 10°C, which offers possibilities to meet low temperatures for engorged females (Zheng *et al*., 2012). In New Zealand, all fed and unfed stages of *H. longicornis* as well as eggs can overwinter (Heath, 2016). All of these demostrated considerable level of cold tolerance of the engorged *H. longicornis*.

Table I.- The effect of temperature on the reproductive parameters of the engorged female *Haemaphysalis longicornis.*

Temp.	Time	Engorgement weights (mg)		Mortality (%)	Pre-ovipositon period (d)		Oviposition period (d)		REI		Incubation period (d)		Hatchment rate (%)	
		Range	Mean± SEM		Range	Mean± SEM	Range	Mean± SEM	Range	Mean± SEM	Range	Mean± SEM	Range	Mean± SEM
23°C	-	215.8-262.5	243.5±14.2	0	4-6	5.0±0.6^{a}	14-15	14.7±0.3^{a}	8.5-9.6	9.1±0.3^{a}	22-24	23.0±0.6^{a}	87.9-97.6	92.8±2.8^{a}
40°C	12h	231.3-238.8	235.3±2.2	0	8-13	10.7±0.5^{b}	13-16	14.0±1.0^{a}	8.9-10.1	9.5±0.3^{a}	16-20	18.3±1.2^{b}	91.6-93.6	92.8±0.6^{a}
	24h	183.6-284.0	220.2±32	0	10-12	11.3±0.7^{b}	7-11	9.3±1·2b	6.3-7.8	7.0±0.5^{b}	21-24	22.0±1.0^{a}	32.5-48.9	40.6±4.7^{b}
	48h	208.8-230.7	221.7±8.5	67	10	10.0	11	11.0	0-8.7	2.9±2.9	24	24.0	0-38.9	13.0±13.0^{b}
	72h	174.3-257.7	225.6±25.9	67	15	15.0	9	9.0	0-5.2	1.7±1.7	NA	NA	NA	NA
37°C	5d	201.1-291.8	255.7±26.2	0	2-4	3.0±0.6^{b}	15-18	16.3±0.9^{b}	9.4-10.2	9.9±0.3^{a}	31-39	36.0±2.5^{b}	4.9-38.4	17.0±10.7^{b}
	10d	195.1-181.2	234.0±25.2	0	2-4	3.0±0.6^{b}	5-17	9.3±3.8^{b}	3.2-6.2	3.8±1.2^{b}	NA	NA	NA	NA
	15d	180.9-243.6	212.2±18.1	67	3	3.0	7	7.0	0.6-7.7	3.0±2.4	NA	NA	NA	NA
	20d	212.1-254.0	237.9±13.0	67	3	3.0	5	5.0	0-4.4	1.5±1.5	NA	NA	NA	NA
4°C	5d	235.3-280.9	257.0±13.2	0	10-11	10.3±0.3^{b}	11-19	13.3±2.8^{a}	10.0-10.1	9.8±0.3^{a}	19-24	21.0±1.5^{a}	70.9-92.6	84.9±7.0^{b}
	10d	224.5-251.9	237.0±8.0	0	15-16	15.3±0.3^{b}	11-13	12.0±0.6^{a}	4.0-10.1	7.2±1.8^{b}	20-23	21.3±0.9^{a}	42.6-80.0	58.8±11.1^{b}
	15d	179.3-294.3	228.0±34.3	0	22-26	24.7±1.3^{b}	10-13	12.0±1.0^{a}	1.8-9.2	4.9±2.2^{b}	21	21.0	0-36.5	15.6±10.9
	20d	228.4-317.7	262.4±27.9	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
0°C	12h	186.1-222.7	208.9±11.4	0	7-8	7.3±0.3^{b}	12-19	14.7±2·2a	10.5-11.1	8.9±0.2^{a}	18-23	20.0±1.5^{b}	85.4-95.9	87.9±5.1^{a}
	24h	232.5-291.7	254.9±18.5	0	7-9	8.0±0.6^{b}	14-16	15.0±0.6^{a}	9.9-10.5	8.2±0.2^{a}	18-19	18.2±0.3^{b}	85.1-95.3	91.2±3.5^{a}
	48h	203.4-238.1	223.0±10.3	0	8-9	8.7±0.3^{b}	12-14	12.7±0.7^{a}	10.7-11.3	8.9±0.2^{a}	18-19	18.2±0.3^{b}	80.0-95.0	85.9±4.6^{a}
	72h	180.1-224.5	208.3±14.2	0	8-10	8.7±0.7^{b}	14-15	14.3±0.3^{a}	9.8-10.7	8.2±0.3^{a}	20-21	20.7±0.3^{b}	81.5-90.8	86.6±2.7^{a}

Means within a column with different superscripts differ significantly ($P<0.05$). NA, not applicable.

Pre-ovipositon periods of *H. longicornis* were prolonged after 0°C, 4°C and 40°C treatment, whereas it was shortened after 37°C stress ($P<0.05$), which indicated that the engorged *H. longicornis* could adapt a wide range of temperatures. This has been proved by the long active months from April to September in the field (Zheng *et al*., 2012). Correspondingly, the tick *Rhipicephalus sanguineus* has been reported to oviposit at 37°C (Jacobs *et al*., 2004), whereas oviposition did not occurr in *H. leachi leachi* (Adejinmi and Akinboade, 2011). Possible explanation may attribute to the different metabolic rates of theses ticks when facing extreme conditions (Adejinmi and Akinboade, 2011).

The oviposition periods of *H. longicornis* were varied among different temperature stress, and the 0°C and 4°C treatment showed no influences, whereas the prolonged exposure to high temperatures (37°C and 40°C) led to a shorter oviposition periods which subsequently resulted in a low REI. Additionally, the 4°C treatment for 10 days and 15 days also resulted in a low REI, which suggested that the low temperature treatments still cause some harmful effects on the tick *H. longicornis* though the exposure time was not long enough to stop oviposition. Similar phenomenon was also observed in *R. sanguineus* stressed at 8°C for 60 days which could also oviposit but without hatchement (Dantas-Torres and Otranto, 2011). Most incubation periods of eggs were shortened after temperature treatment on the engorged *H. longicornis*, and 37°C stressed for more than 10 days resulted in no hatchment. Treatments at 0°C on engorged ticks displayed no effects on egg viability, whereas 4°C, and pronlonged 37°C and 40°C treatments on engorged *H. longicornis* decreased the hatchment rate, and this may due to that the engorged females have started oviposition during temperature stress, and hence the eggs were also subjected to stress.

Conclusions

The results obtained in the current study suggested that the tick *H. longicornis* could adapt a wide range of climate conditions and showed high tolerance to cold and low tolerance to heat. These findings advanced our knowledge on the field adaptation of *H. longicornis* and provided new insights into the epidemiology of *H. longicornis*-borne diseases. However, further investigation remains required to explore the intriguing adaptation of this tick species under background of global climate changes.

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Statement of conflict interest

The authors have no competing interests.

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