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The life cycle of *Haemaphysalis qinghaiensis* (Acari: Ixodidae) ticks under laboratory conditions

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Abstract The developmental stages in the life cycle of *Haemaphysalis qinghaiensis* were investigated under laboratory conditions. The larval, nymphal and adult ticks were fed on sheep at 25–27 °C, 50 % relative humidity (RH) and exposed to daylight. All free-living stages were maintained in an incubator (28 °C with 90 % RH and a 12-h photoperiod). The whole life cycle of *H. qinghaiensis* was completed in an average of 176 days (range 118–247 days). The average developmental periods were 34.44 days for egg incubation; 5.83, 4.20 and 33.70 days for larval pre-feeding, feeding and pre-molting; and 3.88, 5.30 and 46.50 days for nymphal pre-feeding, feeding and pre-molting. The average times for pre-feeding, feeding, pre-oviposition and oviposition of female adult ticks were 2.60, 11.40, 8.50, and 19.35 days, respectively. The results confirmed the positive correlation between the weight of the engorged female and the egg mass laid ($r = 0.557$, $P < 0.05$). The reproductive efficiency index and reproductive fitness index in females were 5.49 and 4.98, respectively. Engorged nymphs moulting to females (4.53 ± 0.16 mg) were significantly heavier ($P < 0.001$) than those moulting to males (3.45 ± 0.19 mg). The overall sex ratio of the adult ticks was 1:1.1 (M:F).

Keywords *Haemaphysalis qinghaiensis* · Sheep · Life cycle · Laboratory conditions

Introduction

Haemaphysalis qinghaiensis, an ixodid tick species, is only recorded in China (Gao et al. 2007a, b, 2008, 2009; Li et al. 2007). It is particularly prevalent in the western plateau,

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including the provinces of Qinghai, Gansu, Sichuan and Tibet (Li et al. 2007; Teng 1991). This three-host tick infests mainly ruminants (Gao et al. 2008) and its debilitating effects on its hosts mainly attribute to blood loss and transmission of pathogens, such as *Theileria uilenbergi*, *Theileria luwenshuni*, *Theileria sinensis* (Li et al. 2007, 2009; Yin et al. 2002a, b, c) and *Babesia* sp. BQ1 (Lintan) (Guan et al. 2002, 2010). Both *Haemaphysalis qinghaiensis* infestation and *H. qinghaiensis*-transmitted protozoal diseases resulted in a major economic burden for the animal husbandry in Northwestern China (Gao et al. 2008, 2009).

The present work was carried out to describe the life cycle of *H. qinghaiensis* under laboratory conditions. The results may provide basic background information for further investigations.

Materials and methods

Collection and rearing of ticks

Unfed adult *H. qinghaiensis* ticks were collected on vegetation in Lintan County (103.35E, 34.7N) of the Gannan Tibetan Autonomous Region during late March when they were actively seeking hosts (Sun et al. 2008). Colonies of the ticks were reared on sheep, and maintained in glass tubes sealed with hydrophilic cotton in an incubator, under controlled conditions (28 °C with 90 % RH and a 12-h photoperiod).

Animals

Six sheep (1-year old Tan mutton breed) were divided into three groups and were infested by larvae, nymph and adult ticks, respectively. They were maintained at 25–27 °C, 50 % RH and exposed to daylight. Muslin cloth bags (25 × 25 cm) were glued to the back of sheep, and ticks were reared in bags. Opening of the bag was bound so that ticks could not come out.

Observation of the biology of larvae and nymph ticks

Two hundred newly hatched larvae and 50 newly emerged nymphs were put onto the back of two sheep daily to determine their pre-feeding periods. Seven days after hatching/moulting, 200 unfed larvae and 50 unfed nymphs were weighed and fed on sheep. Naturally detached engorged ticks were collected, counted, weighed and then placed into separate tubes. These ticks were weighed in pools, in which 10 larvae were a pool and 5 nymphs were a pool with an analytic balance (Denver instrument TB-25, USA). During their non-feeding period, they were maintained in an incubator. The sex was recorded, after engorged nymphs moulting.

Observation of the biology of adult ticks

Freshly emerged adult ticks (25 females and 25 males) were placed on a sheep daily to determine whether they could attach and feed. Freshly males and females were maintained without food for a specified pre-feeding period of 4 days, and then were weighed before put on the back of a sheep for blood-feeding. The engorged females were collected, weighed and put into an incubator. The pre-oviposition and oviposition periods and egg masses laid were observed daily. Daily deposited eggs were collected, counted, and placed into separate glass tubes. In total, 500 eggs were used for observing the incubation period. After hatching, the percentage of hatched larvae was calculated. Finally, the reproductive efficiency index (REI) (amount of eggs/weight of engorged female) (Drummond and Whetstone 1970) and reproductive fitness index (RFI) (amount of eggs incubated to larvae/weight of engorged female) (Chilton 1992) were calculated.

Observation of the longevity of unfed ticks

Freshly emerged larvae (300), nymphs (100) and adults (50) were maintained in an incubator, under controlled conditions (28 °C with 90 % RH and a 12 h-photoperiod), and their longevities were observed.

Data analysis

To reveal the relationship among duration of feeding periods, weight of engorged females, length of oviposition and number of eggs laid, several Spearman's correlations and Student's t tests were applied. The further statistical analyses were performed using SPSS (version 11.5).

Results

Life cycle of *H. qinghaiensis*

Under laboratory conditions, the complete life cycle of *H. qinghaiensis* had a mean duration of 176 days (range 118–247 days), the average developmental periods were 34.44 days for egg incubation, 5.83, 4.20 and 33.70 days for larval pre-feeding, feeding and pre-molting, respectively and 3.88, 5.30 and 46.50 days for the same stages of nymphs, respectively. The average times for pre-feeding, feeding, pre-oviposition and oviposition of female adult ticks were 2.60, 11.40, 8.50, and 19.35 days, respectively. The duration of the stages is shown in Table 1.

Table 1 The duration of various developmental stages of *Haemaphysalis qinghaiensis*

Developmental stage	Period	Number of ticks tested	Duration (days)	
			Range	Mean ± SEM
Egg	Incubation	500	27–43	34.44 ± 1.15
Larva	Pre-feeding	200	4–7	5.83 ± 0.11
	Feeding	200	3–6	4.20 ± 0.06
	Pre-molting	200	26–41	33.70 ± 0.32
Nymph	Pre-feeding	50	2–5	3.88 ± 0.23
	Feeding	50	4–8	5.30 ± 0.16
	Pre-molting	50	28–58	46.50 ± 1.37
Adult female	Pre-feeding	25	1–4	2.60 ± 0.11
	Feeding	25	9–15	11.40 ± 0.54
	Pre-oviposition	25	3–25	8.50 ± 1.66
	Oviposition	25	11–35	19.35 ± 1.50

Table 2 Changes of body weight of larva, nymph and adult *Haemaphysalis qinghaiensis* before and after feeding

Developmental stage	Number of ticks	Unfed (mg) (Mean ± SEM)	Engorged (mg) (Mean ± SEM)	Weight ratio (engorge/unfed)
Larva	200	0.06 ± 0.00	0.55 ± 0.02	9.17
Nymph	50	0.35 ± 0.02	4.03 ± 0.17	11.51
Adult male	25	1.41 ± 0.05	2.15 ± 0.14	1.52
Adult female	25	1.95 ± 0.05	193.72 ± 4.50	99.34

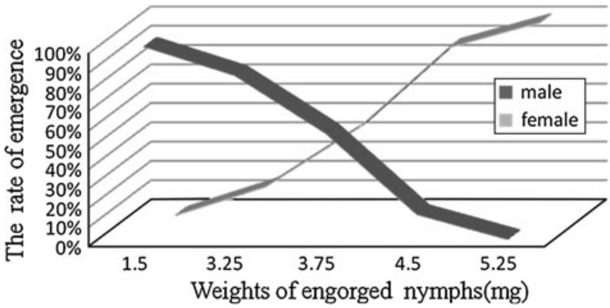

Fig. 1 The emergence rates of males and females after nymph moulting

Feeding biology and changes of body weight

The average weights of the larvae, nymphs and adults before and after feeding are given in Table 2. In addition, the weights of engorged female and male nymphs ranged from 3.7 to 5.4 mg and from 2.4 to 4.6 mg, respectively. The Student's t test revealed that the mean weight of engorged nymphs molting to females (4.53 ± 0.16 mg) was significantly heavier ($P < 0.001$) than that of nymphs molting to males (3.45 ± 0.19 mg). However, their engorged weights overlapped. The relationship between the body weights of *H. qinghaiensis* nymphs and the resultant sex was recorded. The ratio of males to females was 1:1.1. The Fig. 1 illustrated the distribution emergence rates of males and females from engorged nymphs.

Oviposition

The oviposition data are presented in Table 3. Linear regression analysis revealed a positive correlation between the weight of the engorged females and the egg mass laid ($r = 0.557$, $P < 0.05$) (Fig. 2).

The oviposition pattern of *H. qinghaiensis* is presented in Fig. 3 (based on 25 females). The number of eggs laid initially was low but increased rapidly to a peak on day 7, and then gradually declined. The percentage of hatched eggs (hatched eggs/total eggs) was on average 90.7 %. The female reproductive efficiency index (REI) (amount of eggs/weight of engorged female) and reproductive fitness index (RFI) (amount of eggs incubated to larvae/weight of engorged female) were calculated to be 5.49 and 4.98 on the basis of the data in Table 3.

Table 3 Biological characteristics of the engorged females of *Haemaphysalis qinghaiensis* under laboratory conditions

Engorged weight (mg/♀)		Egg mass deposited (mg/♀)		Oviposition (No./♀)	
Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
193.72 ± 4.50	141.5–248.2	112.65 ± 6.12	78.3–195.2	1,063.48 ± 57.78	414–1,843

Data were obtained from 25 females

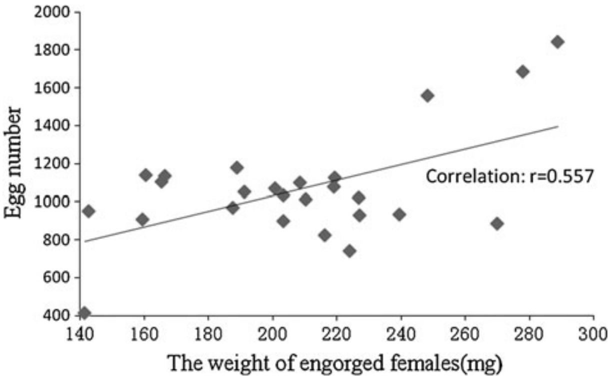

Fig. 2 Relationship between engorged weight and number of eggs laid of *Haemaphysalis qinghaiensis* (based on 25 females)

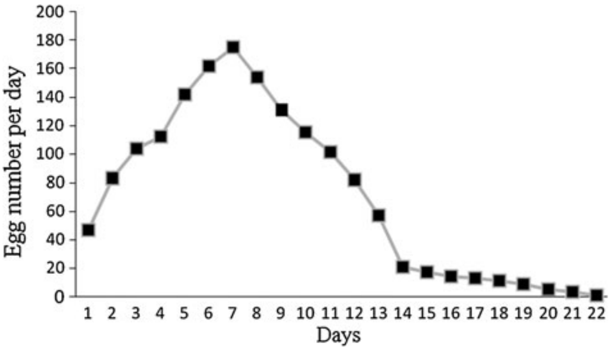

Fig. 3 Mean daily oviposition of engorged females of *Haemaphysalis qinghaiensis* (based on 25 females)

Longevity

Under controlled conditions (28 °C with 90 % RH and a 12-h photoperiod), unfed *H. qinghaiensis* larvae survived for 150–190 days (larvae started to die on the day 150, and they died off on the day 190), nymphs for 170–300 days, and unfed adults for 200–500 days after hatching or molting.

Discussion

Haemaphysalis qinghaiensis, a typical three-host tick, has only been reported from China (Gao et al. 2008). Its natural hosts include sheep, goat, yak, cattle and hare (*Lepus* *oiostolus*) (Teng. 1988). It is known that all stages of the tick could develop in sheep, goat, yak and cattle (Li et al. 2007, 2009; Yin et al. 2002a, b, c; Guan et al. 2002, 2010). However, few studies on its life cycle have been published.

The present work revealed that life cycle of *H. qinghaiensis* needed an average period of 163.39 days (excluding the prefeeding stages of larvae, nymph and adult ticks) under laboratory conditions, which is longer than that of *Haemaphysalis doenitzi* (100.2 days on average) (Chen et al. 2012a), *Hyalomma aegyptium* (147 days on average) (Široký et al. 2011), *Haemaphysalis punctata* (146 days on average) (Yin and Lu 1995), *Hyalomma asiaticum kozlovi* (144.8 days on average) (Chen et al. 2009) and *Rhipicephalus bursa* (142.45 days on average) (Yeruham et al. 2000), and shorter than *Hyalomma rufipes* (173.7 days on average) (Chen et al. 2012b) and *Amblyomma ovale* (190 days on average) (Martins et al. 2012). This discrepancy in life cycle was vastly observed in different tick species, which might also due to different host species (Labruna et al. 2002; Martins et al. 2012) and temperature (Chilton et al. 2000; Yano et al. 1987), ect.

It is known there is a significant relationship between the weight of the engorged nymph and the resulting sex (Chilton et al. 2000), which is often used as an index to predict tick sex. For example, in *Ixodes scapularis*, fully engorged nymphs molting into females weighed between 3.8 and 6.4 mg, while those nymphs molting into males weighed between 2.0 and 3.2 mg, with the difference in nymphal weight being statistically significant (Hu and Rowley 2000). However, despite this discrepancy in the nymphal weight of some other tick species, such as *Dermacentor silvarum*, there was no relationship between engorged nymphal body weight and the resulting sex of the adult (58.4–70.2 mg for males and 57.1–73.3 mg for females) (Liu et al. 2005). In the current work, engorged *H. qinghaiensis* nymphs molting into females were significantly heavier (4.53 ± 0.16 mg) than those molting into males (3.45 ± 0.19 mg). However, the weight range of engorged female nymphs (3.7–5.4 mg) showed some overlaps with that of the engorged male nymphs (2.4–4.6 mg). Similar results are also observed in *Hyalomma asiaticum kozlovi* (Chen et al. 2009), *Dermacentor variabilis* (Hu and Rowley 2000) and *Hyalomma rufipes* (Chen et al. 2012b). In the current study, the adult sex ratio observed was nearly equal, and the ratio of male to female was 1:1.1. Similar results were found for *Haemaphysalis doenitzi* (Chen et al. 2012a) and *H. aegyptium* (Široký et al. 2011). Such a trait could be caused by mating habits with long-lasting host attachment by males (Široký et al. 2011; Siroky et al. 2006).

In comparison with other tick species, *H. qinghaiensis* (193.72 mg of engorged female) is a medium-size tick. Before and after feeding, the average weights of *H. qinghaiensis* adult females increased by approximately 99.34-fold. Other ticks, such as *H. doenitzi*, the increase was 174.42-fold (Chen et al. 2012a), in *H. longicornis* 102.6-fold (Zheng et al. 2011), in *H. rufipes* 51.36-fold (Chen et al. 2012b) and in *D. silvarum* 76.1-fold (Liu et al. 2005). Linear regression analysis revealed a positive correlation between the weights of engorged *H. qinghaiensis* females and the number of eggs laid ($r = 0.557$, $P < 0.05$). The mean number of eggs laid was approximately 1,063 in *H. qinghaiensis*, compared with 2,852 in *H. doenitzi* (Chen et al. 2012a) and 3,335 in *H. punctata* (Yin and Lu 1995). Therefore, the mean number of eggs laid of *H. qinghaiensis* is lower than those of other *Haemaphysalis* species.

The oviposition pattern of *Haemaphysalis qinghaiensis* was similar to other species of *Haemaphysalis* (Chen et al. 2012b; Zheng et al. 2011), laid most of the eggs in the first week. Oviposition peaked at day 5–7 and then declined rapidly throughout the remaining oviposition period. A similar curve has been record for other tick species (Chen et al. 2012b; Rechav and Knight 1983).

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