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The life cycle and occurrence of *Haemaphysalis concinna* (Acari: Ixodidae) under field conditions

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ABSTRACT

The life cycle and occurrence of *Haemaphysalis concinna* were investigated under field conditions from April 2012 to March 2013 in Eerguna National Natural Reserve Area situated on the China-Russia border in Inner Mongolia, China. Under natural conditions, the whole life cycle of *H. concinna* was allowed to complete in a natural tick habitat. With domestic rabbits supplied as hosts, the seasonal occurrence and behaviors of *H. concinna* were also observed in the field plot which was chosen in a natural tick habitat from April to October 2012. Results indicated that the durations of the life cycle from unfed adults to the next generation unfed adults of *H. concinna* ranged from 124 to 186 days (average periods of 153.1 days). The incubation time of eggs ranged from 39 to 57 days (average periods of 41.3 days), which is the longest period among the four developmental stages, followed by the premolt periods for larvae (averaged 37.7 days) and nymphs (averaged 26.0 days). The number of eggs was positively correlated with the weight of engorged females ($r = 0.8562$, $p < 0.001$). Eggs were laid in high amounts in the first week, subsequently, the egg amount declined gradually with small peaks occasionally observed. The female reproductive efficiency index (REI) and reproductive fitness index (RFI) was 6.2 and 4.3, respectively. Observations on the occurrence of *H. concinna* indicated that, in the confined plot under field conditions, larvae appeared in late May and peaked in early July, and nymphs were active during July and August. Therefore, there was an overlap in the occurrence of larvae and nymphs in both June and July.

Introduction

As obligate blood-sucking ectoparasites of various terrestrial vertebrates, ticks are notorious for transmitting the widest variety of pathogens of any blood-sucking arthropod, causing numerous diseases in humans and animals (Telford and Goethert, 2008). The ixodid tick *Haemaphysalis concinna* Koch is widely distributed in China (Teng and Jiang, 1991), Russia, France, Germany, Poland, as well as temperate Eurasia (Nosek, 1971) and can transmit a great variety of pathogens, including *Borrelia* spirochetes (Sun and Xu, 2003), *Coxiella burnetii* (Špitalská and Kocianová, 2003), *Rickettsia sibirica* (Jongejan and Uilenberg, 2004), Russian-spring encephalitis (Ju and Gong, 2010) and Crimean-Congo hemorrhagic fever virus (Tekin et al., 2012). The adult stages of *H. concinna* mainly parasitize on Artiodactyla, and accidently attack humans, while the most important hosts for the immature stages are small- to medium-size mammals (Nosek, 1971). However, recent research has proved that rabbits are suitable hosts for all the active stages of *H. concinna*, as they can complete the whole life cycle in a shortest period when compared with that feeding on dog and cattle under laboratory conditions (Bian et al., 2009). In China, it is reported that outbreaks of *H. concinna*-borne diseases have occurred multiple times since the early 20th century, which caused great economic losses in human health and livestock production (Xian et al., 2000; Sun et al., 2006).

Although the medical and economic significance of *H. concinna* have long been recognized, limited information is available for the design of comprehensive surveillance and control measures for this tick species. Previous studies have indicated that *H. concinna* is abundant from spring to summer and absent during winter (Sun et al., 2006). Under laboratory conditions, *H. concinna* can complete one generation within 120.6 days (Bian et al., 2009). However, knowledge about its field biology, development characteristics and seasonal occurrence is incomplete. Therefore, the present study

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was carried out to investigate the development, survival and fecundity of *H. concinna* under field conditions at the China-Russia border in northeast China. The results obtained in this study will pave the way for the control of this tick and its tick-borne diseases.

Materials and methods

Study site

The field study was carried out from April 2012 to March 2013 in Eerguna National Natural Reserve Area (120°00′26″ to 120°58′02″ E, 51°29′25″ to 52°06′00″ N), situated on the China–Russia border in Inner Mongolia, northeast China. A 150 cm × 150 cm field plot (120°03′15″ E, 51°36′28″ N, and altitude 468 m) was chosen in a natural tick habitat to observe the seasonal occurrence (from April to October 2012), life cycle and biological characteristics (from April 2012 to March 2013) of *H. concinna*. A 15 cm × 15 cm water-filled drain surrounded the plot to avoid tick escape. The plot was also enclosed by a wooden fence to avoid other hosts from entering, and covered with an 8 cm-thick layer of leaf litter containing scattered cluster of vegetation species including *Elymus dahuricus, Geranium maximowiczii, Potentilla longifolia, Chenopodium album, Artemisia mongolica* and *Cannabis sativa.*

Monthly minimum, maximum and mean ambient temperatures and relative humidity were obtained from April 2012 to March 2013 using the hygrothermographs (Qingsheng Electronic Technology Ltd., China) located about 50 cm above the ground in the plot (Fig. 1).

Observations on the biology of immature and adult ticks under field conditions

In April 2012, free-living adults of *H. concinna* (50 females and 50 males) collected from vegetation were weighed individually and fed on the ear of rabbits maintained in iron cages (30 cm × 40 cm × 50 cm) outside the plot as described previously (Yu et al., 2010). In order to facilitate feeding and operation, ticks were released into cloth bags glued on the rabbits' ears, and checked three times daily (8:00, 12:00 and 16:00). All rabbits were fed rabbit pellets and water provided *ad libitum* under natural lighting and climate conditions. The use of rabbits was approved by the Animal Care and Use Committee of Hebei Normal University.

The engorged females were individually collected, weighed, put into separate tubes (1.5 cm × 10 cm), then plugged with gauze netting. These tubes were then placed into the plot where they were exposed to natural conditions, and examined twice daily to record the preovipositional and ovipositional periods, as well as the egg mass laid. The daily deposited eggs were carefully separated from females and weighed. Approximately 1000 eggs, in separate tubes, were placed in the plot and used to determine the incubation period. The reproductive efficiency index (REI) was calculated as the ratio of egg number to the weight of the engorged female and the reproductive fitness index (RFI) was calculated as the ratio of the number of eggs hatched to larvae to the weight of engorged female (Drummond and Whetstone, 1970; Chilton, 1992).

To determine the prefeeding periods of the immature stages of *H. concinna*, freshly hatched larvae ($n = 300$) and nymphs ($n = 200$) were placed on the ear of two rabbits that were glued with cloth bags in iron cages outside of the plot in June and July 2012, respectively, and examined daily to record prefeeding period as defined as the number of days from the hatching or molting to the beginning of attachment. Seven days after hatching or molting, 200 unfed larvae (June 2012) and 200 unfed nymphs (July 2012) were weighed and fed on the rabbits in cages beside the plot. When the larvae or nymphs were engorged, the feeding periods were determined. A total of 150 engorged larvae and 150 engorged nymphs were weighed, placed into individual tubes, plugged with gauze netting, and put into the plot and observed twice daily to record the premolt period.

To test the survival periods of the ticks without feeding, 100 freshly hatched larvae in early-July 2012 and 100 freshly emerged nymphs in mid-July 2012 were placed into ventilated cloth bags and placed on the surface of the soil in the field plot. The length

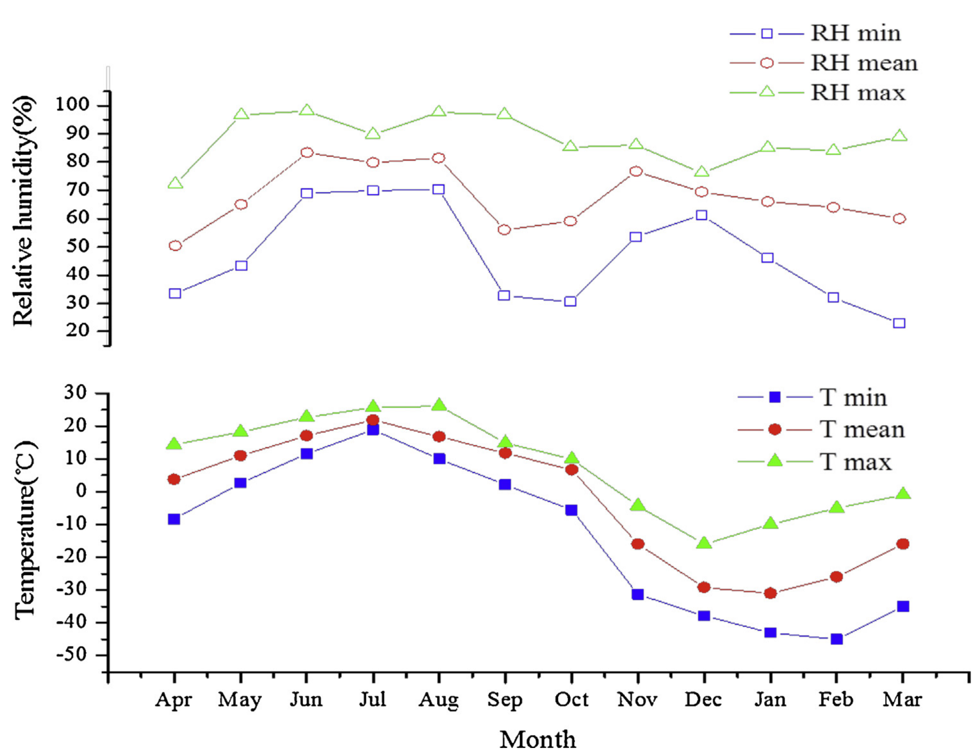

Fig. 1. Minimum (T_{min}), maximum (T_{max}) and mean (T_{mean}) air temperature (°C) and minimum (RH_{min}), maximum (RH_{max}) and mean (RH_{mean}) relative humidity (%) during the experiments from April 2012 to March 2013, Inner Mongolia, northeast China.

of time they could survive without feeding was recorded by daily checking. One hundred newly molted adults confined in large tubes with wire mesh, were put directly into the plot in August 2012. In March 2013, the survival rate was determined after 24 h incubation at 27 ± 1 °C in an incubator (6 L:18 D) supplemented with 90% relative humidity.

Observations on host questing and seasonal occurrence of the H. concinna in the plot

Unfed adults (50 females and 50 males) collected from vegetation in April 2012 were placed into the confined plot. With two rabbits supplied as hosts inside the confined plot, the unfed adult ticks were allowed to quest and attach to the rabbits maintained in the cages, and the engorged females could then detach freely and search for suitable places for oviposition. Once the unfed larvae hatched or the engorged larvae molted, the newly emerged larvae or nymphs could also quest and attach to the rabbits maintained in the plot.

Visual observation was performed daily to observe the questing behavior and feeding at different stages. The plot was observed three times (8:00, 12:00 and 18:00) per day. Tweezers were used to turn the leaves in the plot, and when ticks were found, the distance to the surface of the leaf litter was measured using scales.

Results

Developmental periods of H. concinna under field conditions

Adults of *H. concinna* were collected from vegetation, fed on rabbits, and allowed to develop inside the plot under natural conditions. From April to October 2012, a mean duration of 153.1 days (a range: 124–186 days) was required from the unfed adults to the next generation of the unfed adults (Table 1). The average feeding, preoviposition and oviposition periods of females were 6.7, 9.7 and 15.0 days, respectively.

Oviposition began in late April, and the mean incubation period of eggs lasted for 41.3 days (a range: 39–57 days), which was the longest period among the four developmental stages. The premolt period of the larvae and nymphs was 37.7 and 26.0 days, respectively (Table 1). Larvae and nymphs were first appeared from early June and July, and their feeding period was 5.2 and 4.6 days, respectively.

Characterization of the oviposition of H. concinna under field conditions

Oviposition data from rabbit-fed engorged ticks maintained outside the confined plot were collected by allowing these ticks to oviposition in separate gauze-plugged tubes (1.5 cm × 10 cm) in the confined plot under natural light and climate condition. The mean amount of egg mass laid and the mean weight of engorged females are presented in Table 2. There were no obvious peaks in daily egg numbers (Fig. 2). Eggs were laid in high abundance the first week, and gradually decreased with occasional small peaks. The female reproductive efficiency index (REI) and reproductive fitness index (RFI) was 6.2 and 4.3, respectively (Table 2). The weight of the engorged females was positively correlated with the number of eggs laid (r = 0.8562) (Fig. 3).

Table 1
The life cycle of *H. concinna* under field conditions.

Developmental stages	Number tested	Periods	Duration (d)	
			Range	Mean ± SEM
Egg	500	Incubation	39–57	41.3 ± 4.2
Larvae	200	Prefeeding	2–4	3.1 ± 0.5
	100	Feeding	3–6	5.2 ± 0.2
	100	Premolt	25–42	37.7 ± 1.2
Nymphs	100	Prefeeding	3–5	3.8 ± 0.7
	100	Feeding	3–6	4.6 ± 0.2
	100	Premolt	22–28	26.0 ± 0.4
Adults	50	Feeding	6–9	6.7 ± 0.1
	50	Preoviposition	9–11	9.7 ± 0.2
	50	Oviposition	12–18	15.0 ± 0.2
Life cycle			124–186	153.1

Table 2
The oviposition characteristics of *H. concinna* under field conditions.

Parameters	Mean ± SEM	Range
Engorged weight (mg)	209.3 ± 31.0	74.9–337.9
Egg mass laid (no.)	1293.0 ± 281.5	420.0–2029.2
REI	6.2	
RFI	4.3	

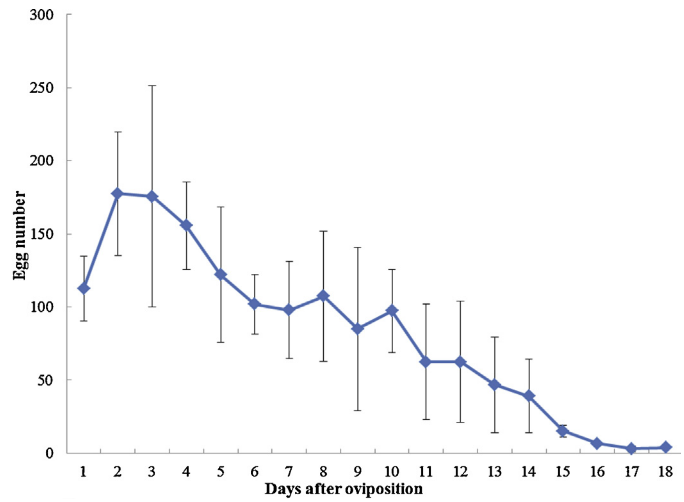

Fig. 2. Mean daily oviposition of engorged females of *H. concinna* (based on 15 females) under field conditions.

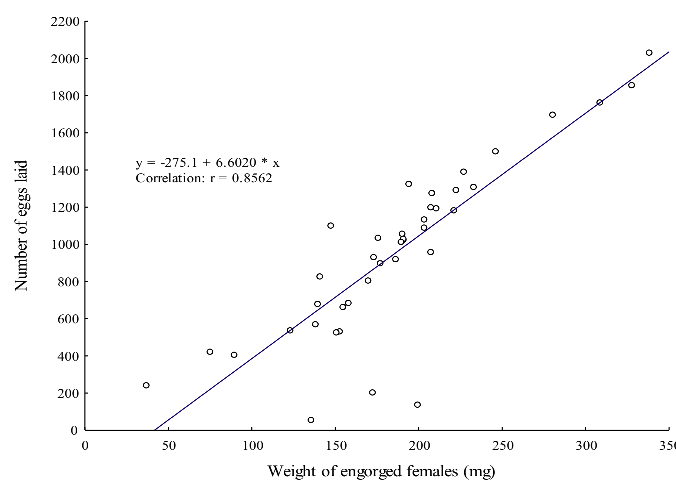

Fig. 3. Relationship between the weight of engorged females and fecundity (based on 40 females) under field conditions.

Observations on the feeding biology of H. concinna in the plot

Both immature and mature *H. concinna* fed slowly in early attachment. After mating, the weight of the female ticks increased rapidly. A large amount of blood was ingested by the females,

Table 3
Changes of body weight of larva, nymphs and adults before and after feeding under field conditions.

Developmental stages	Number tested	Unfed (mg) (mean± SEM)	Engorged (mg) (mean ± SEM)	Weight ratio (engorged/unfed)
Larvae	200	0.03 ± 0.00	0.43 ± 0.00	14.33
Nymph	100	0.25 ± 0.00	2.58 ± 0.50	10.32
Female	50	1.32 ± 0.00	209.33 ± 25.20	158.58
Male	50	1.20 ± 0.00	1.51 ± 0.01	1.26

leading to a 158-fold increase of the weight after engorgement, whereas for males, there was no great weight difference before and after feeding (Table 3). A 14-fold weight increase for larvae and 10-fold weight increase for nymphs were observed before and after feeding under field conditions (Table 3).

Seasonal occurrence and host questing behavior of H. concinna in the plot

Adults were released into the confined plot in April and most of them were able to attach and feed on the rabbits in the confined plot during May and June. The unfed adult ticks that did not find the rabbits, stayed in the grass until mid-July. Following engorgement, the females detached from the host rabbits in the confined plot and spent approximately 2 days in search of a suitable oviposition site. Once onset, the oviposition usually lasts for about one month or so. Larvae appeared in early June and peaked in early July in the confined plot. The unfed nymphs first emerged in early July, and became inactive in late August. The engorged nymphs entered the leaf litter in early August for molting. In September, the emerged adults and nymphs stayed under the leaf litter at a depth of 8–13 cm and kept quiescent.

Under field conditions, the newly emerged larvae usually appeared from early June and remained at the bottom of the grass diurnally. These larvae persisted in the grass roots for about 2–4 days without movement, and then quested diurnally for the host at the bottom of the grass. Most of the nymphs emerged from early July and usually spent about 3–5 days under the leaf litter, then quested mid-height from the grass. Once attached to the host, the larvae and nymphs usually attached to the areas surrounding the eyes or ears, whereas most adults preferred only the ears.

In this study, the unfed larvae could survive for about 16–20 days from July to August, and adults and nymphs survived about 9 and 10 months, respectively. Both adults and nymphs kept quiescence in winter. When observed in March 2013, the survival rates of the adults and nymphs were 86% and 70%, respectively.

Discussion

The 3-host tick *H. concinna* is widely distributed in China, including Inner Mongolia, Heilongjiang, Jilin, Liaoning, Xinjiang, Sichuan and Gansu (Teng and Jiang, 1991), and can infest rabbits, dogs and cows, with rabbits being the most suitable hosts (Bian et al., 2009). In the current study, the life cycle, occurrence and behavior of *H. concinna* were investigated under field conditions with rabbit hosts. We show that *H. concinna* require a mean life cycle (from unfed adults to the next generation of unfed adults) of 153.1 days from April to October 2012, which is significantly longer than that (120.6 days) observed under standard laboratory conditions (Bian et al., 2009). The developmental periods of eggs and larvae (50.4 days and 46.0 days, respectively) were significantly longer than those (24.2 days and 37.0 days, respectively) (Bian et al., 2009). Similar differences were also observed in *H. longicornis* (Zheng et al., 2011) and *Dermacentor silvarum* (Yu et al., 2010), which may be attributed to changing temperature and relative humidity under natural conditions (Fig. 1).

Previous research indicates under field conditions, the tick *H. concinna* requires approximately two years to complete the whole life cycle (Teng et al., 1989). However, this study suggests that, if a host is available, the tick *H. concinna* can complete one generation in one year in northeast China. Observations on the seasonal occurrence of *H. concinna* indicate that, under natural conditions, larvae appear in late May and peak in early June; nymphs appear in early July and became inactive in late August. The overlap activity of larvae, nymphs and adults of *H. concinna* was observed in late June and mid-July, when the temperature and humidity is relatively stable and high (Fig. 1). Similar dynamics of tick life cycle stages are also reported in *H. longicornis* (Zheng et al., 2011), *Amblyomma cajennense* (Oliveira et al., 2003) and *A. parvum* (Nava et al., 2008). A possible explanation of this may be that, the life cycles and seasonal activities of ticks are dependent upon various intrinsic and extrinsic factors specific for each species, and that ticks are most abundant under optimal climatic conditions. Host availability may also play an important role for the maintenance of the population and regulation of seasonal dynamics (Padgett and Lane, 2001; Labruna et al., 2002; Dantas-Torres, 2008; Dantas-Torres et al., 2011).

The long incubation period of eggs and the molting of *H. concinna* larvae and nymphs may also regulate its seasonal occurrence. Hence, this may suggest that under natural conditions, instead of single tick cohort, it is possible *H. concina* is occurring under different and independent population cohorts over the years.

Observations on the host seeking behavior were conducted in the confined plot. Results suggest that the newly emerged larvae usually stay at the bottom of the grass during the day, nymphs occupy the middle of the grass, and the adults often climb to the blade tips. Similar host-seeking behavior is also observed in *I. ricinus* (Mejlon and Jaenson, 1997) and *D. silvarum* (Yu et al., 2010). This may be attributed to different host specificity of the immature and mature stages of *H. concinna* life cycles, as the adult *H. concinna* usually parasitize on Artiodactyla, and the immature stages mainly attack small- to medium-size mammals (Nosek, 1971).

The number of egg masses laid is positively correlated with the weight of engorged females in *H. concinna* ($r = 0.8562$), which has been reported previously in many other tick species, including *D. silvarum* (Yu et al., 2010), *H. longicornis* (Zheng et al., 2011), *H. doenitzi* (Chen et al., 2012), *Hyalomma asiaticum kozlvoi* (Chen et al., 2009) and *H. anatolicum* (Ahmed et al., 2011). Once oviposition begins, large amounts of eggs are laid in the first 5 days, and then gradually decline in number. The pattern of oviposition appears to be affected by the daily temperature and relative humidity (Despins, 1992), and similar patterns are also observed in *D. silvarum* (Yu et al., 2010) and *H. longicornis* (Zheng et al., 2011) under field conditions.

In this study, the unfed larvae, nymphs and adults of *H. concinna* could survive for 16–20 days, 9 and 10 months, respectively. The precipitation and relative humidity are reported to have a great influence on the survival periods during unfed stages (Pegram and Banda, 1990). In order to survive longer, the ticks will seek shelter deep in the leaf litter, especially during winter. According to the premolt periods of larvae and nymphs, in particular the incubation of eggs, the tick *H. concinna* requires at least one year or more to complete its whole life cycle depending on the host availability. Hence, there would be overlap in development and activity of different and independent population cohorts over several years. Due to the experimental confined plot and human intervention, this study may not comprehensively reflect natural events in real field conditions. However, our results will provide a starting point for the investigation of tick developmental dynamics under field conditions.

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