Climate drivers and temporal variation of Ixodes ovatus abundance on a giant panda living in the wild

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Abstract

Background

Ticks and tick-borne diseases have negative impacts on the health of wild animals including endangered and vulnerable species. The giant panda (*Ailuropoda melanoleuca*), one of the iconic flagship species, is threatened by tick infestation as well. Ticks can not only cause anemia and immunosuppression, but also bacterial and viral disease of giant pandas. However, many previous studies about ticks on giant pandas were only limited in scope as case reports of ill or dead animals.

Methods

In this study, an investigation about ticks on a reintroduced giant panda at Daxiangling Reintroduction Base in Sichuan of China was conducted. Ticks were collected daily and identified from the ears of the giant panda from March to September in 2021. A linear model was used to test the correlation between daily tick abundance and climate factors.

Result

All ticks were identified as *Ixodes ovatus*. The daily average of tick population was 6.78 (95% CI: 6.17–7.39) including 1.79 daily males (95% CI: 1.61–1.98) and 4.99 for females (95% CI: 4.53–5.44). Tick abundance was significantly different among months. Tick abundance increased from March and reached the highest point in June and July, then it decreased until September. Results from linear model showed that the temperature positively correlated to tick abundance while air pressure had a negative correlation with tick abundance.

Conclusions

This study investigated tick species and abundance on a giant panda living in the natural environment for the first time, which provided important information for the conservation of giant pandas and other species sharing the same environment.

Background

Ticks are blood-sucking ectoparasites of many vertebrate animals including mammals [1]. Their feeding behavior can cause indirect effects on hosts such as anemia and immunosuppression. The wounds of hosts after tick biting result in a secondary infection of bacterial pathogens [2]. Ticks are also arthropod vectors of medical and veterinary pathogens such as *Anaplasma*, *Borrelia*, *Coxiella*, *Ehrlichia* and *Rickettsia* [3, 4], which threaten not only domestic and wild animals, but also human health.
Within this area of investigation, a number of studies revealed the threats of ticks and tick-borne disease to wild animals [5]. For example, a study in Mississippi, USA revealed that more that 40% of white-tailed deer (Odocoileus virginianus) and raccoons (Procyon lotor) were infected by Ehrlichia chaffeensis while over 50% of raccoons and opossums (Didelphis virginianus) were detected positive for the antibodies of Rickettsia parkeri, which can be explained by the infestation of ticks [6].

Endangered and vulnerable animals, as parts of wild ecosystem, can also be affected by tick infestation. For instance, Italian hares (Manis pentadactyla) were reported to be infected by multiple species of ticks, which potentially limited their survival and fitness [7]. In another study, one subspecies of Manis pentadactyla was reported to carry various ticks and tick-borne pathogens [8]. The infestation of ticks led to a decline of the wild population. One investigation of deaths in a black rhino (Diceros bicornis) population indicated that the tick-borne parasite Babesia bicornis sp. were potentially associated with morality in the those cases [9]. Another study revealed that there was a correlation between the mortality of moose (Alces alces) calves and tick population. In that study, most of the mortalities were correlated to tick infestations [10].

To make matters worse, the range of tick activity seems to be extending as a result of climate change. Ostfeld and Brunner reviewed the distribution of ticks in Europe and indicated this trend [11]. Other studies also observed the seasonal effect of environmental factors on tick abundance [2, 12, 13]. Therefore, it is necessary to evaluate the impacts of tick infestation on endangered and vulnerable animals, especially in small isolated populations, to prevent large scale outbreaks.

The giant panda (Ailuropoda melanoleuca) is recognized as a flagship species and one of the most iconic species for nature conservation. Giant pandas are distributed in six mountain ranges: Minshan, Qionglai, Qinling, Liangshan, Daxiangling, and Xiaoxiangling, mainly in southwestern China [14]. To date, 13 tick species were reported from giant pandas including nine Haemaphysalis spp., three Ixodes spp., and one Dermacentor sp. [15]. A mixed infestation of ticks was reported in most of those previous studies, while single species infestation also occurred in one of the cases [15]. In the past, clinical symptoms like anemia, inflammation, and exhaustion were considered the result of tick infestations [16]. However, most of previous studies on ticks have relied primarily on the investigation of sick or dead giant pandas. Recently, Babesia sp., a blood parasite that are considered to be transmitted by ticks, was reported on a giant panda, which caused a series of symptoms [17].

Furthermore, tick infestation is not only affecting wild giant pandas. Reintroduction of giant pandas is a commonly used strategy to prevent the decline of small natural populations by translocating individuals to their original geographic ranges. The release of captive born giant pandas into these areas exposes these animals to different natural threats, including tick infestation and must be considered in the case of wildlife reintroductions [18].

Therefore, evaluating the potential threats of ticks on giant pandas in the natural environment is vital both for providing better protection for the small isolated wild populations of this species and to provide information for the conservation management including reintroductions. To further safeguard giant
pandas, it is necessary to understand the relationship of ticks and giant pandas in the natural environment including the species and seasonal population of ticks that may affect this vulnerable species. To investigate these factors, a giant panda undergoing reintroduction training was monitored for tick infestations at the Daxiangling Reintroduction Base in Sichuan, China.

**Methods**

**Experimental site and animal information**

This study occurred in an 0.25 km$^2$ site that was enclosed within an iron fence in the Daxiangling Reintroduction Base (29°33'55.076″N–29°32'50.474″N, 102°50'13.866″E–102°51'3.189″E, altitude:1998-2500m) within Daxiangling Natural Reserve in southwestern China which is a part of the Giant Panda National Park [19] (Fig. 1). Mixed coniferous and broad-leaved forest dominate the landscape in this site. One adult female giant panda was kept in this experimental site for pre-release reintroduction training and acclimation. This giant panda was allowed to roam freely and forage naturally, which exposed the pandas to the ticks in the environment. A weather station close to the site (29°33'43.31″N, 102°51'2.46″E) recorded climate information during this study. Climate factors including temperature (°C), Solar radiation (W/m²), gust speed (m/s), precipitation (mm) and pressure (KPa) were collected hourly from the weather station. The daily average values of climate factors were calculated for further analysis.

**Sample collection and identification**

The giant panda body was checked in a daily rotation. Ticks were collected and reported by the Chengdu Research Base of Giant Panda Breeding reintroduction team if the access to the panda was possible. The facial area including face, ears, and neck of the giant panda were checked for ticks during the daily examination. All the ticks in the facial area were collected and were stored on dry ice in the field and then transferred to −20 °C in the lab, and then eventually stored at −80 °C. Tick species and sex identification and population counting were done in lab.

Thirty ticks were selected randomly from 1425 individuals for species identification by both the morphological method and by using Coxidase Subunit I (COI) and 16s rRNA with Basic local alignment search tool (BLAST) using The National Center for Biotechnology Information (NCBI) [20]. After the reliability of morphological identification was verified, the remaining samples were identified with the morphological method alone.

**Statistical analysis**

Due to the fact that the giant panda was free-ranging in the study area and could not always be examined for numerous reasons, such as poor weather conditions, the rolling average value was used to make up for lost sample data that could not be collected. This method has been used in previous research under similar conditions [21, 22, 23, 24]. We used a five-day rolling average value (the average value of previous four days and current date) of both tick abundance and climate data in the analysis.
The difference of tick abundance, female-to-male sex ratio and climate data among months were tested with ANOVA test by “aov” function in R statistical software (version 4.1.2) [25]. A Tukey Honest Significant Differences (HSD) was conducted as the post-hoc test done by the “multcom” R package [26].

A linear regression model was used to explore the correlation between climate factors and the abundance of ticks by the “lm” function of R [25]. We used “step” function to select the best fitted model by dropping independent factors and selecting the model with the lowest Akaike information criterion (AIC). The significance of climate factors in the final model were tested with a t-test method done by “summary” function in the stats package in R. The correlation between female-to-male sex ratio and climate factors was tested as well. Significance test between female and male ticks was done with Welch t-test in R. All analyses met the assumptions of homoscedasticity and normality and were log-transformed where necessary. The effects of correlations between environmental variables on variance inflation factors (VIF) was evaluated, and only non-correlated variables with VIF lower than five were used in the final linear model. The full linear models were:

Tick daily abundance ~ Temperature + Solar radiation + Gust speed + Precipitation + Pressure

Tick sex ratio ~ Temperature + Solar radiation + Gust speed + Precipitation + Pressure

**Results**

**Tick identification and abundance**

A total of 1425 ticks were found and collect from the giant panda’s ears between March 5th and September 30th in 2021 while none were found on the other parts of the facial area. Ticks were all at the adult stage. The research team reported the investigation of ticks for 162 days during the total eight-month experiment (Table 1). Thirty ticks were randomly selected and identified by using both COI and 16s rRNA barcodes and showed that all of the tested ticks are *Ixodes ovatus*. Morphological traits agreed with the molecular identification. The remaining samples were all then identified as *I. ovatus* morphologically (Fig. 2). Daily average abundance of tick was 6.78 (95% CI: 6.17–7.39) including 1.79 daily males (95% CI: 1.61–1.98) and 4.99 for females (95% CI: 4.53–5.44).
Table 1
The average daily tick abundance among months in 2021

<table>
<thead>
<tr>
<th>Months</th>
<th>Days (with tick data collected)</th>
<th>Average daily tick abundance</th>
<th>Daily tick abundance range with 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>21</td>
<td>3.29</td>
<td>1.66–4.91</td>
</tr>
<tr>
<td>April</td>
<td>24</td>
<td>6.58</td>
<td>3.95–9.22</td>
</tr>
<tr>
<td>May</td>
<td>23</td>
<td>10.87</td>
<td>6.27–15.47</td>
</tr>
<tr>
<td>June</td>
<td>26</td>
<td>12.96</td>
<td>6.98–18.95</td>
</tr>
<tr>
<td>July</td>
<td>26</td>
<td>11.85</td>
<td>8.06–15.63</td>
</tr>
<tr>
<td>August</td>
<td>18</td>
<td>10.39</td>
<td>5.93–14.85</td>
</tr>
<tr>
<td>September</td>
<td>24</td>
<td>4.83</td>
<td>3.03–6.63</td>
</tr>
</tbody>
</table>

Daily tick abundance was significantly different among months \( \left( F_{(6, 203)} = 26.8, \ P < 0.01 \) ). Tick abundance increased from March to June and reached the highest point in June and July, then it decreased in the following months. June and July together have the highest average value (averagely 10.50 ticks per day) that was significantly higher than tick abundance in March–April (averagely 3.76 ticks per day) and August–September (averagely 5.01 ticks per day) (Fig. 3A).

**Tick abundance and climate factors**

We found no significant correlation between the sex ratio of ticks and climate factors. But a linear model of daily abundance of ticks and climate factor was selected:

\[
\text{Tick daily abundance} \sim \text{Temperature} + \text{Pressure}
\]

Temperature and air pressure were the important climate factors for this model \( \left( F_{(2, 207)} = 29.03, \ P < 0.001 \right) \). The tick daily abundance was positively correlated with temperature (Coefficient = 0.37, \( R^2 = 0.140, t = 6.22, P < 0.001 \)) but negatively correlated with the air pressure (Coefficient = −5.87, \( R^2 = 0.087, t = −4.49, P < 0.001 \)) (Fig. 4, Fig. 5 and Fig. 6). During this study, the average daily temperature was 10.9 °C (95% CI: 10.28 °C–11.53 °C), while the average daily air pressure was 76.11 KPa (95% CI: 76.09 KPa–76.14 KPa). The daily temperature was significantly different among months. It increased from March to July until reaching the highest point in July and August (average temperature: 15.13 °C; 95% CI: 14.78 °C–15.49 °C), then it decreased in September (Fig. 2C). The air pressure was relatively stable. There were no significant changes from March to August until a decline in September (Fig. 2D).

The daily abundance of male ticks (average value: 1.79, 95% CI: 1.61–1.98) was significantly lower than female ticks (average value: 4.99, 95% CI: 4.53–5.44) through months during this study \( (t = −12.81, P < 0.001) \). The female-male sex ratio of ticks in July was significantly higher than March and August, while
sex ratio of ticks in August was significantly lower than April and May. However, there was no obvious trend of the sex ratio by months (Fig. 2B).

Discussion

Many endangered and vulnerable species, are threatened by tick biting and tick-borne diseases [27]. Giant pandas are one of the most iconic and recognizable wild animal species in nature conservation, and understanding the impacts of ticks and tick-borne diseases on these animals is of the utmost importance. However, a limited number of studies have addressed the effects of ticks on giant pandas. Previous studies about ticks and giant pandas were limited as forms of case reports of dead or diseased individuals and lack of systematic investigation in a wild environment. In this study, we conducted a survey of tick abundance on one giant panda undergoing reintroduction training over seven tick-active months in 2021. We collected and identified ticks from the ears of the giant panda which was living in its natural environment during the entire research period. We explored the changes of tick population and sex ratio over time. The correlation between tick abundance and climate factors during that period was also tested by a linear model.

In this research, we found all the ticks from the ears of the giant panda were from a single species, *I. ovatus*. Although multiple tick species were reported frequently on animals, a number of cases found that animals can be infested by a single tick species. For example, a survey about tick abundance on American black bear (*Ursus americanus*) found that all 1976 ticks collected from multiple areas of 278 black bears were identified as *Ixodes scapularis* [28]. An early case reported more than 2000 ticks as *Haemaphysalis warburtoni* from a two-year old giant panda in a natural reserve in Sichuan, China [29]. Although one meta-analysis study showed that a larger host body size may correlate to a higher diversity of ticks in the neotropical area [30], that study only included data from the neotropical area and did not consider the tick distribution on host body locations.

One previous study about tick species diversity on cattle revealed a significant difference across cattle body parts. Only 1.6% of the total tick species were found on the ears in that study [31]. Another research about tick distribution on horses found that tick species located specifically in different host body parts. In the ear area, only the *Rhipicephalus* spp. was reported in that study [32]. In our study, all the ticks were collected from the ear area of the giant panda in the result of the accessibility to the subject. Therefore, it seems that adult *I. ovatus* was the major species in the ear area of that giant panda in our research field, which agreed with parasitical pattern of ticks’ behavior on mammals in previous studies [28, 32].

*Ixodes ovatus* is the only tick species found in this study. This species was previously reported in North and North-east Asia, including China. Samples were collected within a wide altitudinal range from 1400 m to 4600 m in multiple studies [33]. In our study, the giant panda lived in the wild environment from 1998 m to 2500 m, which is the natural altitudinal range for *I. ovatus* as documented.

*Ixodes ovatus* were mostly reported parasitizing larger domestic and wild mammals including the Bovidae family (cattle, buffalo and goat), rodents, cats, dogs, bears and human [33]. Besides, the only
other case that previously reported *I. ovatus* on giant pandas we could find was reported from one dead giant panda in 1987 in Ganxu province of China [34]. In that case, 103 various ticks were collected in total but only two of them were *I. ovatus*. However, in that study, the location was in the northwest of China, which was not in the same area to our current research site. So, environmental factors can affect the distribution of *I. ovatus* differently disregarding the possibility of the detachment of ticks from the dead animal in that study.

In our research, the average daily abundance of *I. ovatus* increased over time from March to June, then decreased until September. One previous investigation of the seasonal activity of *I. ovatus* in a natural environment [35], using mark-recapture methods on ticks, found adult *I. ovatus* showed high activity from April to mid-July, but decreased significantly in late August. This result showing tick activity peaked in June matches the abundance pattern of *I. ovatus* collected from the giant panda in our study.

Multiple factors may affect the activity of *I. ovatus*. Previous research that examined *I. ovatus* activity in an experimental environment found an increasing activity from April to June followed by a sharp decreasing after June [36]. In that study, ticks were put into plastic cylinders that were placed outdoors where adults were not exposed to sunlight and rain. Another study about the oviposition and development of *I. ovatus* showed that 17–25 °C was the optimal range for the egg hatching and development of *I. ovatus* [37]. However, they only tested five temperatures (15, 17, 20, 25, 27 and 30 °C).

In our study, the average daily temperature was 10.9 °C (95% CI: 10.28 °C–11.53 °C). Even in the months with highest temperature range (15.13 °C; 95% CI: 14.78 °C–15.49 °C), the temperature was lower than the highest point of the optimal temperature tested in that previous study. Studies about other Ixodid species showed that the temperature positively corresponded to the activity of questing ticks [38, 39]. Therefore, our results showed that the chance for *I. ovatus* to actually get on giant pandas may be correlated to the temperature positively. Furthermore, the chance for the giant panda to get *I. ovatus* may be even higher in environments with higher temperatures.

Our study showed a negative correlation between average tick abundance and daily air pressure ($R^2 = 0.087$, $P < 0.001$). One previous investigation found that the cases of severe fever with thrombocytopenia syndrome, one tick-borne disease, negatively correlated to the monthly average air pressure [40], which indicated the negative effect of air pressure to the tick activity.

In short, the results in the current study showed that *I. ovatus* abundance on the giant panda met the pattern of the activity of this species in previous laboratorial and field studies.

**Conclusions**

Our study directly investigated the tick species and abundance on a giant panda in a wild environment for the first time. We found that *I. ovatus* is the only tick species in the ear area of giant pandas. The pattern of the abundance of *I. ovatus* agreed with the previous studies of this tick species. Among environmental factors, temperature was positively correlated to the tick abundance, while air pressure was negatively
correlated to the tick abundance. This study revealed the actually activity of *I. ovatus* on giant pandas. Compared to the previous records of ticks on giant pandas, this current study provided new information about tick species and abundance on a living giant panda, which suggests that the strategy of giant panda conservation should be planned with consideration of local environmental factors. In summary, this study provides knowledge for the conservation and reintroduction of giant pandas in Sichuan China as well as other wild animals that are threatened by tick and tick-borne diseases.

**Abbreviations**

BLAST: Basic local alignment search tool; NCBI: National center for biotechnology Information; COI: Coxidase subunit I; 16s rRNA: 16S ribosomal RNA; ANOVA: Analysis of variance; HSD: Honest significant differences; AIC: Akaike information criterion; VIF: Variance inflation factors; CI: Confidence interval.

**Declarations**

**Acknowledgements**

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**Availability of data and materials**

The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

**Authors’ contributions**

XF and RM contributed to the design of the study, data analysis; RM collected the data and provided samples; XF drafted the work and revised the manuscript; DB contributed to the data analysis; CY, WY, DB and SL revised the manuscript; YL, XY, XS, LL, DZ and HZ contributed to the laboratorial work design; JL and JG contributed to the field work design; DQ, ZY, BY, RH and SL contributed to the establishment of this project.

**Ethics Approval and consent to participate**
Tick samples were collected during the regular body check of the reintroduced giant panda and did not require the use of anesthesia or invasive procedures. Experimental procedures and the use of materials were approved by the Institutional Animal Care and Use Committee of the Chengdu Research Base of Giant Panda Breeding (IACUC number: 2019013).

Consent for publication

Not applicable.

Competing interests

No potential conflicts of interest relevant to this article.

References


Figure 1

Experimental site was located in Daxiangling Natural Reserve, which is a part of the Giant Panda National Park in China. The orange area indicates the range of the Giant Panda National Park. The blue star is the location of this experimental site. Continents outline maps by Vemaps.com.
Figure 2

Ixodes ovatus collected from the giant panda. A: Dorsal of the adult female *I. ovatus*; B: Ventral of the adult female *I. ovatus*; C: Dorsal of the adult male *I. ovatus*; D: Ventral of the adult male *I. ovatus*. 
Figure 3

Boxplot of tick abundance, female-to-male ratio and important climate factors change by months. A: Tick daily abundance; B: Daily female-to-male ratio of ticks; C: Daily temperature; D: Daily air pressure. Variables sharing the same letters among months were not significantly different. The five-day-rolling average values were calculated for all the variables that change by months.
Figure 4

The correlation plot of daily tick abundance and the daily temperature with the trending line. The five-day-rolling average values were calculated for tick abundance and temperature.
Figure 5

The correlation plot of daily tick abundance and the daily air pressure with trending line. The five-day-rolling average values were calculated for tick abundance and the air pressure.

$R^2=0.087$, p-value $< 0.001$
Figure 6

Daily average tick abundance, daily average air pressure and daily average temperature with standard deviations by months. All the values are calculated with five-day-rolling average values.