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RESEARCH ARTICLE

Effects of parasites and predators on nociception: decreases analgesia reduces overwinter survival in root voles (Rodentia: Cricetidae)

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ABSTRACT. Growing evidence suggests that parasite-infected prey is more vulnerable to predation. However, the mechanism underlying this phenomenon is obscure. In small mammals, analgesia induced by environmental stressors is a fundamental component of the defensive repertoire, promoting defensive responses. Thus, the reduced analgesia may impair the defensive ability of prey and increase their predation risk. This study aimed to determine whether coccidia infection increases the vulnerability to predation in root voles, *Microtus oeconomus* (Pallas, 1776), by decreased analgesia. Herein, a predator stimulus and parasitic infection were simulated in the laboratory via a two-level factorial experiment, then, the vole nociceptive responses to an aversive thermal stimulus were evaluated. Further, a field experiment was performed to determine the overwinter survival of voles with different nociceptive responses via repeated live trapping. The coccidia-infected voles demonstrated reduced predator-induced analgesia following exposure to predator odor. Meanwhile, pain-sensitive voles had lower overwinter survival than pain-inhibited voles in enclosed populations throughout the duration of the experiment. Our findings suggest that coccidia infection attenuates predator-induced analgesia, resulting in an increased vulnerability to predation.

KEY WORDS. Analgesic response, coccidian infection, predation effect, small mammal.

INTRODUCTION

In nature, predators and parasites constitute the two primary extrinsic population regulators and play important roles in prey/host population dynamics (Sundell 2006, Tompkins et al. 2011). Predation can increase parasite infection by changing the phenotypic traits of the prey (i.e., morphological and physiologic traits) (Duffy et al. 2011, Caetano et al. 2014,Shang et al. 2019). In turn, increased parasite infection results in hosts vulnerability to predation (Carreon and Faulkes 2014, Shang et al. 2019, Gooding et al. 2020). For example, Møller and Nielsen (2007) showed that prey species with high malaria prevalence have higher predation risk than those with a low prevalence. Moose, *Alces alces* (Linnaeus, 1758), and voles, *Microtus townsendii* (Bachman, 1839), are very different in size, but both are more prone to predation when they have heavy parasite burden (Steen et al. 2002, Joly and Messier 2004). Several possible mechanisms for the synergistic effects of parasites and predators on host/prey mortality have been proposed, such as deteriorated body condition (Wirsing et al. 2002, Hoey and McCormick 2004), reduced escape ability (Alzaga et al. 2008), and increased metabolism and energy output (Haye and Ojeda 1998, Krams et al. 2013). However, the underlying mechanism by which parasite-infected prey are more vulnerable to predation is obscure.

Animals respond to the threat of predation via a series of defensive responses, including flight, freezing, risk assessment, increased alertness and fear, or analgesia (Lima and Dill 1990, Kavaliers and Colwell 1994). In small mammals, analgesia induced by environmental stress factors is a fundamental component of the defensive repertoire, promoting the coordinated expression of other defensive behaviors (Colwell and Kavaliers 1993, Caio 2011). Thus, the decreased analgesia may impair the defensive ability of small mammals and then increase their vulnerability to predation (Ives and Dobson 1987, Tambeli et al. 2012, Lamana et al. 2018).

Experimental evidence from laboratory has shown that predator or predator cues could activate the analgesic system in mice and rats (Kavaliers and Colwell 1991, 1994). Meanwhile, endoparasitic infections such as coccidia or nematodes reduce this analgesia when the rodents are exposed to predator stimuli (Kavaliers et al. 2000). Notably, most studies on nociceptive responses have been performed on mice and rats under laboratory conditions. Whether parasitic infections decrease analgesia in wild rodents exposed to predators and if this affects their population survival remains unclear.

Our previous study showed that the combined effects of coccidia infection and predators decrease the overwinter survival of root voles, *Microtus oeconomus* (Pallas, 1776) (Shang et al. 2019). Coccidia are the most prevalent parasites in root voles (Cao et al. 2014, Nie et al. 2014). The current study aims to evaluate whether coccidia infection increases the predation vulnerability of root voles via decreased analgesia. This work builds on the previous (Shang et al. 2019) to test two hypotheses: 1) coccidia infection reduces predator-induced analgesia in root voles; 2) individuals with reduced analgesia have lower overwinter survival.

MATERIAL AND METHODS

Statement of animal right

The use of animals in this study was in accordance with the guidelines of the regulations of experiments on animals and was approved by the animal Ethics and Welfare committee of the Northwest Institute of Plateau Biology, Chinese Academy of Science.

Laboratory experiments

The laboratory experiments were conducted at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China. Root voles were housed singly in clear polyethylene cages (36 × 20 × 17 cm^3) with wood shavings and maintained at 20 ± 2 °C under a 12:12 h light: dark cycle. Food and water were availed ad libitum. Twenty voles, six months and older, of each sex from a laboratory colony were divided into two groups: coccidia-infected (hereafter PA+) and parasite-free groups (hereafter PA-). Half of the PA+ and PA- groups were exposed to predator odor (hereafter PR+PA+ or PR+PA-), and the other half to a control odor (hereafter PR-PA+ or PR-PA-). Each of the four treatments involved five voles per sex, and the initial vole body mass of the four treatments did not differ ($F_{3,36} = 0.187$, $p = 0.904$).

Parasite infection

Voles in the PA+ group were once orally administered with 2000 coccidia oocysts suspended in 0.1 mL distilled water on June 3rd, 2019. Their oocyte levels were comparable to the oocysts per gram in the feces of coccidia-infected root voles studied by Shang et al. (2019). Meanwhile, each vole in the PA- group was treated with a single orogastric gavage dose of 0.1 mL combinatorial anthelmintic comprising 6.25×10^{-4} mL diclazuril solution (Weierkong, Sichuan) and a 2 mg ivermectin tablet (Weierkong). Combinatorial anthelmintics can effectively expel nematodes, cestodes, and coccidia (Yang et al. 2018).

Our pilot study found that the latency period for coccidia infection in root voles was 6–7 days, and the maximum oocyst output occurred 9–10 days post-infection. Accordingly, we measured nociceptive responses on June 13th, 2019.

Predator odor exposure

Voles were exposed to predator or control odors on June 4–13th, 2019. Silver fox, *Vulpes vulpes* (Linnaeus, 1758), odor was used to stimulate predation risk, while the rabbit odor, *Oryctolagus cuniculus f. domesticus* (Linnaeus, 1758) was used as control (Wang and Liu 2002a, Bian et al. 2005b). Since the major predators in the study area – *Buteo hemilasius* Temminck & Schlegel, 1844 or *Mustela altaica* Pallas, 1811 – are protected animals in China, capturing and collecting fresh feces and urine for 15 consecutive days was challenging. Wang and Liu (2002a, 2002b) found that the silver fox odor could change the behavioral responses of root voles. Thus, our study used silver foxes for predator stimulation instead of natural predators.

Fresh silver fox and rabbit feces and urine were collected in trays under the animal cages daily. Each tray was washed with 500 mL distilled water, and the washing water strained through a filter with a 100 mesh screen (Bian et al. 2005a). Filtered solutions from each animal species collected at different times were thoroughly mixed. At the onset of the laboratory experiment, filter papers infused with predator or control odors were randomly placed in the vole cages three to four times a day between 8:00 am and 11:00 pm. This period was chosen because root voles are primarily diurnal (Sun et al. 1982). Each exposure to predator or control odor lasted 30–60 s.

Nociceptive responses

The nociceptive responses of voles were measured on June 3rd, 2019, prior to parasitic infection. The initial nociceptive response latency did not differ among voles in the four treatments ($F_{3,36} = 0.165$, $p = 0.919$). Nociception was measured based on the latency of foot-lifting or licking responses to an aversive thermal stimulus ("hot plate," CAT.NO.T-91-S, CT, USA). Each measurement was replicated thrice in each individual. The individual was immediately removed from the heated surface following the response display and returned to its cage. If no response was observed within 60 s, the test was terminated, and the vole returned to its cage (Kavaliers and Colwell 1994). In the present study, all voles displayed nociceptive responses within 60 s.

Field experiments

Field experiments were conducted at the Haibei Alpine Meadow Ecosystem Research Station, Menyuan County, approximately 155 km north of Xining, Qinghai Province, China (37°37'N, 101°12'E). The station has an elevation of 3200 m, is surrounded by mountains, and has an average annual temperature and precipitation of -1.6 °C and 560 mm, respectively (Li et al. 2004).

Root vole populations in this area fluctuate annually, usually with relatively low numbers in late winter and spring, increasing throughout the breeding season, and declining after the breeding season; multi-year cycles are weak or absent (Jiang et al. 1991). In this study area, root voles prefer dense vegetation, mainly *Elymus nutans* (Poaceae), in habitat selection. The average population in the habitat ranged from 217 to 280 voles ha^{-1} in autumn, even up to 400 ha^{-1} where grazing activities were limited (Bian et al. 1994, Sun et al. 2002). The breeding season typically lasts from April to October. Juveniles reach puberty and breeding age at approximately 50 and 70 days, respectively (Liang et al. 1982). The primary predators in the study area are falcons, *Falco tinnunculus* Linnaeus, 1758, buzzards, *Buteo hemilasius* Temminck & Schlegel, 1844, and weasels, *Mustela altaica* Pallas, 1811.

Experimental facility

The field experiments were carried out in four 0.15 ha (50 × 30 m) outdoor enclosures located in an old *E. nutans* meadow. Major plants included *E. nutans*, *Poa* spp., *Thalictrum alpinum*, and *Kobresia humilis*. The vegetative cover provided a dense leaf layer, forming a natural refuge for root voles. The enclosures were constructed using galvanized steel panels (1.5 and 0.5 m above and below ground, respectively) but without wire mesh roofs. Further, the enclosures had a series of low panels (~0.3 m high) along the exterior walls every 10 m, allowing terrestrial and avian predators to enter but prevented voles from exiting the enclosures. The vegetation conditions are similar in each enclosure. Each enclosure was equipped with 60 laboratory-made wooden traps (Bian et al. 2011), spaced in 5 × 5 m grids.

Founder populations

Forty-eight voles of each sex, six months or older, from a laboratory colony were used to establish founder populations on October 16, 2017. The voles were divided into two nociception levels according to thermal response latency ("hot plate," CAT. NO.T-91-S, CT, USA); high response latency group (hereafter group H; 53.92 ± 0.11) and low response latency group (hereafter group L; 49.3 ± 0.09). The nociceptive response latency of group H was significantly higher than group L ($F_{1,94} = 1011.93$, $p < 0.001$). Earmarked voles from group H were released into two enclosures, while earmarked voles from group L were released into the other two enclosures. Each enclosure contained 12 voles per sex, and each treatment was conducted in duplicate. The density of the founder population (160 voles ha^{-1}) was in line with natural densities in autumn (Bian et al. 1994, Sun et al. 2002).

Prior to the experiment, all voles were treated with a combinatorial anthelmintic to eliminate parasites and ensure homogeneity. Besides, all enclosures were trapped for two weeks to remove small resident mammals. We also ensured the initial vole body mass did not differ between the enclosures ($F_{1,94} = 0.004$, $p = 0.95$).

Vole trapping

Live trapping began on October 28th, 2017, after the voles had acclimated to the enclosures for two weeks, and lasted for 141 days (at the end of March 17th, 2018). Standard capture-record-recapture methods were used throughout the present study. Six trapping sessions were conducted, each consisting of three trapping days. The time interval between two trapping sessions was approximately one month. Each trap was baited with carrots, set between 8:00 am and 5:30 pm, checked every two hours and closed when trapping did not occur. Following capture, the individual was identified and their sex recorded.

Survival rate and population size estimations

We estimated the apparent survival (hereafter "survival") and recapture probability (hereafter "recapture") using the standard open population Cormack-Jolly-Seber model (Lebreton et al. 1992) in the MARK program (White and Burnham 1999). The recapture probability was evaluated under the assumption that the individual was alive and in the sample (Cooch and White 2006). The data comprised a capture history of 96 voles in six trapping sessions from October 28th, 2017, to March 17th, 2018. First, RELEASE in the MARK program was used to conduct a goodness-of-fit test for the global models, namely ΦTR × T, with both vole survival and recapture dependent on treatment, "TR," and time, "T." The goodness-of-fit of the global model was assessed by testing the assumptions of independence and homogeneity of individuals in the enclosures. The goodness-of-fit tests were not significant for voles (tests 2 and 3, RELEASE: $\chi^2 = 12.64$, df = 18, p = 0.81), suggesting that voles in the enclosures were independent and that the model fits were acceptable. We then used a bootstrap-based goodness-of-fit test to estimate the c-hat value (a variance inflation factor; 1.92), which was adjusted to 1.92 in the global models.

Second, we selected the models as described in our previous study (Yang et al. 2018). Briefly, parsimonious models were selected based on the QAICc values, which allows a compromise between bias and precision when the global model does not fit the data (Anderson et al. 1994) and incorporates a variance inflation factor. Third, we tested the hypothesis that nociception influences vole overwinter survival. For the test, a parsimonious model containing the treatment factor was compared with neighboring populations without the factor, using QAICc values. Subsequently, the model average was estimated from the mean monthly apparent survival probability.

We used the minimum number known alive method to estimate population sizes across trapping sessions in each enclosure. Mark-recapture sampling trials of known populations in the enclosures showed that the minimum number known alive was the best estimate of the actual population size relative to other estimators (Chambers et al. 1999, Bian et al. 2011). The rate of population change for each enclosure was calculated using the following equation: $r_t = (1/T) \ln (N_{t+1}/N_t)$, where N_t is the population density at time t, N_{t+1} is the population density during the subsequent trapping session, i.e., at time t +1, while T is the time interval between trapping sessions (Klemola et al. 2002).

Statistical analysis

The vole population size (Poisson distribution) was analyzed using generalized linear mixed models, with log link functions in the SPSS v. 20 program (IBM, Armonk, NY, USA). Continuous variables were analyzed using a linear model. Data sampled repeatedly were analyzed using the repeated measures method, and all models were simplified by eliminating non-significant ($p > 0.05$) interactions. Post hoc comparisons of significant effects were computed using the sequential Bonferroni post hoc procedure.

In the analyses of nociceptive responses, treatments were input into the models as fixed factors, and individual IDs were put as the random factors. In the analyses of population change rate and density, treatments and trapping sessions were input as fixed factors to test the primary and interaction effects separately. Meanwhile, enclosures were input as random factors. Since no sex differences were found for any parameter, the data for males and females were pooled during analyses.

RESULTS

Laboratory experiments

We found an effect of treatment on vole nociceptive responses ($F_{3,34} = 8.89$, $p < 0.001$). Compared with uninfected voles exposed to the control odor (50.70 ± 0.41; range from 49.07 to 53.7), uninfected voles exposed to the predator odor had increased nociceptive latencies (53.65 ± 0.41; range from 51.73 to 55.77), indicating the induction of analgesia (PR-PA- vs. PR+PA-, $p < 0.001$). However, the response latencies of infected voles after exposure to the predator odor (52.11 ± 0.41; range from 51.40 to 53.23) were lower than those of uninfected voles (PR+PA+ vs. PR+PA-, $p < 0.05$; Fig. 1). Thus, coccidia infection altered the vole response to predator odor.

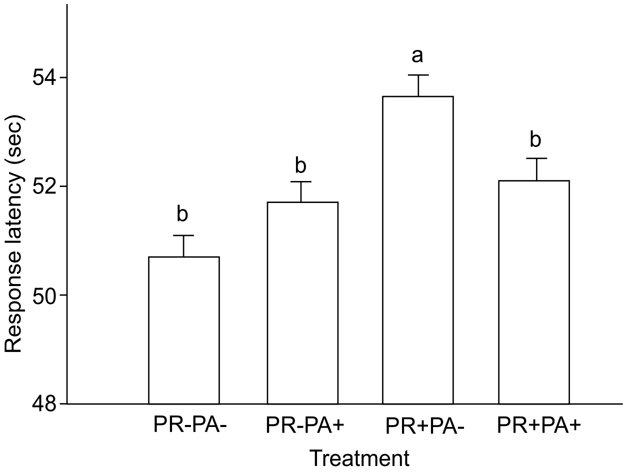


Figure 1. Vole nociceptive latencies among the four treatments. N=10, 10, 10 and 10 for the PR+PA+, PR+PA-, PR-PA+ and PR-PA- groups, respectively. Different letters represent significant difference between four treatments ($p < 0.05$). Data were expressed as the mean ± SE.

Field experiments

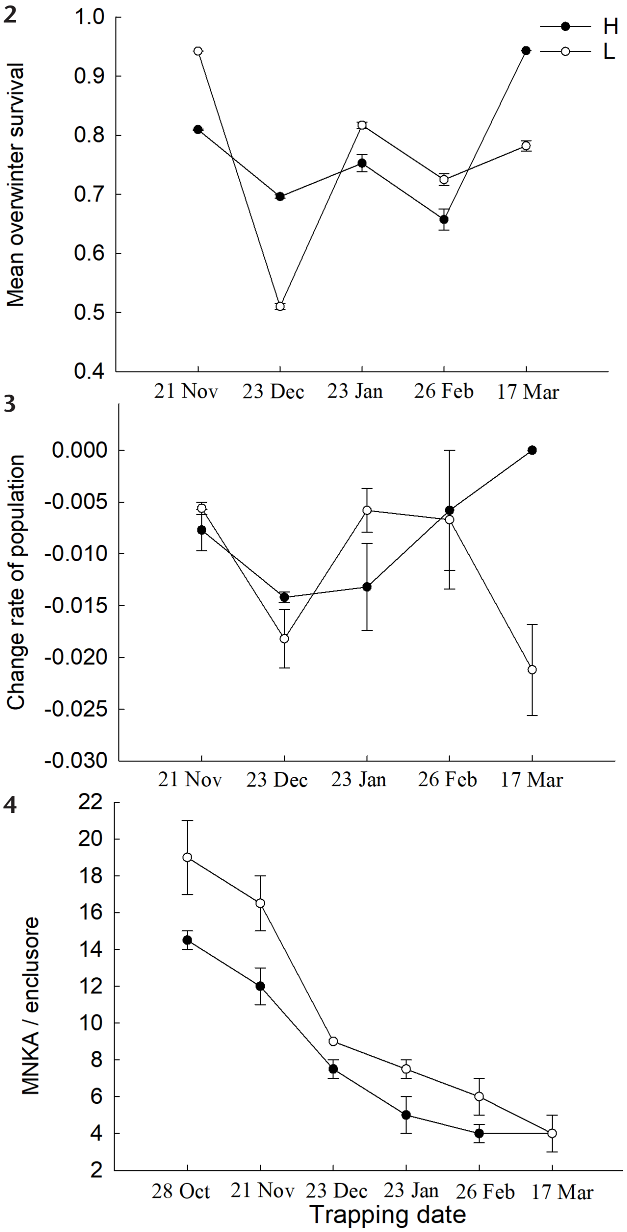
Among the various models describing survival, model 1, 2 and 3 were parsimonious models (Table 1). The differences of QAICc value between models 1, 2 and 3 are less than 2 (the differences between model 1 and 2, 3 is 0.11 and 1.71, respectively), thus these models are considered equally valid models. The model 1, 2 and 3 included the effect of time, treatment and interaction between time and treatment, indicating that time and treatment affected vole overwinter survival (Table 1). The average overwinter survival rates in group H and group L were 0.772 ± 0.01 and 0.755 ± 0.01, respectively (Fig. 2).

The population change rate was affected by time ($F_{5,12} = 10.277$, $p < 0.05$) and the interaction between time and treatment ($F_{5,12} = 0.785$, $p < 0.05$). However, no effect of treatment alone was found ($F_{1,12} = 0.06$, $p = 0.81$), indicating that only

Table 1. Best model structures for modeling survival of the root vole population. The model with the lowest QAICc is reported for the first time. The model structure for recapture remained the best model {P(TR + TR . S + TR . T)}. The effect of treatment is abbreviated TR; time effect, T; sex effect, S. The main effects are symbolized by a plus sign (+) and specific interactions are symbolized by a dot (.), and models including all combinations of additive and interaction effects are represent by an asterisk (*).

	Model number	Model	Number of parameters	QAICc	QAICc weight	QDeviance
General models	1	Φ T + TR . T	12	237.91	0.3696	56.26
	2	Φ T	10	238.02	0.3494	61.06
	3	Φ TR + T + TR . T	13	239.62	0.1573	55.57
	4	Φ TR + S + T + TR . T	14	241.96	0.0488	55.47
Global models	5	Φ TR * T * S	27	269.26	0	47.52

time and its interaction with the treatment affected population change rate. The average population change rates in the group H and group L were -0.008 ± 0.01 and -0.012 ± 0.01, respectively (Fig. 3). Meanwhile, the population density was affected by time ($F_{5,12} = 53.203$, $p < 0.001$) and treatment ($F_{1,12} = 14.735$, $p < 0.05$), but not the interaction between time and treatment ($F_{5,12} = 0.736$, $p = 0.611$). The group L vole populations demonstrated a higher density than group H ($p < 0.05$). Although the former had a 23.7 % higher density than the latter during the adaptive phase (the first 13 days), its population declined sharply to a size similar to group H at the end of the experiments (March 2018, Fig. 4).



Figures 2–4. Monthly apparent survival probability (2), population change rate (3) and population size (4) of root voles during the live-trapping sessions under two different groups. H signifies that root voles with high thermal responses latency; L signifies that root voles with low thermal responses latency. n = 48 and 48 for H and L groups. Data were expressed as the mean ± SE.

DISCUSSION

The primary finding of this research was that coccidian infection in voles reduces analgesia induced by predator risk, resulting in a lower overwinter survival in root voles.

In small mammals, analgesia can promote defensive responses to stimuli and is advantageous in real-time or potentially dangerous situations (Kavaliers 1988, Rodgers 1995, Kavaliers et al. 2000). Laboratory studies have shown that mice and rats display analgesic response when exposed to predator or predator odors (Kavaliers 1990, Rebecca 2003). For instance, Furuya-da-Cunha et al. (2016) found that mice reduce pain reactivity when exposed to predators. However, parasitic infections in mice reduce the analgesia following exposure to predators (Kavaliers et al. 1997). Our results supported our first hypothesis that coccidia infection reduces predator-induced analgesia in root voles. To our knowledge, the present study is the first to test this hypothesis in wild rodents.

Although various reports have highlighted the positive role of analgesia induced by stress in animal defense responses (Amit and Galina 1986, Butler and Finn 2009, Thomson et al. 2020), the causal correlation between nociception and survival at the population level is obscure. Our study is the first to examine whether nociceptive responses affect the overwinter survival of small mammal populations. We found that pain-sensitive voles have lower overwinter survival than pain-inhibited voles. Firstly, the average overwinter survival rate of pain-sensitive voles was lower than it of pain-inhibited voles. Secondly, some pain-inhibited voles died during the two weeks of acclimatization (October 16–28th, 2017) prior to the mark-recapture experiment. Subsequently, the population of pain-sensitive voles had a higher density than pain-inhibited voles during the first trapping session. However, the population of pain-sensitive populations declined sharply at the end of the experiment (March 17th, 2018) to a size comparable to that of the pain-inhibited populations. This result indicates that pain-sensitive voles had lower survival throughout the experiment, which last approximately five months. Finally, the pain-sensitive voles had a higher population change rate than pain-inhibited voles. Root voles do not breed in winter (Liang et al. 1982) and were prevented from entering or leaving the enclosures throughout the experimental period. Thus, the higher population change rate of pain-sensitive voles was only due to lower overwinter survival. These findings support our second hypothesis that voles with reduced analgesia have lower overwinter survival.

Numerous studies have found that extrinsic factors, including parasites (Ryberg et al. 2020), predators (Sheriff et al. 2020), climate (Rödel et al. 2004), and food (Pedersen and Greives 2008), can directly or indirectly affect animal mortality. In our field experiments, parasites are removed in both treatments, and food and climatic conditions are consistent in all four enclosures. Moreover, some laboratory studies have shown that increased analgesia can enhance the anti-predator responses, increasing the survival probability (Ornstein and Shimon 1981, Lichtman and Fanselow 1990, Tambeli et al. 2012). For instance, predator-induced analgesia promoted anti-predator behaviors in mice, which decreased mortality when exposed to cats (Ornstein and Shimon 1981). Notably, voles prefer routes with higher vegetation cover to avoid predation risk (Merken et al. 1991, Taraborelli et al. 2008). In this study, vegetation cover was low in winter, which may have enhanced predation risk by decreasing natural shelter. Therefore, in the present study, the lower survival of voles with reduced analgesia was due to the increased vulnerability to predator, which may relate to the decreased anti-predator behaviors.

Growing evidence suggests that predators and parasites can have non-additive effects on a shared group of prey or hosts, which can influence the population dynamics (Ramirez and Snyder 2009, KrkoŠek et al. 2011, Duffy et al. 2011, Marino and Werner 2013). Shang et al. (2019) found that predators increase both the prevalence and intensity of coccidian infection in voles through immune suppression induced by predation stress. Meanwhile, the increased coccidia infection increases the predation risk, reducing the overwinter survival and population density of voles. The number of individuals present at the beginning of the spring breeding period depends on overwinter survival. Thus, the reduced overwinter survival plays a key crucial role in subsequent population fluctuations (Shang et al. 2020). The present study provides possible insights on how coccidia infection increases the infection vulnerability of root voles. We demonstrate that coccidian infection attenuates predator-induced analgesia, and the reduced analgesia increases the susceptibility to predation.

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