Infection With The Pinworm *Blatticola Blattae* And Survival of Starving German Cockroaches, *Blattella Germanica*

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Abstract

The German cockroach, *Blattella germanica*, is often infected with the pinworm, *Blatticola blattae*. To investigate the effect of pinworm infection on cockroach survival, we artificially manipulated the pinworm infection status of two lines of German cockroaches kept in the laboratory and compared survival rates in starvation. Of the two lines of German cockroaches WATn and NIIDp bred in the laboratory, the WATn line was not infected with pinworm, and the NIIDp line was naturally infected with pinworm *B. blattae*. The WATp line was created by artificially infecting the pinworm *B. blattae* with this WATn line. The NIIDn line was created by treating the NIIDp line with anthelmintic drugs. The 50% survival days of uninfected WATn line of starving cockroaches was 8 days, and infected WATp line of starving cockroaches was 13 days. The 50% survival days of naturally infected NIIDp line of starving cockroaches was 6 days, and that of anthelmintic-treated NIIDn line of cockroaches was 4 days. The survival days were significantly longer in pinworm-infected cockroaches than uninfected or anthelmintic-treated cockroaches. These results suggest that pinworm infection enhances the survival of cockroaches under starving conditions.

Introduction

The German cockroach, *Blattella germanica*, is frequently infected with the pinworm, *Blatticola blattae* (Kobayashi et al. 2021). Although pinworm infection is common among cockroaches worldwide, few reports have described the effects of parasite infection on the insect host. In order to investigate the effects of nematode infection in cockroaches, it is necessary to control the pinworm infection. Kobayashi et al. (2021) reported that German cockroaches can be easily infected with *B. blattae* via contact with feces from pinworm-infected cockroaches. Anthelmintics of pyrantel pamoate and pyrvinium pamoate were reported to be effective for deworming *B. blattae* in the German cockroaches, without causing mortality for the host (Kobayashi et al. 2020). Based on these reports, we established a line of German cockroaches that originated from a pinworm-free line and artificially infected the cockroaches with pinworms. We also established an anthelmintic-treated line of German cockroaches that originated from a line naturally infected with pinworms.

The purpose of this study was to investigate the effect of *B. blattae* infection on the survival of German cockroaches under starvation conditions. We compared the number of days until the cockroach survival rate reached 50% for cockroaches that artificially changed the pinworm infection status.

Materials And Methods

German cockroach

Four lines of German cockroach (WATn, WATp, NIIDn, and NIIDp) were used in the study. WATn is originally a pinworm-uninfected WATARIDA strain, and is abbreviated as WATn by adding “n” which means pinworm-negative. WATp is a cockroach strain that artificially infected the WATARIDA strain with *B. blattae*, and is designated as WATp by adding “p”, which means pinworm- positive. WATn cockroaches

were artificially infected with *B. blattae* by exposing them to feces of infected cockroaches for 6 months in the breeding environment. The prevalence of *B. blattae* infection was 0% (0/40) in the WATn line and 83% (20/24) in the WATp line.

NIIDp is naturally pinworm-infected NIID strain, with "p" meaning pinworm-positive. The NIIDn strain was artificially generated by treating pinworm-infected NIIDp line with an anthelmintic (2000 ppm pyruvium pamoate solution) for 16 days, and the negative meaning "n" was added to make NIIDn. The prevalence of *B. blattae* was 93% (40/43) for the NIIDp line and 6.7% (1/15) for the NIIDn line.

Adult German cockroaches 3 to 30 days old for the WATp and WATn lines and 24 to 50 days old for the NIIDp and NIIDn lines were used in the study.

Cockroach breeding environment

A total of 10 to 15 adult German cockroaches were bred in a plastic cage (size: 175 × 140 × 105 mm) with a porous cover to allow air entry. Escape of cockroaches was prevented by applying a 50-mm wide layer of Vaseline (manufactured by Kanto Chemical Co., Inc.) to the upper surface of the inner wall of the container. A piece of folded paper (approx. 70 × 70 mm in size) was placed in the cage to provide a hiding place. Water and food (control area only) were also provided, and the cages were kept in a constant temperature environment (25 ± 1°C).

Comparison of survival rate

Pinworm-infected cockroaches (WATp & NIIDp), uninfected cockroaches (WATn), and anthelmintic-treated cockroaches (NIIDn) were bred separately in two groups, a normal feeding group and a non-feeding group. The number of surviving cockroaches was recorded at the end of each day. Feces excreted by the cockroaches during the test period were not removed until the end of the experiment.

Statistical analysis

For each group, the 50% survival days (the number of days until the survival rate reached 50%) was determined from the survival rate in the elapsed days. The 50% survival days was calculated according to the Kaplan-Meier method and the log-rank test was used to examine the significance of the differences. The analysis was performed in a high-level test using Statcel4 software (OMS Publishing, Tokyo, Japan) and a *P* value of ≤0.05 was considered significant.

Results

Table 1 summarizes the results of a comparison of the number of surviving cockroaches between the pinworm-infected lines (WATp and NIIDp) and uninfected line (WATn) or anthelmintic-treated line (NIIDn). WAT line was used in Experiment 1 and NIID line was used in Experiment 2. When fed a normal diet, 100% of the cockroaches in both experiments survived to the end of the study period. In the absence of food, survival of WATn cockroaches reached 50% after 8 days, whereas 13 days were required for survival of

WATp cockroaches to reach 50%. In Exp. 2, survival of anthelmintic-treated NIIDn cockroaches declined to zero in 6 days, but NIIDp cockroaches continued to survive even after 10 days.

Table 1. Comparison of the number of surviving German cockroaches, *Blattella germanica*, between pinworm (*Blatticola blattae*)-infected lines and un-infected or anthelmintic-treated lines.

	Diet ¹⁾	B. blattae	Cockroach line ²⁾	Days after treatment						
				1	5	7	9	13	14	15
Exp. 1	Normal	Infected	WATp	50	50	50	50	50	50	50
		Free	WATn	50	50	50	50	50	50	50
	None*	Infected	WATp	53	53	50	41	23	10	6
		Free	WATn	51	47	32	10	0	0	0
				Days after treatment						
				1	4	6	7	10		
Exp. 2	Normal	Infected	NIIDp	15	15	15	15	15		
		Treated	NIIDn	16	16	16	16	16		
	None*	Infected	NIIDp	15	10	6	5	1		
		Treated	NIIDn	16	2	0	0	0		

1) For the normal diet, approximately 3 g of solid feed for mice (MF Oriental Yeast Co., Ltd., Tokyo, Japan) was given. For 'None', no feed was given.

2) The prevalence of *B. blattae* in the WATp and WATn lines was 83% (20/24) and 0% (0/40), respectively. WATp cockroaches were experimentally infected with pinworms via exposure to feces from pinworm-infected cockroaches in the breeding environment. The prevalence of *B. blattae* in the NIIDp and NIIDn lines was 93% (40/43) and 6.7% (1/15), respectively. The NIIDn group is a line in which the infection rate was reduced by treatment of pinworm-infected NIIDp roaches with an anthelmintic agent (pyrvinium pamoate 2000 ppm solution) for 16 days.

*WATp vs. WATn: $P=3.89 \times 10^{-8}$, NIIDp vs. NIIDn: $P=0.048$ (log-rank test)

Figure 1 shows cockroach survival curves for Exp. 1 and Exp. 2 without food. In Exp. 1, the 50% survival period was 13 days for pinworm-infected WATp cockroaches and 8 days for uninfected WATn roaches. A significant difference in survival was noted between the WATp and WATn lines (log-rank test: $P=3.89 \times 10^{-8}$). In Exp. 2, the 50% survival period was 6 days for pinworm-infected NIIDp cockroaches and 4 days for anthelmintic-treated NIIDn roaches. A significant difference in survival was also noted between the NIIDp and NIIDn lines (log-rank test: $P=0.048$).

Discussion

The results of this study suggest that pinworm-infected cockroaches survive longer than uninfected cockroaches under starvation conditions. The mechanism underlying this difference will need to be investigated in further studies. We speculate that pinworm-infected cockroaches have higher tendency to engage in coprophagy (i.e., ingesting feces as a nutrient source), which consequently enables them to survive longer than uninfected cockroaches.

The host-parasite relationship between German cockroaches and *B. blattae* pinworms may represent a unique model for studies of relationship between parasitic nematodes and the hosts. Based on our results, we speculate that the German cockroach and *B. blattae* have a mutually beneficial relationship. The German cockroach is an important sanitary insect pest that is widely found in urban human living spaces worldwide. In addition, numerous studies have indicated that *B. blattae* is a common nematode parasite of the German cockroach (Adamson and Waerebeke 1992; Jarry 1964; Tsai and Cahill 1970; Kobayashi et al. 2021). The results of the present study suggest that *B. blattae* infection enhances the survival of German cockroaches.

In conclusion, the present study demonstrated for the first time that pinworm-infected German cockroaches can survive longer than uninfected cockroaches under starvation conditions. Our results suggest that the German cockroach and pinworm *B. blattae* have a mutually beneficial relationship.

Declarations

Conflict interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Survival rate of German cockroaches, *Blattella germanica*, under starvation conditions. Exp. 1: WATp cockroaches were infected with *Blatticola blattae* pinworms, and survival was compared with uninfected WATn cockroaches (WATp vs. WATn: $P=3.89 \times 10^{-8}$ [log-rank test]). Exp. 2: NIIDp cockroaches were naturally infected with *B. blattae*, and the infection rate of NIIDn cockroaches was reduced by administration of the anthelmintic agent pyvinium pamoate (NIIDp vs. NIIDn: $P=0.048$ [log-rank test]).