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Parasitology

Prevalence of *Blatticola blattae* (Thelastomatidae) in German cockroaches *Blattella germanica* in Japan

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ABSTRACT. A survey for nematode infection in German cockroaches captured in restaurants in various areas of Japan as well as the laboratory-bred colony was carried out. The nematodes were then identified morphologically and molecularly. Of the 320 German cockroaches collected at 79 restaurants in 26 prefectures in Japan, 66.6% (213/320) were found to be parasitized by a single species of pinworm in the hindgut. The mean number of pinworms per cockroach was less than 1.6. Of the three laboratory-bred lines of cockroaches examined, 2 lines (NIID and NK) were found to be infected with a single species of nematode. The prevalence was 93.0% (40/43) and 84.8% (39/46), respectively. The other laboratory line (WAT) was found to be free of the nematode infection. The nematode detected in this study was identified as *Blatticola blattae*. This is the first report of *B. blattae* infection in German cockroaches in Japan. Our study showed that *B. blattae* is distributed all over Japan together with its host *Blattella germanica*. An experimental infection with *B. blattae* in nematode-free cockroach by contaminating the rearing environment with infected-cockroach feces showed that once the environment of the cockroach is contaminated with *B. blattae* eggs, the pinworm infection could spread easily.

KEY WORDS: *Blatticola blattae,* cockroach, distribution, experimental infection, pinworm

German cockroach *Blattella germanica* inhabits in the same environment as with humans including livestock farms, and is one of the most concerned sanitary pests in restaurants and food processing factories. In contrast with the American cockroach *Periplaneta americana* or smoky brown cockroach *Periplaneta fuliginosa*, the German cockroach does not inhabit outdoor, such as river-terrace or bush. In Japan, infection of nematodes in American cockroach and smoky brown cockroach has been reported [17–19, 22], however, there is no reports for German cockroaches.

Since cockroaches are commonly seen in shed of livestock farms and often predated by farm animals such as pigs and chickens, this insect has a high potential to transmit pathogenic organisms to the livestock animals. In fact, cockroaches have been known to play a role in the transmission of pathogens, such as bacteria and parasites, to animals [12, 20]. In experimental studies on coccidia, *Eimeria tenella* oocysts taken orally by cockroaches remained infectious in the cockroach for 3–4 days, and thus cockroaches were involved in the mechanical transmission of *E. tenella* in chickens [10]. Other experimental studies also demonstrated that *Sarcocystis muris*, *Toxoplasma gondii* or *Toxocara canis* can be transmitted to mice via the German cockroach [5, 21].

Since German cockroaches are in close proximity to human, it is of importance to know their parasites fauna. Pinworm *Blatticola blattae* is known to infect the German cockroaches in Europe and USA [4, 6, 8, 9, 25], but the status of the parasitic infection in German cockroaches in Japan is still unknown.

The present study aims to investigate the prevalence of nematodes infection in wild-caught and laboratory-bred German cockroaches in Japan, and also to identify the nematodes species. We also conducted an experimental infection study with pinworms in German cockroaches to confirm the route of infection.

MATERIALS AND METHODS

German cockroach Blattella germanica

Wild German cockroaches were captured in 79 restaurants spanning 26 prefectures in Japan from August 2019 to March 2020

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by using a commercial sticky trap.

Laboratory-bred lines of German cockroach in Japan, which have been used for experimental researches, have been passaged in 3 different institutes (NIID, WAT, NK) were examined for nematodes. All these lines have been kept for more than 30 years in each of the institutes. These 3 lines of cockroach were given to our laboratory, and kept for more than 2 years. Each line of cockroaches was reared in plastic cages (380 × 230 × 250 mm) at a constant room temperature of 25 ± 5°C with 16 hr light and 8 hr dark condition. The cockroaches were given commercial pellet feed for mice (MF Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*.

Morphometry of parasites

Nematodes were collected from the digestive tract of cockroaches in physiological saline, according to Kobayashi *et al*. (2020) [11]. Morphological measurement was conducted using a bio-imaging software (DP-2BSW, Olympus Corp., Tokyo, Japan) after taking pictures by a microscope digital camera (DP25, Olympus Corp.). The body length and width, the length of the esophagus, and the distance from the excretory pore to the anterior end, and the distance from the nerve ring to the anterior end of adult males and females were measured. Feces of cockroaches were mixed with a small drop of physiological saline on a slide-glass, covered by a cover-glass, and observed under a light microscope. The length and width of eggs were measured.

Molecular identification of parasites

Genomic DNA was extracted from 6 pinworms from different cockroaches (NIID line) using Nucleo Spin Tissue XS (Macherey-Nagel., GmbH and Co., KG, Duren, Germany). The D2 / D3 region of rDNA was amplified using a primer pair; forward: D2a (5 '-ACA AGT ACC GTG AGG GAA AGTTG-3 ') and reverse: D3b (5′-TCG GAA GGA ACC AGC TAC TA-3′) [23].

The total reaction volume used for PCR was 50 µl; Taq DNA polymerase (TaKaRa Ex Taq]; manufactured by Takara Bio Inc.) 0.25 µl (0.5 U), 10 × PCR Buffer 5 µl, 2.5 mM of dNTP 4 µl, template DNA 2 µl, primer D2a 0.25 µl, primer D3b 0.25 µl, which was adjusted with sterile distilled water (38.25 µl). PCR reaction conditions were set at 94°C for 2 min. After pre-incubation, heat denaturation at 94°C for 45 sec. annealing 55°C for 30 sec. and elongation reaction of 35 cycles were carried out for 1 min and 30 sec. The amplified product was electrophoresed on a 2% agarose gel, bromide staining was performed, and bands were confirmed under UV light. Amplicons were sent to Macrogen Japan Co., Ltd. (Kyoto, Japan) to determine the nucleotide sequence. To analyze the obtained nucleotide sequences, Molecular Evolutionary Genetics Analysis (MEGA 7) was used. The sequence was subjected to BLAST (Basic Local Alignment Search Tool) search in NCBI (National Center of Biotechnology and Information), and the homology with the registered sequences in GenBank was examined.

Experimental infection of pinworm

Approximately 1-gram feces of pinworm-infected German cockroach (NIID line) was placed into the cage containing 50 pinworm-free cockroaches (WAT line). To confirm that the WAT line was free of pinworm infection, 20 cockroaches were necropsied, and no nematodes were found. Fecal examination of the WAT line using feces in the cages conducted at least twice per year had been also carried out, and no parasite eggs were found for the past 3 years. At days 0, 10, 20 and 30 after the contamination of feces, 14–20 cockroaches were sacrificed, and examined for any stages of pinworm in their gut.

RESULTS

Distribution of pinworms in wild cockroaches

A total of 320 German cockroaches from 79 commercial restaurants in 26 prefectures were collected in Japan. The prefectures in which the cockroaches were collected, and the prevalence of pinworms is shown in Fig. 1. Distribution of *B. blattae* in naturally infected German cockroach *B. germanica* in Japan is shown in Table 1. Pinworm *B. blattae* infection was seen in German cockroaches in all the examined prefectures with the exception of two, namely Hiroshima and Oita. Overall, prevalence of the pinworm was 66.6% (213/320), and the mean number of pinworm per infected cockroaches was 1.6 ± 0.75 (SD). Of the detected pinworms, 89.4% (319/357) were adult females.

Prevalence of pinworms in laboratory-bred cockroaches

Prevalence of *Blatticola blattae* in the hindgut of laboratory-bred *B. germanica* in Japan is shown in Table 2. Of the three *B. germanica* lines, NIID and NK lines of cockroaches were found to be infected with a single species of pinworm *B. blattae,* and the prevalence were 93.0% (40/43) and 84.8% (39/46), respectively. The average number of pinworms per infected cockroach for NIID and NK was 1.8 and 1.5, respectively. The remaining WAT line was found not to be positive for pinworm infection.

Morphology of pinworms detected from cockroaches B. germanica

Morphometry of *B. blattae* detected from the cockroaches *B. germanica* is shown in Table 3. Photographs of the adult female, the egg and the posterior part of the adult male worm are presented in Fig. 2. The body surface of this pinworm was almost transparent. Tails of both males and females were conical. The length of adult female was 1,890–2,892 µm, and the width 200–276 µm. The esophagus consists of a club-shaped anterior part, a muscular bulb at the posterior part, and the length was 212–360 µm. The vulva was located in the posterior part of the body (Fig. 2a). The egg was oval with a slightly pointed tip. Eggs were found in the cockroach feces collected from the cage of the infected German cockroaches (Fig. 2b). The length of the eggs was 106–139 µm and the width was 44–53µm. The male body length was 676–931 µm, and the width was 53–70 µm. The esophageal length was 99–153 µm, and

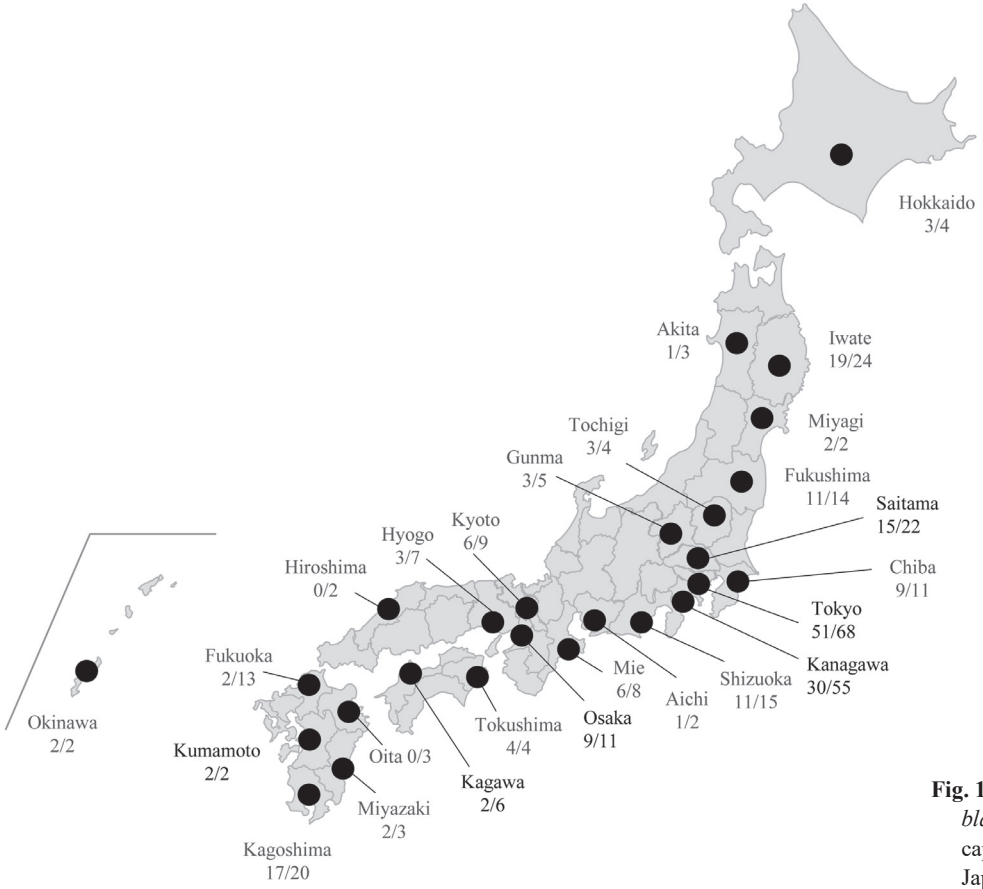

Fig. 1. Distribution of pinworms *Blatticola blattae* in cockroaches *Blattella germanica* captured in restaurants in several areas of Japan (No. of positive / No. of examined).

Table 1. Distribution and prevalence of pinworm *Blatticola blattae* infected in wild German cockroach *Blattella germanica* in Japan

Prefecture	Number of captured area	Total number of cockroach examined	Total number of positive cockroach	Prevalence of pinworm (%)	Mean number of pinworm/ positive cockroach (SD)		Total number of pinworm detected	Total number of adult female pinworm
Hokkaido	2	4	3	75.0	1.7	(0.94)	5	4
Iwate	6	24	19	79.2	1.5	(0.75)	29	24
Miyagi	1	2	2	100.0	1.5	(0.50)	3	2
Akita	2	3	1	33.3	2.0	(0.00)	2	3
Fukushima	2	14	11	78.6	1.8	(0.83)	20	14
Tochigi	1	4	3	75.0	2.0	(1.41)	6	4
Gunma	1	5	3	60.0	1.3	(0.47)	4	5
Saitama	4	22	15	68.2	1.7	(0.79)	25	22
Chiba	3	11	9	81.8	1.9	(0.87)	17	11
Tokyo	14	68	51	75.0	1.7	(0.72)	88	68
Kanagawa	13	55	30	54.5	1.4	(0.55)	41	55
Shizuoka	4	15	11	73.3	1.6	(0.64)	18	15
Aichi	1	2	1	50.0	2.0	(0.00)	2	1
Mie	2	8	6	75.0	1.7	(0.47)	10	8
Kyoto	3	9	6	66.7	1.7	(0.47)	10	9
Osaka	2	11	9	81.8	1.9	(0.87)	17	11
Hyogo	2	7	3	42.9	1.7	(0.47)	5	7
Hiroshima	1	2	0	0.0	0.0	(0.00)	0	2
Tokushima	1	4	4	100.0	1.5	(0.50)	6	4
Kagawa	1	6	2	33.3	1.0	(0.00)	2	6
Fukuoka	3	13	2	15.4	2.5	(0.50)	5	13
Kumamoto	1	2	2	100.0	2.5	(0.50)	5	2
Oita	1	5	0	0.0	0.0	(0.00)	0	5
Miyazaki	1	3	2	66.7	1.5	(0.50)	3	3
Kagoshima	6	20	17	85.0	1.9	(0.87)	33	20
Okinawa	1	1	1	100.0	1.0	(0.00)	1	1
Total	79	320	213	66.6	1.6	(0.75)	357	319

Table 2. Prevalence of pinworms *Blatticola blattae* in the hind-gut of laboratory-bred cockroaches *Blattella germanica* in Japan

Cockroach	Lines	Number of examined	Number of positive	Prevalence of pinworm (%)	Mean number of pinworm/infected cockroach
B. germanica (German cockroach)	NIID	43	40	93.0	1.8
	NK	46	39	84.8	1.5
	WAT	40	0	0	0

[a)] Laboratory-bred lines of cockroaches. Each line has been maintained for more than 30 years in different institutes in Tokyo, Japan.

Table 3. Morphometrical comparison of pinworm *Blatticola blattae* detected from cockroaches *Blattella germanica*

Subject		Present study			Chitwood (1932)	Groschaft (1956)	Guzeeva and Spiriddonov (2012)	Bozeman (1942)
		Min.–Max.	Average	(SD)	Min.–Max.	Min.–Max.	Min.–Max.	Average
Female	No. examined	12			-	-	-	-
	Body length (μm)	1,890–2,892	2,549.8	(506.90)	2,000–3,000	2,100–2,800	-	2,000
	Body width (μm)	200–276	247.4	(44.80)	110–165	200–360	-	130
	Esophagus length (μm)	212–360	328.8	(54.50)	158–280	290–320	-	-
	Excretory pore-anterior end (μm)	498–661	610.8	(115.40)	564–846	-	-	-
	Nerve ring-anterior end (μm)	150–259	182.8	(46.60)	110–220	-	-	-
	Tail length (μm)	20–80	45.2	(16.30)	-	-	-	-
	Vulve-anterior end (μm)	1,357–2,376	1,280.9	(698.60)	1,650–2,500	1,660–2,480	-	-
Egg	No. examined	21			-	-	-	-
	Length (μm)	106–139	126.6	(9.34)	122–126	120–129	105–130	-
	Width (μm)	44–53	47.5	(3.27)	38–42	42–48	45–56	-
Male	No. examined	8			-	-	-	-
	Body length (μm)	676–931	814.8	(91.80)	780–1,000	-	-	800
	Body width (μm)	53–70	66.7	(14.70)	54–75	-	-	60
	esophagus length (μm)	99–153	145.9	(31.09)	132–170	-	-	-
	Excretory pore-anterior end (μm)	133–312	202.2	(86.60)	218–280	-	-	-
	Nerve ring-anterior end (μm)	77–108	93.1	(29.46)	85–100	-	-	-
	Spicule length (μm)	18.3–20.0	18.8	(0.68)	20			
	Tail length (μm)	10–19	14.6	(3.89)	-	-	-	-

-: No data was presented.

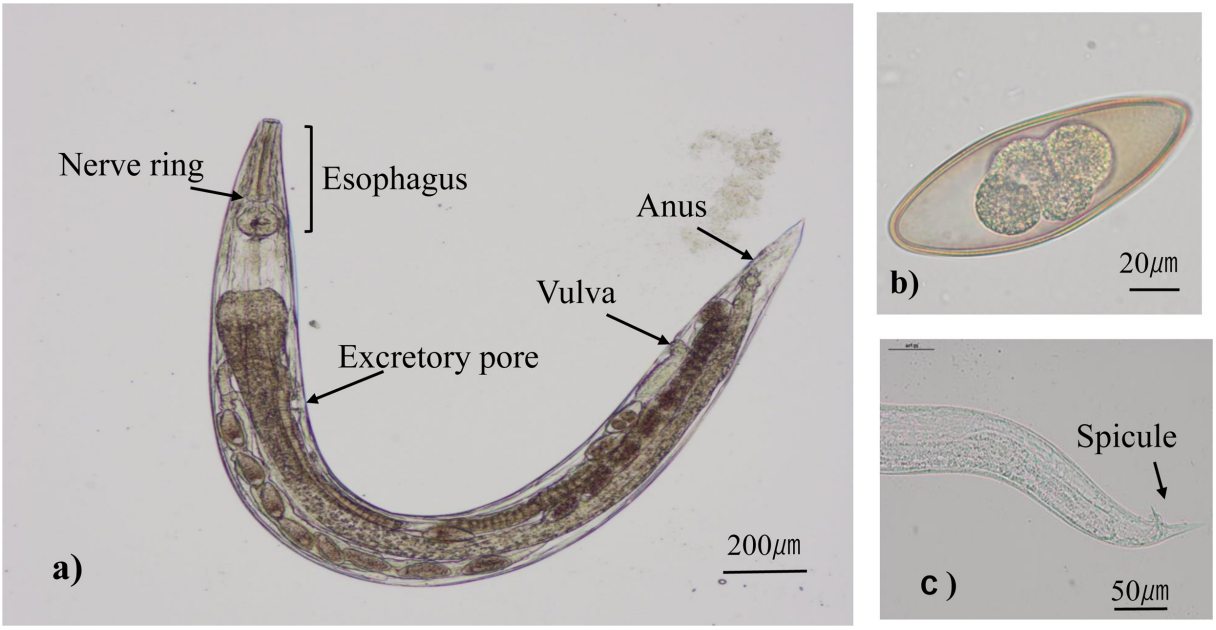

Fig. 2. *Blatticola blattae* infected in German cockroaches *Blattella germanica*. a) Adult female detected from the hindgut of host, b) an egg collected from the feces of host, c) posterior part of an adult *B. blattae* male.

Table 4. Experimental infection of pinworm *Blatticola blattae* in pinworm-free German cockroach (WAT line) by an artificial contamination with feces of pinworm-infected German cockroach (NIID line) in the breeding cage

Cockroach (line)	Subject	Days after treatment[a)]			
		0	10	20	30
Blattella germanica (WAT)	Number of infected/examined	0/40	8/14	13/16	10/14
	Prevalence of pinworm (%)	0.0	57.1	81.3	71.4
	Number of gravid female pinworms/infected cockroach	0.0	0/8	3/13	9/10

[a)] Days after the day of infected-cockroach feces setting in the breeding cage.

the male has a spicule (Fig. 2c). The spicule length was 18.3–20.0 µm. The characteristic shape and the morphometric measurement of the pinworm collected were in accordance with those of the previous reports [3, 4, 6, 7]. Thus, the pinworm was identified as *B. blattae*. The pinworm from the laboratory lines and wild cockroach were morphologically identical, and all the pinworms detected in the present study were identified as *B. blattae*.

Molecular identification of pinworms

We amplified the D2/D3 region of rDNA of 6 individual nematodes obtained from different German cockroaches (NIID line). The resulting 751 bp amplicon was sequenced and then subjected to a search with BLAST. The amplicon sequences were found to be 100% identical to *B. blattae* (Accession number: GQ368472), registered by Spiridonov and Guzeeva (2009) [23].

Experimental exposure of B. blattae in pinworm-free cockroach

Results of experimental exposure of *B. blattae* in pinworm-free *B. germanica* (WAT line) is shown in Table 4. The prevalence of *B. blattae* in the pinworm-free cockroaches gradually increased from days 10 to 20 post exposure, and more than 70% of the cockroaches were infected after day 20. Ninety percent of the nematodes detected in the hindgut of the cockroaches after 30 days were gravid females.

DISCUSSION

Our study presents the first record of *Blatticola blattae* infection in German cockroaches *B. germanica* in Japan. *Blatticola blattae* is a pinworm belonging to the family Oxyroidea, Thelastomatidae [1]. Cockroaches of the species, *Blatta aegyptiaca*, *Blattella germanica*, *Ectobia laponica*, and *E. livida* had been reported to be the host of *B. blattae* in Europe as well as North and South America [4, 6, 8, 9, 25]. Tsai and Cahill (1970) reported that 96% of German cockroaches in New York city were infected with *B. blattae* [25]. *Blatticola blattae* has been suggested to be a native intestinal nematode of German cockroaches in the Eurasian continent and limited to the Ectobiidae family (Chaeroberaceae). It is considered to be distributed worldwide through the German cockroach *B. germanica* [1, 9]. In our survey, German cockroach in Japan was found to be infected with *B. blattae.* There was no significant difference in the prevalence of the nematode among the hosts collected in the different region of Japan. *B. blattae* was found to infect an average of 66.6% of the German cockroaches caught in local restaurants from Hokkaido to Okinawa. Although Hokkaido is a prefecture in subarctic northern part of Japan, stable temperature inside the building provided by human activities enable the German cockroaches to survive even the temperature falls below freezing point [2, 16]. Our results suggested that *B. blattae* is distributed all over Japan together with the German cockroach.

Müeller-Graf *et al*. (2001) reported that *B. blattae* infection had a significant impact on the survival of the cockroach nymphs and their maturity, but no effect on the survival of the adults [15]. Thus, pathogenic effects of *B. blattae* infection on their cockroaches host need to be further investigated in future studies. Since *B. blattae* has a haplodiploid mode of reproduction, the females can produce parthenogenetically haploid male [15]. According to Morand and Rivault (1992), older larvae and adult cockroaches are more likely to be infected with mature female *B. blattae*. Their experimental study showed that it takes 7 days after the exposure for the larvae to be established in the non-infected cockroaches. Male oxyurids appeared between 1 and 2 weeks after the beginning of the experiments and females became dominant after 3 weeks. The first gravid females were observed 5–6 weeks after the beginning of the experiment [14]. The results of our experimental infection study were almost consistent with previous reports, i.e., prevalence of *B. blattae* increased to 57% at 10 days and 81% at 20 days after exposure and at 30 days 90% of the worms were gravid female.

We have demonstrated that non-infected cockroaches can easily turned positive for pinworm infection when the feces of infected cockroaches were introduced into the breeding cage. Gravid females of *B. blattae* were detected in the previously non-infected cockroaches at 20 days after exposure. Since German cockroaches do not generally eat their own feces [24], it was thought that cockroaches orally ingested the pinworm eggs through contamination of their food and drinking water by the feces. German cockroaches indiscriminately excrete feces everywhere, including the place where they eat or drink water in their living environment. Once German cockroach is infected with *B. blattae*, the gravid female of pinworm is continued to lay 10 to 20 eggs per day for about 100 days, which led the *B. blattae* eggs to accumulate rapidly in their living environment. These eggs can survive up to 120 days in the external environment [15]. Thus, we speculated that the laboratory-reared cockroach lines (NIID and NK) used in this study had been infected with the pinworm since the introduction of the cockroaches into the laboratory, and the pinworm has been passaged together with cockroaches up to now. On the other hand, the non-infected line of cockroach (WAT) was originally

captured in Watarida district of Kanagawa Prefecture, Japan, in 1968 [13]. This WAT line of pinworm-free cockroach indicated that it is possible to keep cockroach free from pinworm infection for a long time under normal breeding management, if no individual is infected in the colony or no contamination with the feces of infected cockroaches is occurred.

In conclusion, our study indicated that *B. blattae* infection in the German cockroach occurs very commonly throughout Japan, and there are no regional differences in the prevalence of *B. blattae*. Experimental infection with *B. blattae* in the German cockroach could be established by contamination of the rearing cage with the feces of infected cockroaches.

CONFLICT OF INTEREST. The authors have no conflicts of interest directly relevant to the content of this article.

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