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Screening of Helminths on the External Surface of Cockroaches (Family: Blattidae) collected from Residential Areas in Senawang, Negeri Sembilan

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Abstract

Cockroaches are common pests which live in close contact with humans. It is believed that cockroaches are parasitic vectors of helminths that can colonize humans or animals. However, the records on the prevalence of parasites in domestic cockroaches are very limited. This study aimed to identify helminths on external surface of cockroaches collected from residential areas in Senawang, Negeri Sembilan. A total of 41 cockroaches were collected from three households randomly selected in Senawang, Negeri Sembilan, Malaysia. Cockroaches were caught in kitchens and toilets using modified plastic bottles. An overall percentage of helminths presence on cockroach samples of 39.02 % was recorded. Cockroaches caught in kitchens carried more helminths (50.00 %) compared to those from toilets (26.32 %). Four genera of helminths were identified from the specimens. The identification of parasites showed that the highest percentage of parasites found was *Strongyloides* (19.51 %), followed by *Ascaris* (14.63 %), *Schistosoma* (2.44 %) and *Dipylidium* (2.44 %). The diversity index and richness index of parasites were higher on cockroaches collected in kitchens (H'=1.121 and R'=1.251, respectively) than those collected in toilets (H'=0.673 and R'=0.621, respectively). Meanwhile, the evenness of parasites was higher on cockroaches collected in toilets (E'=0.9801) than those collected in kitchens (E'=0.7669). These findings support the cockroaches' ability as parasitic vectors. Hence, increasing the sanitation level of these areas can help in preventing cockroach infestation and reduce the risk of parasitic disease transmission. In the future, further study should be conducted by incorporating Polymerase Chain Reaction (PCR) method to precisely differentiate between parasite species.

Keywords: cockroach, helminth, host, parasite, vector

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Introduction

Helminths are multicellular parasitic worms that belong to a wide taxonomic group of Nematoda (roundworms) and Platyhelminthes (flatworms) with the latter subdividing into cestodes (tapeworms) and trematodes (flukes). These helminths can easily cause infections, especially in tropical countries. According to Mpairwe and Amoah (2019), about 1.5 billion people worldwide are infected with at least one chronic soil-transmitted helminth infection, and 240 million people are infected with the watery helminth disease. More so, helminth infection can have an effect on female reproductive health and susceptibility to sexually transmitted diseases (Chetty et al., 2020) as well as classified as biological carcinogen for certain helminth infections (the trematodes *Opisthorchis viverrini* and *Clonorchis sinensis*, and the blood fluke-*Schistosoma haematobium*) (Brindley and Loukas, 2017). With the worm infection being typically long-lived and associated with a variety of morbidities, including anemia, growth retardation, cognitive impairment, and elephantiasis (Pabalan et al., 2018; Drurey and Maizels, 2021), these parasites have a complex life cycle that necessitates definitive hosts and intermediate host or vectors. Cockroaches have the potential to be the mechanical vectors of various pathogens including parasitic worms. They are involved in human intestine parasites and intermediate hosts (Nasirian 2017). This pest is most found in residential buildings near human daily life, and also any poor hygiene and inadequate sanitation places such as the urban sewer environments, toilets, septic tanks, and drains. These places are also known to be linked to the sources of helminths' presence (Atiokeng *et al*., 2017). Correspondingly, Abbar et al (2022) observed the infestation rates of cockroaches are higher compared to bed bugs and house mice when they conducted the prevalence rate of pests in low-income residences in New Jersey, USA. There are several research conducted on host diversity compared to their corresponding parasites (Civitello *et al*., 2015; Johnson *et al*., 2015). To the best of author's knowledge, the data available for the presence of parasites on cockroaches in Malaysia only comes from a study conducted by Afzan (2018) in Kuantan, Malaysia. The information is insufficient to analyse and compare the parasites found on cockroaches in other areas. In addition, a study conducted on parasitic infestation on the external body surface of cockroaches in Fresh Market, Thailand resulted in high prevalence of this pest with half of its findings being helminths (Dokmaikaw and Suntaravitun, 2020). The high probability of human exposure to cockroaches increases the potential for disease transmission, especially in tropical country like Malaysia. Hence, this study is focused on the isolation and identification of helminths on external surface of cockroaches, and this was the first study conducted to identify helminths on cockroaches in Senawang, Negeri Sembilan. Discoveries from this study demonstrate the identification and comparison between diversity, richness and evenness of helminths on the external surface of cockroaches. From the public health perspective, the findings of this research were intended to create awareness for local residents of Senawang and other people for the negative health effects potentially caused by cockroaches. Besides, the importance of practicing good sanitation techniques to prevent cockroach infestation is emphasized.

Methods

Sample Collection

This study was conducted in three different family households from residential areas located in Senawang district of Negeri Sembilan (2.6859° N, 101.9833° E). Cockroaches were caught from different sites (kitchens and toilets) by using modified plastic bottles (Figure 1). Biscuit crumbs were used as food attractant. Each trap was placed on the floor of kitchens and toilets near to the water sources where cockroaches live. All of the trapped samples were kept alive before transporting to the Biology Laboratory in University Teknologi MARA Kuala Pilah Campus.

Isolation and Identification of Parasites

The cockroaches were euthanized by using cotton soaked in chloroform. Then, each cockroach was individually placed into a 15 mL centrifuge tube filled with 5 mL of 0.9 % saline solution (Tatfeng *et al*., 2015). The cockroach was well-shaken in solution for two minutes to remove any possible adhering parasites. It was then centrifuged at 3000 rpm for five minutes (Bala and Sule, 2012). Following centrifugation, the preparation was kept stagnant for a few minutes to allow parasites float on the surface of supernatant. The supernatant collected from centrifugation was placed on a clean glass slide by using Pasteur pipette. Then, the supernatant was mixed with one drop of Lugol's iodine. Immediately, cover slides were carefully placed on top of the mixture. The preparation was examined using light microscope at 40x and 100x magnification (Salehzadeh *et al*., 2007). Parasites were identified based on morphology, color and length using keys by Cheesbrough (1998).

Statistical Analysis

Descriptive statistics were used to analyze the presence of helminths while the diversity, richness and evenness indices analyzed using the Paleontological statistics software package for education and data analysis (PAST) were used to evaluate the statistical difference of helminths observed between two trap sites. In addition, the diversity of helminths on cockroaches in kitchen and toilet were subjected to statistical analysis using t-test.

Result and Discussion

Sample Collection

The two months sampling successfully trapped 41 cockroaches (22 from kitchens and 19 from toilets) from different parts of Senawang, Negeri Sembilan. The cockroaches were trapped for both day and night as the traps were set open the whole day.

Presence of Helminths

Helminths were present on at least 15 cockroaches harboring eggs and larvae stages. Results of the present study isolated 16 parasites (39.02 %) which is incomparable to a previous study conducted by Ajero *et al*. (2011) in Nigeria that recorded a much higher prevalence of parasites on cockroaches which is 98 %. The differences in prevalence obtained in this study and earlier study is largely attributed to number of sampling site, sanitation and environmental hygiene since these vectors tend to inhabit areas with poor hygiene and sanitary conditions. The low prevalence demonstrated by our study might result from the low number of sampling sites and also the residents in that area may have heightened awareness of the dangers of cockroaches in their environment and also the residents in Malaysia are more likely to practice personal and environmental hygiene than Nigeria which is usually associated with having poor facilities such as contaminated water supply, insufficient water and poor waste management (Shehu & Nazim, 2022; Akpabio, 2012). Single and double parasitic associations were observed on cockroaches in this study whereby fourteen cockroaches carried one parasite and the remaining carried two parasites. The cockroach that carried two parasites were those trapped from kitchen. A study conducted by Atiokeng *et al*. (2017) recorded multiple parasites carried by each cockroach including *Ascaris* spp. eggs (33.76 %), *Trichuris* spp. (11.97 %) and *Capillaria* spp. (6.16 %) whereas Al-Mayali and Al-Yaqoobi (2010) recorded *Entamoeba* spp. (33.0 %), *Nyctotherus* spp. (65.3 %), *Hammersmiditiella* spp. (83.3 %), *Thelastoma* spp. (15.4 %), *Gordius* spp. (1.3 %), *Enterobius* spp. (2 %) and *Ascaris* spp. (1.3 %). Hence, the mixed infestations suggest that cockroaches are not a specific vector that can carry any number of parasites. The presence of parasites on cockroaches is linked to their filthy habits of crawling in and out of dirty places to forage (Nalyanya *et al*., 2001). This heightened the possibility of them encountering pathogenic microorganisms including parasites (Cotton *et al*., 2000; Pai *et al*., 2003; Salahzadeh *et al*., 2007). This is evident by the findings recorded by Bala and Sule (2012) in which 77.24 % of cockroaches carried various parasites on their external surface.

Prevalence of Helminths

Table 1 showed the comparison between prevalence of helminths from cockroaches trapped in kitchens and toilets. The result showed the prevalence is higher on cockroaches trapped in the kitchens (50.00 %) than cockroaches trapped in toilets (26.32 %). The high prevalence of helminths on cockroaches trapped in kitchens could be due to poor sanitation practices in the kitchens. The preferred humidity levels for cockroaches are found in kitchens and toilets and if food, water and shelter are available; the cockroach population can increase rapidly (Abdel-Gahny *et al*., 2019). Leftover or rotten food that were not properly covered or disposed may be the source of nutrition for cockroaches and the source of pathogenic agents that would eventually be picked up by them. In addition, the traps were placed in damp and contaminated areas in the kitchens such as under the sink and near the sink hole. The results were quite alarming as kitchens are the place where human food is prepared and consumed. The presence of cockroaches carrying helminths there is viewed as a health threat to humans. These results, however, were opposite to those recorded by Tatfeng *et al*. (2015) which found more helminths on cockroaches caught in toilets compared to cockroaches caught in kitchens. The result was expected since cockroaches feed on human faeces. While they feed on the faeces, helminths were encountered on their body through physical touch and eventually transmitted to human.

Table 1. Prevalence of helminths grouped by the trap sites

Trap Sites	Samples trapped (n)	Samples carrying helminth (n)	Helminth observed (n)	Prevalence of helminths (%)
Kitchens	22	10	11	50.00
Toilets	19	5	5	26.32
Total	41	15	16	

Identification of Helminths

Table 2 showed the prevalence of helminths based on the parasite genera identification. Four genera of helminths were isolated from cockroaches in this study which were *Strongyloides*, *Ascaris*, *Schistosoma* and *Dipylidium*. The prevalence of *Strongyloides* spp. (19.51 %) was the highest followed by *Ascaris* spp. (14.63 %), *Schistosoma* spp. (2.44 %) and *Dipylidium* spp. (2.44 %). The high prevalence of *Strongyloides* spp. and *Ascaris* spp. were linked with their thickness and tough, resistant eggshell. Both *Strongyloides* spp. and *Ascaris* spp. are from the genus of nematodes. The eggshell of nematodes is known as the most resistant biotic structures (Wharton, 1986). This provides high protection for the egg and allows long-term survival on hosts. Besides, a single female *Ascaris* lays approximately 200,000 eggs per day which could lead to the high prevalence of *Ascaris* in the environment (Abdel-Gahny *et al*., 2019). This study is compatible with the study conducted by Afzan (2018) in Indera Mahkota, Kuantan and Maji and Ahmed (2023) in Jigawa, Nigeria which also recorded highest prevalence of *Strongyloides* spp. and *Ascaris* spp. on external surface of cockroaches. Bala and Sule (2012) revealed the presence of human intestinal parasite which is *Schistosoma mansoni* (9.48 %) on cockroaches. Some cockroaches may be infected with this parasite which might have been picked up in the toilet (Isaac *et al*. 2014). Cockroaches can transfer the parasite on their body surfaces through personal contact, vomit, or faeces on any nearby surface, including man's food, kitchen utensils, and areas where meals are prepared. This happens as they migrate between toilet and human food sources. The detection of *Dipylidium* spp. (2.44 %) egg on the external surface of cockroaches was surprising as there has not been any record regarding the presence of this parasite on cockroaches. The presence of *Dipylidium* spp. egg might be due to physical contact with animals in the environment.

Table 2. Prevalence of helminths based on parasite genus observed on cockroaches (n=41)

Genera of Parasites	No. positive	No. negative	Prevalence (%)
Strongyloides	8	33	19.51
Ascaris	6	35	14.63
Schistosoma	1	40	2.44
Dipylidium	1	40	2.44

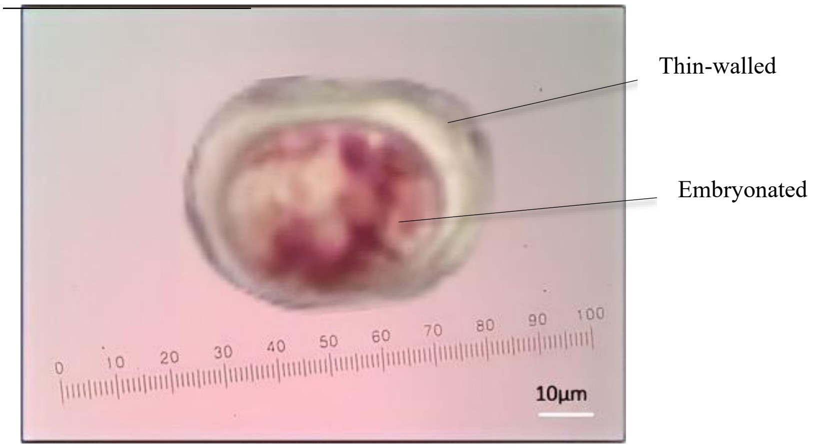

Figure 1. Structure of *Strongyloides* spp. egg under 100x magnification

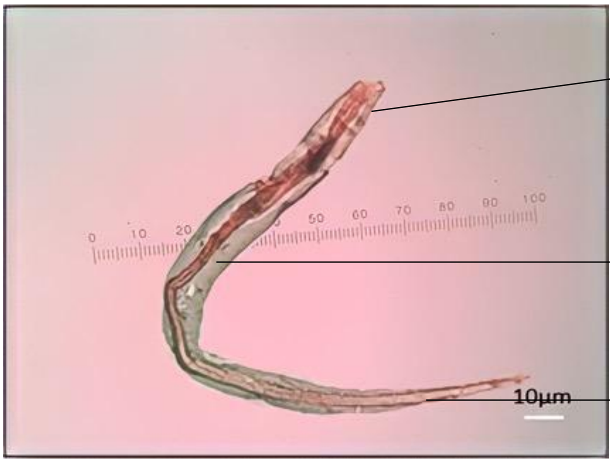

Figure 2. Structure of rhabditiform larvae of *Strongyloides* spp. under 40x magnification (Cheesbrough, 1998)

Strongyloides genus of nematodes are common parasites of terrestrial vertebrates (Viney, 2017). Humans and animals are common vectors of *Strongyloides* especially animals that consume wet, rotting substances such as cockroaches and flies (White *et al*., 2019). *Strongyloides* and *Ascaris* can survive in unfavorable environmental conditions because they both have an inner shell layer of lipoprotein and are air-borne (Atiokeng *et al*., 2017; Ngwamah *et al*., 2021). This explains the higher prevalence of these species on cockroaches. The morphology that identifies the eggs as *Strongyloides* spp. include ellipsoid in shape, measuring 40–85µm in length, embryonated and brown in color covered with thin wall as shown in Figure 1. Microscopic examination of the egg observed measures 55 µm. Microscopic examination of the rhabditiform larvae of *Strongyloides* showing the structure of short buccal cavity, esophagus and a short, pointed tail (Figure 2). Generally, rhabditiform larvae measures 250-300 µm (Nakamura *et al*., 1990). According to Nakamura *et al*. (1990), the size of filariform larvae is bigger measuring about 520-600 µm in length. Other structures of filariform larvae include a long oesophagus and notch at the tail which cannot be observed in this study probably due to low magnification.

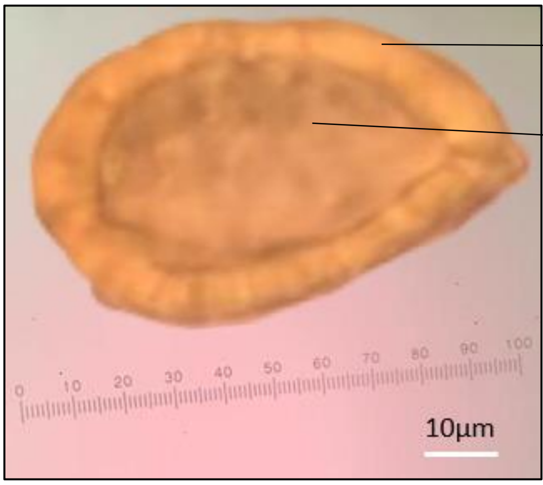

Figure 3. Structure of infertile egg of *Ascaris* spp. under 100x magnification (Cheesbrough, 1998)

Figure 4. Structure of fertile egg of *Ascaris* spp. under 100x magnification (Cheesbrough, 1998)

The genera *Ascaris* is a parasitic nematode. *Ascaris* infects several animals including ruminants, rodents and humans. The widespread *Ascaris* species are *Ascaris lumbricoides* which infect humans and *Ascaris suum* which infect pigs (Leles *et al*., 2012). *Ascaris lumbricoides* was the most prevalent gastrointestinal parasites in the study reported by Belema *et al*. (2020) and Wahedi *et al*. (2020). Both infertile and fertile egg were observed from external surface of cockroaches. The general egg measurement of *Ascaris* is 90 µm long. Figure 3 showed the microscopic examination of infertile egg observed in this study which displays the structure of albuminous coating and central mass of large granules, measuring 95 µm (Cheesbrough, 1998). The color of the egg is yellowish brown. Meanwhile, microscopic examination of fertile egg observed showed no albuminous coat since it is a decorticated egg (Figure 4). The decorticated egg measures 50 µm and is brown in color.

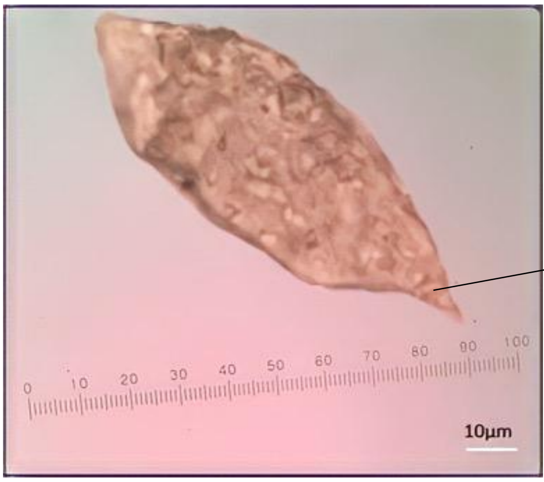

Figure 5. Structure of fertile egg of *Schistosoma spp*. under 100x magnification (Cheesbrough, 1998)

The genera *Schistosoma* is a parasitic trematode. The widespread species of *Schistosoma* is *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma intercalatum* and *Schistosoma mekongi* (Cheesbrough, 1998). According to Ikeh *et al*. (2023), *Schistosoma mansoni* is life-threatening and is responsible for many human pathological conditions and diseases. The presence of *Schistosoma mansoni* on cockroaches indicates that it is a possible vector of the schistosomiasis (Obisike *et al*., 2019). *Schistosoma* also infects ruminants and rodents. The main structure that identifies the egg as *Schistosoma* is the location of the spine which is on the terminal. Other structure including elongated pale yellow coloured egg, measuring from 50-80 µm (Cheesbrough, 1998). Microscopic examination of the egg in Figure 5 showed all the structures previously described and is 80 µm long.

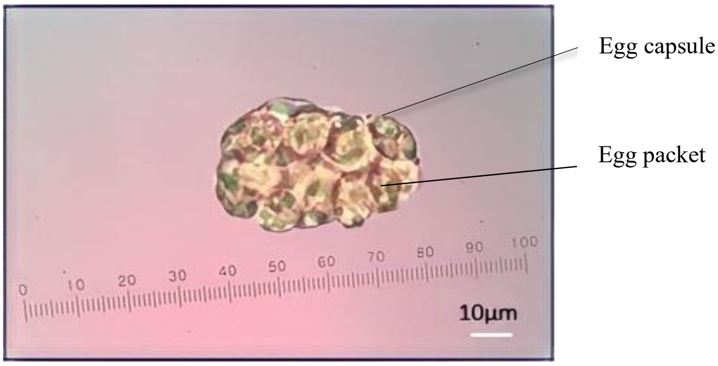

Figure 6. Structure of fertile egg of *Dipylidium spp. egg* under 40x magnification (Cheesbrough, 1998)

The genera *Dipylidium* is a parasitic cestode. It is a common tapeworm of dogs, foxes and cats. Insects are used to carry the eggs to complete its life cycle (Cabello *et al*., 2011). The widespread species of *Dipylidium* is *Dipylidium canimum*. Microscopic examination of the egg observed from the study showed structure of egg capsule containing eight to five egg packets and yellowish in color (Figure 6). According to Cheesbrough (1998), the egg capsule measures 60 µm-100 µm. Each egg packet is 35 µm in diameter Cheesbrough (1998).

Diversity, Richness and Evenness of Helminth on Cockroaches

The cockroaches from kitchen harbored more diversity of helminths species than toilet but the difference in helminths species was not statistically significant (p>0.05). *Schistosoma* spp. and *Dipylidium* spp. was not found in the samples from toilets and present in samples from kitchen with only just one individual respectively. The diversity index of helminths is higher for cockroaches trapped in kitchens (H'=1.121) than the cockroaches trapped in toilets (H'=0.673). This is understandable as kitchens identified higher number of helminths. Since the diversity index exceeds one, within the limits of the sampling of this study, it is said that kitchens had more diversified helminths communities than toilets. Furthermore, the highest richness index of helminths also is recorded on cockroaches trapped in kitchens with R'=1.251 whereas that of cockroaches trapped in toilets is R'=0.621. For instance, the size of the surrounding area influence species richness of flea where larger area results in higher species richness (Krasnov *et al*. 2004a). It is speculated that the cockroaches in kitchens have greater accessibility to the surrounding environment and thus, would pick up more parasites on their body. According to Oyeyemi *et al*. (2016), female cockroaches harbor more parasites (94.0 %) than male cockroaches (80.9 %). This could influence helminths species richness but the sex of the cockroaches was not identified in this study. Conversely, the evenness index of helminths is the highest on cockroaches trapped in toilets (E'=0.9801) in comparison to those trapped in kitchens (E'=0.7669). This is due to the distribution of common species of helminths on cockroaches trapped in toilets.

Conclusion

The findings showed the presence of helminths on external surface of cockroaches with overall percentage of 39.02 %. A total of four genera of helminths were identified, *Strongyloides*, *Ascaris*, *Schistosoma* and *Dipylidium*. The diversity and richness of parasites is higher on cockroaches trapped from kitchens, while it is more even on cockroaches trapped from toilets. The results agreed that kitchens may be unsanitary due to poor sanitation practices in the studied area. Besides, the findings also supported the supposition that cockroaches could be carriers of parasitic agents. Hence, it is suggested that cockroach control measures should be implemented urgently to minimize cockroach infestation. Additionally, DNA barcoding and agar plate culture methods should be incorporated to precisely differentiate between parasite species to allow researchers to recognize important reservoir hosts, as well as distinguishing morphologically similar species that cause distinct pathologies.

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Author Contribution

Nur Syamimi Zamzuri – study design, data collection, analysis and interpretation of results, and manuscript preparation
Sarah Shazwani Zakaria – conceptualization, data curation, supervision, review and editing
Nur Hashimah Ramli – supervision and review

Conflict of Interest

Authors declare no conflict of interest.

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