SHORT COMMUNICATION

Parasitic *Gregarine blattarum* Found Infecting American Cockroaches, *Periplaneta americana*, Population in Penang, Malaysia

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Running head: *Gregarine blattarum* Infecting *P. Americana*

**Abstract:** A study on the prevalence of common endoparasite in wild population of American cockroach in was conducted in Penang Island using trapping method at several sampling sites on the island. *Gregarine blattarum* was found in the digestive tract of 5 out of 115 or 4.35% of the wild American cockroaches, *Periplaneta americana*, sampled. This is the first finding of *Gregarine blattarum* in local American cockroaches reported in Malaysia.
**Keywords:** American cockroach, Endoparasite, Gregarines

*Gregarina blattarum* von Siebold, 1839, originally described from *Blatta orientalis*, has been reported to infect at least six other cockroach species worldwide. However, despite the morphological similarity, Clopton and Gold (1996) demonstrated experimentally that *G. blattarum* infecting laboratory colonies of *Blatella germanica* was unable to infect four other cockroach species from which this gregarine had previously been reported (Smith & Cook, 2008). This parasite which belongs to the Order Eugregarinida (Protista: Apicomplexa) are typically distinguished using characters (Smith & Cook, 2008) and this protozoa are obligate intestinal parasites of a large number of invertebrates, including annelids, crustaceans, echinoderms, arachnids, pelagic tunicates and insects (Clopton and Gold, 1996). Arthropods such as German cockroaches, *Blatella germanica*, was found to be a specific host for *Gregarine blattarum* (Lopez & Alves, 2005). This is the first report of *Gregarine blattarum* infection observed in the American cockroaches sampled in Penang Island, Malaysia. Previously, *Gregarine* sp. infecting local American cockroaches was never been reported or observed in Malaysia.

Trapping method was done according to method suggested by Kinfu et al. (2008). Clean and empty jars were coated with Vaseline on the inside using a suitable cotton meanwhile on the outside of the jars were wrapped with a crumpled papers. A piece of bread was dipped in the fermented liquid or beer and was placed inside the trap. Fermented liquid or beer produced a strong smell and could attract more cockroaches to the traps. The purpose of coated with Vaseline is to ensure the inside wall was slippery so that the trapped cockroaches would not climb out of the jar. The outside traps were wrapped with the crumpled papers to ensure the cockroaches from the surrounding could easily climb and get contact with the bait provided inside the trap. Some traps were tied with ropes on both sides to enable those traps to be tied at suitable holder or corner when traps were placed in the sewers, in the drains or ditches or any other crucial places. This method to ensure that those traps were not remove easily by locals or other animals and also were not moving or carrying by water flow from drains or by rain. A large hole about half from the size of the bottle covers was made for each trap so that cockroaches could be attracted to the baits inside and in the same time other larger animals such as rats won’t easily get into the traps. For some traps, double tapes were used to stick the bottom of traps to the floor at the sampling sites. The traps were then brought to the sampling sites and were put at the suitable places with high potential of population of cockroaches.
All trapped cockroaches from the sampling sites were brought to the laboratory for identification. Identification was made according to the description and general characteristic appearances observed after collected the samples from the sampling sites. Only the species from American cockroaches, *P. americana*, a whole and live captured samples were proceed to the next methods for examination. Other species will be discarded. The samples were divided according to adult males, adult females and nymphs. This classification facilitated the next steps during the examination to determine which sex and stage of cockroach’s life stage were most numerous and having a higher and lower of parasitic infection.

American cockroaches morphologically have shiny red to chocolate brown exoskeleton and the nymphs were uniformly brown in colour and almost resembling the adult cockroaches except they were wingless. This species had long and tapered cerci for all stages and the presence of white mark on their antennae. Male cockroaches had reproductive system known as stylus and for female cockroaches were known as ovipositor. Meanwhile, a nymph cockroach was wingless and had not fully developed reproductive system (Lee & Ng, 2009).

The dissections of the cockroaches were done in order to examine any presence of parasites inside the digestive tract and the alimentary canal. Before the cockroaches were dissected, the head and both wings were pinned on the dissecting tray using fine pins to ensure the sample did not move during the dissection process. The legs were removed using pointed forceps or scissors and the abdomen was exposed to the upper side. Then a dissection scissors were used to cut the ventral side along the abdomen to remove the exoskeleton. The internal part of the sample was exposed after removing the exoskeleton and the body fat that surrounds the abdominal organs was removed slowly using pointed forceps and sharp needles to expose the internal organs of the cockroaches and isolated the alimentary canal. Then the alimentary canal of the cockroaches were removed carefully by cut at the end near the esophagus and cut at the other end near the anus/ cloaca and placed onto a Petri dish. Under the dissecting microscope, the alimentary canal was macerated using sharp scissors and fine needles on the petri dish that contained 2 ml of normal saline.

Any presences of parasites such as nematodes, helminthes, protozoa or the eggs of parasites under the dissecting microscope were recorded. If any, pipette was used to transfer the endoparasites to be observed under the compound microscope for identification. The nematodes found were fixed in 70% alcohol for further identification. For the examination of any presence of ova/ cysts/ eggs of
parasites, 1 ml from the internal washing result was centrifuged at 2000 rpm for 5 minutes and the deposit was examined after staining with 1% Lugol's iodine. Parasite identification was based on the morphology and characteristic appearances. The ova and cysts of the parasites were identified under compound microscope included with the measurement and the identification was according to the length, width and special characteristics shown (Melvin et al., 1964)

A total of 115 samples of cockroaches were collected during sampling period and five of them or 4.35% of the samples were infected by parasitic gregarines. The cockroaches infected with G. blattarum were collected from Gelugor, Bukit Jambul, Bayan Baru, Bayan Lepas, Batu Lanchang dan Tanjung Bungah, while samples collected from Univerisiti Sains Malaysia (USM) Main Campus and Sungai Dua, Penang were uninfected by G. blattarum. Most of the gregarines observed were in gamont form (Figure 1). Gregarines were reported as one of the most diverse groups of organisms and have been reported found in the invertebrate hosts including cockroaches, dragonflies, beetles and crustaceans (Clopton and Gold, 1996; Wise et al., 2000; Lopes and Alves, 2005; Clopton et al., 2008; Smith and Cook, 2008; Roberts and Javony, 2010; Bunker et al., 2013). The gregarines infected invertebrate insects were grouped under order Eugregarinorida and suborder Septatorina. Gregarines infected the cell of intestine of American cockroaches was identified as Gregarina blattarum.

The gamont of G. blattarum was cylindrical in shape with 100 µm to up to 2000 µm in length and 30 µm to 1500 µm width. Each of gamont was attached to the host intestinal epithelium. A pair of gamonts will undergo syzygy and detached from the intestinal epithelium. The association of the two gamonts then form gametocyst wall in which the process of gametogenesis, fertilization and division of zygote happened. Then, the production of an oocyst occurred which also known as process of sporulation. Gametocyst with oocysts encapsulate inside were passed out with the cockroach's feces. When the other cockroaches consumed the infected feces, oocysts of G. blattarum would also infect them. In the intestine of infected host, the oocyst will undergo exsporulation and penetrate the cell of epithelium by sporozoite. Then sporozoite will growth became the trophont. Trophont growth to gamont that attached to the intestinal cell of epithelium and the process repeated. According to Lopes and Alves (2005), the infection of gregarines to the population of cockroaches can caused mortality and this was the observation recorded in this study. Many of the cockroaches sampled which were later found to be infected with G. blattarum were dead before being dissected.
The gregarine life cycle can be divided into four phases: a) excystation, b) infection and development, c) assortment and syzygy and, d) mixis and sporogony. Mechanism enforcing host specificity may operate during any one of this life cycle phase (Clopton & Gold, 1996) and the oocysts in the environment or from the cadaver of the infected host are accidentally consumed by the American cockroaches. However, some of the cockroaches infected with *Gregarine* sp. were alive and well during trapping and collecting, probably due to the low number of gamonts found in the digestive tract. According to Lopez *et al.* (2005), gregarines were also found in insects with healthy appearance but in low numbers (1–10 gamonts), demonstrating the chronic aspect of the disease. The occurrence of chronic infections of gregarines in cockroaches were reported in colonies maintained under laboratory conditions (Clopton, 1995; Clopton and Gold, 1996).

Since the population of the American cockroaches in the sampling areas normally share the same habitat with other species of cockroaches such as German cockroaches (*Blatella germanica*), Lobster cockroaches (*Nauphoeta cinerea*), Brown-banded cockroach (*Supella longipalpa*) and many more, the American cockroaches could have been consuming the contaminated fecal or body of the dead infected host, thus were infected by horizontal transmission of the parasite.

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**REFERENCES**


**Figure 1:** Gamont of *G. blattarum* is cylindrical in shaped. Note the presence of nucleus (N).