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Isolation and Diagnosis of Parasites from Domestic Cockroach *Periplaneta americana* in Mosul city, Iraq

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Abstract

Cockroaches are the greatest potential mechanical vector of various pathogenic microorganisms. some of which cause serious diseases in humans and domestic animals. The high prevalence of human pathogens carried by the cockroach has been reported in many countries. This study aimed to determine the prevalence of internal and external parasites parasitizing the American cockroach, *Periplaneta americana* and the effect of the sex of the cockroach and the area of sample collection on The type, number and types of parasites isolated, The current study was conducted for the period from July 2023 until April 2024, A samples of 111 male *Periplaneta americana* cockroaches were collected from some homes and schools in the city of Mosul. After the samples arrived at the laboratory, by placing them in clean, sterilized tubes in a refrigerator set at -4°C for 10 minutes Subsequently, 10 ml of normal saline solution was added to each tube. The tubes were then vigorously shaken for 5 minutes to dislodge parasites a salt solution was added to centrifuge tubes, which were spun at 2000 rotations per minute for 5 minutes. This mixture was shaken for 5 minutes and then, Cockroaches were dissected to isolate internal parasites The contents of the digestive tract were taken, and the solution was filtered through a mesh. Each sample was prepared by placing a drop of sediment on a glass slide, adding Lugol's iodine dye, and examining it under a microscope. The majority of the samples were infected with one or more types of parasites on their body surfaces and within their digestive tracts, infection rate of 96.40 %. This study involved the isolation and the diagnosis of 19 parasitic species, including five types of worms: *Ancylostoma duodenale*, *Leidynema*, *Strongyloides stercoralis*, *Hammerschmidtiella* male, and *Thelastoma*. and six types of protozoan parasites, including both active and Nyctotherus ovalis cyst, active and cyst stages of Lophmonas, cyst stage of Entamoeba coli, and Gregarine. and Seven types of eggs, comprising eggs of each of *Ancylostoma duodenale*, *Thelastoma*, *Leidynema*, *Strongyloides stercoralis*, *Hammerschmidtiella* , *Gryllophila*, and in addition to the external parasitic Mite, The highest infection rate was recorded in worms, with 91 out of 111 samples i.e. (81.98%) This was followed by protozoa, with a rate of 72% out of 80 samples. Finally, external mites, 52 samples i.e. (46.84%). In this study, it was observed that the number of parasites harbored by cockroaches in their digestive tracts exceeds more than that found on their external surfaces. Cockroaches collected from households were found to harbor a greater number of parasites compared to those collected from schools, underscoring the necessity of raising awareness about the dangers of cockroach presence in homes and keeping them away from food sources due to the parasites they carry, which may cause various diseases.

Keywords: *Parasite diagnosis, American cockroach, Digestive tract, External parasites*

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I. INTRODUCTION

Parasites are one of the most significant pathogenic causes that spread worldwide, especially in developing countries. They cause many diseases in both humans and animals alike. Their prevalence increases in tropical and subtropical regions (Hajare *et al.,* 2021) Parasites can be transmitted through various means depending on the type of parasite. Some are transmitted orally after ingesting contaminated food or drinks or through contamination with ova, cyst stages and larval stage, which represents the infective stage of the parasites (Petersen *et al.*, 1988). Also, it is transmitted by arthropods, the animal phylum that is the largest in number among the animal species present on the surface of the Earth and the most successful among all animals of medical and veterinary importance in the terrestrial ecosystem, some of which play an important role in the transmission of disease pathogens (Giovanni *et al.*, 2021).

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Cockroaches serve as an important reservoir for many pathogenic agents, including protozoa, parasitic worms, bacteria, and viruses, due to their feeding on sewage systems (Fotouhi-Ardakani *et al.*, 2022). Many studies have been conducted to clarify the role of insects in transmitting pathogens. One of these studies was conducted by Hamoo and Alnuri (2019) where 140 houseflies were collected randomly from butcher shops, sweet markets, school lavatories, and grocery stores in the city of Mosul. The results revealed the presence of 11 species of parasites among them *E. coli*, *E. histolytica* cyst. The percentage of each of them reached 17.02%. The lowest prevalence was *Isospora sp*, i.e. (2.1%) of the total examined parasites. There are other parasites such as *Cryptosporidium, Giardia Lamblia*, *Fasciola hepatica*, and *Toxocara spp*, with varying proportions represented by the eggs of *Enterobius vermicularis*, *Ascaris lumbricoides*, *hookworm*, and *Hymenolepis nana*. Another study was conducted by Otu-Bassey *et al*. (2019) where the parasites of Cockroach *Periplaneta americana* in Nigeria were examined. The study examined the external surface and the contents of the digestive tract of 240 cockroaches. The study on parasites revealed that the highest rate of mechanical distribution of parasites on the external body surface was 45%. It is higher in comparison to the intestinal content of parasites at 10%. The highest percentage of parasites obtained was *Isospora belli* at 50.8%, followed by *Enterobius vermicularis* at 43.1%. Then, *Ascaris lumbricoides* and *E. histolytica /dispar* were both at 3.1% each. On the other hand, Abdel-Gahny *et al*. (2019) collected in their study several samples belonging to two families and four species from 7 different locations. The number of parasites was six, five types of parasitic worms, *Ascaris sp*., *Trichuris sp*, *Hymenolepis sp*, *Entrobius sp*., and *Taenia sp*., and one type of parasitic protozoan, *Entamoeba histolitica.* The rate of spread of parasites on the body surface was 12.1% and in the intestines 13.2%. Another study, done by Maji and Ahmed (2023) in Nigeria, identified parasites inside cockroaches. They evaluated the role of the cockroach in carrying parasites of medical importance. From different locations inside homes, 150 cockroaches were collected and all were identified as *Periplaneta americana* species. Out of 150 cockroaches examined, 79 cockroaches were found to be infected with parasites that included *Hookworms* 22% *Entamoeba histolytica* % 19.3 *Taenia sp* % 26.5, *Ascaris lumbricoides* %68.8 *Wuchereria Bancroft* %15. All of these types were obtained from outside and inside the cockroach's body. Approximately, there are 4,000 species of cockroaches distributed throughout the world. About 30 of them are considered to have a global distribution and inhabit human homes. The most widespread cockroaches are the American domestic ones followed by the German cockroach (*Blattella germanica*), and the Oriental cockroach *(Blatta orientalis*) (Robinson, 2005; Hamu *et al*., 2014).

The American cockroach is one of the largest species of insects that live near humans. It is a widespread urban health pest found in warm and humid areas around the world. It is closely associated with human activities and it is believed to have originated in Africa. It was introduced to the Americans through everyday activities in the early 16th century. Then, it becomes common world widely. The American cockroach is a social insect that prefers to live in dark environments with high moisture content, such as sewers, bathrooms, drainage systems, and kitchens (Bell and Adiyodi, 1982). It is known that cockroaches are nocturnal creatures and feed indiscriminately on garbage and sewage water. Through their feeding and random movement between dirty places and food storage areas, the American cockroach can transmit a variety of disease-causing pathogens and allergenic substances that can induce allergies and asthma in some individuals (Govindaraj *et al*., 2016). Thus, it has great medical importance, although it does not bite or sting, because it transmits a large number of disease-causing microorganisms, represented by bacteria, fungi, viruses, parasitic protozoa, and parasitic worms, internally and externally (Zhang, 2011).

Despite the huge number of studies done globally, the researchers felt that more deep studies should be conducted locally due to the scarcity of studies done on it, especially at the University of Mosul. Due to this fact, it is decided to conduct this study to find out the types of parasites carried and transmitted by the American domestic cockroach in the city of Mosul, Iraq to show the danger of its presence around. Residential populations, including homes, schools, and hospitals, contain parasites that cause many other diseases.

II. Materials And Methods

A. Sample collection

From September 2023 until April 2024, 111 samples of male *Periplaneta americana* cockroaches were collected from homes and schools in the city of Mosul / Iraq. After they were caught, they were placed in sterile tubes as shown in Figure 1. Then, they were transferred to the research laboratory, College of Education for Girls, Department of Biology to be examined.

Figure 1. A picture showing the bottles for collecting and storing samples

B. Isolation and diagnosis of parasites

1. Isolation and diagnosis of external parasites

After the samples arrived at the laboratory, they were euthanized by placing them in clean, sterilized tubes in a refrigerator set at -4°C for 10 minutes to induce anaesthesia, following the method described by Chamavit *et al.* (2011). Subsequently, 10 ml of normal saline solution was added to each tube. The tubes were then vigorously shaken for 5 minutes to dislodge parasites (eggs, larval stages, adult stages) from the external surface of the bodies, as noted by Bala and Sule (2012). After shaking, the samples were transferred from the saline solution to 70% ethanol to prevent contamination. They were then placed on sterilized slides and allowed to dry at room temperature (25°C). Following drying, the samples were washed with normal saline solution for 2-3 minutes according to Abdolmaleki *et al*. (2019). Finally, a salt solution was added to centrifuge tubes, which were spun at 2000 rotations per minute for 5 minutes. The floating fraction was then discarded, and a drop of the sediment was taken and placed on a glass slide, 5 slides for each sample containing a drop of Lugol's iodine dye. They are then moved and examined under a microscope to diagnose parasites (Montresor, 2019).

2. Isolation and diagnosis of internal parasites

The dissection began by placing the cockroach in a wax-lined Petri dish. Appendages, including legs and wings, were removed. Using scissors, the cockroach's body was opened laterally, and the body segments were separated and folded back, secured with pins in the dish. The digestive tract and the fatty bodies, which were isolated from cockroaches using brushes and forceps, were then rinsed with distilled water and processed by emptying its contents into a sterile test tube containing 10 mL of normal saline solution. This mixture was shaken for 5 minutes. Then, the solution was filtered through a mesh (Atiokeng Tatang *et al*., 2017).

The filtered solution was then centrifuged at 2000 revolutions per minute for 5 minutes. The supernatant was discarded, and the sediment was collected for analysis. Each sample was prepared by placing a drop of sediment on a glass slide, adding Lugol's iodine dye, and examining it under a microscope. This procedure was repeated for five slides per sample, following the techniques of (Dokmaikaw and Suntaravitun, 2019; Ejimadu *et al*., 2015; Yahaya *et al*., 2017) depicted in Figure 2.

The percentage was calculated based on the Excel program and according to the equation shown below: (Part / Total value) x 100.

Figure 2. The complete digestive tract of *Periplaneta americana*

III. RESULTS

A. Isolation and diagnosis of parasites from Periplaneta americana males

Microscopic examination of 111 samples from adult male *Periplaneta americana*, collected from both their external surfaces and digestive tracts, and sourced from various homes and schools in Mosul city, yielded significant findings. Each of the 111 samples underwent five replicates of testing. The results, documented in Table 1, indicated parasitic infections in 107 of these samples, accounting for 96. 40% of the total examined.

Table 1. Number of Infected Samples obtained from male *Periplaneta Americana*

Total number of samples	Number of infected samples	%	Number of non-infected samples	%
111	107	96.40	4	3.60

B. Percentage of parasitic species isolated from male Periplaneta americana

Concerning Table 2, it shows that worms had the highest infection rate, affecting 91 out of 111 samples (i.e. 81.98%). Protozoa followed with 80 infected samples (i.e. 72%).

Table 2. Infection Rate and isolated parasitic Species obtained from male *Periplaneta americana*

Parasitic organisms	Number of infected samples	%
Worms	91	81.98

parasitic protozoa	80	72
Mites	52	46.84

C. *Percentage of parasites carried on the external surface and digestive tract of male Periplaneta americana in the city of Mosul*

The results of the study, during the examination of both the external surface and the digestive tract of the cockroach, showed that most of the samples were infected with parasites internally and externally, but the percentage of internal infection was higher than the external one, as shown in Table 3.

Table 3. Percentage of parasites carried on the external surface and digestive tract of male *Periplaneta americana*

Parasitic organisms	Number of Samples	Number of Samples infected	%
External Infected	111	71	63.96
Internal Infected	111	103	92.79

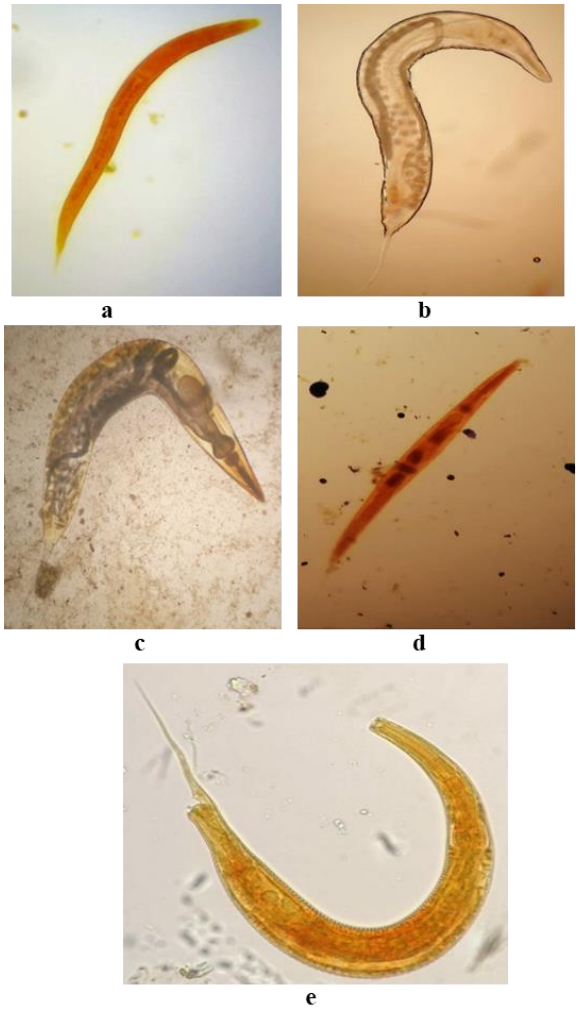

Figure 3. a- *Ancylostoma duodenale* 10x, b-*Thelastoma* 4x, c- *Leidynema 4* x, d- *Strongyloides stercoralis* 10x, e- *Hammerschmidtiella male 10* x.

D. *Parasitic species recorded in males in homes and schools in the city of Mosul*

In present-day studies, 20 types of parasites were isolated, Tables 4 and 5, and five types were diagnosed as worms, represented by *Ancylostoma duodenale, Thelastoma, Leidynema, Strongyloides stercoralis, and Hammerschmidtiella* male. shown in Figure 3 (a, b, c, d, e) respectively Among the household samples, 6 species of parasitic protozoa were isolated and diagnosed in both active and cyst stages, including the active and cyst stages of *Nyctotherus ovalis*, the active and cyst stages of *Lophmonas*, the cyst stage of *Entamoeba coli*, and *Gregarin*. The cyst stage of both *Entamoeba coli* and *Lophmonas* was not observed in the samples taken from the schools shown in Figure 4 (a, b. c, d, e. f respectively. As for the eggs, seven types were isolated belonging to *Ancylostoma duodenale*, *Thelastoma*, *Leidynema*, *Strongyloides stercoralis*, *Hammerschmidtiella*, *Gryllophila*, and *Schistosoma hematobium*, Figure 5 (a, b. c, d, e, f, g) respectively which appeared only in the samples collected from the schools. Additionally, Figure 6 (a, b) respectively the external parasite Mite and its eggs were identified.

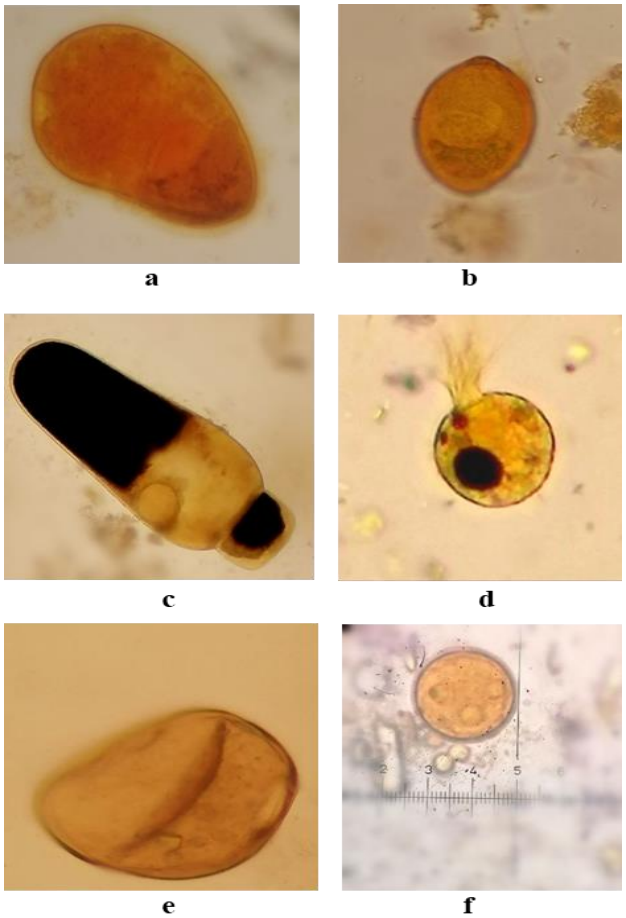

Figure 4. a-*Nyctotherus ovalis* Trophzout 40x, b-*Nyctotherus ovalis* cyste 40x, c- *Gregarine* 40x, d-*Lophomonas* Trophzout 100x, e-*Lophomonas* cyste 40x, f- *Entamoeba coli* 100x.

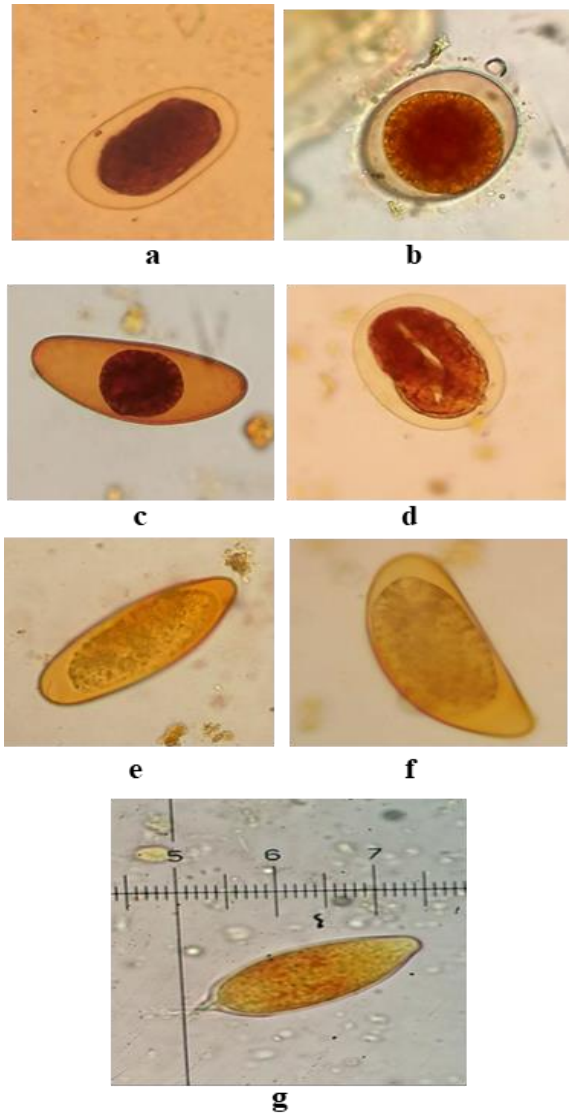

Figure 5. *a- Ancylostoma duodenale* eggs 40x, b- *Thelastoma* eggs 40x, *c-Leidynema* eggs 40x, d-*Strongyloides stercoralis* eggs 40x, e-*Hammerschmidtiella* eggs 40x, f-*Gryllophila* eggs 40x, g-*Schistosoma hematobium* eggs 100x.

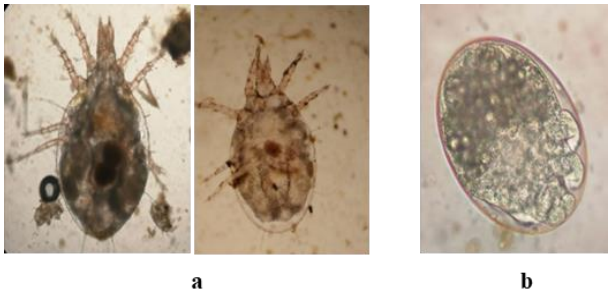

Figure 6. a- Mite 40x, b- Mite eggs 40x.

Table 4. The percentage and number of parasitic species isolated from male *Periplaneta americana* in homes

Parasitic species	Number of infected samples(76)	%
Leidynema eggs	68	89.47
Mites	63	82.89
Thelastoma eggs	53	69.73
Thelastoma	50	65.78
Nyctotherus ovalis cyste	47	61.84
Ancylostoma duodenale	46	60.52
Leidynema spp	38	50
Strongyloides stercoralis	36	47.36
Strongyloides stercoralis eggs	36	47.36
Ancylostoma eggs	36	47.36
Hammerschmidtiella eggs	34	44.73
Gregarine spp	30	39.47
Gryllophila eggs	24	31.57
Hammerschmidtiella male	24	31.57
Nyctotherus ovalis trophzoit	19	25
Entamoeba coli cyste	16	21.05
Lophmonas spp cyste	12	15.78
Lophomonas spp trophozoit	8	10.52
Mites eggs	1	1.31

Table 5. The percentage and number of parasitic species isolated from male *Periplaneta americana* in schools

Parasitic species	Number of infected samples (35)	%
Leidynema eggs	29	82.85
Nyctotherus ovalis cyste	29	82.85
Ancylostoma eggs	29	82.85
Mites	29	82.85
Nyctotherus ovalis trophzoit	27	77.14
Thelastoma eggs	26	74.28
Lophmonas trophzoit	25	71.42
Mites eggs	22	62.85
A. duodenale	15	42.85
Thelastoma spp	14	40
S. hematobium	11	31.42
Leidynema spp	8	22.85
Gregarine spp	5	14.28
Gryllophila eggs	5	14.28
Hammerschmidtiella male	3	3.57
Strongyloides stercoralis female	2	5.71

IV. Discussion

Table 1 shows the presence of parasitic infections in 107 of these samples, representing 96.40% of the total samples examined. These infection rates align with findings from various studies, including that of Abu-Zaid (2018) in Al-Zawiya, Tripoli, Libya, observed a high infection rate among cockroach samples collected from residential rooms, bathrooms, and sewage inspection areas, with 87.2% of adult male cockroaches carrying parasites. Similarly, Pai *et al*. (2003) in their study in China, aimed at determining the role of cockroaches in transmitting pathogens, reported an

infection rate of 96.9%. Furthermore, Al-Aredhi (2014) research in Diwaniyah Governorate indicated that out of 50 examined cockroaches, most harbored one or more types of parasites, resulting in an infection rate of 78%. Otu-Bassey *et al*. (2019) also found a high infection rate of 95.7% among cockroaches. These consistently high percentages across different studies suggest a widespread prevalence of parasitic infections in cockroaches. The percentage of infection around the world with parasites may reach 3.5 billion people, with 450 million of them afflicted with chronic diseases (Who,1998). In contrast to what Salehzadeh *et al*. (2007) found during their study on the diagnosis of the most important microorganisms, bacteria, viruses and parasites, which are transmitted by cockroaches in Iran from hospitals and homes, where no major infections were recorded, as previously mentioned, except in a small percentage. Concerning Table 2, it shows that worms had the highest infection rate, affecting 91 out of 111 samples (i.e. 81.98%). Protozoa followed with 80 infected samples, (i.e. 72%. These findings align with Abdel-Gahny *et al*. (2019), who highlighted the role of cockroaches in mechanically transmitting human parasites in Egypt's Ismailia Governorate. Similarly, Taimeh *et al*. (2012) reported the most frequent infections were with worms, followed by protozoan parasites. Al-Aredhi *et al.* (2014) noted an infection rate of 78% with worms and 62% with protozoa. Additionally, a significant incidence of external parasitic mites, specifically 52 samples with an infection rate of 46.84%, was noted. This rate of mite infestation had not been previously reported in studies conducted in Iraq. The results of the study also showed, during examination of both the external surface and the digestive system of the cockroach, that most of the samples were infected with parasites internally and externally, but the percentage of internal infection was higher than external.

Accordingly, and based on this table, it is evident that the rate of internal parasite prevalence is higher than that of external surface parasites on the body. This is indicated by studies conducted by Al-Aredhi (2014) in Al-Diwaniyah, Iraq, Abu- Zaid (2018) in Tripoli, Libya, and Siagian *et al*. (2017) in Indonesia, during their study on diagnosing parasites transmitted by cockroaches, in contrast to the research conducted by Otu-Bassey *et al*. (2019) in Nigeria, Dokmaikaw and Suntaravitun (2019) in Thailand, and

Atiokeng Tatang *et al*. (2017) in Cameroon, which focused on parasitic survey of domestic cockroaches, examining both the external surface and the contents of the digestive tract revealed that the external parasites carried by cockroaches outnumber those harbored within their digestive tracts, The presence of parasites inside the digestive tract may be higher than those carried on the external surface because the eggs of worms and the sacs of parasites on the external body surface of cockroaches may fall off during their friction and movement from one place to another. Consequently, they can spread what is present on the surface of their bodies, including adult parasites, eggs, and sacs, onto objects they pass through during their wanderings. Meanwhile, what they carry inside their digestive tract may be attributed to the feeding habits of the American cockroach, as it may be contaminated with these eggs or sacs (Al-Aredhi, 2014).

In present-day studies, 20 types of parasites were isolated, and five types were diagnosed as worms, six species of parasitic protozoa as for the eggs, seven types were isolated and the external parasite Mite and its eggs were identified. The number of parasites obtained exceeded that reported by Nedelchev *et al*. (2013) during their study on cases of primary parasitism and helminth infections in the American cockroach in Bulgaria, where they diagnosed five cases of primary parasitism and 2 cases of helminth parasitism. Similarly, Tetteh-Quarcoo *et al*. (2013) found the most prevalent parasites to be nematodes. Singh *et al*. (2014), who isolated and identified worms such as *Thelastoma*, *Leidynema appendiculata*, and *Hammerschmidtiella* , and Ozawa and Hasegawa (2018), who isolated and diagnosed infection with the threadworm *Leidynema appendiculatum* in the *Periplaneta fuliginosa* cockroach, reported findings. Woerden *et al*. (2020) recorded parasites capable of transmission to humans or animals in Spain, including *Nyctotherus sp*. and *Lophomonas blattarum*. However, in Shaibu *et al*. study (2019), infection with hookworms, *Strongyloides stercoralis* larvae, and mites was reported, which was in stark contrast to the study conducted by Debash *et al*. (2022), where the species identified in our study did not appear in their research.

Comparison of values and proportions of infections with major and isolated parasites in adult male *Periplaneta americana* cockroaches shown in Table 4 indicates a clear difference in the numbers of samples infected with each parasite type for samples collected from households. *Leidynema* eggs had the highest number of infected samples, with 68 samples infected, (i.e 89.47%) infection rate, followed by Mites with an 82.89% infection rate, then *Thelastoma* eggs and worms with a 69.73% and 65.78% infection rate respectively. The cyst stage of *Nyctotherus ovalis* accounted for 61.84% of infections, followed by the *Ancylostoma duodenale* worm at 60.52%. Subsequently, Leidynema worm infection was reported at a rate of 50%. The female *Strongyloides stercoralis* and eggs of *Strongyloides stercoralis* and *Ancylostoma* were both found at an infection rate of 47.36 %. Then, *Hammerschmidtiella* eggs were present in 44.73% of cases, followed by *Gregarine,* with 30 samples infected at a rate of 39.47%, then, eggs of *Gryllophila* and males of *Hammersmiditiella* were recorded at a rate of 31.57% each. This was followed by the active stage of *Nyctotherus ovalis*

and the cyst of *Entamoeba coli* at a rate of 25 %, 21.05%. Subsequently, the active and cyst stages of *Lophmonas* were recorded at rates of 15.78% and 10.52% respectively. Finally, the lowest infection rate was recorded for mite eggs, with only 1 sample infected at a rate of 1.31%. No infection with *Schistosoma hematobium* eggs was observed. However, in samples collected from schools, where the number of infected samples reached 11, the infection rate was (9.90 %), In Table 5, it is observed that no eggs were recorded for both *Hammerschmidtiella* and *Strongyloides stercoralis*, as well as the cyst stage for both amoeba and *Lophmonas*. The highest infection rate was recorded for *Leidynema* eggs and the cyst stage of *Nyctotherus ovalis*, as well as *Ancylostoma* eggs and Mites, with 29 infected samples for each type, accounting for 82.85% of the total. Following closely, the active stage of *Nyctotherus ovalis* showed an infection rate of 77.14%, and *Thelastoma* eggs were recorded at 74.28%, and then, the eggs of the defectors accounted for 31.42%, followed by *Leidynema* worm at 22.85%. After that, *Gregarine*, *Gryllophila* eggs, and the mentioned of *Hammerschmidtiella* worm recorded infection rates of 14.28%, 14.28%, and 3.57% respectively. Meanwhile, the lowest infection rate was recorded for the female.

V. Conclusion

The present study demonstrates the danger of the diversity of parasites found on and within the bodies of household and school cockroaches, which may come into direct contact with humans in their daily life activities. It underscores the importance of these cockroaches in the mechanical transmission of parasites shared between humans and animals, especially intestinal worms such as *Strongyloides stercoralis* and *Ancylostoma*. These parasites constituted 54.95% and 14.4% of infections, respectively. This calls for caution and avoidance of their interference with human food and consumer products.

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