

Original Research Paper

## Preliminary results on biodiversity of intestinal parasites carried by the external body of cockroaches at different fast food locations: case of Ain Témouchent city, Algeria

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**Article history:** Received: 12 January 2022; Revised: 31 March 2022; Accepted: 7 April 2022

### Abstract

Cockroaches are vectors and harborages of several microorganisms such as bacteria, viruses, protozoa, and helminths which most of them are known to be causative agents of gastro-intestinal intoxications. Due to the lack of information in Algeria about the intestinal parasites intoxication and their transmission by cockroaches, this work aims to examine the intestinal parasites present on the bodies of twenty-eight cockroaches collected from different sites (Fast food, bakeries, supermarkets, kitchens) in Ain Témouchent city (Algeria). However, no cockroach was found in the university restaurants of the girls' and boys' residences to be collected. The cockroaches identified belong to the four genera, namely American, German, Striped, and Nymph, with prevailing of the male sex. Identification results of intestinal parasites and their different stages showed that the quality of the parasite does not depend on the species of cockroaches and/or the collection site. In fact, all cockroaches load helminth eggs followed by *Giardia* spp. and *Entamoeba* spp. (each one at 60%), *Ascaris* eggs (50%), hookworm (40%), nematodes (20%), *Endolimax* spp. (5%) and others that they could not be identified. Therefore, these results are of great interest in food risk communication and management.

**Keywords:** Cockroaches, Intestinal parasites, Food Intoxication, Algeria

### المخلص

الصراصير المنزلية هي نواقل ومأوى للعديد من الكائنات الحية المجهرية مثل البكتيريا والفيروسات والأوليات والديدان الطفيلية التي يعرف معظمها بأنها مسببة للتسمم المعدي المعوي. بسبب نقص المعلومات في الجزائر حول تسمم الطفيليات المعوية وانتقالها بالصراصير، يهدف هذا العمل إلى فحص الطفيليات المعوية الموجودة على أجسام ثمانية وعشرين صرصوراً تم جمعها من مواقع مختلفة (محلات الوجبات السريعة، مخازن، محلات تجارية، مطاعم الجامعة) بمدينة عين تموشنت (الجزائر). باستعمال مصيدة خاصة، لم يتم العثور على أي صرصور في مطاعم الجامعة بإقامات البنات والأولاد. بعد فحص العينات، الصراصير التي تم تحديدها تنتمي إلى الأنواع الأربعة وهي الأمريكية والألمانية والمخطط والحورية، مع انتشار الجنس الذكر. أظهرت نتائج التقسيم والتعرف على الطفيليات المعوية ومراحلها المختلفة أن نوع الطفيليات لا يعتمد على نوع الصراصير أو موقع أخذ العينة. في الواقع، تحمل جميع الصراصير بيض الديدان الطفيلية تليها *Giardia* spp. و *Entamoeba* spp. (كل واحدة منها بنسبة 60%)، بيض الأسكاريس (50%)، الدودة الشصية (40%)، الديدان الخيطية (20%) *Endolimax* spp. (5%) وأنواع أخرى لم يتم التعرف عليها. لذلك، فإن هذه النتائج تعتبر ذات أهمية كبيرة في الإعلام والتحسيس وإدارة المخاطر المرتبطة بهذه الحشرات الملوثة للأغذية.

**الكلمات المفتاحية:** الصراصير المنزلية، الطفيليات المعوية، التسممات الغذائية، الجزائر

## Introduction

Cockroaches (order *Blattodea*) are a common pest. They are flat bodied insects, usually with two pairs of wings folded flat on the back. However, most of the species can rarely fly but can move on their legs very quickly. Their color ranges from light brown to black. Depending on the species, their length is between 2-3 mm, but it could reach more than 80 mm, allowing them to live in buildings (Rozendaal, 1997). To date, scientists have described more than 4,000 species of cockroaches, about 25 of which seek human proximity (Anticimex, 2014) namely the American cockroaches (*Periplaneta americana*), German cockroaches (*Blattella germanica*), Oriental cockroaches (*Blatta orientalis*) and Striped cockroaches (*Supellalongipalpa*). They are encountered in residential buildings, hospitals, hostels, hotels, restaurants and households (Bala and Sule, 2012, Etim et al., 2013). Their presence is abundant in warm and humid environmental conditions, especially in the kitchen, toilets, drainage systems and even sewers (Afzan, 2018). They are nocturnal and omnivorous species (Salehzadeh et al., 2017); they consume garbage, rotten food, and even fecal waste. Generally, building users intend to control cockroaches with insecticides, as they are unaware of the danger associated with their presence. Unfortunately, they are considered a likely source of microbiological hazards to food either through direct contact and/or through contact with kitchen utensils and surfaces as well as other areas. As reported by several authors, cockroaches are considered potential mechanical vectors of various pathogenic microorganisms such as *Salmonella* spp. and *Shigella* spp. as bacteria (Ojiezeh and Odunayo. 2015), rotavirus (Tetteh-Quarcoop et al., 2013) and parasites (Tatang et al., 2017; Afzan, 2018; Dokmaikaw and Suntaravitun, 2019). Furthermore, these insects are silent vectors of parasites that are not well studied yet as causal agents of food poisoning, especially in Algeria. Therefore, this work aims to identify microscopic parasites that can be carried by cockroaches, opening thus a deep discussion about these hazardous insects in Algerian public health.

## Materials and Methods

### *Cockroaches collection*

A total of twenty-eight cockroaches were collected from different locations in Ain Témouchent city (Algeria), (35° 17' 22" north, 1° 08' 28" west) for two-month (February and March 2020). Table 1 describes the distribution of cockroaches collected from the different sites. The number of samples was limited due to the cold weather during the collection period, which unfortunately coincided with the Covid-19 pandemic, where most of the Algerian restaurants were closed during that critical period.

The cockroaches were collected using a cockroach trap supplemented with special cockroach food and put in a damp and dark place. Cockroaches are attracted to the food at the bottom of the trap, once inside, they will be unable to escape because of a barrier that locks them in (Figure 1).

### *Transport and cockroach identification*

Each hunted cockroach was put separately in a vial tube and then transported in an ice bag to the laboratory for identification and parasitological analysis.

Cockroaches were identified based on morphological and morphometric characteristics (size, color) using standard taxonomic keys (Lane and Crosskey. 1993).

### *Isolation of parasites from the outer surface of cockroaches*

The investigation and detection of parasites and their various stages were carried out according to the procedure described by Bala et al. (2012).

### *Cockroaches' preparation*

After the identification, each cockroach was placed in a sterile test tube containing 2 mL of sterile physiological solution (0.9% NaCl). The solution was vigorously mixed for 2 min to remove the ectoparasites fixed on the outer surface of the cockroaches. Then, the solution tubes were centrifuged at 3000 rpm for 5 min. The pellets were recovered for microscopic examination.

### *Smear preparation*

For microscopic identification, several fresh and colored smears were prepared:

**Fresh smear:** A drop of pellet from each cockroach's solution tube was placed on a clean slide with a sterile Pasteur pipette and covered with a cover glass.

**Lugol staining:** This stain was used to observe cysts and oocysts (England Public Health, 2019). First, a drop of physiological water was placed at one end of a clean slide, and at the other end of the slide, a drop of Lugol 1% was placed. Then, using a sterile Pasteur pipette, a drop of the specimen was added to the mixture and mixed until the suspension became homogeneous. After that, the suspension was delicately covered with a cover glass to avoid the production of air bubbles.

**Ziehl-Neelsen staining:** To observe coccidial parasites, a modified Ziehl-Neelsen staining was used according to Henriksen and Pohlenz's (1981) method. A drop of the specimen was placed on the slide and was air-dried. Then, the slide was submerged with Fuchsin, heated slightly, and left to cool for 5 min before distilled water rinsing. Thereafter, the slide was discolored by alcohol 3% for 30 seconds and rinsed with distilled water. After that, the slide was colored with green malachite for about 1 min then rinsed with distilled water and left to dry.

### *Microscopic observation*

The previously prepared smears were examined gradually under a light microscope (Zeiss Primo star) using magnifications from 10× to 100×.

### *Parasite identification*

The morphological characteristics were used for each sample to identify parasites based on the key identification as described by Soulsby (1974) according to shape, size, and number of cysts or oocysts nucleus.

## **Results and Discussion**

### *Distribution and identification of cockroaches*

This study shows that 66% of collection sites in Ain Témouchent city contain cockroaches, but at a lower number during the period of February-March for the current year due to the low temperature recorded for these months ranging from 17°C to 27°C ([www.accuweather.com](http://www.accuweather.com)). Results demonstrated a heterogeneous distribution of cockroaches varying from one site to another and even absent in some (Table 1). This variability is due to several reasons: (1) sampling site temperature, (2) regular sanitary treatment against insects, (3) rearrangement of restaurants and sale points, (4) continuous improvement of hygienic conditions...etc. Indeed, most of these insects have been hunted at bakeries and kitchens compared to other locations. This can be explained by the hot and humid microclimates in these places due to the use of ovens. However, in the university restaurants of the girls' and boys' residences, no cockroaches have been collected due to the regular use of insecticides. Moreover, at this level of study, it is difficult to judge the abundance of cockroaches due to the lack of information on the number of ubiquitous individuals and their spread.

Each collected cockroach has been identified and reported in Table 2. Accordingly, four types of cockroaches have been collected and identified belonging to the American, German, Nymph, and Striped species. Moreover, the results of this study show that male cockroaches were more collected compared to females for the three species of American, Nymph and German cockroaches. Otherwise, females were mostly collected for Striped cockroaches. The distribution of German was observed only in Fast-food premises and kitchens. This distribution may be depending on the feeding habits of the cockroaches.

### *Parasites species identification and their prevalence*

Results of the prevalence and identification of each parasite stage carried by cockroaches' bodies collected from different points in the Ain Témouchent city are presented in Table 2.

Our finding revealed a low presence of protozoa (50%) compared to metazoan cysts/oocytes. However, these latter are ubiquitously found on all examined cockroaches' bodies, firstly because they have a

strong ability to bind to the surface of these insects, and secondly, they are resistant to environmental conditions contrary to protozoa. These results are similar to those reported by Afzan (2018) and Adenusi et al. (2018). It has been stated that a higher prevalence of helminth may be due to poor waste disposal practices in their study area.

**Table 1.** Distribution of cockroaches collected from different places in the city of Ain Témouchent (Algeria).

Collection site	Number of site	Cockroaches number
Fastfood	1	05
Bakeries	1	13
Super markets	1	03
Kitchens	1	07
Collective catering (University city girls)	1	0
Collective catering (University city boys)	1	0
Total	6	28

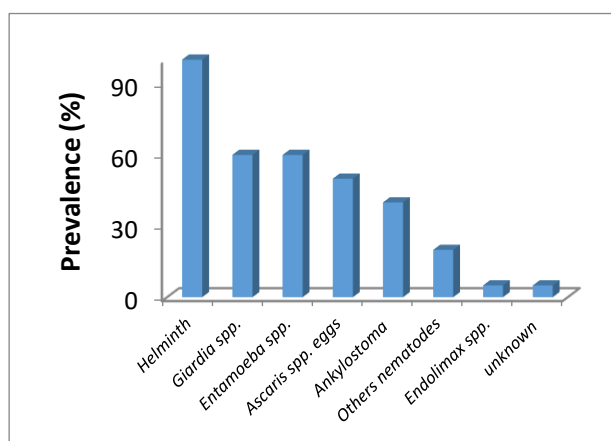


**Figure 1.** Cockroach trap used for cockroach's collection.

Regarding the identification results, they are shown in Figure 2. Accordingly, 100% of cockroaches tested positive for the presence of different eggs of the helminth (100%) followed by *Giardia* spp. and *Entamoeba* spp. each one at 60%, *Ascaris* spp. eggs (50%), *Ancylostoma* (40%), other nematodes (20%), *Endolimax* spp. (5%), and unknown (5%). The variability in the results is consistent with those previously reported by Kinfu and Erko (2010) and Afzan et al. (2018). Furthermore, this parasites' list is not exhaustive, and probably other types could be detected. In fact, Adenusi et al. (2018) demonstrated the occurrence of *Enterobius vermicularis* (17.2%), *Trichuris trichuria* (55.8%), *Strongyloides stercoralis* (11.7%), *Cryptosporidium* sp. (13.8%), *Hymenolepis nana* (11.6%) and *Taenia/Echinococcus* spp. (10.5%). On the other hand, other studies reported the presence of *Nyctotherus ovalis* (65.3%), *Hammersmidtella diesingi* (33.3%), *Thelastoma bulhoe* (51.4%) and *Gordius robustus* (Al-Mayali et al., 2010).

In addition, it was found that the parasites' species did not depend on the collection site of cockroaches nor on their species and sex. The parasite load is intimately dependent on the degree of hygiene and sanitation of the habitat level of these cockroaches.

As shown by Benouis et al. (2013), parasites were assigned to 20% of the tested population (1042 people). They were as follows: *Blastocystis hominis* (47.17%), *Entamoeba coli* (18.95%), *Giardia intestinalis* (15.32%), *Endolimax nana* (5.24%), *Entamoeba histolytica* (4.83%), *Pseudolimax butschlii* (4.43%), *Enterobius vermicularis* (2.82%), *Cryptosporidium* sp. (0.4%), *Ascaris lumbricoides* (0.4%) and *Taenia saginata* (0.4%). Among these mentioned parasites, four genera (*Ascaris*, *Endolimax*, *Entamoeba*, *Giardia*) were identified in this study (Figure 2).



**Figure 2.** Distribution of parasites present on examined cockroaches' bodies.

**Table 2.** Distribution of different parasite stages according to cockroach type and sex.

Collection site	Genus	Number/sex	Cockroaches load a parasites (%)	Presence (%)	
				Protozoa	Helminthes
Fast- food	German	3 males	100	60	100
		0 females	-		
		0 male	-		
Bakeries	Striped	2 females	100	50	100
		5 males	100		
	Nymph American	0 females	-		
		2 males	100		
		6 females	100		
Super markets	Striped	2 males	100	0	100
		0 females	-		
	American	1 male	100		
		0 females	-		
Kitchens	Nymph	7 males	100	50	100
		0 females	-		
		0 females	-		
Total	28	28	100	50	100

## Conclusion

From the research that has been carried out, it is possible to conclude that cockroaches act as potential mechanical vectors for a range of enteric parasites such as helminths eggs and other protozoa (e.g. *Giardia* spp. and *Entamoeba* spp.) which might be involved in gastroenteric problems. Therefore, the results of this study hoped to raise awareness among food premises owners to fight the cockroach infestation by increasing the level of sanitation in the surrounding area. On the other hand, to our knowledge, this work is the first report on cockroaches as foodborne vectors of parasites, especially in food locations. Some studies have been previously carried out in Algeria on cockroaches; however, they were undertaken from a zootechnical point of view.

In this context, this paper could thus open an interesting scientific discussion about the foodborne insects carried to humans and encouraged more research in this field, since studies have not yet been extensively performed in this research axis.

Finally, it is recommended to (1) respect the hygienic conditions in the food distribution rooms, (2) establish regular protocols for cleaning and disinfection, and (3) protect and keep food safe away from cockroaches to prevent food intoxication related to parasite contamination.

As perspectives, future work will involve more specific coloration protocols and analyses to better identify these enteric parasite agents.

## Author's Contributions

As for research topic was choose by ZIANE Mohammed and AFZAN MAT Yusof. ALI-BACHA Kawther and ABED-BRIXI Soumia contributed in cockroaches' harvest and laboratory assay with help of Dr ZIANE Mohammed. As for writing and English correction were ensuring by ZIANE Mohammed, BOUAMRA Mohammed, BERROUKECHE Farid, BEN BRAÏEK Olfa, AFZAN MAT Yusof.

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