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Filth Flies As Carriers of Intestinal Parasites And Fungi in a Tertiary Institution in Ghana

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ABSTRACT

Introduction: Filth flies can mechanically transmit pathogens, some of which can cause significant diseases in humans and animals. **Methods:** This study aimed at isolating and identifying pathogenic fungi and intestinal parasites from flies sampled with sweep nets and fly traps from different dumpsites in a tertiary institution. Dumpsites at the various halls of residence were designated A, B, C, and D. **Results:** Pathogens were mostly isolated from the body surfaces of the flies. The 605 captured filth flies belonged to the two families, Calliphoridae (77.69%) and Muscidae (22.31%). Three genera of fungi were identified, with *Aspergillus* (91.69%) as the most predominant, followed by *Penicillium* (5.23%) and *Rhizopus* (3.08%). The intestinal parasites identified from only the external body surfaces of the sampled flies were protozoans, *Cryptosporidium parvum (95%)*, and *Entamoeba histolytica/dispar (*0.83%), as well as helminths including *Ascaris lumbricoides (*3.34%) and *Strongyloides stercoralis* (0.83%). The percentage occurrence of fungi (57.54%) and intestinal parasites (85.83%) isolated from flies caught at dumpsites D and B were higher than those isolated from flies caught in any of the other study sites. **Conclusion:** This study confirms filthy flies as mechanical transmitters of pathogens and emphasizes adopting control measures to prevent the possible spread of infections within the university community.

INTRODUCTION

True flies, which typically have a pair of wings and halteres, play an ecological role by impacting both humans and the environment [1]. These flies can serve as pollinators, decomposers, predators, and prey and can be involved in spreading disease-causing pathogens. Many species grouped as true flies have been classified as filth flies due to their location and source [1]. These filthy flies have been found to dwell in unsanitary areas compared to locations with proper sanitary conditions [2]. The flies can be a nuisance and are frequently observed in and around human dwellings [3]. Worldwide, filthy flies have been found to play an essential role in the spread of microbes due to their random movement, breeding habit, and mode of feeding [4]. Reports indicate that diverse pathogenic microorganisms, including bacteria, parasites, and fungi [5] can be carried and transferred by flies through their body parts' secretions [6]. These pathogenic infections result in diseases such as cholera, diarrhea, typhoid, and anthrax in men and animals [7].

Although refuse dumps are widely used, they serve as a source of disease-causing pathogens, spread foul scents, and may expose nearby inhabitants to environmental health complications [8]. These refuse dumps also provide food for flies as they attract high densities of human waste and garbage [9]. As non-biological carriers, the role of pathogen transmission to humans and animals by flies cannot be overlooked [10–12]. It has been observed that, on campus, the environmental and hygienic status varies, especially among the various halls of residence. The closeness of the refuse dumps to the halls can facilitate the activities of flies and consequently increase pathogen transmission. Thus, this study focused on the filth flies present at dumpsites in a tertiary institution to identify the intestinal parasites and fungi they carry to determine the potential risk of transmission to academic members.

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MATERIAL AND METHODS

Study area. Samples were collected from four refuse dumpsites at various residence halls labeled A, B, C, and D within the tertiary institution.

Collection and identification of filth flies from sampling sites. Filth flies were collected using hand-made bottle traps and sweep nets. The traps were made from disposable plastic water bottles, as indicated by El-Sherbini [13], and baited with 2-day-old tainted cow meat, mango, and stinking fish mixed with chloroform to knock them down. The designed traps with bait were glued to the refuse container at the dumpsite for 4 hours before collection. Sweep nets were also used to collect flies at the various refuse dumps. The sampled flies from each site were transferred into sterile plastic containers labeled with the date, time of collection, and location of the site and sent to the Microbiology Laboratory of the Department of Theoretical and Applied Biology for further analysis. The filth flies were identified morphologically with a magnifying glass using taxonomic keys [14]. 5-10 flies were pooled based on genera and respective sampling sites. Each pool was stored in a test tube containing 10 ml of peptone water and shaken thoroughly for 2 min to dislodge microbes from their bodies.

Fungal isolation and identification from collected fly samples. Nine ml of sterile peptone water was added to 1 ml of the solution (15 gr/1000 ml distilled water), and the suspension was serially diluted and down to 10^{-5}. From each appropriately diluted suspension tube, 1ml was pipetted into a sterilized petri dish before adding potato dextrose agar (PDA) at about 35 ºC containing chloramphenicol to inhibit bacterial growth. The agar was allowed to solidify at room temperature, and the inoculated plates were incubated at 37 ºC for 4 days and checked for fungal colonies. The total number of colonies retrieved from the flies' external bodies was obtained by counting the colony-forming units per 1ml [15].

The colonies were counted, then distinct colonies were randomly selected and sub-cultured on PDA using a sterile loop and incubated at 37 ºC for 2 days in an inverted position to obtain pure culture and reactivate their macro morphological features for identification of various genera.

Fungal colonies were stained with iodine glycerol to observe microscopic morphological features that would assist in their identification. The isolated stained fungal colonies were mounted on the microscope slides and identified based on morphological details described by others [16] and confirmed by an expert from the Department of Theoretical and Applied Biology.

Detection of intestinal parasites from the external body surface of filth flies. The remnant of the suspension was centrifuged at 2000 RPM for 5 min, and the supernatant was discarded. The sediment was examined with a light microscope under 10 x and 40 x objectives after adding a drop of saline or iodine to detect parasite larvae, eggs, cysts, and oocytes [17].

Furthermore, as described previously [18], the formalin-acetone concentration technique was also employed in detecting parasites. Each sediment was mixed thoroughly in 7ml of 10% formol water in a conical centrifuge tube, centrifuged at 3000 RPM for 5 min, and the supernatant decanted. Then 4ml of acetone was added and mixed before centrifuging at 2000 RPM for 5 min. The top three layers were decanted, and the sediment was mixed with the small amount of fluid that remained using a pipette. The resulting sediment was then examined microscopically using the 10 x and 40 x objective lenses after adding a drop of saline or Lugol's iodine on a labeled slide.

The modified Ziehl-Neelsen (mZN) staining was further used to identify some protozoan parasites [19]. A drop of each sediment was placed on a glass slide and air-dried. Methanol was added for 3 min and stained with unheated carbol-fuchsin for 15 min. After staining, the preparation was rinsed with running tap water, and acid alcohol was added to decolorize it. This was further rinsed with water after 15 sec and counterstained for 30 sec with methylene blue. After rinsing and allowing the slide to dry, it was examined using 40 x and 100 x objectives.

Identification of pathogens from the gut content of flies. Flies were placed in conical tubes containing 70% alcohol after external washing, rinsed with distilled water, and wholly air-dried to prevent cross-contamination. Each gut was dissected by forceps, and the gut content was put into 10 ml peptone water in a test tube and processed as described above to identify fungi and intestinal parasites. Slides were prepared in triplicates for each sample to increase the chance of parasite detection.

The data obtained were analyzed using the chi-square test at a significant $P<0.05$.

RESULTS

Six hundred-five filthy flies were captured from four dumpsites in the tertiary institution. The sampled flies were abundant in A, B, D, and C dump sites. The results showed that blowflies (77.69%) were the most predominant flies caught in all four sites during the sampling period (Table 1). A significant difference was observed between the two families of flies collected from the collection sites ($P<0.05$).

Fungal identification from sampled flies. A total of 325 fungal colonies were isolated from the sampled flies. From the colonies, three fungal genera were isolated. *Aspergillus* (91.69%) was the highest-occurring fungal genera, followed by *Penicillium* (5.23%) and *Rhizopus* (3.08%) as the least isolated (Fig. 1). Sampled blowflies (60.92%) had the highest fungi as compared to houseflies (39.08%). Fungi isolated from the flies sampled at the various dumpsites were determined as follows; D (57.54%), A (19.39%), B (12%), and C (11.08%).

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Table 1. The proportion of flies at the various dumpsites

Location of dumpsites	Houseflies (%)	Blowflies (%)	Total
A	27 (11.25)	213 (88.75)	240
B	21 (16.28)	108 (83.72)	129
C	34 (30.36)	78 (69.64)	112
D	53 (42.74)	71 (57.26)	124

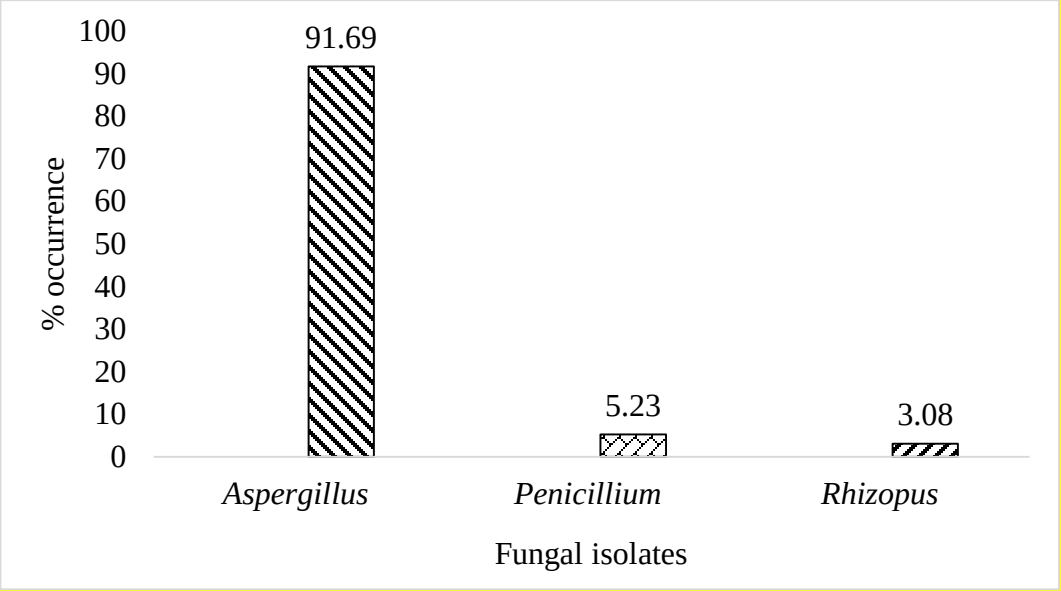

Fig. 1. Genera of fungi isolated from sampled flies

It was observed that most of the fungi identified were from the external body surface of the flies as compared to their gut contents. However, in the case of dumpsite C, fungi were only isolated from the external body surface of flies and none from the gut content (Fig. 2).

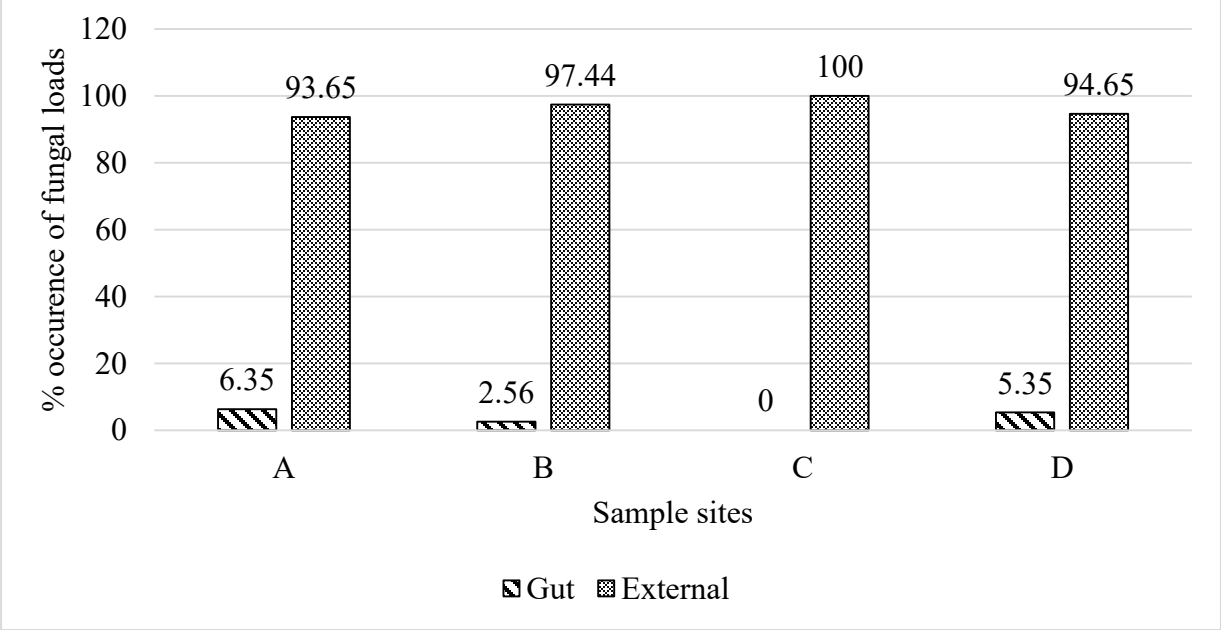

Fig. 2. Frequency of fungal isolates from the flies' gut and external body surfaces.

Parasite identification from sampled flies. Both intestinal helminth and protozoan parasites of humans were detected on the external surfaces of the flies. However, none of these parasites was isolated from their gut content. Parasites were primarily isolated from blowflies (89.17%) compared to houseflies (10.83%). *Cryptosporidium parvum (95%)* was the most predominant, followed by *Ascaris lumbricoides (*3.34%),

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*Entamoeba histolytica/dispar (*0.83%) and *Strongyloides stercoralis* (0.83%) as the least occurring parasite (Fig. 3). The parasites identified were found to be highest in flies sampled from dumpsite B, followed by C, A, and D dump sites. There was a significant difference in parasites from flies collected from the various dumpsites ($P < 0.05$) (Fig. 4).

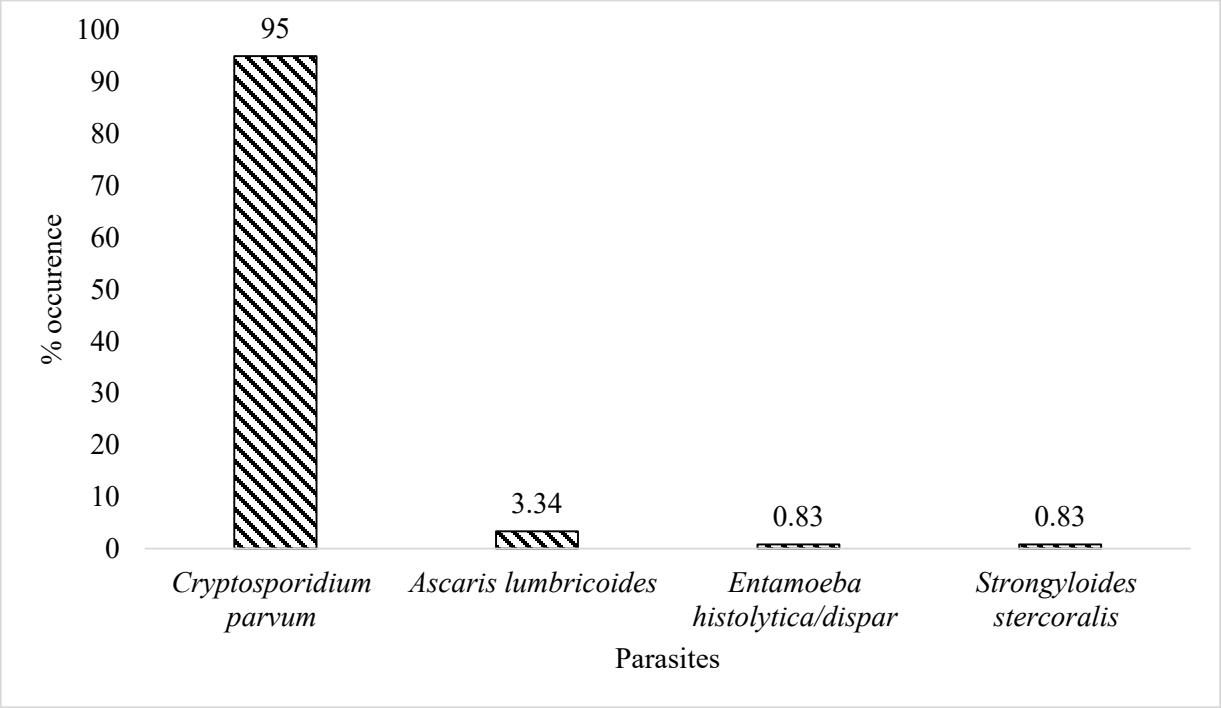

Fig. 3. The distribution of parasites identified from sampled flies.

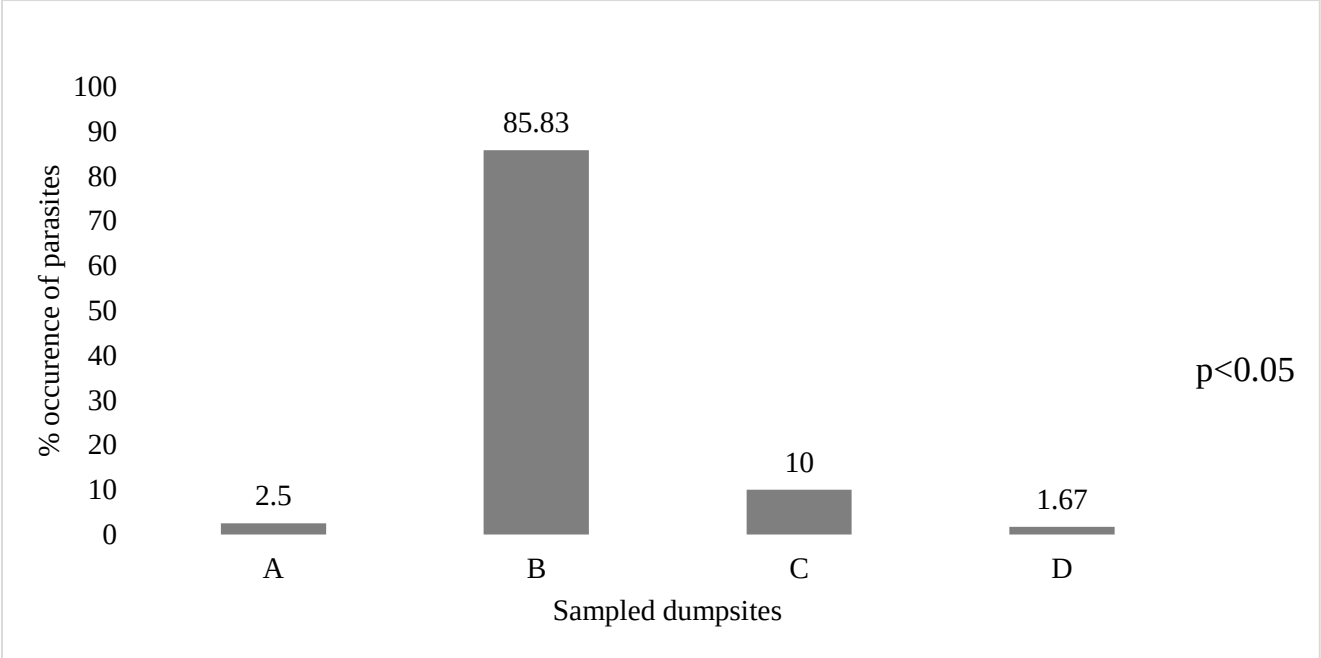

Fig. 4. The occurrence of parasites across the sampling sites.

DISCUSSION

In this study, filth flies were found to carry three genera of fungi, including *Aspergillus*, *Penicillium*, and *Rhizopus*. Furthermore, from the external body surface of the flies, parasites such as *Cryptosporidium parvum*, *Entamoeba histolytica/dispar*, *Ascaris lumbricoides*, and *Strongyloides stercoralis* were identified.

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Various dumpsites have been located at strategic sites within the tertiary institution to maintain proper hygienic and sanitary conditions. However, these dumpsites can be a source of infection when insects that can facilitate pathogen transmission are present. Filth flies are among a group of insects capable of transmitting disease-causing pathogens from dumpsites to individuals nearby. These flies can travel long distances [20] in search of food, usually waste products and human foods [21], increasing the likelihood of pathogen transmission to humans [22]. The usual movement of these flies from the dumpsites to kitchens, marketplaces, halls, and sometimes students' rooms further increases the risk of pathogen transmission.

The abundance of filth flies in the present study demonstrates that the tertiary institution can support various flies, prompting high species diversity in the areas. More flies were captured in dumpsite A than in the other sites. This may be due to favorable breeding sites and climatic conditions that increase flies' rate of development [23]. In this study, two families of Diptera (Muscidae and Calliphoridae) were identified, with Calliphoridae (blowflies) being the predominant family in all the sites. Although the collection sites used for this study are known to be suitable for Muscidae [2], more Calliphoridae were collected, probably due to the use of 2-day-old tainted meat and rotten mango, a more suitable bait for blowflies [24,25].

Detecting pathogenic fungi from the sampled filth flies indicates their potential role in transmitting pathogens [26,27]. The fungi were primarily isolated from the flies' external body surface, which could be due to their feeding habits. During feeding, pathogens can stick to their spongy mouthparts, wings, and other body parts. With their random movement from waste products and other potentially infected places to food and drinking water of humans and animals, the risk of human infection increases [28]. Isolating pathogens from the external body surface of these flies also suggests their role as mechanical carriers. *Aspergillus* was the predominant fungi identified from the sampled flies. The prevalence of *Aspergillus* in our study could be due to their ability to use various organic substrates and efficiently adapt to various environmental conditions [29]. *Aspergillus* has been documented as a significant cause of severe fungal diseases [30]. In immunocompromised individuals, invasive fungal diseases such as pulmonary aspergillosis can occur due to *Aspergillus fumigatus* infection [31]. These fungi grow in an individual's lungs, causing tissue damage and sometimes coughing blood [32]. Identifying the specific *Aspergillus* species in the tertiary institution will be necessary to determine the risk of infections. The role of filth flies in spreading these pathogens also needs to be fully established, and preventive measures should be adopted to avoid infections.

There are reports of the isolation and transmission of helminths and protozoan parasites such as *Toxoplasma gondii*, *G. lamblia*, and *C. parvum* by filth flies [4,33,34]. In Nigeria, houseflies captured from markets and residential areas were found to carry *Toxocara* ova, *S. stercoralis* larvae, and *A. lumbricoides* ova [35]. More recently, a study in Sudan suggested houseflies were involved in the mechanical transmission of intestinal parasites prompting a need for improved hygiene and sanitation [5]. In this study, four different intestinal parasites which can infect humans were isolated from the external body surface of the sampled flies, with none from the gut contents. The most prevalent parasite was *Cryptosporidium parvum*, a pathogen known to cause diarrhea in humans, with transmission occurring through the fecal-oral route and consuming contaminated food and water [36]. About 30% to 50% of deaths in children worldwide are caused by *Cryptosporidium* infection, the second-highest cause of diarrhea and death in children after rotavirus [37]. In sub-Saharan Africa, ~2.9 million *Cryptosporidium* infections have been reported in children less than 24 months old [38]. Detecting high *Cryptosporidium parvum* infections in the sampled flies indicates their essential role in parasite transmission on campus and a need to improve sanitary conditions. Although the other identified parasites were low prevalence, they could still threaten individuals.

Filth flies sampled from the various sites are potential transmitters of pathogens that could cause significant infections in individuals within and around the halls of residence. The role of filth flies in pathogen transmission cannot be ignored; hence, control measures need to be employed to prevent the possible spread of infections.

This study identified two families of filth flies from the sampled sites and confirmed them as mechanical carriers. The fungal load on flies' external body parts was higher than the gut content. The intestinal parasites identified were only found on the external body surface of the flies and could be easily transmitted. Filth flies at a tertiary institution are of medical importance; hence, a comprehensive study is needed to ascertain the risk of human infections.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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