Public health importance of non-biting cyclorrhaphan flies

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Summary This study was carried out to determine the role of non-biting cyclorrhaphan flies as carriers of human intestinal parasites at Woreta, northwestern Ethiopia. In total, 6530 flies were collected from four breeding sites and then examined for human intestinal parasites, mainly using the formol–ether concentration method. Fly species identified were Musca domestica (32.9%), Chrysomya rufifacies (32.6%), Musca sorbens (23%), Lucina cuprina (4.7%), Calliphora vicina (2.8%), Chrysomya bezziana (2.3%) and Wohlfahrtia magnifica (1.7%). Intestinal parasites such as Ascaris lumbricoides (36.9%), Trichuris trichiura (38.8%), hookworm (13.0%), Hymenolepis nana (0.6%), Taenia spp. (8.4%), Strongyloides stercoralis (1.7%), Entamoeba histolytica/dispar (48.1%), Entamoeba coli (24.7%), Cryptosporidium spp. (16.7%) and Giardia lamblia (10.4%) were isolated from both external and gut contents of the flies. Trichuris trichiura and A. lumbricoides among the helminths and E. histolytica/dispar and E. coli among the protozoans were the dominant parasites identified. It was observed that more parasites were isolated from gut contents than the external surfaces of the flies examined ($P < 0.001$). Chrysomya rufifacies were found to carry more helminths than M. sorbens and M. domestica. Musca sorbens were the highest carriers of protozoan parasites followed by M. domestica and C. rufifacies. The significance of filth flies as carriers of human intestinal parasites has been highlighted.

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1. Introduction

Non-biting flies are equipped with special sensory cells on their antennae that can detect strong compounds such as ammonia and carbon dioxide emitted from faeces and other decomposing organic materials. The free access of flies to such sites ensures that they are laden with disease-causing organisms on their mouthparts, body hairs and sticky pads of their feet, stomach, faeces and vomit. Although other routes of transmission, such as contaminated water, carriers and food handlers, might be major possibilities, the likelihood of non-biting flies mechanically transmitting these parasites cannot be excluded. Several non-biting cyclorrhaphan flies are involved in the mechanical...
transmission of pathogens in different parts of the world.\(^4\)

Several studies showed that non-biting flies carry different stages of helminths and protozoan parasites. According to Sulaiman et al.,\(^5\) for example, eggs of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (*Necator americanus*) were isolated from *Chrysomya*, *Sarcophaga* and *Musca* fly species collected from refuse dump and peri-domestic sites. Filth flies were also found to carry cysts of *Entamoeba histolytica* and eggs of cestodes and different nematodes.\(^6\) Similarly, Doiz et al.\(^7\) incriminated houseflies as carriers of eggs of *Enterobius vermicularis*, *Toxocara canis*, *Strongyloides stercoralis* and cysts and triphozoites of *Entamoeba coli*, as well as *Giardia* and *Trichomonas* spp.

Most protozoan and helminth parasites are prevalent in urban and rural areas of Ethiopia,\(^8,9\) affecting the health of the community. The housefly (*Musca domestica*), the eye-seeking fly (*Musca sorbens*) and other filth flies have been foraging on and breeding in rubbish and waste matter, playing a great role as mechanical carriers of different helminths and protozoan parasites in Addis Ababa, Ethiopia.\(^3\)

Therefore, this study was carried out to assess the significance of non-biting cyclorrhaphan fly species as carriers of intestinal human parasites in Woreta, northwestern Ethiopia.

### 2. Materials and methods

#### 2.1. Study area

The study was carried out in Woreta, northwestern Ethiopia. Woreta, the capital city of Kemkem District, is located at an altitude of 1950 m a.s.l. near Lake Tana, having an annual precipitation of more than 650 mm per year and an estimated population of 27 563.

In the area, the majority of inhabitants had pit latrines, which were not properly maintained. Open-air defecation was commonly observed among children, a few adults and homeless people, particularly in peripheral areas. Small market areas, slaughtering in open fields and inappropriate disposal of wastes are also common in the area. Flies were collected from randomly selected potential foraging and breeding sites, such as rubbish heaps, open defecating grounds, open-air small markets and butcheries, close to human dwellings.

#### 2.2. Fly collection and identification

Flies were collected monthly for 9 months during the dry season (October 2006—June 2007) from all foraging and breeding sites by investigators and field assistants using a sweeping net for 2 h in the morning (9:00 to 11:00 h) when most flies were active. Trapped flies were placed in small groups in plastic bags and test tubes, labelled and transported to the laboratory in a cooler box with ice packs and stored in the refrigerator at −4 °C until identification and processing for parasite examination.

Species of collected flies were identified using reference keys given by Crosskey and Lane.\(^10\) All trapped flies were killed by chloroform and then separated into specified species and sex groups, counted and pooled into batches of 10 flies of the same sex and species.

### 2.3. Processing flies for parasite isolation

Each batch of pooled flies was immersed in sterile physiological saline in a separate container and stirred with an applicator stick for 5 min then vortexed for 2—3 min to wash off any parasitic eggs, cysts or larvae from the external body of the flies.

These flies were then transferred to other containers in their batches in sterile conditions using forceps and further processed for examination of gut contents. The whole gut of each washed fly was dissected out with the others from its batch on an autoclaved microscope slide under a stereoscopic microscope using entomological needles and was macerated to liberate the lumen contents.

Suspensions from both the external surface and gut content of each fly batch were processed for microscopic examination using the formol—ether concentration method.\(^11\) Smears prepared from the concentrations were examined under the microscope using 10× and 40× magnifications for helminth ova/larvae and protozoan cysts, respectively. The data obtained were recorded per batch of pooled flies in reference to the external surface and gut content of the flies.

A modified Ziehl–Neelsen staining method\(^12\) was applied for the detection of *Cryptosporidium* spp. Air-dried smears of the external surface and gut content suspensions were fixed with methanol and stained with carbol—fuchsins for 30 min. These were then washed with tap water, decolorized with 1% acid alcohol for 1 min, washed with tap water again, counterstained with 1% Methylene Blue for 1 min, rinsed in tap water and then air-dried. The slides were examined under a microscope to count the number of cysts per batch of pooled flies.

Species and type of intestinal parasite were identified based on the description and techniques stated by WHO.\(^13\)

#### 2.4. Data analysis

Data analysis was carried out using SPSS version 12.0 for Windows. A \(\chi^2\) test was used to compare different independent variables with carriage of the parasites. Degree of parasite contamination was determined calculating their relative frequencies. A \(P\)-value of <0.05 was considered statistically significant.

### 3. Results

#### 3.1. Flies and their breeding sites

In total, 6530 non-biting cyclorrhaphan flies, which pooled into 653 batches, were collected from four potential fly breeding sites: butchery, rubbish, market and defecating ground areas (Figure 1).

The non-biting cyclorrhaphan flies identified in this study area were: *Musca domestica* (32.9%), *C. rufifacies* (32%), *Musca sorbens* (23%), *Lucina cuprina* (4.7%), *Calliphora vicina*
3.2. Parasite species identified

The relative burden of helminths and protozoans in 1306 fly batches (653 external surface and 653 gut contents) are summarized in Supplementary Tables 1 and 2, respectively. Both the external surface and gut contents of the flies were positive for helminth and protozoan parasites.

Identified helminth parasites were *T. trichiura* (38.8%), *A. lumbricoides* (36.9%), hookworm (13.7%), *T. taenia* spp. (8.4%), *S. stercoralis* (1.7%) and *H. nana* (0.6%). The protozoan parasites isolated from different species of flies were *E. histolytica/dispar* (48.1%), *E. coli* (24.7%), *Cryptosporidium* spp. (16.7%) and *G. lamblia* (10.4%).

*Chrysomya rufifacies*, *M. sorbens* and *M. domestica* were found to be more contaminated with one or more intestinal parasites than were other fly species (Table 1): *C. rufifacies* carried more helminths, followed by *M. sorbens* and *M. domestica*; *M. sorbens* carried more protozoan parasites, followed by *M. domestica* and *C. rufifacies*.

Parasite burden in relation to the sex of the flies was investigated, and there was no significant difference (*P* = 0.343) in degree of contamination between male and female flies.

The results of this study showed a highly significant difference (*P* < 0.001) in the extent of contamination between external body surface and gut content of flies: the gut content of the flies was found to contain more parasites than did the external surface of the flies.

4. Discussion

The present study shows that *M. domestica*, *C. rufifacies* and *M. sorbens* are dominant and abundant species, as shown by Getachew et al. Moreover, it demonstrates that each fly species prefers specific breeding sites. The high abundance of *C. rufifacies* in butcheries and defecating grounds may be due to the scavenger feeding habit of the fly and its high preference for liquid faecal matter. According to
Boonchu et al.,\textsuperscript{15} pork viscera is more attractive to metallic flies than to Sarcophagidae and Muscidae flies. The high abundance of *M. domestica* in rubbish dumps is perhaps due to the ability of the fly to feed on drier food substrates. Human faeces are attractive to houseflies in their drier solid state,\textsuperscript{16} and rubbish provides preferred conditions for their breeding.\textsuperscript{17} Eesa and El-Sibae\textsuperscript{18} showed that *M. domestica* were abundant in rubbish dumps, cattle markets and slaughterhouses. *Musca sorbens* was abundant in market areas, and this may be mainly due to the preference of the fly to feed on human secretions\textsuperscript{19} and faecal material in market areas and areas where human defecation is left exposed.\textsuperscript{16}

In this study, female flies were more numerous than males at all breeding sites. This may be due to the females' ability to locate food and favourable oviposition sites. The study indicates that sex does not affect the parasite carriage potential of the flies; supporting the results of studies carried out by Monzon et al.\textsuperscript{20} and Getachew et al.\textsuperscript{1, 1} that both sexes have equal significance in intestinal parasite transmission.

The present work shows that non-biting cyclorrhaphan flies carry *A. lumbricoides*, *T. trichiura*, *S. stercoralis*, *H. nana* and *Taenia* spp., and *E. histolytica*, *E. coli*, *G. lamblia* and *Cryptosporidium* spp. Thus, this indicates that filth flies with access to substrates containing these parasites can contaminate themselves and the food of inhabitants near the sites where flies have been collected. It has been shown that houseflies caught on rubbish dumps in six areas of Ibadan (Nigeria) were found to harbour intestinal parasitic cysts and eggs in their alimentary canal, which were similar to those found in the faeces of the community living in that environment.\textsuperscript{21}

Other studies have shown that non-biting flies carry different stages of helminth and protozoan parasites. For example: flies were found to be contaminated with eggs and larvae of *Ancylostoma caninum*;\textsuperscript{2, 1} Monzon et al.\textsuperscript{20} in the Philippines and deOliveira et al.\textsuperscript{21} in Brazil isolated eggs of *Ascaris* and *Trichuris* from *M. domestica* and *Chrysomya megacephala*; Umeche and Mandah\textsuperscript{22} obtained ova of *A. lumbricoides*, *S. stercoralis*, *Ancylostoma doudenale* and *T. canis* from *M. domestica*; Graczyk et al.\textsuperscript{23} confirmed *M. domestica* as a vector of *Sarcocystis* spp., *Toxoplasma gondii*, *Isospora* spp., *Giardia* spp., *E. coli*, *E. histolytica*, *Endolimax nana*, *Trichomonas* spp., *Hammondia* and *Cryptosporidium parvum*. Refuse and promiscuous landing synanthropic filth flies were also recognized as transport hosts for *Sarcocystis* spp.,\textsuperscript{24} *T. gondii*,\textsuperscript{25} *Isospora* spp. and *Eimeria tenella*.\textsuperscript{26}

*Cryptosporidium* spp. were detected from both the external surface and gut content of flies collected at different sites in Woreta. Therefore, this work demonstrates that adult flies with access to materials containing the oocysts contaminate themselves and carry the parasite. It has been confirmed that filth flies could transport infectious oocysts of *C. parvum* on their external surfaces and in their digestive tracts.\textsuperscript{7, 25, 26}

The gut contents of flies were found to carry more intestinal parasites than the body surface of flies. Experimentally infected *M. domestica* carried larvae of *A. caninum* longer in the gut than on the external surface of the fly.\textsuperscript{25} Ingestion and defecation of parasites is one of the most important potential routes of contamination, as the infectious agent is protected while it is in the gut of the fly and maintained for a certain periods of time.\textsuperscript{10} This may increase the importance of flies as a source of human food contamination, with the pathogens they carry when landing on it.

The study results have revealed that *M. sorbens*, *M. domestica* and *C. rufifacies* carry more intestinal parasites than other flies identified in this area. These flies forage predominantly on human faeces,\textsuperscript{27, 28} and if infected individuals defecate in exposed situations, the flies might get chance to forage on positive faeces. The authors observed overcrowded conditions, lack of proper sanitation and health education, and human faeces were commonly seen scattered on the ground in the area. Dipeolu\textsuperscript{21} demonstrated that houseflies in low-income areas carried more parasitic cysts and eggs than those in high-income residential areas. Moreover, filth flies associated with cattle barns and municipal landfills have shown that more *C. parvum* and *G. lamblia* were carried by flies from cattle barns.\textsuperscript{29}

The external body parts of flies (antennae, mouthparts and legs), and their gut contents, vomit and faeces carry pathogens that cause diseases.\textsuperscript{25} The present study demonstrates that non-biting cyclorrhaphan flies carry eggs, larvae and cysts of intestinal parasites naturally acquired from unhygienic sources. Thus they can be involved in the deposition of these parasites on visited surfaces. Mechanical transfer of intestinal parasites by flies could be achieved through defecation, regurgitation or mechanical dislodgement.\textsuperscript{4, 10}

Parasite eggs and cysts adhering to the surface of the fly are quickly removed, because the fly autosterilizes itself immediately as it withdraws from the surface on which it has been feeding.\textsuperscript{3} This creates favourable conditions for contaminating human food with the pathogens carried by flies when they land on it, and transmission of the parasites is readily ensured if the food is inadequately cooked and consumed. Therefore, it is crucial to establish immediate control mechanisms for these flies, through mass education to improve the standards of sanitation in the area.

**Authors’ contributions:** TF and NW conceived and designed the project and study methodology, carried out the data collection and analysis, drafted and revised the manuscript, and read and approved the final version. TF is guarantor of the paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2008.08.010.

References