CLINICAL MICROBIOLOGY REVIEWS, Jan. 2005, p. 128–132 Vol. 18, No. 1
0893-8512/05/$08.00+0 doi:10.1128/CMR.18.1.128–132.2005

Mechanical Transmission of Human Protozoan Parasites by Insects

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INTRODUCTION

The feeding mechanisms and filthy breeding habits of synanthropic insects such as flies, cockroaches, and coprophagic beetles make them efficient vectors and transmitters of human enteric protozoan parasites (18). In particular, domestic filth flies (some species in the families Sarcophagidae [flesh flies], Muscidae [house flies and latrine flies], and Calliphoridae [blow flies and bottle flies]) have evolved to live in close association with humans (synanthropic flies) as annoying pestiferous scavengers (5, 15). Filth flies breed in animal manure and human excrement, i.e., coprophagic flies, and garbage, animal bedding, and decaying organic matter, i.e., saprophagous flies (5, 15).

Synanthropic insects are abundant in urban and rural areas where unsanitary conditions prevail and are usually scarce when sanitary conditions are enforced (14). Outbreaks and cases of food-borne diarrheal diseases in urban and rural areas are closely related to the seasonal increase in abundance of filth flies, and enforced fly control is closely related to reductions in the number of cases of such diseases (14). Over 50 species of synanthropic flies have been reported to be associated with unsanitary conditions and involved in dissemination of human enteropathogens in the environment. Of these, 21 species of filth flies have been listed by regulatory agencies concerned with sanitation and public health as causative agents of gastrointestinal diseases in people based on synanthropy, endophily (the preference of insects to enter buildings), communicative behavior, and strong attraction to filth and human food (22). These species include *Hermetia illuscens*, *Megaselia insulana*, *Eristalis tenax*, *Piophila casei*, *Fannia canicularis*, *Musca domestica*, *Muscina stabulans*, *Stomoxys calcitrans*, *Calliphora viscina*, *Calliphora vomitoria*, *Chrysomya putoria*, *Cynomyopsis cadaverina*, *Cochliomyia macellaria*, *Phaenicia cuprina*, *Phaenicia sericata*, *Phormia regina*, *Sarcophaga crassipalpis*, *Sarcophaga carneria*, and *Sarcophaga haemorrhoidalis*.

Cockroaches frequently feed on human feces, and therefore they can disseminate cysts of enteric protozoans in the environment (7, 16, 18, 24). Cockroaches have been epidemiologically involved in toxoplasmosis, giardiasis, sarcocystosis, and intestinal amoebiasis, (16, 18, 24, 27, 30).

TRANSMISSION MECHANISMS

Mechanical Transmission

Transmission of human protozoan parasites by synanthropic insects is predominantly mechanical. In adult flies it occurs via mechanical dislodgement from the exoskeleton, fecal deposition, and regurgitation, i.e., vomit (14). Flies can carry human pathogens on the sponging mouthparts, on body and leg hairs (i.e., setae), or on the sticky pads of the feet (i.e., tarsi). Fine hairs on the pads of a fly's feet are coated with a sticky substance which improves the fly's ability to adhere while resting or climbing on nonhorizontal surfaces. This substance also enhances the adhesion of particles, i.e., viruses, bacteria, and protozoan cysts, to fly legs, which then can be directly transported to the next visited surface and dislodged. Small particles readily adhere to a fly's exterior surfaces due to their electrostatic charge (14). Fly exoskeletons have certain electrostatic charges, and any particle with a different charge or a neutral charge will adhere to the fly surface.

The effectiveness of feces in enhancing the transmission of infectious agents by house flies is much greater than that of any other substrate or medium (9). This is a result of fecal viscosity, which increases the efficiency of tarsi and bristles in trapping particles suspended in the feces (9). Protozoan parasites can pass through the fly gastrointestinal tract without alteration of their infectivity and can be subsequently deposited on visited surfaces in "fecal spots" (9). Alternatively, the parasites present in fly alimentary tracts can be regurgitated, i.e., vomit

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drops, on a surface perceived by a fly as a meal (regurgitation always precedes feeding). Frequent meals on contaminated substrates together with alternating regurgitation and ingestion cause progressive accumulation of human pathogens in the fly alimentary system (14). Human pathogens can also be transmitted as airborne particles for short distances from fly-electrocuting traps, as electrocuting traps do not alter the infectivity of pathogens transported by flies (22). There are size limitations regarding the transmittal from the contaminated sites. Bigger particles such as helminth eggs are transported by flies on their external surfaces, i.e., exoskeletons, while small cystic stages of human-infectious intestinal protozoa can be ingested as well as transported on the exoskeleton.

Transtadial Transmission

It is unlikely that human protozoan parasites are transmitted transtadially through the larval and pupal stages of synanthropic insects to the adult stages of species whose larval stages, e.g., maggots, breed in contaminated substrates (9, 15). Transtadial transmission is the passage of an infectious agent from the egg to the adult insect stage. This is because the pupation process involves intense reorganization of the digestive tract tissue, resulting in the development of a new digestive system and production of the meconium (9, 15), accumulated intestinal wastes that remain behind in the puparium. This has been confirmed in laboratory experiments. *Toxoplasma gondii* oocysts were isolated from larvae and pupae of house flies reared in infectious cat feces but not from newly emerged adult house flies (31). Also, *Cryptosporidium parvum* oocysts were present in the alimentary canals of maggots reared on a contaminated substrate, inside the pupae, and in the meconium but not in or on adult flies (9). However, even if flies are sterile when they emerge from the pupa, they will acquire pathogens rapidly from contaminated substrates in which they develop by direct contact (9).

COCKROACHES AS VECTORS OF HUMAN ENTERIC PROTOZOA

Cockroaches frequently feed on human feces, and therefore they can disseminate cysts of enteric protozoans in the environment if such feces are contaminated (7, 16, 24, 25, 30, 31, 32). The important epidemiological role of cockroaches in transmission of intestinal *Entamoeba histolytica*-associated amoebiasis was demonstrated in 1971 (25) and in giardiasis in 1981 (16). German cockroaches (*Blatella germanica*) spread infectious *Entamoeba histolytica*/*Entamoeba dispar* and *Giardia lamblia* cysts at their visited sites (16, 30). A field survey carried out in 11 primary schools in an urban area of South Taiwan showed that over 25% of American cockroaches (*Periplaneta americana*) and 10% of *Blatella germanica* were positive for infectious *Entamoeba histolytica*/*Entamoeba dispar* cysts on the cuticle and in their digestive tracts (24). Laboratory experiments in which *Blatella germanica* and *Periplaneta americana* were exposed to *Toxoplasma gondii* and *Sarcocystis* oocysts demonstrated that these oocysts were infectious (as per mouse bioassay) while transported by cockroaches (27). *Sarcocystis* oocysts remained infectious on *Periplaneta americana* for at least 20 days after initial exposure to contaminated feces and for 5 days on *Blatella germanica* (27). However *Toxoplasma gondii* oocysts were infectious up to 10 days on *Periplaneta americana* and only immediately after the exposure on *Blatella germanica* (27).

DUNG BEETLES AS HOSTS OF HUMAN PROTOZOAN PARASITES

Dung beetles (*Onthophagus* spp.) exposed to cat feces containing *Toxoplasma gondii* released oocysts for 3 consecutive days (26). Furthermore, the oocysts present on the body surface of these beetles remained infectious for several months (26). Also, in the field survey, *Isospora* oocysts were recovered from dung beetles collected from fecal matter (26). Testing of infectivity of *Cryptosporidium parvum* oocysts ingested by dung beetles, *Anoplotrupes stercorosum*, *Aphodius rufus*, and *Onthophagus fracticornis*, demonstrated that the oocysts passed unaltered through the mouthparts and gastrointestinal tracts of these beetles (20). Thus, coprophagic beetles can be involved in the epidemiology of cryptosporidiosis by transmission of infective oocysts of *Cryptosporidium* (20).

FILTH FLIES AND HUMAN FOOD-BORNE PROTOZOAN DISEASES

There are 108 families of Diptera containing over 120,000 species, of which approximately 350 fly species in 29 families have been potentially associated with the spread of food-borne diseases (22). Over 50 species of synanthropic flies have been reported to be associated with unsanitary conditions and involved in dissemination of human pathogens in the environment (22). Of these, 21 species of filth flies have been involved in transmission of human gastrointestinal diseases (22) (see the Introduction). Flies from these families breed in animal manure and human excrement, garbage, animal bedding, and decaying organic matter (5). The ecology and biology of breeding, indiscriminate traveling between filth and human food, and feeding habits of nonbiting flies are similar (5).

Promiscuous-landing synanthropic flies are recognized vectors for a variety of protozoan parasites of public health importance (15, 22). Synanthropic flies, particularly the common house fly (*Musca domestica*), have been identified as vectors of protozoan parasites such as *Sarcocystis* spp. (19), *Toxoplasma gondii* (31), *Isospora* spp. (17), *Giardia* spp. (3, 13, 16, 29), *Entamoeba coli* (17), *Entamoeba histolytica*/*Entamoeba dispar* (17), *Endolimax nana* (17), *Pentatrichomonas hominis* (17), *Hammondia* spp. (17), and *Cryptosporidium parvum* (2, 10, 11, 13, 29). Despite intensive efforts to test synanthropic flies for *Cyclospora* spp., this pathogen has not yet been recovered from flies (23). *Toxoplasma gondii* can be mechanically transmitted by *Musca domestica* and *Chrysomya megacephala* (31). The flies were able to contaminate milk with *Toxoplasma gondii* oocysts 48 h after last contact with infectious feces, and infectious oocysts were isolated from flies up to 72 h after contact with contaminated fecal material (31).

The biology and ecology of *Musca domestica* ensure efficient transmission of human protozoan parasites. Adult female flies can live 15 to 25 days (5) and lay five to six batches of 75 to 150 eggs (5, 15). In temperate climates there can be 10 to 12 fly

generations in the summer (5, 15). Winter usually ends the breeding cycle; however, indoors, i.e., barns and houses, flies can develop several generations during the winter months (5, 15). Cattle barns, for example, are sites where house flies can breed throughout the winter (15). Individual flies can travel as far as 20 miles (21); however, the vast majority, over 88%, do not travel more than 2 miles (5), and their movement is generally oriented toward unsanitary sites (15).

Current hazard analysis and critical control point and good manufacturing practice regulations require the exclusion of flies from sites where food is produced or stored (22). However, a reasonable approach to excluding flies from such areas requires differentiation between fly species of public health importance and other species which are not involved in transmission of pathogens (22).

Filth Flies and *Cryptosporidium* spp.

The involvement of nonbiting flies in mechanical transmission of *Cryptosporidium parvum* has been discovered recently (2, 9–11, 13, 29). *Cryptosporidium parvum* is an anthropozoonotic protozoan parasite which significantly contributes to the mortality of immunocompromised or immunosuppressed persons (8). Diarrheal disease is initiated by a microscopic stage of this parasite, the oocyst. The pathogen also debilitates healthy, i.e., immunocompetent, individuals, in which the disease can be caused by as few as 10 oocysts (8). It is believed that in those with impaired immune systems, a single oocyst can initiate infection (8). *Cryptosporidium parvum* is particularly prevalent in preweaned cattle, and cattle manure is a source of the oocysts (8).

Animal manure is a recognized source of anthropozoonotic parasites such as *Cryptosporidium* spp. and is also a favorite breeding place, food source, and landing site of filth flies (4, 14, 15). Coprophagic and saprophagous flies are proficient vectors of *Cryptosporidium* spp. because of their breeding and feeding ecology; they also act as an epidemiologic link between animals and humans (14, 15). *Cryptosporidium parvum* oocysts can be transported by filth flies not only from cattle sources but from any unhygienic or contaminated source, i.e., toilets, abattoirs, trash, carcasses, and sewage (13, 29). Because wild filth flies carry viable *Cryptosporidium parvum* oocysts acquired naturally from unhygienic sources, they can be involved in the epidemiology of cryptosporidiosis (11). Filth flies can cause human or animal cryptosporidiosis via deposition of infectious oocysts on visited foodstuff (2, 6, 9–13, 23, 29). However, such epidemiologic involvement is difficult to prove, as cryptosporidiosis cases that result from fly visitations on food items or raw, preprocessed food products will be classified as food borne (12). Interestingly, food-borne cases of cryptosporidiosis have been extensively documented (1). Winter usually ends the breeding cycle of synanthropic flies; however, indoor flies can develop several generations (14, 15).

The involvement of filth flies, i.e., house flies, in mechanical transmission of *Cryptosporidium parvum* was first described in 1999 (9), although it had been suggested in 1987 (28). Other insects, i.e., dung beetles, have also been reported to mechanically carry *Cryptosporidium parvum* oocysts acquired from animal manure or other unhygienic sites (20). Subsequent reports confirmed that filth flies can transport infectious oocysts of *Cryptosporidium parvum* on their external surfaces and in their digestive tracts (2, 4, 6). Thus, nonbiting flies can serve as mechanical vectors for the human parasite against which no effective prophylaxis or therapy exists (8).

Exposure of adult house flies, *Musca domestica*, to bovine diarrheal feces with *Cryptosporidium parvum* oocysts resulted in intense deposition of the oocysts through fecal spots and vomit drops on the visited surfaces, with an average of 108 oocysts per cm^2 (9, 10). On average, 267, 131, 32, 19, and 14 oocysts per house fly were eluted from its exoskeleton on days 3, 5, 7, 9, and 11 after emergence, respectively (9). Approximately 320 *Cryptosporidium parvum* oocysts per pupa were eluted from the external surface of the pupae derived from maggots that bred in a substrate with the bovine feces; oocysts were numerous on the maggots (approximately 150 oocysts/maggot) (9). In another study, over the course of 6 months, wild filth flies (families *Muscidae*, *Sarcophagidae*, and *Calliphoridae*) were collected in barns with and without a calf shedding *Cryptosporidium parvum* oocysts in diarrheic feces (11).

Oocysts of *Cryptosporidium parvum* transported on the flies' exoskeletons and eluted from their fecal and vomit droplets were infectious to neonatal mice, which are always used in *Cryptosporidium* bioassays (11). The mean number of oocysts carried by a fly varied from 4 to 131, and the total oocyst number per weekly collection varied from 56 to approximately 4.56×10^3 (11). Molecular data showed that the oocysts shed by infected calves were carried by flies for at least 3 weeks (11).

In the next study, wild synanthropic flies (Muscidae, Calliphoridae, Lauxaniidae, and Anthomyiidae) caught at cattle dairy farms and cattle waste facilities were tested for *Cryptosporidium parvum* on their exoskeletons and in their digestive tract by a technique that allows assessment of oocyst viability, fluorescent in situ hybridization (13, 29). Fluorescent in situ hybridization employs fluorescently labeled oligonucleotide probes targeted to species-specific sequences of 18S rRNA (29). As rRNA has a short half-life and is only present in high copy numbers in viable organisms, fluorescent in situ hybridization allows differentiation between viable and nonviable cells and eliminates the need for fluorogenic dyes (13, 29). The vast majority of oocysts, >80%, were viable, and more oocysts were located within the digestive tract than on the exoskeleton (13, 29).

CONCLUSIONS

Synanthropic insects such as flies and cockroaches can significantly contribute to the spread of food-borne protozoan diseases in both developing and developed countries. With the capacities of modern synanthropic insect control, the detrimental impact of these insects on public health is minimized, which gives a false sense of security about the infectious disease threat from them. Populations of synanthropic insects can grow rapidly, and any temporary inattention to continuous and proper sanitation and control can allow transmission of human food-borne diseases associated with synanthropic insect infestations.

As synanthropic insects harbor human protozoan parasites acquired naturally from unhygienic sources, it is necessary to prevent these insects from gaining access to human food. A sanitary, insect-free environment is of the highest priority in

modern food sanitation programs and in food-processing facilities. The proper venue for controlling the transmitters or vectors of food-borne pathogens is an effective sanitation and pest exclusion program which would control or eliminate pests from food-processing areas.

In areas where valid health statistics are not available, microbiological studies of synanthropic insects can provide essential epidemiologic information on human enteropathogens.

Not all nonbiting fly species are associated with unsanitary conditions and pathogen transmission or involved in the epidemiology of human diseases. Sanitation and pest control professionals should recognize the adult and larval stages of nonbiting flies that are potential public health threats and should be able to link the fly species with a potential hazard for food-borne disease. Only flies classified as filth flies pose potential health hazards and require regulatory actions or pest control programs to be effectively neutralized or eliminated. These programs and corrective actions instituted through them will help to prevent or correct potential microbial contamination or cross-contamination from these flies. Filth flies share certain attributes that have been recognized by entomologists and account for their strong association with human food-borne diseases, such as synanthropy, endophily, communicative behavior, and attraction to both excrement and human food products.

Fly populations can increase quickly in a relatively short time, particularly during the summer. The flies associated with transmission of human pathogens often exhibit clustering and swarming behaviors. As a result, the sites of attraction, filth or food items, are visited by large numbers of flies. This causes the cumulative effect of parasite deposition, which is much greater that the pathogen-carrying capacity of a single fly. High densities of flies proportionally increase the load of pathogens on surfaces visited by the flies.

Mechanical transfer of *Cryptosporidium parvum* oocysts by filth flies can be achieved through defecation, regurgitation, or mechanical dislodgement, and the vast majority of transported oocysts are viable and thus capable of infection. The biology and ecology of synanthropic filth flies indicate that their potential for mechanical transmission of *Cryptosporidium parvum* is high. The epidemiologic involvement of nonbiting flies in transmission of *Cryptosporidium parvum* is difficult to prove, as cases of cryptosporidiosis resulting from fly visitations on foodstuffs are classified as food borne. As fly eradication or control of synanthropic fly populations coincides with sharp reductions in outbreaks and cases of diarrheal diseases, it is most likely that food-borne cases of cryptosporidiosis could be even more drastically reduced by enforcing fly control.

Mechanical transmission of pathogens by nonbiting flies and epidemiological involvement of synanthropic flies in human food-borne diseases have not received adequate scientific attention. Further research is necessary to elucidate the mechanisms involved in retaining the infectivity of pathogens vectored by flies, the efficiency of various transport types, e.g., exoskeleton versus gastrointestinal tract, and the temporal and spatial dispersal of pathogens by flies from contaminated sites.

ACKNOWLEDGMENTS

The studies on mechanical transmission of *Cryptosporidium parvum* were supported by the Maryland Sea Grant, College Park, Md. (grant no. R/F-88), U.S. Environmental Protection Agency, Washington, D.C. (grant no. R824995), the Center for a Livable Future, Johns Hopkins University, Baltimore, Md. (grant no. H040-951-0180), and the NATO Collaborative Linkage Grant, Brussels, Belgium (grant no. CLG 979765).

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