Abstract. Refuse and promiscuous-landing synanthropic filth flies, such as house flies (*Musca domestica*), are recognized as transport hosts for a variety of protozoan and metazoan parasites in addition to viral and bacterial pathogens of public health importance. Exposure of adult *M. domestica* to 20 ml of bovine diarrheal feces containing *Cryptosporidium parvum* oocysts (2.0 × 10^8 oocysts/ml) resulted in intense deposition of the oocysts through fly feces on the surfaces visited by the flies (mean = 108 oocysts/cm²). *Cryptosporidium parvum* oocysts were detected by immunofluorescent antibodies on the exoskeleton of adult flies and in their digestive tracts. An average of 267, 131, 32, 19, and 14 oocysts per adult fly were eluted from its exoskeleton on days 3, 5, 7, 9, and 11 after they emerged, respectively. Approximately 320 *C. parvum* oocysts per pupa were eluted from the external surface of the pupae derived from maggots that breed in a substrate contaminated with the bovine feces; the oocysts were numerous on maggots (approximately 150 oocysts/maggot). Adult and larval stages of house flies breeding or having access to *C. parvum*-contaminated substrate will mechanically carry the oocysts in their digestive tracts and on their external surfaces.

*Cryptosporidium* spp. are protozoan parasites infecting primarily the gastrointestinal, and to lesser extent, the respiratory, and renal epithelium of vertebrates. The infectious stage, the oocyst, is transmitted by the fecal-oral route and is often waterborne. Of eight valid *Cryptosporidium* species, only *Cryptosporidium parvum* infects humans and represents a public health threat. This zoonotic and anthropotic parasite significantly contributes to the mortality of immunocompromised or immunosuppressed people and can severely debilitating healthy, i.e., immunocompetent, populations in which infections can be caused by as few as 30 oocysts. In people with impaired immune systems, a single oocyst can initiate infection.

Insects, particularly refuse and promiscuous-landing synanthropic flies, i.e., house flies (*Musca domestica*), are known transport hosts for a variety of pathogens of public health importance. Synanthropic flies, including pestiferous house flies, were identified as transport hosts of *Sarcocystis* sp., *Toxoplasma gondii*, *Isospora* spp., and *Giardia* spp. Refuse house flies have been incriminated in transmission of helminth eggs, i.e., *Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis*, *Toxocara canis*, and *Strongyloides stercoralis*, protozoan cysts and trophozoites, i.e., *Entamoeba coli*, *Giardia* spp., and *Trichomonas* spp., and bacteria such as *Shigella* sp., *Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas*. *Eimeria tenella*, the coccidian parasite of poultry, can be mechanically transmitted by house flies.

*Cryptosporidium parvum* is prevalent in preweaned cattle in which the infections can produce high oocyst output sometimes exceeding 10^10 oocysts per day. Cattle manure is recognized source of *C. parvum* oocysts and is also a favorite breeding place, food source, and descending site of house flies. Although house flies come into direct contact with manure, and it has been demonstrated that small particles readily adhere to the exterior surfaces of the flight, the role of house flies as the transport hosts for *C. parvum* oocysts has not been elucidated. The purpose of the present study was to determine if house flies can serve as transport hosts for infectious *C. parvum* oocysts.

**MATERIALS AND METHODS**

**Bovine feces.** A watery, diarrheal feces originated from a Holstein calf experimentally infected with *C. parvum* strain AUCP-1 as described previously. To determine the number of oocysts per milliliter of feces, the fecal specimen was vortexed, and 10 50-ml aliquots were processed with immunofluorescent antibodies (IFA) of the MERIFLUOR® *Cryptosporidium/Giardia* test kit (Meridian Diagnostic, Inc., Cincinnati, OH). Oocysts were counted and determined to be 2.0 × 10^8 oocysts/ml. Diarrheal feces from a calf not infected with *C. parvum*, which was confirmed as oocyst-negative by the same techniques, were used as the negative control. Both fecal specimens were stored approximately 2 weeks at 4°C prior to the experiments, and were thoroughly mixed before use.

**Adult *M. domestica.*** All experiments with the adult, maggots, and pupae of laboratory-reared *M. domestica* (F58WT2 strain) were conducted indoors in the same room (12:12 hr L:D) with controlled temperature (21°C) and humidity (65%). Approximately 250 old (dark-colored) pupae obtained from Insect Control & Research, Inc., (Baltimore, MD) were placed in each of six 1-gallon paper mosquito cages. Moisturized cotton was placed on top of each cage, and the cages were covered half-way up with a polyethylene bag to retain moisture. Each of 5 cages contained a 6-cm-diameter petri dish with 20 ml of bovine feces with 2.0 × 10^8 *C. parvum* oocysts/ml and 5 glass microscope slides located on the bottom of the cage. The sixth cage was similarly equipped but contained oocyst-negative feces. Three days after the flies emerged, the petri dish was removed from all cages (longer exposition of feces caused their desiccation). One slide was removed from each cage on days 3, 5, 7, 9, and 11 after flies emerged.
Glass slides with cumulative fly fecal spots were placed on the top of the IFA slide (MERIFLUOR®) and the number of spots that appeared in three 13-mm-diameter wells (113 mm² each) were counted. The slides were fixed with methanol, and a 1 cm-diameter black circle was marked in the central area of each of the slides with fly fecal spots on the other side. Fifty microliters of IFA were applied on each slide within circle, and the slides were processed as described above. The slides were examined by immunofluorescence microscopy and the number of C. parvum oocysts in at least 4 fecal spots per slide were counted. Several randomly selected slides were processed with acid-fast stain (AFS) and examined by light microscopy.

Thirty flies were removed from each cage by aspirator on days 3, 5, 7, 9, and 11 after they emerged. They were killed by exposure to low temperature (~20°C), placed into a plastic tube with 50 ml of eluting fluid, and vortexed. The eluant with the flies was filtered onto the surface of a 25-mm-diameter cellulose acetate membrane (CAM). Flies were collected from the CAM, and the CAM was processed as described previously. The digestive tract was extracted from the abdomen of randomly selected flies at each time-point. The digestive tracts were air-dried and processed using the MERIFLUOR® test. In addition, 10 flies from each time-point were ground in an Eppendorf tube (VWR, Piscataway, NJ) in 300 µl of phosphate-buffered saline in groups of five. Fifty microliters of the homogenate were collected, placed on the MERIFLUOR® slide, air-dried, and processed according to the manufacturer’s instructions.

**Maggots of M. domestica.** Approximately 300 eggs obtained from Insect Control & Research, Inc. were placed into each of two 100 ml-capacity beakers filled to 5 cm with fly larvae medium (fly larvae media; PMI FEEDS, Inc., St. Louis, MO) thoroughly mixed with 50 ml of bovine diarrheal feces containing 2.0 × 10⁵ C. parvum oocysts/ml. To facilitate pupation, a 2 cm-high perlite (Pennington, Inc., Lebanon, OR) layer was placed on top of the fly larvae medium. The perlite was also provided to prevent secondary contact with the fecal matter of any flies that may emerge. The beakers were located inside a 1-gallon paper mosquito cage, and the cage was covered half-way up with a polyethylene bag to retain moisture. With the aid of forceps, 30 maggots were collected 4 days later, and 10 maggots (in their third instar) and 30 old pupae were collected 4 days after the initial maggot collection. Maggots were placed on a glass slide and gently pressed-to-rupture by another slide to produce symmetrical image on both slides. One set of slides was stained with IFA, and several randomly selected slides from the second set were processed with AFS. The pupae were placed in a plastic tube containing 15 ml of eluting fluid and vortexed. The fluid with the pupae was filtered by the CAM, the pupae were collected from the surface of the CAM, and the membrane was processed by the CAM-filter dissolution method. The pupae were ruptured on IFA slides that were subsequently processed using the MERIFLUOR® test according to the manufacturer’s instructions. The slides were examined by immunofluorescent microscopy and the number of C. parvum oocysts were counted. Using Pearson’s correlation coefficient (R). The numbers of C. parvum oocysts recovered by the CAM-filter dissolution method was adjusted for the method recovery efficiency of 78.8%. The number of fly fecal spots counted in the circle area of 113 mm² were adjusted to 100 mm² (= 1.0 cm²) (Figure 1). Mean ± SD values are reported, and statistical significance was considered to be a P value < 0.05.

**RESULTS**

**Adult M. domestica.** Fly fecal spots were distributed homogeneously over the area of each glass microscope slide and the number significantly increased over time (R = 0.95, P < 0.01) (Figure 1), despite the fact that the number of flies progressively decreased due to the sampling. A mean ± SD of 14.0 ± 6.8 fecal spots were counted per 1.0 cm² of glass slide. Slides collected 3 and 5 days after flies emerged had smaller and darker fecal spots than those on slides from days 7, 9, and 11. The fecal spots on slides from days 9 and 11 overlapped (although they were still clearly identifiable and countable), resulting in almost total coverage of the slide surface. The appearance and abundance of the fecal spots of flies exposed to oocyst-negative calf feces were similar.

Cryptosporidium parvum oocysts were present on the surface of all slides from cages with flies and oocyst-positive feces. In general, the oocysts were associated with the fly fecal spots (Figure 2); however, oocysts were observed that were not associated with fly feces. These were particularly frequent on slides collected on days 9 and 11 after flies emerged. Fly fecal spots without C. parvum oocysts were rarely observed; however, these were present on all slides and their numbers were stable over time. The mean number of C. parvum oocysts per fecal spot observed for the duration of the experiment ranged from 4 to 12 (Figure 1) (mean ± SD = 7.0 ± 3.2 oocysts/fecal spot). A maximum of 20

![Figure 1](image-url)
Cryptosporidium parvum oocysts was observed in some spots. The mean number of oocysts per fly fecal spot did not correlate with the increasing number of spots or show a significant pattern, e.g., decrease or increase, over time \( (P > 0.05) \). Analysis of the data presented in Figure 1 demonstrated that approximately 20, 40, 96, 228, and 180 \( C. \) parvum oocysts/cm\(^2\) (only feces-associated oocysts) were observed on slides collected from cages on 3, 5, 7, 9, and 11 days after flies emerged, respectively \( (\text{mean} \pm \text{SD} = 108.0 \pm 86.0 \text{oocysts/cm}^2) \). The number of oocysts per 1.0 cm\(^2\) deposited on the slides by flies through their feces significantly increased over time \( (R = 0.89, P < 0.03) \). Screening of control slides with IFA yielded negative results.

The IFA-stained oocysts associated with fly fecal spots, those not associated with spots, and those in calf feces all displayed similar intensity of fluorescence and morphology. In the acid-fast staining, fly-derived oocysts displayed non-uniform bright pink coloration and contained characteristic black granules similar to oocysts in calf feces.

From 1 to 8 oocysts were detected in 17 (68\%) of 25 digestive tracts of flies exposed to feces with oocysts. The digestive tracts of all 5 flies collected 11 days after they emerged contained \( C. \) parvum oocysts. Fifteen (60\%) of 25 homogenate samples of flies that were eluted with detergents and subsequently ground were positive for \( C. \) parvum oocysts (approximately 5 oocysts/fly). There was no apparent temporal pattern in oocyst numbers in these flies.

\textit{Cryptosporidium parvum} oocysts were detected by IFA on the external surface of house flies exposed to contaminated feces, i.e., legs and wings (Figure 2). All samples of external surface eluants were positive for \( C. \) parvum oocysts. The number of such oocysts progressively significantly decreased over time \( (R = -0.89, P < 0.03) \). An average of 267, 131, 32, 19, and 14 \( C. \) parvum oocysts were collected from a single fly 3, 5, 7, 9, and 11 days after emergence, respectively.

\textit{Maggots of} \textit{M. domestica}. \textit{Cryptosporidium parvum} oocysts were numerous on slides obtained from ruptured maggots, and were present in all maggot samples; approximately 150 oocysts were recovered per maggot. Oocysts were also detected on the glass behind the third instar maggots before they were ruptured; these oocysts were probably from the external surface of maggots.

The eluant of the pupae external surface was positive for \( C. \) parvum oocysts. Approximately 320 oocysts were eluted from the external surface of a single pupae. \textit{Cryptosporidium parvum} oocysts were also detected by IFA on the slides obtained by crushing of surface-eluted pupae; 21 (70\%) of 30 slides were positive for the oocysts and their numbers varied considerably from 10 to 94 oocysts per pupae \( (\text{mean} \pm \text{SD} = 34.0 \pm 28.1 \text{oocysts/pupae}) \).

The oocytes from maggots and pupae (external surface and ruptured pupae) stained with AFS and IFA appeared similar to oocysts in calf feces.

**DISCUSSION**

The present study demonstrated that adult flies with access to a substrate containing \( C. \) parvum oocysts can transport these oocysts in their digestive tracts and on their external surfaces. Thus, adult house flies can serve as mechanical carriers of \( C. \) parvum in the environment.

\textbf{FIGURE 2}. \textit{Cryptosporidium parvum} oocysts (arrows) detected by immunofluorescent antibodies in the fecal spots (A) and on the exoskeleton of house flies (\textit{Musca domestica}) (B and C) exposed to bovine diarrheal feces containing \( C. \) parvum oocysts. A, fly fecal spot; B, leg (tibia) with \( C. \) parvum oocyst captured by the hairs; and C, posterior margin of the wing; note the \( C. \) parvum oocyst captured by the wing bristles. Bars = 50 mm.
vectors for *C. parvum*, and can be involved in human and animal cryptosporidiosis. Mechanical transmission of *C. parvum* oocysts by flies could be important although it is difficult to prove this mode of transmission. *Cryptosporidium parvum* oocysts can be deposited on food products if such are accessible for promiscuous-landing flies, which upon consumption would cause cryptosporidiosis. However, under such circumstances the *C. parvum* infections will be classified as foodborne, which, as a matter of fact, have been extensively documented.\(^{18,19}\) Maggots and pupae that internally and externally harbor *C. parvum* oocysts can be involved in animal cryptosporidiosis.

The house fly, domestic fly, or coprophilic fly (families Muscidae, Anthomyiidae, Sarcophagidae, and Calliphoridae) have evolved to live in close association with humans (synanthropic flies) as annoying pestiferous scavengers.\(^{18±20}\) Outbreaks of diarrheal diseases are closely correlated with the seasonal increase in abundance of filth flies, and fly control has been closely correlated with a decrease in the incidence of such diseases.\(^{19}\) All filth flies breed in animal manure, human excrement, garbage, animal bedding, and decaying organic matter, and their breeding biology is similar.\(^{19,20}\) Based on the results of the present study, it is highly likely that flies displaying similar habits and frequently similar habitats may also be assumed to carry oocysts of *Cryptosporidium*.

It has been demonstrated herein that adult house flies are efficient mechanical vectors of *C. parvum*. A 3-day-exposure of flies to feces with oocysts resulted in deposition for next 8 days (only through fly feces) of an average of more than 100 oocysts/cm\(^2\) of the surface visited by flies, and a single fly can carry on its external surface more than 200 oocysts. Effectiveness of feces in enhancing transmission of infectious agents, e.g., rotaviruses, by house flies is much greater than any other substrate or medium,\(^{24}\) and is a result of feces viscosity that increase efficiency of the fly hairs and bristles in trapping particles suspended in the feces.\(^{25}\) Since the fecal substrate used in the present laboratory experiments is a natural breeding substrate of house flies, we conclude that the results obtained can be directly applied to the natural situation in which adult house flies have access or breed in the feces with *C. parvum* oocysts.

The features of the house fly biology and the results of the present study indicate that the potential of mechanical transmission of *C. parvum* oocysts by adult flies may be high. Female flies can live 15–25 days,\(^{19}\) and lay 5–6 batches of 75–150 eggs.\(^{19,20}\) In temperate climates, there can be 10–12 fly generations in the summer.\(^{19}\) Winter usually ends the breeding cycle of the house fly. However, indoors flies can develop several generations during winter;\(^{18,19}\) and cattle barns are one site where house flies can breed throughout the winter.\(^{18}\) Ecologic monitoring of house flies flight range showed that individual flies can travel as far as 20 miles;\(^{20}\) however, the vast majority of flies, i.e., more than 88%, do not travel more than 2 miles,\(^{19,20}\) and their movement is oriented toward unsanitary sites.\(^{18}\)

Transmission of human pathogens by adult house flies occurs via fecal deposition, mechanical dislodgement of pathogens from the exoskeleton, or through regurgitation of ingested particles.\(^{21}\) In the present study, oocysts not associated with fecal spots were most likely dislodged from the external surface of the fly. The numbers of non-feces-associated oocysts were lower than number of oocysts eluted from fly exoskeleton. This it may be due to the possibility that oocysts trapped by hairs and bristles do not detach easily.

*Cryptosporidium parvum* oocysts were present inside the pupae (which were surface-eluted), indicating that they had been ingested by the maggots. In flies, the pereparation process involves intense re-organization of the digestive tract tissue resulting in development of a new digestive system and production of the meconium,\(^{19}\) i.e., accumulated intestinal wastes. Thus, it is unlikely that in the house fly, *C. parvum* oocysts are transmitted transnodally from the larval stages to the adult. However, even if adult flies are *C. parvum*-sterile when they emerge, by breeding in the contaminated substrate, adult flies could acquire the oocysts by direct contact.

The oocysts recovered from adult and larval stages of house flies in the present study had similar cellular morphology when stained with AFS and IFA as the infectious *C. parvum* oocysts in the bovine feces, indicating that their infectivity was not altered by mechanical passage in the fly. The CAM-filter dissolution method\(^{27}\) used in the present study offers advantages for the recovery of coccidian, e.g., *Cryptosporidium*, oocysts from insect external surfaces. The use of the eluting fluid assures detachment of the oocysts from the exoskeleton and their dispersion, the recovery efficiency of oocysts is high, i.e., 77.8%,\(^{27}\) and the procedure does not alter infectivity of *C. parvum* oocysts.\(^{32}\) The CAM-filter dissolution method can be applied to recover particles from the external surface of wild-caught insects, i.e., flies.

Wild-caught coprophilic flies carried oocysts of *Isospora* or *Hammondia* (not positively identified).\(^{8}\) Based on these results and the observation that these flies moved constantly between bovine manure and cattle, it has been postulated that flies may play a role in distributing coccidian parasites in the environment.\(^{11}\) The present study supports this possibility with regard to house flies (*M. domestica*) and *C. parvum*.

Acknowledgments: We thank N. Spero (Insect Control & Research Inc., Baltimore, MD) for providing house flies, and R. Knight (Johns Hopkins University, Baltimore, MD) for consultation.

Financial support: This study was supported in part by the Maryland Sea Grant (College Park, MD) (grant no. R/F-88); the Maryland Zoological Society (Baltimore, MD) (grant no. H680-951-2118); and the AKC Fund of New York (New York, NY) (grant no. H630-951-2002).

Authors’ addresses: Thaddeus K. Graczyk, Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205. Michael R. Cranfield, Division of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205. Ronald Fayer, Immunology and Disease Resistance Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705. Heather Bixler, St. Charles Veterinary Clinic, 530 Dunham Road, St. Charles, IL 60174.

Reprint requests: Thaddeus K. Graczyk, Department of Molecular Microbiology and Immunology, School of Hygiene and Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205.
REFERENCES