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MECHANICAL TRANSMISSION OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS BY FLIES

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ABSTRACT. Long term field studies and laboratory experiments demonstrated that synanthropic filth flies can mechanically transmit infectious oocysts of *Cryptosporidium parvum*, an anthropozoonotic protozoan parasite which significantly contributes to the mortality of immunocompromised or immunosuppressed people. *C. parvum* oocysts are acquired from unhygienic sources, and can pass trough fly gastrointestinal track without alteration of their infectivity and can be subsequently deposited on visited surfaces. Transmission of the oocysts by adult flies occurs *via*: (1) mechanical dislodgement from the exoskeleton; (2) fecal deposition; and (3) regurgitation, i.e., vomits. Filth flies can cause human or animal cryptosporidiosis *via* deposition of infectious oocysts on the visited foodstuf, and the biology and ecology of synanthropic filth flies indicate that their potential for mechanical transmission of *C. parvum* is high.

Key words: cryptosporidiosis, *Cryptosporidium parvum*, FISH, oocysts, synanthropic flies.

INTRODUCTION

Domestic filth flies (families *Sarcophagidae*, *Muscidae*, and *Calliphoridae*) have evolved to live in close association with man (synanthropic flies) as annoying pestiferous scavengers (Graczyk et al. 2001). 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 (Graczyk et al. 2001). *Cryptosporidium parvum* is an anthropozoonotic protozoan parasite which significantly contributes to the mortality of immunocompromised or immunosuppressed people (Graczyk et al. 2003). Diarrheal disease is initiated by a microscopic stage of this parasite, the oocyst. The pathogen debilitates also healthy, i.e., immunocompetent, individuals in which the disease can be caused by as few as 10 oocysts. It is believed that in people with impaired immune systems, a single oocyst can initiate infection.

Cryptosporidium parvum is particularly prevalent in pre-weaned cattle and cattle manure is a source of the oocysts (Graczyk et al. 2000).

MATERIALS AND METHODS

Laboratory experiments were carried out with bovine diarrheic feces (20 ml) that contained 2.0 x 105 oocysts/ml (Graczyk et al. 1999a, b). Feces were placed in petri dishes in each of 5, 4-liter-capacity paper cages with approximately 250 pupae of laboratory-reared house flies (*Musca domestica*) and flies were allowed to emerge (Graczyk et al. 1999a, b). Three days after the flies emerged the petri dishes were removed. Each cage contained several glass microscope slides on which flies defecated. Thirty flies aspirated from each cage on day 3, 5, 7, 9, and 11 after emergence were eluted and the eluants were processed by the cellulose acetate membrane (CAM)-filter dissolution method (Graczyk et al. 1999a). The digestive tract dissected from randomly selected flies and glass slides with fly excreta were examined by immunofluorescent antibody (IFA), and *C. parvum* oocysts were counted (Graczyk et al. 1999a). Maggots of *M. domestica* were reared in fly larvae medium contaminated with calf diarrheic feces (50 ml) containing 2.0 x 105 *C. parvum* oocysts/ml (Graczyk et al. 1999a). Resulting pupae were eluted, eluting fluid was processed by the CAM-filter dissolution method, and *C. parvum* oocysts were identified by IFA and counted (Graczyk et al. 1999a, b). Diarrheic feces from a *C. parvum*-uninfected calf were used as negative control in similar experiments.

Ten flying insect traps of the Victor(r) type were baited with rotten fish flesh and placed inside a barn (approximately 880 m2) in which a male Holstein calf experimentally (and clinically) infected with *C. parvum* was housed (Graczyk et al. 2000). The traps were emptied weekly, the flies were counted, identified, and the inside surface of traps (that contained fly excreta) along with the flies were eluted with 200 ml of eluting fluid (Graczyk et al. 2000). The eluting fluid was filtered through a CAM. The membrane was processed as described previously, *C. parvum* oocysts were identified by IFA and counted (Graczyk et al. 2000).

Wild non-biting flies associated with dairy cattle operations and animal and municipal waste processing sites were caught at several locations in North America (USA) and Europe (Poland) (Graczyk et al. 2003, Szostakowska et al. 2004). Flies were killed, identified to the family taxon level, and preserved in 75% ethanol. Flies were surface-eluted, homogenized and the eluant and the homogenate were processed by the CAM-filter dissolution method (Graczyk et al. 2003, Szostakowska et al. 2004). Elution ensures recovery of particles from the fly's exoskeleton, and homogenization from their guts. The resulting pellets were processed by IFA specific and fluorescent *is situ* hybridization (FISH) with oligonucleotide probes specific to *C. parvum* (Graczyk et al. 2003, Szostakowska et al.

2004). Positive and negative controls were prepared as described previously (Graczyk et al. 2003, Szostakowska et al. 2004).

RESULTS AND DISCUSSION

Exposure of adult *M. domestica*, to bovine diarrhoeal feces with *C. 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 (Graczyk et al. 1999a, b). On average 267, 131, 32, 19, and 14 oocysts per a housefly were eluted from its exoskeleton on day 3, 5, 7, 9, and 11 after they emerged, respectively (Graczyk et al. 1999a). Approximately 320 *C. parvum* oocysts per pupa were eluted from the external surface of the pupae derived from maggots that breed in a substrate with the bovine feces; the oocysts were numerous on maggots (approximately 150 oocysts/maggot) (Graczyk et al. 1999a). In another study, over the course of six months wild filth flies (families: *Muscidae*, *Sarcophagidae*, and *Calliphoridae*) were collected in a barn with, or without, a calf shedding *C. parvum* oocysts in diarrhetic feces (Graczyk et al. 2000). The oocysts of *C. parvum* transported on the flies' exoskeletons and eluted from their fecal and vomit droplets were infectious to suckling mice (Graczyk et al. 2000). The mean number of oocysts carried by a fly varied from 4 to 131, and the total oocyst number per a weekly collection varied from 56 to approximately 4.56 x 103 (Graczyk et al. 2000). Molecular data showed that the oocysts shed by infected calves were carried by flies for at least 3 wk (Graczyk et al. 2000). In the next study, wild synanthropic flies (*Muscidae*, *Calliphoridae*, *Lauxaniidae*, and *Anthomyiidae*) caught at cattle dairy farms and cattle waste facilities were tested for *C. parvum* on their exoskeletons and in their digestive tracks by technique that allows assessment of oocyst viability, i.e., FISH % (Graczyk et al. 2003, Szostakowska et al. 2004). The vast majority of oocysts, i.e., > 80%, were viable and more oocysts was located within the fly's digestive tract than on the fly's exoskeleton (Graczyk et al. 2003, Szostakowska et al. 2004).

Transmission of *Cryptosporidium* oocysts by adult flies occurs *via*: (1) mechanical dislodgement from the exoskeleton; (2) fecal deposition; and (3) regurgitation, i.e., vomits. Bristles on fly legs are coated with sticky substance which form cushion-like structure that enhance fly adherence to the surface. This substance also enhance adhesion of oocysts to fly legs which then can be directly transported to the next visited place and dislodged. As small particles readily adhere to fly exterior surfaces due to its electrostatic charge, *C. parvum* oocysts on fly legs can also originate from the exoskeleton as a results of frequent grooming with involvement of the legs. Effectiveness of feces in enhancing transmission of oocysts by houseflies is much greater than of any other substrate or medium. This is a direct result of feces viscosity that increase efficiency of the fly hairs and bristles in trapping particles suspended in the feces (Szostakowska et al. 1999a, 2000, 2003; Graczyk et al. 2003).

Cryptosporidium parvum oocysts can pass trough fly gastrointestinal track without alteration of their infectivity and can be subsequently deposited on visited surface in fecal spots. Alternatively, the oocysts present in fly alimentary tract can be regurgitated, i.e., „vomit drops", on the surface perceived by a fly as a meal (regurgitation always precede feeding). Frequent meals on contaminated substrate together with alternated regurgitation and ingestion, cause progressive accumulation of *C. parvum* in fly alimentary system (Graczyk et al. 2001).

It is unlikely that *C. parvum* oocysts are transmitted transtadially from the larval stage to the adult stage when maggots breed in a contaminated substrate. This is because the puparation process involves intense re-organization of the digestive tract tissue resulting in development of a new digestive system and production of the meconium, i.e., accumulated intestinal wastes. *C. parvum* oocysts were present in alimentary canals of maggots breed on contaminated substrate, inside the pupae, and in the meconium but not in or on adult flies (Graczyk et al. 1999a). However, even if flies are sterile when they emerge from pupa, they will acquire the oocysts from contaminated substrate (in which they bred) by a direct contact (Graczyk et al. 1999a).

Filth flies can cause human or animal cryptosporidiosis *via* deposition of infectious oocysts on the visited foodstuff. However, such epidemiologic involvement is difficult to prove as cryptosporidiosis cases that result from fly visitations on food items or raw, pre-processed food products will be classified as foodborne. Interestingly, foodborne cases of cryptosporidiosis have been extensively documented (Graczyk et al. 2000).

The biology and ecology of synanthropic filth flies indicate that their potential for mechanical transmission of *C. parvum* is high. Adult female flies can live 15 to 25 days, and lay 5 to 6 batches of 75 to 150 eggs. In temperate climates there can be 10 to 12 fly generations in the summer. Winter usually ends the breeding cycle; however, indoor, i.e., barns, houses, flies can develop several generations. Cattle barns, for example, are one site where houseflies can breed throughout the winter. Individual flies can travel as far as 20 miles; however, their vast majority, i.e., over 88%, do not travel more than 2 miles, and their movement is always oriented toward unsanitary sites.

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