Report as of FY2006 for 2006DC80B: "Assessment of Waterborne Contamination with Human Pathogens in Tributaries of the Anacostia River using the Asiatic Clam (Corbicula fluminea)"

Publications

- Other Publications:
  - Graczyk, Thaddeus K., Deirdre Sunderland, Leena Tamang, Timothy M. Shields, Frances E. Lucy, Patrick N. Breysse, 2007, A Quantitative Evaluation of the Impact of Bather Density on Levels of Human-Virulent Microsporidian Spores in Recreational Water, Applied and Environmental Microbiology, published online ahead of print on 4 May 2007.
  - Graczyk, Thaddeus K., Leena Tamang, Richard Pelz, 2007, the Effect of a Taste Enhancement Process for Cold-Stored Raw Shell-Stock Oysters (Crassostrea virginica) on the Spillage of Human Enteropathogens, 2007.
- Articles in Refereed Scientific Journals:
  - Graczyk, Thaddeus K., Cynthia McOliver, Ellen K. Silbergeld, Leena Tamang, Jennifer D. Roberts, 2007, Risk of Handling as a Route of Exposure to Infectious Waterborne Crystosporidium parvum Oocysts via Atlantic Blue Crabs (Callinectes sapidus), Applied and Environmental Microbiology, Vol.73 (12), p. 4069-4070.
  - Graczyk, Thaddeus K., Deirdre Sunderland, Ana M. Rule, Alexandre J. da Silva, Iaci N. S. Moura, Leena Tamang, Autumn S. Girouard, Kellogg J. Schwab, Patrick N. Breysse, 2007, Urban Feral Pigeons (Columba livia) as a Source for Air-and-Waterborne Contamination with Enterocytozoon bieneusi Spores, Applied and Environmental Microbiology, published online ahead of print on 4 May 2007.
- unclassified:
  - Jedrzejewski, Syzmon, Thaddeus K. Graczyk, Anna Slodkowicz-Kowalska, Leena Tamang, Anna C. Majewska, 2007, Quantitative Assessment of Contamination of Fresh Food Produce of Various Retail Types by Human-Virulent Microsporidian Spores, Applied and Environmental Microbiology, Vol. 73 (12), p. 4071-4073.

Report Follows

Award Number: GF4136F201

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Peer-reviewed publications resulted from the Award Number: GF4136F201: five (5). (Attached)

Final Report

Description of Experiments

1. Depuration of *Corbicula fluminea* clams collected from the Anacostia River.
2. Determination of the bioaccumulation rate of human waterborne parasites by *Corbicula fluminea* clams from the Anacostia River and comparison with *Dreissena polymorpha* mussels.

Corbicula fluminea, 2.0 to 2.5 cm shell length were obtained from Anacostia River, and *Dreissena polymorpha*, 2.0 - 3.5 cm shell length, were obtained from the St. Lawrence River. Clams and mussels were depurated for 3 weeks (4), and after depuration 30 randomly selected clams and mussels were individually tested for *Cryptosporidium* and *Giardia* (4). Depurated *C. fluminea* clam were tested for *Cryptorporidium*, *Giardia*, and human-virulent microsporidia as described previously (6). Three, 38-l aquaria (approximately 10-gallon), i.e. aquarium A, B, and C, were filled with dechlorinated drinking water filtered by the Filterite 10-µm-pore yarn-wound cartridge (Memtec America Corp., Baltimore, Maryland). Each aquarium was equipped with a Fluval filter (model 403) (Askoll, Italy) and two air-stones. Two hundred-twenty specimens of *C. fluminea* or *D. polymorpha* were placed separately in aquarium A and B, respectively, and 110 of each bivalve species were placed in aquarium C. Shellfish in aquaria were maintained as described previously (4).

Cryptosporidium parvum oocysts and *G. lamblia* cysts originated from experimental infection of a calf and were purified by $CsCl_2$ gradient centrifugation (7). Oocysts and cysts were enumerated by flow cytometry (2). Water in each aquarium was spiked daily in the early morning with 106 oocysts and 304 cysts for 31 consecutive days. The inoculum size was calculated to produce the concentration of oocysts and cysts reported from surface water, i.e., 28 oocysts/10 liters, and 304 cysts/10 liters (1).

Thirty bivalves were sampled 7 times at weekly intervals in the late afternoon with the

first sampling timepoint, i.e., week 1, on three days after the first water contamination event. The fifth sampling timepoint, i.e., week 5, occurred the day of the last water contamination timepoint. Each time the sampled bivalves included 30 clams (aquarium A), 30 mussels (aquarium B), and 15 of each species (aquarium C). The bivalves were opened (4), the soft tissue and hemolymph from 30 shellfish was pooled, homogenized with a doubled volume (w/v) of phosphate-buffered saline (PBS) (pH 7.4), and the homogenate was sieved, sedimented (5, 8), and purified over $CsCl_2$ gradient (7). The oocyst and cyst-containing fraction of $CsCl_2$ was centrifuged (1,000 *g*; 3 min; 4°*C*), and the pellet resuspended in 4 ml of deionized water. Approximately 500 µl of resuspension was placed in each of eight wells on an 8-well-chamber tissue culture glass slide (Nalge Nunc International, Naperville, IL, USA). After 3 hr incubation at 20°*C*, the fluid was aspirated from each well, the plastic dividers were removed, and the slide was air-dried. *Cryptosporidium parvum* oocysts and *G. lamblia* cysts were visualized by immunofluorescent antibody (IFA) of the MER***IF****LUOR*™ test kit (Meridian Diagnostic, Cincinnati, OH) and enumerated (5). The overall numbers of oocysts and cysts were adjusted for the method recovery efficiency, i.e., 51.1% (5). Sediments from all aquaria were tested for *Cryptosporidium* and *Giardia* (4) every time the bivalves were sampled. Efforts were made to collect all sediments. All water from all aquaria was filtered by the cellulose acetate membrane disk; 393-mm diameter, 3.0-µm pore size (Millipore Corp., Bedford, MA) (3) every time the bivalves were sampled. After total aquarium drainage the filtered water was recirculated back to the aquarium. The membranes were processed to detect *C. parvum* and *G. lamblia* (9,10). To confirm the recovery efficiency of this method 5 38-l water samples were processed as described above except that each sample was spiked with 106 *C. parvum* oocysts and 304 *G. lablia* cysts.

Statistical analysis was carried out with Statistix 4.1 (Analytical Software, St. Paul, Minnesota). The variables were examined by the Runs test to determine conformity to a normal distribution. The degree of linear association between variables was evaluated using Pearson's correlation coefficient (R), two-sample t-test was used to assess the significance of differences between mean values, and fractions were compared using the G-heterogeneity test. Mean values (x) were associated with standard deviation (SD). Statistical significance was considered to be $P < 0.05$.

Results

The numbers of human pathogens identified in *C. fluminea* clams form the Anacostia River are presented in Figure 1.

The numbers of *C. parvum* oocysts and *G. lamblia* cysts identified in shellfish tissue increased progressively through week 5, and both parasites were identified for the first time, i.e., on week 1, in *D. polymorpha* tissue (Fig. 2). There was a significant correlation observed in all three experiments between the cumulative numbers of *C. parvum* oocysts seeded to the water and identified in bivalve tissue (Pearson correlation; $P = 0.94$, $P < 0.02$). This was also the case for *G. lamblia* in two experimental options, i.e., aquarium A and B (Pearson correlation; $P = 0.96$, $P < 0.01$). The parasite levels decreased on the week 6 after cessation of water contamination, but *C. parvum* and *G. lamblia* were still detected in *D. polymorpha*, i.e., aquarium B and C, two

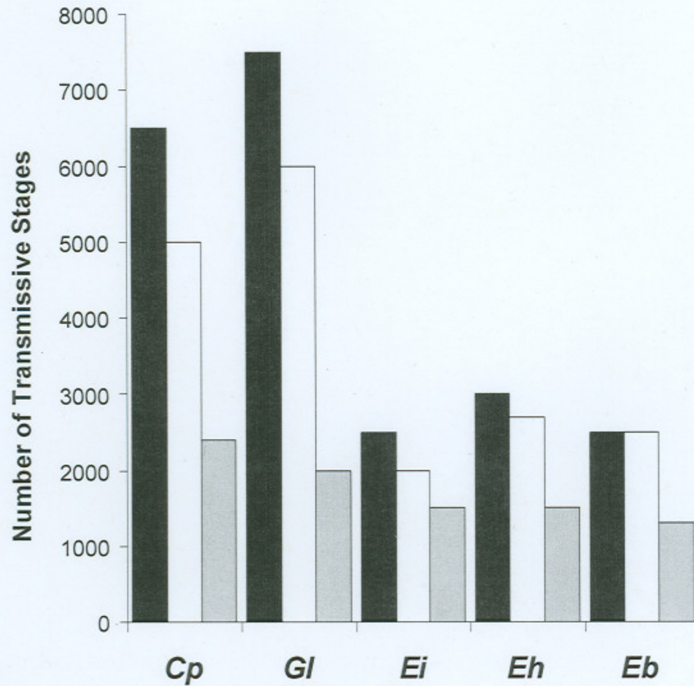

Figure1. Number of *Cryptosporidium parvum* (Cp), *Giardia lamblia* (Gl), *Encephalitozoon intestinalis* (Ei), *Encephalitozoon hellem* (Eh), and *Enterocytozoon bieneusi* (Eb) recovered from 30 *Corbicula fluminea* clams from the Anacostia river during first (black), second (white), and third (gray) week of depuration.

weeks after the last water contamination event.

In general, more cystic stages of both parasites were identified in the tissues of *D. polymorpha* (aquarium B) than *C. fluminea* (aquarium A). In aquarium C in which equal numbers of each bivalve species were kept (and sampled), most parasites were identified in the *D. polymorpha* tissue. Based on the data from all three 7-week-long experiments, on average 48 $\pm$ 24.9 pathogen cystic stages (both *C. parvum* and *G. lamblia*) were identified in the tissue of 30 *C. fluminea* clams, and 70 $\pm$ 25.8 in 30 *D. polymorpha* mussels. Analysis of these results by two-sample *t*-test demonstrated that significantly higher numbers of parasites were identified in *D. polymorpha* than in *C. fluminea* ($t = 3.03$, $P < 0.05$).

On average, from 7% to 32% (mean, 17.8%) of all *C. parvum* oocysts added to the water were identified in the bivalve tissue for the 31 day duration of water contamination (Fig. 3). This level was significantly higher than the level of *G. lamblia* cysts (range: 1 - 5%; mean, 1.7%) (two-sample *t*-test; $t = 59.2$, $P < 0.01$). Overall, for all three 7-week-long experiments 35.0% and 16.3% of the parasite cystic stages seeded into the water were identified in *D. polymorpha* and *C. fluminea*, respectively (*G*-heterogeneity test: $G = 6.8$, $P < 0.01$).

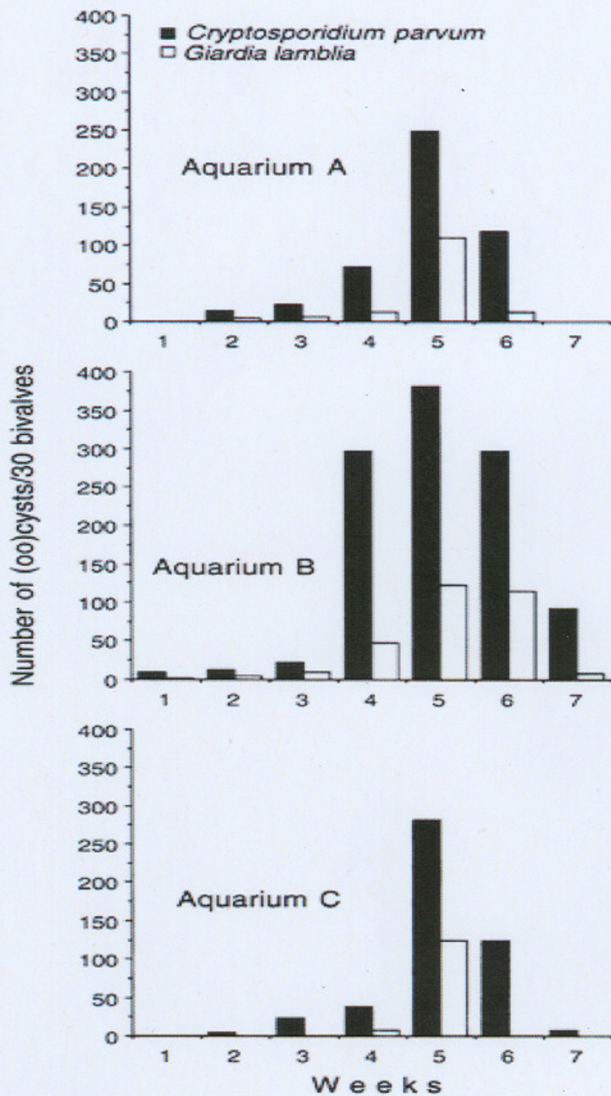

Fig. 2. Identification of *Cryptosporidium parvum* oocysts and *Giardia lambia* cysts recovered from artificially contaminated water by freshwater bivalve mollusks, *Corbicula fluminea* (aquarium A), *Dreissena polymorpha* (aquarium B); aquarium C contained equal numbers of both bivalve species which were sampled equally. Water in each 38-l aquarium seeded daily for 31 consecutive days, i.e., up to week 5, with 106 oocysts and 304 cysts. Aquarium C; *Cryptosporidium parvum* and *G. lamblia* identified in *D. polymorpha* tissue only. Oocysts and cysts identified by immunofluorescent antibody.

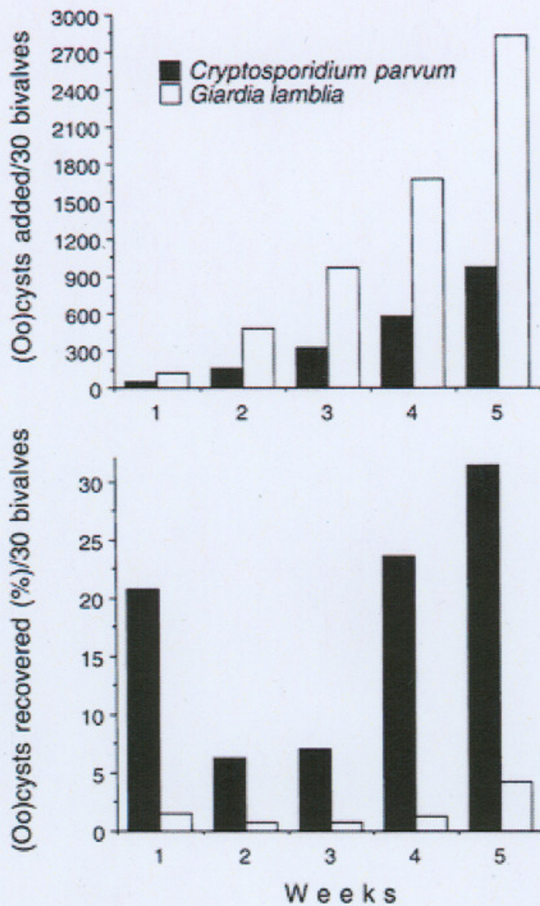

Fig 3. ***Upper panel*** **The theoretical cumulative numbers of** ***Cryptosporidium parvum*** **oocysts and** ***Giardia lamblia*** **cysts seeded to the water in three 38-l aquaria with freshwater mollusks,** ***Corbicula fluminea*** **clams and** ***Dreissena polymorpha*** **mussels (aquarium A, B, and C, as described in Fig. 1.** ***Lower panel*** **The overall mean percentage of oocysts and cysts identified in the tissue of bivalves maintained in aquaria with** ***Cryptosporidium parvum*** **and** ***G. lamblia*****-seeded water.**

Conclusions

- *Corbicula fluminea* collected from the Anacostia River are highly contaminated with humans waterborne pathogens such as *Cryptosporidium parvum*, *Giardia lamblia*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem* , and *Enterocytozoon bieneusi.*
- Anacostia River waters is contaminated with human pathogens.

- *Corbicula fluminea* clams are able to bioaccumulate waterborne parasites recovered from contaminated water in proportion to ambient concentrations.
- *Corbicula fluminea* clams can be used as bioindicators for waterborne contamination and for sanitary assessment of water quality.
- *Corbicula* clams have an important role in aquatic habitats because of filtering suspended particles, thereby clarifying the water and improving water quality.
- *Corbicula fluminea* clams are convenient for biomonitoring because they form dense populations, do not have economic value, are easily collected, have a relatively small size, and occur in large numbers that facilitate collection of a large sample.

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