Cryptosporidium in Bivalves as Indicators of Fecal Pollution in The California Coastal Ecosystem

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Fecal pollution flowing from land to sea poses major health risks. New methods using bivalves (such as clams and mussels) as bio-indicators of fecal contamination were designed and evaluated in our lab. We then successfully applied these methods to monitor fresh and marine water quality in coastal California ecosystems. Our results suggest that humans and animals ingesting fecal-contaminated water and shellfish may be exposed to both host-specific and anthropozoonotic *Cryptosporidium* genotypes of public health significance.

Fecal pathogen pollution at the terrestrial-aquatic interface poses health risks to both humans and animals. Sensitive and specific detection of zoonotic fecal protozoa, such as *Cryptosporidium* spp., is needed to investigate fecal pathogen pollution flowing from land to sea. Environmental monitoring for fecal protozoa can be problematic, partly because of the dilution effect that occurs as oocysts are disseminated from the feces of animals and humans to terrestrial and aquatic ecosystems, and also because particulate matter can inhibit or interfere with detection methods. Bivalve molluscs can concentrate protozoal oocysts from fecal-contaminated aquatic environments, and may therefore be useful for monitoring water quality.

The objective of Dr. Woutrina Miller's PhD research was to obtain data on the epidemiology of the fecal pathogen *Cryptosporidium* in freshwater, estuarine, and nearshore marine ecosystems in California. Goals were to evaluate innovative *Cryptosporidium* detection techniques, to assess the genotypes of *Cryptosporidium* flowing from land to sea, and to identify risk factors for fecal contamination in coastal ecosystems.

First, a real-time TaqMan polymerase chain reaction (PCR) system that allows large scale, semi-quantitative detection of *Cryptosporidium* spp. was compared to more conventional detection methods in mussels (*Mytilus californianus*) using tissue spiking experiments. Next, tank exposure experiments and then sentinel bivalve studies in the field were conducted to evaluate clams (*Corbicula* spp.) and mussels (*Mytilus* spp.) as bioindicators of fecal contamination in freshwater and marine ecosystems, respectively, in California.

Our results indicate that TaqMan PCR, conventional PCR, and fluorescent antibody techniques can all be used to detect *Cryptosporidium* in bivalves. The most sensitive method was immunomagnetic concentration of bivalve digestive gland followed by fluorescent antibody detection. Applying these methods in controlled tank exposure experiments confirmed that mussels and clams can concentrate *Cryptosporidium* oocysts from inoculated waters, and that oocysts are detectable for days to weeks post-exposure.

Our use of sentinel clams in the field was the first report worldwide utilizing *Corbicula* clams to detect *Cryptosporidium* and *Giardia* in natural riverine ecosystems in California. *Cryptosporidium* oocysts and *Giardia* cysts were both detected significantly more often in the wet season than in the dry season.

Additionally, our use of sentinel clams in the field was the first multi-year project using bivalves to detect *Cryptosporidium* on the Pacific coast. We successfully detected zoonotic as well as host-specific genotypes of

Dr. Woutrina Miller's PhD project involved collecting mussels near Bodega Bay.

Cryptosporidium. Exposure to freshwater outflow and recent precipitation preceding bivalve collection were identified as risk factors associated with protozoal detection in mussels.

These findings indicate that, in California, fecal contamination is flowing from land to sea and clams and mussels can be used to monitor water quality. Humans and animals ingesting fecal-contaminated water and shellfish may be exposed to both host-specific and anthropozoonotic *Cryptosporidium* genotypes of public health significance. Continued investigation is needed to better understand the dynamics of fecal pathogen pollution and how to manage it in order to minimize health risks.

Professional Presentations

Smith, W.A., E.R. Atwill, H.M. Fritz, R.P. Hedrick, A.C. Melli, K.D. Arkush, and P.A. Conrad, Detecting environmental levels of *Cryptosporidium* in clams (*Corbicula fluminea*), International Association for Aquatic Animal Medicine, Kona, Hawaii, May, 2003.

Miller, W.A., M.A. Miller, I.A. Gardner, E.R. Atwill, M. Harris, J. Ames, D.A. Jessup, K. Worcester, D. Paradies, A.C. Melli, N.M. Barnes, P. Olin, and P.A. Conrad, *Cryptosporidium* epidemiology in fecal impacted coastal California ecosystems, using mussels (*Mytilus* spp.) as bioindicators. International *Giardia* and *Cryptosporidium* Congress, Amsterdam, Netherlands, September, 2004.

Miller, W.A., E.R. Atwill, I.A. Gardner, M. Miller, M. Harris, J. Ames, D. Jessup, S. Jang, B. Byrne, A. Melli, D. Paradies, K. Worcester, P. Olin, and P. Conrad, Habitat quality in California: risk factors for detecting fecal pathogens in coastal ecosystems. International Association for Aquatic Animal Medicine Conference, Seward, Alaska, May, 2005.

Miller, W.A., P. Conrad, M. Miller, I.A. Gardner, E.R. Atwill, M. Harris, J. Ames, D. Jessup, D. Paradies, K. Worcester, A. Melli, N. Barnes, P. Olin, and P.A. Conrad, Detection of pathogenic protozoa in marine ecosystems using mussels as bioindicators. International Wildlife Disease Association, Cairns, Queensland, Australia, June, 2005.

Miller, W.A., E.R. Atwill, I.A. Gardner, M. Miller, B. Byrne, M. Harris, J. Ames, D. Jessup, S. Jang, A. Melli, D. Paradies, K. Worcester, and P.A. Conrad. Risk factors for detecting protozoal and bacterial fecal pathogens in coastal California ecosystems, World Assoc. for the Advancement of Veterinary Parasitology, Christchurch, New Zealand, October, 2005.

Publications

Miller, W.A., E.R. Atwill, I. Gardner, M. Miller, H. Fritz, R. Hedrick, A. Melli, N. Barnes, and P.A. Conrad, Clams (*Corbicula fluminea*) as bioindicators of fecal contamination with *Cryptosporidium* and *Giardia* in freshwater ecosystems of California, *International Journal for Parasitology,* 2005, 35(6):673-684.

Miller, W.A., M.A. Miller, I.A. Gardner, E.R. Atwill, A.C. Melli, M. Harris, J. Ames, K. Worcester, N. Barnes, D. Jessup, and P.A. Conrad, New genotypes and factors associated with *Cryptosporidium* detection in mussels (*Mytilus* spp.) along the California coast, *International Journal for Parasitology,* 2005, June 30 [Epub].

Miller, W.A., I.A. Gardner, C.M. Leutenegger, M. Miller, E.R. Atwill, R. Hedrick, A.C. Melli, N. Barnes, and P.A. Conrad, Evaluation of methods for improved detection of *Cryptosporidium* spp. in mussels, *Journal of Microbiological Methods*, 65(3):367-379, 2005.

Sentinel freshwater clams just before placement in the San Lorenzo River.

Collaborative Efforts

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