Zoonotic protozoa in the marine environment: a threat to aquatic mammals and public health

Edited by: R. Fayer, D. Lindsay


Abstract

This collection of abstracts provides an account of four presentations at the 19th International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (held in New Orleans, LA, USA from 10–14 August 2003) in a symposium session on zoonotic protozoan parasites found in the marine environment and chaired by Ronald Fayer and David Lindsay. The focus was on three genera of parasites of veterinary and public health concern—Toxoplasma, Giardia, and Cryptosporidium with emphasis on their epidemiology in the marine environment.

Keywords: Toxoplasma; Giardia; Cryptosporidium; Shellfish; Oysters; Mussels; Pinnipeds; Seals; Whales; Sea otters; Epidemiology; Detection

Giardia duodenalis and Cryptosporidium parvum infections in pinnipeds

M.E. Olson, A. Appelbee, L. Measures

University of Calgary, Calgary, Alta., Canada; Fisheries and Oceans Canada, Maurice Lamontagne Institute, Mont-Joli, Que., Canada

Sixteen beluga whales (Delphinapterus leucas) and 15 ringed seals (Phoca hispida) from the western Arctic region of Canada were examined for giardiosis and cryptosporidiosis.


(Olson et al., 1997). Contents from the rectum and colon were collected. *Giardia* sp. cysts were identified in three of 15 (20%) seals, implicating them as a potential reservoir for this zoonosis in the arctic. Cysts of *Giardia* sp. were also detected in feces from 20 of 74 (28%) seals examined from the eastern coast of Canada in 1997 and 1998 using a monoclonal antibody (Measures and Olson, 1999). Seal species included 15 adult harp seals (*Phoca groenlandica*), 4 adult grey seals (*Halichoerus grypus*), and 1 juvenile harbor seal (*Phoca vitulina*). Cysts were not detected in seal pups less than 1 year of age. The highest prevalence (50%) was found in adult harp seals near the Magdalen Islands in the Gulf of St. Lawrence. The overall prevalence of *Giardia* sp. in adult grey and harbor seals from the Gulf and St. Lawrence estuary was 23%. Feces from 11 beluga whales (*Delphinapterus leucas*) and 1 northern bottle-nosed whale (*Hyperoodon ampullatus*) stranded in the St. Lawrence estuary were negative for *Giardia* sp. cysts. All *Giardia* isolated belonged to the Assemblage A genotype, which is considered zoonotic. Immunoglobulins (IgG) specific for *Giardia* could be demonstrated in the serum and milk of seals. *Cryptosporidium* oocysts could not be demonstrated in the seals or whales. Weanling harp seals pups were experimentally challenged with *Giardia duodenalis* (Assemblage A, sheep origin) and *Cryptosporidium parvum* (Cattle genotype). Both *Giardia* and *Cryptosporidium* are capable of infecting harp seal pups through direct and indirect routes. This is the first report of cryptosporidiosis in phocids and also demonstrated indirect, water-borne transmission of *Giardia* and *Cryptosporidium* from experimentally inoculated to non-inoculated seals via fecal contamination of the tank salt water. The significance and origin of *Giardia* and *Cryptosporidium* in marine mammals is unknown. Infections may result from pathogen pollution as seals are infected with zoonotic genotypes and the St. Lawrence receives sewage and agricultural runoff. It is unknown whether seals become clinically ill following infection or infections make them susceptible to starvation, predation or other infectious diseases. These findings have significant impacts on public health, environment and wildlife conservation.

References


*Toxoplasma gondii* in California sea otters (*Enhydra lutris nereis*): past and present

R.A. Cole*\(^a\), D.S. Lindsay\(^b\), J.P. Dubey\(^c\), N.J. Thomas\(^a\)

\(^a\)USGS, NWHC, Madison, WI, USA; \(^b\)Virginia Tech, Blacksburg, VA, USA; \(^c\)USDA, ARS, Beltsville, MD, USA

In the early 1990’s, National Wildlife Health Center Researchers began a comprehensive necropsy and diagnostic investigation into mortality factors affecting the southern sea otters. This effort was in response to a declining otter population that had comparable birth rates to a growing Alaskan subspecies population. Disease was found to be one of the major causes of mortality. *Toxoplasma gondii* was identified in 1994 and isolated in cell culture in 1995. Biological and molecular characterization was conducted on the strains isolated from the
brains and heart tissue of 15 southern sea otters after isolation in cell cultures (Cole et al., 2000). These strains were used to infect mice and mouse brains containing cysts were fed to cats that excreted oocysts that were infectious for mice. Molecular analysis indicated that 13 strains were Type II, those associated with the majority of human cases, but were genetically distinct from one another. The overall picture that emerges from this study is that oocysts of *Toxoplasma* are capable of entering and surviving in seawater while retaining the ability to initiate infection in susceptible hosts. Initial questions as to the oocyst’s ability to sporulate in seawater were raised and cats implicated as a most likely source of oocysts. New data have demonstrated that *T. gondii* oocysts can sporulate in sea water and that they can remain infectious for several weeks in sea water. Recent work (Lindsay et al., 2001) on the eastern oyster (*Crassostrea virginica*) indicated that oysters can act as phoretic agents removing oocysts from seawater and carrying infectious oocysts for up to 3 weeks as confirmed in a mouse bioassay system. Other filter feeding marine bivalves may also serve as paratenic hosts that assimilate and concentrate *Toxoplasma* oocysts. Experimentally exposed mussels (*Mytilus galloprovincialis*) were found to contain *Toxoplasma*-specific ssrRNA as long as 21 days post-exposure and mice could be infected with mussel tissue for at least 3 days post-exposure (Arkush et al., 2003). These experimental data on marine bivalves provide a basis for considering that contaminated free-living shellfish may well act as a phoretic agents and “collectors” of oocysts thus exposing the otters that prey on them.

**References**


**An update on Toxoplasma gondii infections in California sea otters**

M. Miller a,b,⁎, P. Conrad a, J. Gardner a, C. Kreuder a, J. Mazer a, David Jessup b, Erin Dodd b, Mike Harris b, Jack Ames b, Karen Worcester c, David Paradies c, Michael Grigg d

a School of Veterinary Medicine, UC Davis, CA, USA; b CDFG, Santa Cruz, CA, USA; c Water Quality Board, San Luis Obispo, CA, USA; d Stanford Medical School, Palo Alto, USA

*Toxoplasma gondii* infection is associated with fatal meningoencephalitis in southern sea otters (*Enhydra lutris nereis*), a federally-listed threatened species (Kreuder et al., 2003; Thomas and Cole, 1996). Sea otters live and feed exclusively in the nearshore marine environment, and the source of *T. gondii* infection for these animals is unknown. Aside from cats, no other hosts have been identified that shed *Toxoplasma* oocysts, and otters rarely, if ever, consume recognized intermediate hosts. Since 1997, necropsies were completed on freshly dead California sea otters, along with serological testing, parasite isolation, and brain immunohistochemistry. Because of the apparent terrestrial origin of *T. gondii*,
we expected to find that natural infections of sea otters were uncommon. However, *T. gondii* infections were detected in 36% of freshly dead otters examined between 1997 and 2001. An indirect fluorescent antibody test (IFAT) for *T. gondii* was developed and validated (Miller et al., 2002a) and used to screen sera from live, free-ranging sea otters from California, Washington, and Alaska (Miller et al., 2002b); 36% of free-ranging California sea otters were seropositive for *T. gondii,* compared to 38% of Washington otters and 0% of Alaskan otters. To investigate the apparent emergence of *T. gondii* infections in California otters, spatial, environmental, and demographic data from 223 sea otters were examined for associations with *T. gondii* exposure. Risk factors associated with *T. gondii* seropositivity included male gender and older age class. Spatial analysis revealed two “high-risk” sites for *T. gondii* exposure. Most importantly, otters sampled near heavy freshwater outflow were almost three times more likely to be seropositive to *T. gondii* than otters sampled near areas of low flow.

The association between anthropogenic environmental disturbance, pathogen pollution and the emergence of infectious diseases in wildlife has been postulated, but not always well supported by epidemiologic data.

This study provides specific evidence of contamination of the coastal marine ecosystem with the zoonotic pathogen, *T. gondii* and reveals extensive infection of the threatened California southern sea otter populations along the California coast. It also supplies statistical evidence implicating land-based surface runoff as a source of *T. gondii* infection for sea otters. Sea otters and humans compete for some of the same benthic invertebrate prey. Thus, if otters are ultimately found to be exposed to *T. gondii* and other pathogens through consumption of filter-feeding prey, this study has potent implications for human health.

**References**


**Atlantic and Gulf Coast study of Cryptosporidium in shellfish**

Ronald Fayera, Earl J. Lewisb, James M. Trouta, Lihua Xiaoc, Dorothy W. Howardb, Robert Palmera, Kristie Ludwiga, Suzanne S. Tylerb

a USDA, Agriculture Research Station, ANRI, Animal Waste Pathogen Laboratory, Beltsville, MD 20705, USA; b NOAA, NOS, Coastal Center for Environmental Health and Biomolecular Research, Oxford, MD 21654, USA; c Centers for Disease Control and Prevention, Chamblee, GA 30341, USA
Studies in the late 1990s showed that oocysts of Cryptosporidium could survive for long periods in seawater (Fayer et al., 1998), could be filtered from seawater by experimentally exposed oysters (Fayer et al., 1997), and were found in a high prevalence within tissues, on gill surfaces, and in hemocytes of oysters at 13 sites in Chesapeake Bay, MD including 9 sites open to shellfish harvest (Fayer et al., 1999). A later 3 year study of the Bay indicated higher numbers of infected oysters after rainfall events (Fayer et al., 2002). Molecular analyses identified four species of Cryptosporidium in oysters, two of which have been found in outbreaks affecting humans (C. hominis and C. parvum). Cryptosporidium has also been found in hard clams, bent mussels, and zebra mussels (Graczyk et al., 1999, 2001). These findings indicate the presence of human and/or animal feces possibly from sewage outfalls leaky septic systems, or farm runoff in shellfish growing waters.

To determine the distribution of Cryptosporidium over a greater geographic area, USDA, NOAA, and CDC scientists obtained oysters and hard clams from retail markets in Atlantic and Gulf coast locations in 2001 and 2002 (Fayer et al., 2003). Twenty-five shellfish were examined at each of 49 collection sites (33 oyster and 16 clam) from New Brunswick, Canada to Florida to Texas. Oocysts were detected in shellfish from New Brunswick, in 11 Atlantic, and 1 Gulf coast states. Of 1225 oysters and clams examined by IFA microscopy 4% were found to harbor Cryptosporidium. PCR detected Cryptosporidium DNA in 35.2% of 185 pools of gill washings from Atlantic shellfish and 1.7% of 60 pools of Gulf shellfish. Cryptosporidium parvum, C. hominis, and C. meleagridis, all infectious for humans, were found in commercial shellfish from 59% of the sites by either IFA or PCR. These specimens, acquired at one time point during a time of drought, may or may not be indicative of the long-term prevalence of Cryptosporidium in coastal shellfish. Despite these and other findings of Cryptosporidium in shellfish, there have been no reports of cryptosporidiosis linked to eating raw shellfish.

References