
Neospora caninum Infection in Marine Mammals Stranding in Northeastern Pacific Ocean Region

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We used immunohistochemistry and PCR to identify *Neospora caninum* in 6 infected marine mammal species, including 2 pups, that stranded in the northeastern Pacific Ocean. Our findings suggest the expansion of this parasite's host range to marine mammals, underscoring the effect of terrestrial pathogens that flow from land to sea.

Researchers using immunohistochemistry and PCR-DNA sequencing analyses have confirmed high infection rates of the protozoan parasites *Toxoplasma gondii* and *Sarcocystis neurona*, often as mixed infections, in a range of stranded pinniped, cetacean, and mustelid species (1,2). Parasite transmission is closely linked to land-to-sea pathogen flow (3,4). We identified *Neospora caninum*, a protozoan pathogen known to affect reproductive fitness in livestock, in 6 different species of marine mammals stranded in the northeastern Pacific Ocean region. The emergence of *N. caninum* illustrates a third terrestrially sourced parasite (also referred to as a pollutagen) flowing from land to sea to infect marine mammals in this region. This parasite is distinct from previously reported *N. caninum*-like parasites that circulate between pinnipeds in a marine cycle (5).

In cattle, *N. caninum* is considered among the most efficient pathogens to be transmitted transplacentally (6). This pathogen is a major contributor of

reproductive loss in the dairy industry worldwide. Dogs and wild canids, including foxes, wolves, and coyotes, are among the recognized hosts, both definitive and intermediate, for this parasite. Unlike most coccidian parasites that have a limited host range, *N. caninum* is increasingly detected in a wide array of terrestrial and avian species (7). Although reports have identified antibodies to *N. caninum* in prior serosurveys of marine mammals in Australia, Japan, and the United States (8–10), those assays were not validated for wildlife. Cross-reactivity with related coccidian parasites that commonly circulate between marine mammals in a marine cycle may have confounded interpretation of the results (11). We report 8 confirmed cases of *N. caninum* infection in 6 marine mammal species, including 2 pups and a pregnant female.

The Study

Throughout the northeastern Pacific region, local and regional marine mammal stranding networks respond to live stranded and dead marine mammals. In this case series, wildlife officials delivered a California sea lion (*Zalophus californianus*) for rehabilitation. Despite supportive care, the animal declined and was euthanized. We identified a solitary *N. caninum* infection using PCR, observing no discernible parasites by histopathology. As a result of

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that finding, we conducted a retrospective database analysis on 410 stranded marine mammals previously screened by PCR-DNA sequencing at the internal transcribed spacer (ITS) 1 marker. We identified 7 additional cases of *N. caninum* infection in another 5 species of marine mammals. The geographic range of the *N. caninum*-infected animals (Figure 1) suggested multiple points, rather than a single point source, of parasite exposure.

We documented the stranding location, recorded signalment, compiled morphometrics, and performed a necropsy for all 8 cases (Table). We also harvested representative tissues for histopathology and immunohistochemistry and froze samples for ancillary diagnostic studies. We conducted PCR-DNA sequencing using pan-Apicomplexan primers that amplify across the ITS1 region for speciation. We carried out immunohistochemistry for *N. caninum*, *T. gondii*, and *S. neurona* on available tissues from 4 PCR-positive animals, following previously reported protocols (1,12).

Phylogenetic analysis at the ITS1 locus established unequivocally that all 8 animals were infected with an identical sequence type that resolved as *N. caninum* (GenBank accession nos. PX529932–8) (Figure 2). For comparison, we included in the tree sequences recovered from other pinnipeds infected by *N. caninum*-like parasites that commonly circulate within a marine transmission cycle (5). We conducted PCR testing, determining 7 of the 8 *N. caninum*-infected animals had polyparasite infections with 1 or 2 other terrestrially sourced coccidian agents (*T. gondii*, *S. neurona*) in their tissues. Infected animals were 2 sea otters (*Enhydra lutris*), 2 harbor seals (*Phoca vitulina*), 1 northern elephant seal (*Mirounga angustirostris*), 1 Guadalupe fur seal (*Arctocephalus townsendi*), and 1 Steller sea lion (*Eumetopias jubatus*) (Table).

We diagnosed nonsuppurative and necrotizing encephalitis (n = 2), meningoencephalomyelitis (n = 1), and meningoencephalitis (n = 1) by histology of the brain for 4 of 6 cases, including the pup and subadult harbor seals, adult Steller sea lion, and yearling California sea lion. We conducted immunohistochemistry in 4 encephalitic cases, observing *N. caninum* antigen in brain samples from the harbor seal pup and *T. gondii* and *S. neurona* (one or both) antigens in the other 3 animals (Figure 3). We found no pathognomonic lesions in this case series directly attributed to *N. caninum* infection. The cause of death for all 8 animals was independent of *N. caninum* infection (Appendix Table, <https://wwwnc.cdc.gov/EID/article/32/2/25-1507-App1.pdf>).

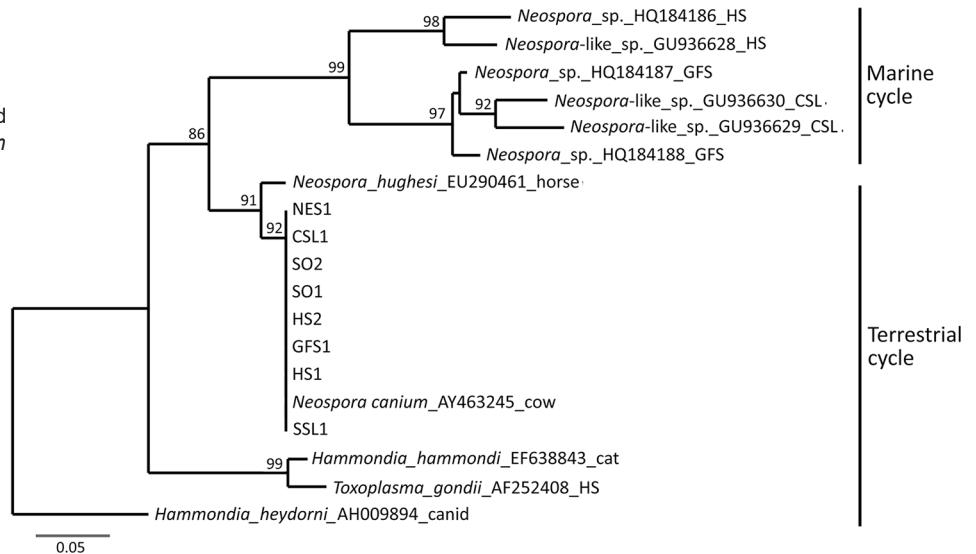


Figure 1. Stranding locations and animal species in study of *Neospora caninum* infection in marine mammals stranding in the northeastern Pacific Ocean region. NES, northern elephant seal (*Mirounga angustirostris*); CSL, California sea lion (*Zalophus californianus*); HS, harbor seal (*Phoca vitulina*); GFS, Guadalupe fur seal (*Arctocephalus townsendi*); SSL, Steller sea lion (*Eumetopias jubatus*); SO, sea otter (*Enhydra lutris*).

Conclusion

We identified 8 animals from 6 marine mammal species unequivocally infected with *N. caninum*. Infection by this parasite alone was confirmed in 1 animal, a yearling California sea lion, and the infection represents another example of land-to-sea flow of a pollutant of parasite origin. The contribution of this pathogen to marine mammal health is unknown. All infected animals we studied were from species with varying degrees of philopatry, with either short natal dispersal distances or extended oceanic to pelagic migrations. Seasonal haul out associated with pupping and breeding, coupled with accounts of wild canids scavenging at rookeries (13), provide insights into potential parasite introduction, persistence, and dissemination. For sea otters, bioaccumulation of parasites by invertebrate prey species may be inferred from observations of transmission dynamics with *T. gondii* (4). On the basis of gross and microscopic findings, we determined *N. caninum* infection to be unre-

Figure 2. Phylogenetic relationships among *Neospora caninum*-like species that circulate in a marine cycle compared with terrestrial-sourced species from study of *N. caninum* infection in marine mammals stranding in the northeastern Pacific Ocean region. Neighbor-joining consensus tree shows marine strains that commonly infect pinnipeds and terrestrial strains (GenBank accession numbers PX529932–8) that transferred from land to sea to infect 5 species of pinnipeds. Evolutionary distances computed using the Tamura-Nei genetic distance model at the complete internal transcribed spacer 1 locus. Tree inferred using the outgroup sequence from *Hammondia heydorni* to root the tree; 1,000 bootstrap values listed at supported nodes. NES, northern elephant seal (*Mirounga angustirostris*); CSL, California sea lion (*Zalophus californianus*); HS, harbor seal (*Phoca vitulina*); GFS, Guadalupe fur seal (*Arctocephalus townsendi*); SSL, Steller sea lion (*Eumetopias jubatus*); SO, sea otter (*Enhydra lutris*).



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lated to the proximate cause of death in the examined animals. Nevertheless, detection of *N. caninum* in a pregnant Steller sea lion, as well as in a harbor seal pup and northern elephant seal pup (weanling), is concerning. Recrudescence of latent infection during pregnancy contributes to parasite reactivation that results in vertical transmission in cattle and dogs (6), and we propose this phenomenon might be occurring sporadically in marine mammals.

Documented reports have confirmed land-to-sea transmission of protozoan parasites, and serosurveys for *N. caninum* have detected titers in marine mammal

species. A recent review documents the global extent of land-to-sea pathogen flow (14). Seals and sea lions are monophyletic, members of the order Carnivora, and share a common ancestry with terrestrial canids. This evolutionary relationship may predispose these marine mammals to pollutants defecated by canids. Cross reactivity with previously described nonpathogenic *N. caninum*-like parasites that circulate among pinnipeds in a marine transmission cycle, including coccidia types A and B (with California sea lions as definitive hosts) (5), may have contributed to false-positive results in prior serosurveys. The contribution of these *N. caninum*-like parasites to immune protection of hosts against *N. caninum* is unknown. In this limited series, we confirmed *N. caninum* infection in 2 sea otters, 2 harbor seals, 1 California sea lion, 1 northern elephant seal, 1 Steller sea lion, and 1 Guadalupe fur seal, none of which had been infected with previously described *N. caninum*-like parasites. Those findings extend the host range and ecologic niche for *N. caninum*. Infections were predominantly mixed with *T. gondii*, *S. neurona*, or both. Climate change, ecologic marine regime shifts, rural and urban development, weather events, and other environmental perturbations may lead to incursion of previously recognized land-based pathogens into the marine environment.

In conclusion, our results indicate that further investigations to characterize the life history of *N. caninum* in the marine environment, the role of polyparasitism in disease manifestation, and the potential pathogenicity in susceptible host species are

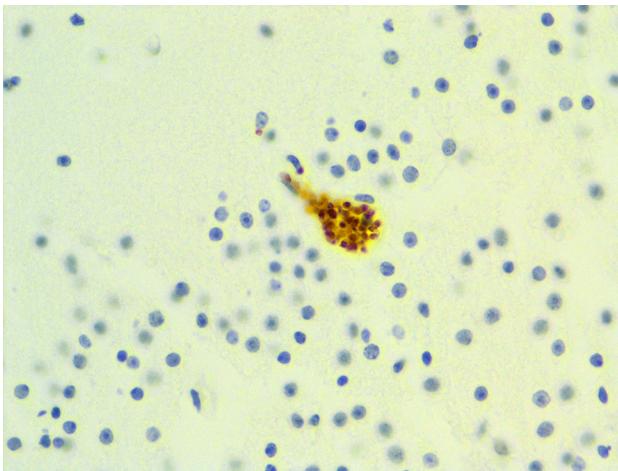


Figure 3. Immunohistochemistry of a harbor seal pup with *Neospora caninum* antigen localized within a neuron from study of *N. caninum* infection in marine mammals stranding in the northeastern Pacific Ocean region. Original magnification $\times 600$.

warranted. Whereas we cannot definitively attribute *N. caninum* as the cause of illness or death for the animals described here, future investigations of similar unexpected deaths, particularly those involving abortion storms among coastal breeding marine mammals in the northeastern Pacific, should consider molecular screening for this pathogen.

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References

- Gibson AK, Raverty S, Lambourn DM, Huggins J, Magargal SL, Grigg ME. Poly-parasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. *PLoS Negl Trop Dis*. 2011;5:e1142. <https://doi.org/10.1371/journal.pntd.0001142>
- Miller MA, Shapiro K, Murray MJ, Haulena M, Raverty SA. Protozoan parasites of marine mammals. In: Gulland FMD, Dierauf LA, Whitman KL, editors. *CRC handbook of marine mammal medicine*, 3rd edition. Oxford: CRC Press; 2018;chap 20:p. 1-46.
- Burgess TL, Tinker MT, Miller MA, Smith WA, Bodkin JL, Murray MJ, et al. Spatial epidemiological patterns suggest mechanisms of land-sea transmission for *Sarcocystis neurona* in a coastal marine mammal. *Sci Rep*. 2020;10:3683. <https://doi.org/10.1038/s41598-020-60254-5>
- Conrad PA, Miller MA, Kreuder C, James ER, Mazet J, Dabritz H, et al. Transmission of *Toxoplasma*: clues from the study of sea otters as sentinels of *Toxoplasma gondii* flow into the marine environment. *Int J Parasitol*. 2005;35:1155-68. <https://doi.org/10.1016/j.ijpara.2005.07.002>
- Carlson-Bremer D, Johnson CK, Miller RH, Gulland FM, Conrad PA, Wasmuth JD, et al. Identification of two novel coccidian species shed by California sea lions (*Zalophus californianus*). *J Parasitol*. 2012;98:347-54. <https://doi.org/10.1645/GE-2752.1>
- Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin Microbiol Rev*. 2007;20:323-67. <https://doi.org/10.1128/CMR.00031-06>
- Almeria S. *Neospora caninum* and wildlife. *ISRN Parasitol*. 2013;2013:947347. <https://doi.org/10.5402/2013/947347>
- Dubey JP, Zarnke R, Thomas NJ, Wong SK, Van Bonn W, Briggs M, et al. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis canis*-like infections in marine mammals. *Vet Parasitol*. 2003;116:275-96. [https://doi.org/10.1016/S0304-4017\(03\)00263-2](https://doi.org/10.1016/S0304-4017(03)00263-2)
- Omata Y, Umeshita Y, Watarai M, Tachibana M, Sasaki M, Murata K, et al. Investigation for presence of *Neospora caninum*, *Toxoplasma gondii* and *Brucella*-species infection in killer whales (*Orcinus orca*) mass-stranded on the coast of Shiretoko, Hokkaido, Japan. *J Vet Med Sci*. 2006;68:523-6. <https://doi.org/10.1292/jvms.68.523>
- Wong A, Lanyon JM, O'Handley R, Linedale R, Woolford L, Long T, et al. Serum antibodies against *Toxoplasma gondii* and *Neospora caninum* in southeast Queensland dugongs. *Mar Mamm Sci*. 2020;36:180-94. <https://doi.org/10.1111/mms.12629>
- Donahoe SL, Lindsay SA, Krockenberger M, Phalen D, Šlapeta J. A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. *Int J Parasitol Parasites Wildl*. 2015;4:216-38. <https://doi.org/10.1016/j.ijppaw.2015.04.002>
- Barbosa L, Johnson CK, Lambourn DM, Gibson AK, Haman KH, Huggins JL, et al. A novel *Sarcocystis neurona* genotype XIII is associated with severe encephalitis in an unexpectedly broad range of marine mammals from the northeastern Pacific Ocean. *Int J Parasitol*. 2015;45:595-603. <https://doi.org/10.1016/j.ijpara.2015.02.013>
- Reid RE, Gifford-Gonzalez D, Koch PL. Coyote (*Canis latrans*) use of marine resources in coastal California: a new behavior relative to their recent ancestors. *Holocene*. 2018;28:1781-90. <https://doi.org/10.1177/0959683618788714>
- Song L, Hu C, Zhang X, Liu Y, Song G, Wu J, et al. Effects of feeding on microbial community structure and pathogen abundance in marine aquaculture ponds. *Mar Environ Res*. 2025;210:107319. <https://doi.org/10.1016/j.marenvres.2025.107319>

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