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Protozoal Meningoencephalitis in Sea Otters (*Enhydra lutris*): a Histopathological and Immunohistochemical Study of Naturally Occurring Cases

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Summary

Protozoal meningoencephalitis is considered to be an important cause of mortality in the California sea otter (*Enhydra lutris*). Thirty nine of 344 (11.3%) California (CA) and Washington state (WA) sea otters examined from 1985 to 2004 had histopathological evidence of significant protozoal meningoencephalitis. The aetiological agents and histopathological changes associated with these protozoal infections are described. The morphology of the actively multiplicative life stages of the organisms (tachyzoites for *Toxoplasma gondii* and merozoites for *Sarcocystis neurona*) and immunohistochemical labelling were used to identify infection with *S. neurona* ($n = 22$, 56.4%), *T. gondii* ($n = 5$, 12.8%) or dual infection with both organisms ($n = 12$, 30.8%). Active *S. neurona* was present in all dual infections, while most had only the latent form of *T. gondii*. In *S. neurona* meningoencephalitis, multifocal to diffuse gliosis was widespread in grey matter and consistently present in the molecular layer of the cerebellum. In *T. gondii* meningoencephalitis, discrete foci of gliosis and malacia were more widely separated, sometimes incorporated pigment-laden macrophages and mineral, and were found predominantly in the cerebral cortex. Quiescent tissue cysts of *T. gondii* were considered to be incidental and not a cause of clinical disease and mortality. Protozoal meningoencephalitis was diagnosed more frequently in the expanding population of WA sea otters (10 of 31, 32.3%) than in the declining CA population (29 of 313, 9.3%). Among sea otters with protozoal meningoencephalitis, those that had displayed neurological signs prior to death had active *S. neurona* encephalitis, supporting the conclusion that *S. neurona* is the most significant protozoal pathogen in the central nervous system of sea otters.

Keywords: Enhydra lutris; sea otter; immunohistochemistry; meningoencephalitis; *Sarcocystis neurona*; *Toxoplasma gondii*

Introduction

Case reports have described protozoal meningoencephalitis in a variety of marine mammals including several pinniped and dolphin species, West Indian manatee (*Trichechus manatus*), Beluga whale (*Delphinapterus leucas*), and sea otter (*Enhydra lutris*) (Dubey *et al.*, 2003a; Honnold *et al.*, 2005). The protozoan reported in most cases was *Toxoplasma gondii*, but *Sarcocystis neurona* has been found in Pacific harbour seals (*Phoca vitulina*) (Lapointe *et al.*, 1998; Miller *et al.*, 2001a) and sea otters (Lindsay *et al.*, 2000; Miller *et al.*, 2001b), and an unidentified apicomplexan protozoan was described in a Pacific harbour seal (Lapointe *et al.*, 2003). More is known about these infections in sea otters because they have been investigated in serological studies and through necropsy surveillance. Protozoal meningoencephalitis was cited as an important cause of mortality in sea otters (Thomas and Cole, 1996; Kreuder *et al.*, 2003).

The sea otter is a large mustelid that feeds on invertebrates in the near-shore marine environment along

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the coasts of California, Washington, British Columbia, Alaska and Russia. The California (CA) subspecies (*E. lutris nereis*) is listed as threatened under the Federal Endangered Species Act. This population has had slow growth throughout its history and a notable decline during the second half of the 1990s (Estes *et al.*, 2003), while the population of Washington (WA) sea otters (*E. lutris kenyoni*) grew more rapidly and expanded its range during the same period (Laidre *et al.*, 2002). Infectious disease was reported to be the most common cause of mortality in CA sea otters, with protozoal meningoencephalitis causing between 8.5% and 22.9% of mortality (Thomas and Cole, 1996; Kreuder *et al.*, 2003). Two causative agents were identified: *T. gondii* and *S. neurona* (Rosonke *et al.*, 1999; Cole *et al.*, 2000; Lindsay *et al.*, 2000; Miller *et al.*, 2001b).

Both *T. gondii* and *S. neurona* are apicomplexan coccidial parasites that have indirect two-host life cycles. In a suitable intermediate host these parasites asexually reproduce in host tissues and eventually form resting tissue cysts. The role of the sea otter in this cycle is that of the intermediate host. The life cycles of these parasites are completed when tissue containing a tissue cyst is ingested by the definitive host (felids for *T. gondii*; opossum for *S. neurona*). Oocysts from these definitive hosts are shed with faeces into the environment to potentially infect additional intermediate hosts and continue the life cycle (Dubey *et al.*, 1998, 2001d). There has been considerable interest in the mechanisms by which oocysts shed by terrestrial mammals become ingested by sea otters, and how these mechanisms might be managed to decrease sea otter exposure.

In order to guide effective management of this problem it is important to provide an assessment of the roles of the different agents in disease and mortality. However, protozoal meningoencephalitis in sea otters presents, to some degree, a diagnostic conundrum. Two different agents have been found to cause this condition and a dual infection with both agents has been reported (Lindsay *et al.*, 2001a). The problem is compounded by the fact that these agents are capable of producing chronic latent infection in suitable intermediate hosts. Previous studies in which *T. gondii* was isolated from the brains of sea otters without significant encephalitis provide evidence that latent infections also occur in sea otters (Cole *et al.*, 2000; Miller *et al.*, 2004). Latent *T. gondii* can become reactivated to cause disease recrudescence in an intermediate host. Serological surveys show that exposure to *T. gondii* is 60–80% in both CA and WA sea otters (Miller *et al.*, 2002b; Dubey *et al.*, 2003a), so the potential for latent infections and possibly even the residual "footprints" of inflammation from the acute infection may also be high. Less is known about *S. neurona* exposure in sea otters because no serological survey has been done. However the course of infection with *S. neurona* may be less complicated than with *T. gondii* because there is no evidence that encysted *Sarcocystis* spp. can become reactivated to cause disease recurrence.

In a previous study, *T. gondii* meningoencephalitis was determined to be either the primary cause of death or a significant contributor to the deaths of 27.6% of 105 CA sea otters examined from 1998 to 2001 (Kreuder *et al.*, 2003). The National Wildlife Health Center (NWHC) performed necropsies on 344 sea otters from 1985 to 2004. In this study we retrospectively review NWHC cases of protozoal meningoencephalitis in two populations of sea otters, one that declined and one that was expanding during the time period. Protozoal meningoencephalitis has been previously reported in both populations (Lindsay *et al.*, 2000, 2001a). The aims of the present study were to identify the aetiological agents of protozoal meningoencephalitis in these cases and to characterize the lesions and key diagnostic features that accompany active infection. Because mild meningitis, perivascular lymphocytic cuffs and latent *T. gondii* tissue cysts are common in sea otters, we also evaluated the prevalence of these lesions in a subset of otters that had not been diagnosed with significant encephalitis or protozoal meningoencephalitis and died from other causes.

Materials and Methods

Case Selection

Necropsy records of sea otters found dead or terminally ill along the coasts of CA and WA from 1985 to 2004 and examined at the National Wildlife Health Center (NWHC) of the US Geological Survey were reviewed; only cases in which brain histopathology was performed were included ($n = 344$). During this period the NWHC received all fresh dead sea otters found in WA, occasional otter carcasses from CA (1985–1991), all fresh dead otters found in CA (1992–1996), and every fourth fresh dead otter found in CA from 1997 to 2002. Most carcasses had been shipped refrigerated by overnight commercial carrier and necropsies were performed on arrival; however, several frozen carcasses were also examined. Cases were selected for histopathological review if protozoal meningoencephalitis had been diagnosed by necropsy as a primary cause of death, or was of sufficient severity to have been considered a significant cofactor in death. Cases were also selected if there was a diagnosis of significant nonsuppurative meningoencephalitis of unknown aetiology. In addition, sections of brain from 80 randomly selected CA sea otters were examined in order to determine the presence of inflammation and/or protozoa.