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EPIDEMIOLOGY AND PATHOLOGY OF *TOXOPLASMA GONDII* IN FREE-RANGING CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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ABSTRACT: The coccidian parasite *Toxoplasma gondii* infects humans and warm-blooded animals worldwide. The ecology of this parasite in marine systems is poorly understood, although many marine mammals are infected and susceptible to clinical toxoplasmosis. We summarized the lesions associated with *T. gondii* infection in the California sea lion (*Zalophus californianus*) population and investigated the prevalence of and risk factors associated with *T. gondii* exposure, as indicated by antibody. Five confirmed and four suspected cases of *T. gondii* infection were identified by analysis of 1,152 medical records of necropsied sea lions from 1975–2009. One suspected and two confirmed cases were identified in aborted fetuses from a sea lion rookery. Toxoplasmosis was the primary cause of death in five cases, including the two fetuses. Gross and histopathologic findings in *T. gondii*-infected sea lions were similar to those reported in other marine mammals. The most common lesions were encephalitis, meningitis, and myocarditis. The antibody prevalence in stranded, free-ranging sea lions for 1998–2009 was 2.5% (±0.03%; IgG titer 640). There was an increase in odds of exposure in sea lions with increasing age, suggesting cumulative risk of exposure and persistent antibody over time. The occurrence of disseminated *T. gondii* infection in aborted fetuses confirms vertical transmission in sea lions, and the increasing odds of exposure with age is consistent with additional opportunities for horizontal transmission in free-ranging sea lions over time. These data suggest that *T. gondii* may have two modes of transmission in the sea lion population. Overall, clinical disease was uncommon in our study which, along with low prevalence of *T. gondii* antibody, suggests substantially less-frequent exposure and lower susceptibility to clinical disease in California sea lions as compared to sympatric southern sea otters (*Enhydra lutris nereis*).

Key words: California sea lions, marine mammal, pathology, epidemiology, protozoal disease, *Toxoplasma gondii*, *Zalophus californianus*.

INTRODUCTION

Toxoplasma gondii infects humans and warm-blooded animals worldwide (Dubey and Beattie 1988; Tenter et al. 2000; Dubey and Jones 2008). Despite a complex life cycle involving felid definitive hosts and terrestrial warm-blooded intermediate hosts, *T. gondii* occurs in aquatic mammals (Dubey et al. 1970; Dubey and Beattie 1988; Miller 2008). *Toxoplasma gondii* has contributed to sea otter (*Enhydra lutris*) mortality both directly through encephalitis and indirectly by increasing the risk of shark attack (Kreuder et al. 2003). Detailed investigations of sea otter *T. gondii* infections are possible because collection of biologic samples is feasible, and resources are available to manage this federally threatened species (Johnson et al. 2009). Several species of mussels, oysters, and fish accumulate oocysts under natural and experimental conditions, demonstrating the potential of marine prey to serve as vectors for transmission to a range of marine mammal

species (Lindsay et al. 2001, 2004; Miller et al. 2008; Massie et al. 2010; Esmerini et al. 2010).

Published reports on *T. gondii* antibody titers in California sea lions (*Zalophus californianus*, hereafter referred to as CSL), a marine mammal with a range overlapping with and extending beyond that of the southern sea otter, indicate they are exposed to the parasite, and clinical toxoplasmosis has been reported in captive CSLs (Ratcliffe 1951; Migaki et al. 1977, Dubey et al. 2003; Miller 2008). We performed a retrospective study of archived cases to summarize the clinical and pathologic findings associated with *T. gondii* infection in CSLs. We also investigated *T. gondii* exposure in CSLs and potential risk factors and health-related outcomes associated with exposure.

MATERIALS AND METHODS

Cases

To identify *T. gondii* infections in CSLs we reviewed archived pathology records, and slides when available, from 1,152 animals that died during rehabilitation between 1975 and 2009 at The Marine Mammal Center (TMMC) in Sausalito, California. All CSLs had stranded along the central California coast (37°42′N, 123°05′W to 35°59′N, 121°30′W) and were transported to TMMC for evaluation and rehabilitation. Demographic and clinical information was obtained from medical records. We also evaluated pathology records and slides from 10 aborted CSL fetuses collected during a routine population survey at the San Miguel Island rookery in May 2004.

Pathologic evaluation

Complete necropsies were performed on all animals and representative, standardized samples from all major organs including brain, heart, lymph nodes, skeletal muscle, and liver were fixed with 10% buffered formalin and sent to either the Pathology Service, Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, California; the Zoological Pathology Program, University of Illinois at Urbana-Champaign, Illinois; or the Armed Forces Institute for Pathology (AFIP), Washington, DC for routine processing and histologic assessment. All case material was re-reviewed by a single pathologist (KMC), and cases of suspect or confirmed protozoal infection were identified based on the presence of protozoa or lesions consistent with protozoal infection including meningoencephalitis, myocarditis, myositis, hepatitis, vasculitis, and lymphadenitis (Thomas et al. 2007; Miller 2008). When archived material was available, tissues containing protozoa or lesions consistent with protozoal infection were evaluated using immunohistochemistry, PCR, or culture. Cases were further classified as confirmed cases of *T. gondii* infection when *T. gondii*-like tissue cysts or zoites were identified on histopathologic examination and at least one additional diagnostic test (immunohistochemistry, culture, or PCR) was positive for *T. gondii*. Suspected cases were defined as CSLs with *T. gondii*-like tissue protozoa observed histologically but when a confirmation of *T. gondii* using immunohistochemistry, culture, or PCR was not possible. For all confirmed and suspect cases, if present, inflammation in the brain, heart, skeletal muscle, lymph nodes, or other tissue with protozoal infection was graded subjectively as mild, moderate, or severe.

Material was available from nine cases for immunohistochemistry (IHC). We performed IHC for *T. gondii* (rabbit polyclonal antibody, AR125-5R, produced from strain C56 culture derived tachyzoites; Biogenex Laboratories, Inc., San Ramon, California, USA; or rabbit polyclonal antibody produced from strain ME49 culture-derived tachyzoites, California Animal Health and Food Safety Lab, Davis, California, USA) on tissues with evidence of protozoal-related inflammation (Suedmeyer et al. 2001). In addition, IHC testing was performed for *Sarcocystis neurona* (monoclonal clone 2G5-2T75 described in Marsh et al. 2002) on six cases and for *Neospora caninum* (rabbit polyclonal, produced from bovine fetal isolate no. 66, California Animal Health and Food Safety Lab, Davis, California, USA, described by Conrad et al. [1993]) on four cases using described methods (Suedmeyer et al. 2001; Marsh et al. 2002).

When available, sera collected postmortem from cases were tested for IgG antibodies to *T. gondii*, *S. neurona*, and *N. caninum* as described for the population serosurvey. For two CSLs (cases 4 and 6), brain tissue was collected at necropsy and processed for parasite isolation as described by Miller et al. (2001). Cell cultures were considered positive when intracellular protozoal parasite clusters, extracellular zoites, or both were observed by inverted light microscopy. For positive cultures, protozoan isolate identity was confirmed

through parasite morphology in cell culture and molecular characterization (Miller et al. 2001; Conrad et al. 2005). Cell cultures were maintained for at least 1 mo before being classified as negative.

For two cases, archived tissues were available for molecular analysis. For one of these, archived brain tissue stored at −80 C was submitted in triplicate to the Real-Time PCR Core Diagnostic Facility (University of California, Davis, California, USA) for analysis. Five hundred microliters of stabilization solution (DX Binding Solution [DXB], Qiagen, Valencia, California, USA) were added to each 50–100 mg sample of tissue. DNA extraction and real-time quantitative PCR (qPCR) were conducted as described by Dabritz et al. (2007). The Real Time TaqMan® PCR assays for the *T. gondii* (GenBank accession EF 472967.1) small subunit 18S rDNA gene were designed using AB Primer Express 3 by the Real-Time PCR Diagnostic Core Facility. Human 18s (HS99999901_s1) was ordered directly from Applied Biosystems (Foster City, California, USA) as an inventoried gene expression assay. Assays were run with both negative and *T. gondii*-positive DNA controls. Fluorescent signals were collected during the annealing temperature, and cycle threshold (Ct) values were exported with a threshold of 0.1 with a baseline of 3–10 for human 18S and with a threshold of 0.06 with a baseline of 3–15 for *T. gondii*. For the second case, DNA was extracted from formalin-fixed, paraffin-embedded cardiac tissue as described (Gozalo et al. 2007). DNA was screened for *T. gondii* by amplification and restriction fragment length polymorphism (RFLP) analysis of the *T. gondii SAG1* gene product (Grigg and Boothroyd 2001; Miller et al. 2004). PCR analysis targeting the ITS1 region and B1 gene of *T. gondii* were conducted as described by Rejmanek et al. (2010). PCR products were separated electrophoretically on a 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light along with a negative and a *T. gondii* DNA-positive control.

Serosurvey

Serum samples were collected at or near the time of admission (Dierauf and Gulland 2001) and archived at −70 C. The serum sample collected closest to admission was selected for testing, and samples from restranded animals were excluded. Stratified random sampling was used to select a representative sample and avoid potential exclusion of age-sex classes due to the preponderance of younger CSLs admitted to TMMC (Greig et al. 2005). Serum samples were stratified by year (1998–2009) and then by age-sex class within years (male-pup, female-pup, male-yearling, female-yearling, juvenile [males only], male-subadult, female-subadult, male-adult, female-adult). For each stratum, 20 samples were randomly selected from the serum archive using a random number generator. If fewer than 20 samples were available in a class, all samples were used.

Demographic and stranding information was extracted from medical records. Age class was determined as described by Greig et al. (2005). Age classes for females were estimated as: pup 0–1 yr old; yearling 1–2 yr old; subadult 2–5 yr old; adult ≥5 yr old. Age classes for males were estimated as: pup 0–1 yr old; yearling 1–2 yr old; juvenile 2–4 yr old; subadult 4–8 yr old; adult ≥8 years old.

Sera were tested for IgG antibodies to *T. gondii*, *S. neurona*, and *N. caninum* with the indirect fluorescent antibody test (IFAT) as described by Miller et al. (2002a) using a fluorescein isothiocyanate (FITC)-conjugated rabbit anti-canine IgG (Bethyl Laboratories, Montgomery, Texas, USA). *Toxoplasma gondii* exposure, as defined by serologic antibody titer, was investigated as a risk factor for other clinical diagnoses in the CSL population. Evaluation of potential associations with other diagnoses was restricted to CSLs that died or were euthanized and received a gross necropsy to reduce information bias associated with decreased diagnostic sensitivity and specificity in animals that survived or were not necropsied. Diagnoses were obtained from medical records and included malnutrition, domoic acid toxicosis, shark bite, gunshot injury, other trauma, leptospirosis, myopathy, and carcinoma.

Statistical analyses

Yearly antibody prevalence was estimated using a weighted proportion of the sample stratum proportions (Lohr 1999). Period prevalence for 1998–2009 was also calculated using this method in which age-sex strata were collapsed over years. Serologic titers from confirmed cases were used to establish a titer 640 as the cutoff for an antibody-positive classification. Titers are expressed as the reciprocal of the highest dilution giving a positive result. Associations between *T. gondii* antibody positivity and risk factors, as well as between *T. gondii* antibody positivity and other diagnoses, were evaluated with the chi-square (χ^2) test of independence or Fisher's exact test when a cell expected frequency was less than five (Daniel 2005).

TABLE 1. Demographic and stranding information for California sea lions (*Zalophus californianus*) with confirmed or suspected *Toxoplasma gondii* infections. Cases confirmed by additional diagnostic testing are indicated by an asterisk (*) next to the case number. Antibody titers for protozoal pathogens were determined using the indirect fluorescent antibody test.

Case	Animal ID	Age class	Sex	Date of stranding	*T. gondii* IgG titer[a]	Cause of death
1*	CSL 4210	Adult	Female	10 October 1998	n/a	Domoic acid toxicosis
2	CSL 5026	Subadult	Female	3 August 2001	5,120	peritonitis
3	CSL 5443	Yearling	Female	27 December 2002	10,240	Malnutrition
4*	CSL 5531	Adult	Female	19 October 2003	81,920	Domoic acid toxicity
5*	CSL 6009	Yearling	Female	18 December 2003	40,960	Protozoal encephalitis
6*	CSL 6464	Subadult	Female	13 December 2004	10,240	Protozoal encephalitis
7	CSL 6540	Adult	Male	25 May 2005	n/a	Trauma
8	CSL 7065	Juvenile	Male	12 December 2006	n/a	Protozoal encephalitis
9*	CSL 9201	Adult	Female	8 October 2009	640	Domoic acid toxicity
10*	ZCSMI04-PP1	Neonate	Unknown	15 May 2004	n/a	Disseminated *T. gondii*
11	ZCSMI04-PP3	Neonate	Unknown	15 May 2004	n/a	Sepsis
12*	ZCSMI04-PP9	Neonate	Unknown	17 May 2004	n/a	Disseminated *T. gondii*

[a] n/a = not available, test not performed.

When indicated, the strength of an association was estimated by the odds ratio (OR) and associated 95% confidence limits. The score test for trend was used to evaluate trends in odds across age class categories (Clayton and Hills 1993).

RESULTS

Cases of *Toxoplasma gondii* infection in California sea lions

Five confirmed and four suspected *T. gondii* infections were identified from archived case material from animals that died at TMMC. Three additional cases of *T. gondii* infection (two confirmed) were identified in prematurely aborted CSLs sampled on the San Miguel Island rookery (Tables 1–3).

Clinical, gross necropsy and histologic findings: Protozoal infection was the cause of death in cases 5, 6, 8, 10, and 12 (Table 1). Cases 5, 6, and 8 all exhibited neurologic symptoms prior to death including ataxia, seizures, and abnormal mentation. Contributing causes of death in two CSLs included chronic domoic acid toxicosis (case 5) and verminous pneumonia (case 8). In other cases, protozoal-related inflammation was considered mild to moderate, and death was attributed to other disease processes (Table 1).

Grossly, lymphadenopathy was observed in three animals (cases 4, 6, and 7). Histologically, lesions consistent with protozoal infection or associated with protozoal organisms were noted in brain, heart, skeletal muscle, urinary bladder, pancreas, spleen, lung, liver, and lymph nodes in CSLs with confirmed or suspected *T. gondii* infection (Tables 2–3). The most-common microscopic lesions observed were encephalitis, meningitis, and myocarditis (Table 2a, b). Encephalitis was observed in the majority of cases (10/12) with mild to severe lesions noted throughout the cerebrum and cerebellum. Lesions were most common in the grey matter of the frontal and temporal lobes. Lesions were characterized by multifocal, discrete, nodular accumulations of macrophages, lymphocytes, plasma cells, and glial cells with variable necrosis of the parenchyma and frequent broad lymphocytic perivascular cuffing (Fig. 1A). Associated meningitis was common. Rare protozoal tachyzoites or cysts were observed in or adjacent to brain lesions in nine of 11 cases, and in six cases organisms were confirmed as *T. gondii* via immunohistochemistry. Organisms consisted of individual 1–2-μm zoites, or clusters of up to 20 μm in diameter (Fig. 1B), and cysts ranging

TABLE 2. Severity of protozoal-related inflammation, location of protozoa, and additional diagnostic testing results in confirmed and suspected cases of *Toxoplasma gondii* in California sea lions (*Zalophus californianus*) receiving full gross necropsies and histologic evaluations (1975–2009). Confirmed cases are indicated by an asterisk (*) next to the case number. Severity of pathologic lesion is reported as not observed (−), mild (+), moderate (++), or severe (+++).

Case	Meningitis	Encephalitis	Myocarditis	Myositis	Lymphadenitis	Other	Location of parasite	Additional testing[a]
1*	−	++	−	−	−	−	Brain: tachyzoites	IHC brain (+)
2	+	−	−	+	−	−	Skeletal muscle: cysts	n/a
3	+	+	−	−	−	−	Heart: cysts	n/a
4*	+	+	+	−	−	−	Brain: cysts	IHC brain (−)[b]
							Heart: cysts	IHC heart (+)
							Lung: tachyzoites	IHC lung (−)[b]
								Culture brain (+)
								PCR brain (−)
5*	++	++	+	++	+++	+	Brain: cysts, tachyzoites	IHC brain (+)
							Lymph node: tachyzoites	IHC lymph node (+)
6*	+++	+++	+++	+	+++	++	Brain: cysts, tachyzoites	IHC brain(+)
							Heart: cysts, tachyzoites	IHC heart (+)
							Lung: tachyzoites	IHC lung (+)
								IHC liver (−)
								IHC pancreas (−)
								IHC lymph node (−)
								Culture brain (−)
								PCR brain (+)
7	+++	−	−	−	−	−	Brain: cysts	IHC brain (−)[b]
8	−	++	−	+	+++	−	Brain: cysts, tachyzoites	n/a
9*	−	++	−	−	−	−	Brain: cysts, tachyzoites	IHC brain (+)
10*	+++	+++	+++	−	−	++	Brain: tachyzoites	IHC brain (+)
							Heart: cysts	IHC heart (+)
								IHC liver (−)
11	−	−	++	−	−	+	Heart: cyst	IHC heart (−)[b]
								IHC liver (−)
12*	−	++	+	−	−	++	Brain: tachyzoites	IHC brain (+)
								IHC lung (−)

[a] (+) = positive; (−) = negative; IHC = immunohistochemistry; culture = in vitro cultures of *T. gondii* tachyzoites; PCR = PCR amplification of *T. gondii* DNA from tissue; n/a = archived material not available.

[b] Protozoa cysts were observed on H&E but additional tissue sections did not contain visible cysts or tachyzoites and were negative on immunohistochemistry.

TABLE 3. Distribution of IgG antibody titers to *Toxoplasma gondii* by indirect fluorescent antibody test stratified by year for California sea lion (*Zalophus californianus*) serum samples collected by The Marine Mammal Center, Sausalito, California, USA.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	Total
<40	142	79	108	107	71	95	80	95	71	62	81	103	1,094
40	9	11	10	11	30	33	40	21	41	46	46	34	332
80	0	1	1	0	3	6	4	11	10	21	16	14	87
160	0	1	1	0	2	0	1	2	15	12	8	4	46
320	0	0	1	2	2	0	2	0	5	5	3	5	25
640	1	0	0	0	2	3	0	0	2	1	2	1	12
1,280	0	0	0	0	0	2	0	0	0	1	0	0	3
2,560	0	0	0	0	0	3	0	2	0	0	1	0	6
5,120	0	0	0	0	0	1	0	0	1	0	1	4	7
10,240	0	0	0	0	0	0	0	1	1	0	1	3	6
20,480	0	0	0	0	0	0	0	1	1	3	0	1	6
40,960	0	0	0	0	0	0	0	0	1	0	1	0	2
81,920	0	0	0	0	0	0	0	0	3	1	0	0	4
Total	152	92	121	120	110	143	127	133	151	152	160	169	1,630

from approximately 40–75 μm in diameter with a thin eosinophilic wall and numerous, crescent-shaped bradyzoites (Fig. 1C). Cysts were occasionally located distant from areas of inflammation.

Myocarditis (6/12 cases) ranged from mild to marked, with predominantly lymphoplasmacytic or histiocytic inflammation, necrosis, and mineralization in more-severe cases (Fig. 2). Intralesional protozoal cysts or tachyzoites, similar to those noted in brain lesions, were observed in four cases of myocarditis and confirmed as *T. gondii* via IHC in three cases. In the fourth case, additional cut sections for IHC did not contain protozoa and results were negative. Case 3 had myocardial protozoal cysts that were not associated with inflammation. Though archived material was not available for additional analysis, serology was positive for *T. gondii* and negative for *S. neurona* and *N. caninum* (Table 1). Mild to moderate lymphoplasmacytic and histiocytic myositis was observed in skeletal muscle (cases 6 and 8), diaphragm (case 6), esophagus (cases 5 and 6), pharynx (case 3), and tongue (case 2). In cases 2 and 3, protozoal cysts were observed in skeletal muscle and heart, respectively, without concurrent inflammation.

Five cases had disseminated infections with the most-severe lesions noted in case 6. Other lesions included lymphoplasmacytic cystitis (cases 5 and 6), histiocytic and lymphoplasmacytic pancreatitis (case 6), histiocytic splenitis (case 6), necrotizing or histiocytic hepatitis (cases 6, 11, and 12), necrotizing lymphadenitis (cases 5, 6, and 8), and interstitial pneumonia (case 13). Lesions were characterized by small, randomly distributed nodular foci of nonsuppurative inflammation and necrosis. Lymphoid hyperplasia was noted in multiple lymph nodes in four cases (2, 5, 6, and 7), two of which had a grossly noted lymphadenopathy. Lymph node sections from case 5 and lung tissue from case 6 had rare *T. gondii* tachyzoites, both confirmed by IHC.

Immunohistochemistry, serology, culture, and PCR: Seven of the 12 cases of *T. gondii* infection in CSLs were confirmed with additional diagnostic testing, most commonly IHC (Table 2). In cases 7 and 11, protozoal cysts morphologically consistent with *T. gondii* were observed with H&E staining, but additional sections did not contain the previously observed protozoal cysts and were negative for *T. gondii* on IHC. By IHC, cases 1, 5, 6, and 10–12 were negative for *S. neurona* and cases 5 and 10–12 were negative for *N. caninum*. For case 4, brain tissue culture was positive for

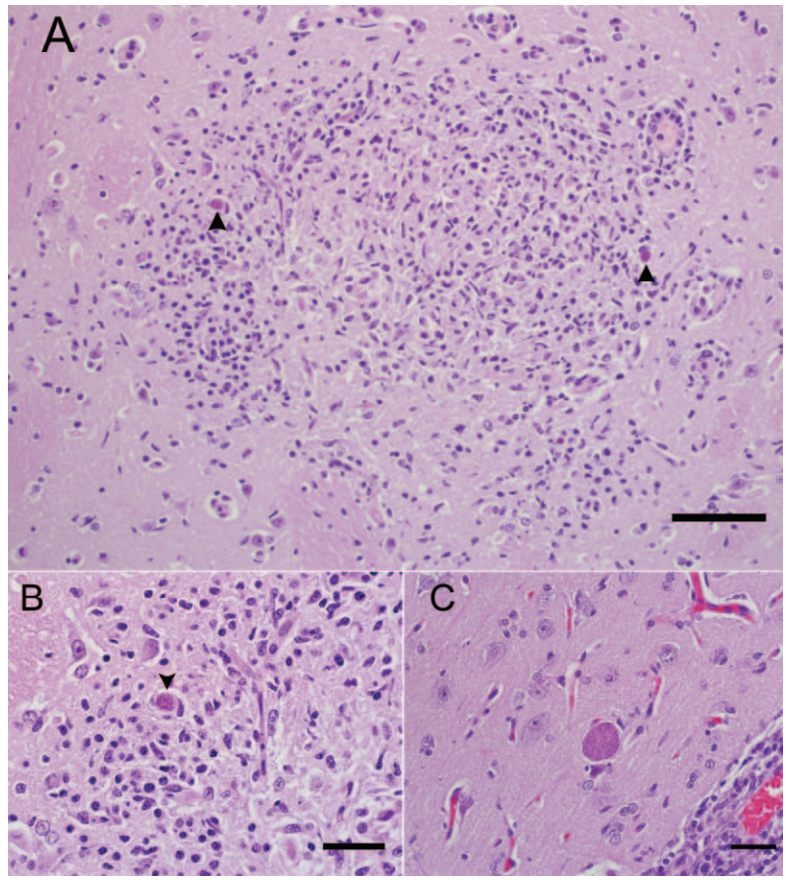

FIGURE 1. Hematoxylin and eosin-stained histologic sections of a subadult female California sea lion (*Zalophus californianus*) brain (case 6) infected with *Toxoplasma gondii*. A. Focal area of histiocytic and lymphocytic encephalitis and necrosis with two clusters of protozoal tachyzoites (arrowheads). Bar=100 μm. B. Higher magnification of protozoal tachyzoites within an area of inflammation (arrowhead). Bar=20 μm. C. Focal protozoal cyst and an adjacent blood vessel cuffed by lymphocytes and plasma cells. Bar=50 μm.

T. gondii; however, in this study, *T. gondii* DNA was not amplified from frozen brain tissue by qPCR (Ct>35). For case 6, DNA extracted from brain tissue was positive for *T. gondii* using amplification of the ITS1 region and *B1* gene and PCR-RFLP analysis of the *T. gondii SAG1* gene. For cases 4, 5, and 6, serum *T. gondii* titers increased fourfold to ninefold within 3 wk. Serologic titers for *S. neurona* (cases 2–6) and *N. caninum* (2–3, 6) were negative (≤80).

Serosurvey of stranded sea lions

Serum samples from 1,630 CSLs were tested for *T. gondii* IgG antibody (Table 3). Forty-six of the serum samples were antibody-positive using a cutoff titer of 640. The period prevalence for 1998–2009 in the CSL population, in which strata for each age class were collapsed across years, was 2.5% (±0.03%). The yearly adjusted antibody prevalence in CSLs varied from 0.0% in years 1999–2001 and 2004 to 6.2% (±1.7%) in 2003. Antibody positivity was not significantly associated with sex. *Toxoplasma gondii* exposure status differed by age class ($\chi^2=10.22$, $P=0.04$) with an increasing trend in odds of *T. gondii* exposure with increasing age (score test for trend in odds $\chi^2=3.25$, $P=0.07$). Sea lions exposed to *T. gondii* were 3.6 times

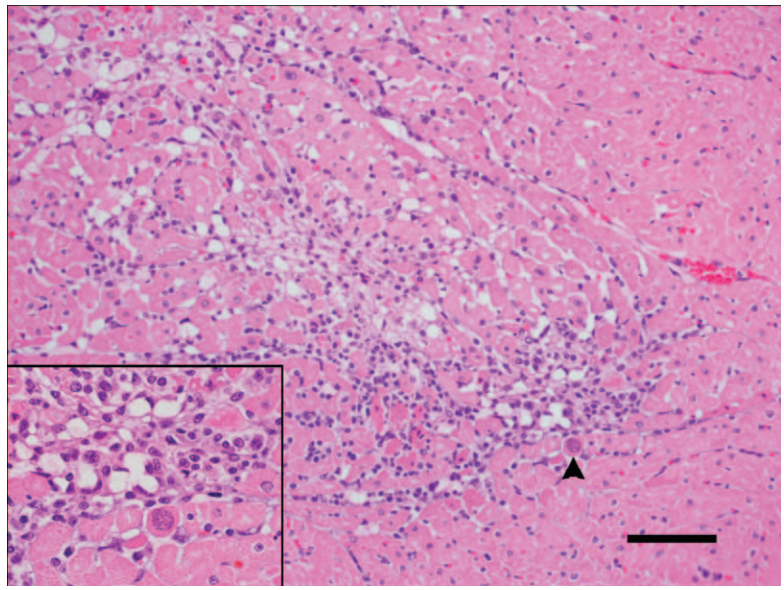

FIGURE 2. Hematoxylin and eosin-stained section of a California sea lion (*Zalophus californianus*) heart (case 6) with severe *Toxoplasma gondii* infection demonstrating marked lymphocytic and histiocytic myocarditis, loss of cardiac myocytes, and myocyte necrosis. Clusters of protozoal zoites are within an affected myocyte (arrow). Bar=100 μm. Inset: Higher magnification of protozoal zoites.

more likely to have a gunshot injury diagnosed on admission to TMMC compared to unexposed CSLs (OR_{MH} 95% CI 1.2, 10.7; $P=0.02$). All other associations between *T. gondii* exposure and risk factors or diagnoses of interest were not statistically significant ($P>0.05$).

DISCUSSION

Toxoplasma gondii infection in free-ranging, stranded CSLs in central California was uncommon during the study period. We estimated a population-level antibody prevalence of 2.5% using a large sample size, adjusting for the bias in age class for stranded, free-ranging CSLs. Dubey et al. (2003) reported *T. gondii* antibody prevalence of 61% in CSLs based on 18 serum samples collected from captive CSLs using the modified agglutination test. Though direct comparisons between different methods cannot be made, the previous estimate of antibody prevalence was based on a low antibody titer cutoff (25) compared to our study (IFAT 640). While our more-conservative cutoff may have misclassified some individuals exposed to this pathogen, we based our cutoff on serologic data from histologically confirmed cases. Lowering the cutoff to 320 increased the period prevalence from 2.5% to 4.0%, but all statistical associations remained similar. The limited number of confirmed-positive infections prevented the validation of the IFAT in CSLs. The *T. gondii* antibody prevalence in CSLs in this study was substantially lower than that reported for sympatric sea otters with this same serologic test. From 1997–2001, antibody prevalence in sympatric sea otters was 42% for live otters and 62% for dead otters (Miller et al. 2002b), possibly representing variable exposure to infectious oocysts, differences in susceptibility to infection, or both, between the two species.

Our findings suggest that *T. gondii* may have two modes of transmission in the CSL population. The occurrence of disseminated *T. gondii* infection in aborted fetuses confirms transplacental transmis-

sion in free-ranging CSLs, and the increasing odds of exposure with age is consistent with cumulative risk of exposure over time and persistent antibody response to infection that may stimulate a humoral response. The second-highest odds of exposure to *T. gondii* occurred in pups, second only to adults, which is consistent with *T. gondii* exposure early in life. Female CSLs give birth between mid-May and the end of June each year and wean pups 6–11 mo later (Peterson and Bartholomew 1967). All of the pups that were positive for *T. gondii* antibody in this study stranded between March and May, a time when CSL pups start to make foraging trips and are first exposed to prey species and the California mainland coast line. Even though CSL pups may still be nursing, it is unlikely that the serologic titers represent maternal antibodies because maternal antibodies are likely only transferred in colostrum in the days following birth (Omata et al. 1994).

Toxoplasma gondii was rarely observed on histopathologic examination of CSLs at TMMC, with tissue stages identified in only nine of the 1,152 CSLs examined; however, histopathologic examination of tissues and IHC may not be highly sensitive for confirming *T. gondii* infection in sea lions. Protozoa are often rare, sparsely distributed, and can be missed on additional sections cut for IHC. Chronic *T. gondii* infection may result in meningoencephalitis in sea lions with no identifiable organisms via routine staining or IHC. Three CSLs with elevated *T. gondii* antibody titers had meningoencephalitis morphologically consistent with protozoal infection but were not included as confirmed or suspect cases, as organisms were not detected histologically and ancillary testing (IHC, PCR) was negative.

The patterns of lesions in *T. gondii*-infected CSLs were similar to those previously reported in other marine mammals. In most cases protozoal organisms were found in association with necrosis and nonsuppurative inflammation. Lymphoid tissue is a target organ for *T. gondii*, and lymphadenopathy, the only common gross pathologic finding observed in these CSLs, has been reported in cetaceans with disseminated *T. gondii* infection (Miller 2008). However, unlike some cetaceans, encephalitis was also common (Roe et al. 2013). In the most-severe disseminated cases, protozoa were noted in lung and lymph node, in addition to brain and muscle, but not in other tissues in which likely protozoal-related inflammation was present. This suggests that visible organisms may be infrequent even in disseminated cases. Meningoencephalitis observed in CSLs in this study was morphologically similar to lesions described in previous reports (Migaki et al. 1990; Kreuder et al. 2003; Miller 2008).

While it is generally accepted that *T. gondii* tissue cysts do not provoke a significant inflammatory response (Miller 2008), tissue cysts in this study were typically observed within the general proximity of inflammatory lesions on histopathologic examination. Two suspected *T. gondii* cases (cases 2 and 3) had tissue cysts observed without inflammation in muscle; however, these CSLs also had high serum antibody titers to *T. gondii*, indicating an acute exposure. These findings may suggest that some animals are sufficiently immunocompetent to control parasite proliferation, resulting in an incidental infection, while a few experience widespread pathology with associated disease.

Neurologic signs observed in CSLs with *T. gondii* as the primary cause of death were consistent with signs observed in a captive CSL and other marine mammal species infected with *T. gondii* (Migaki et al. 1977; Miller 2008). Based on our data, a high or rising titer to *T. gondii* should alert medical staff to the likelihood of a *T. gondii* infection and prompt appropriate interventions. Domoic acid intoxication can also present with similar neurologic signs (Gulland et al. 2002) and

may be difficult to distinguish from *T. gondii* infection in the clinical setting; however, a fourfold and a ninefold increase in *T. gondii* titers for cases 4 and 5, respectively, supports acute and clinically relevant protozoal infections and suggests that the neurologic signs could have been due, at least in part, to protozoal encephalitis. Case 2 suggests that CSLs with chronic infections may have slightly elevated titers but only mild, subclinical encephalitis and no visible protozoa in the brain.

Transplacental infections have been reported in both early- and late-term fetuses in cetaceans (Jardine and Dubey 2002; Resendes et al. 2002) as well as in a CSL neonate (Ratcliffe and Worth 1951). In these cases, as well as in the CSL fetal cases reported here, infection was disseminated. This is the first report of *T. gondii*-induced, late-term abortion and fetal infection in CSLs.

Based upon the literature and clinical experience at TMMC, we suspected that mortality primarily due to *T. gondii* would not have a substantial negative impact on CSL population trends. We investigated associations between *T. gondii* and other clinical outcomes to assess potential comorbidities that may be linked to *T. gondii* infection. Kreuder et al. (2003) found that sea otters with protozoal encephalitis were more likely to be attacked by sharks than were otters without the condition, suggesting that encephalitis-induced abnormal behavior attracted more attention or increased risky behaviors, making affected otters more susceptible to attack. Sea lions with subclinical *T. gondii* infections may also exhibit abnormal behaviors, or be less wary of people, accounting for the positive association we observed between exposure and gunshot.

Clinical disease was uncommon in our study, and low prevalence of *T. gondii* antibodies suggests decreased frequency of exposure compared to sea otters. Standardized use of PCR in future investigations may improve detection of *T. gondii* infection and provide additional data to understand the ecology of this parasite in marine mammals. There has been significant speculation regarding the life cycle of *T. gondii* in the marine environment and the ways in which many marine mammal species occupying a variety of ecologic niches are exposed (Miller et al. 2002b, Conrad et al. 2005). Exposure to *T. gondii* among California sea otters was highly influenced by individual animal prey choice and habitat use (Johnson et al. 2009). Sea lions and sea otters have overlapping ranges in the coastal marine environment, yet have different feeding ecologies and migratory patterns, which likely results in different patterns of exposure within the same ecosystem. Potential theories of exposure in the marine environment include infective *T. gondii* oocysts being transported from domestic and wild feline feces on land to the sea (Miller et al. 2002b; Vanwormer et al. 2013a, b) or an undiscovered marine cycle.

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