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EVALUATION OF AN INDIRECT FLUORESCENT ANTIBODY TEST (IFAT) FOR DEMONSTRATION OF ANTIBODIES TO *TOXOPLASMA GONDII* IN THE SEA OTTER (*ENHYDRA LUTRIS*)

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ABSTRACT: An indirect fluorescent antibody test (IFAT) for detection of *Toxoplasma gondii* infection was validated using serum from 77 necropsied southern sea otters (*Enhydra lutris nereis*) whose *T. gondii* infection status was determined through immunohistochemistry and parasite isolation in cell culture. Twenty-eight otters (36%) were positive for *T. gondii* by immunohistochemistry or parasite isolation or both, whereas 49 (64%) were negative by both tests. At a cutoff of 1:320, combined values for IFAT sensitivity and specificity were maximized at 96.4 and 67.3%, respectively. The area under the receiver-operating characteristic curve for the IFAT was 0.84. A titer of 1:320 was used as cutoff when screening serum collected from live-sampled sea otters from California (n = 80), Washington (n = 21), and Alaska (n = 65) for *T. gondii* infection. Thirty-six percent (29 out of 80) of California sea otters (*E. lutris nereis*) and 38% (8 out of 21) of Washington sea otters (*E. lutris kenyoni*) were seropositive for *T. gondii*, compared with 0% (0 out of 65) of Alaskan sea otters (*E. lutris kenyoni*).

After near-extinction from overhunting in the maritime fur trade, the southern sea otter (*Enhydra lutris nereis*; Wilson et al., 1991) population has recovered slowly, punctuated by several periods of decline (Estes, 1990). The sluggish growth and recent decline of this population appear to be the consequence of elevated mortality (Estes et al., 1996; Monson et al., 2000). A high proportion of southern sea otter mortality (40%) has been attributed to infectious disease (Thomas and Cole, 1996). Protozoal meningoencephalitis is a frequent postmortem finding in southern sea otters stranded along the California coast (Thomas and Cole, 1996). Two different protozoa, *Toxoplasma gondii* and *Sarcocystis neurona*, have been isolated from affected animals (Thomas and Cole, 1996; Cole et al., 2000; Lindsay et al., 2000; Miller, Crosbie et al., 2001) and are considered to be an important cause of mortality. Isolates of *T. gondii* obtained from otters were similar or identical to those isolated from terrestrial animals and humans, and produced oocysts in cats fed mice inoculated with brain tissue from infected otters (Cole et al., 2000). Most reports on *T. gondii* infection in North American marine mammals provide detailed information on histopathological abnormalities (Van Pelt and Dieterich, 1973; Migaki et al., 1977; Buergelt and Bonde, 1983; Holshuh et al., 1985; Cruickshank et al., 1990; Inskeep et al., 1990; Migaki et al., 1990) but do not include evaluation of antibody response to *T. gondii*. Existing *T. gondii* serological surveys of marine mammals in North America have been limited to screening individuals diagnosed with fatal protozoal meningoencephalitis or opportunistically collected serum samples with limited internal controls (Lapointe et al., 1998; Rosonke et al., 1999; Lindsay et al., 2000; Mikalelian et al., 2000; Miller, Sverlow et al., 2001). To date, no studies on the true prevalence or seroprevalence (or both) of *T. gondii* in sea otters in different geographical areas has been reported.

In the present study, an indirect fluorescent antibody test (IFAT) for diagnosis of *T. gondii* infection in sea otters was validated. The IFAT was developed in response to the need for a screening test that could be used on hemolyzed serum samples, such as those collected at necropsy or samples from live animals with hemolysis attributable to conditions of collection, transport, or storage. Sera collected from necropsied southern sea otters with confirmed *T. gondii* infection status were used to validate this test and to examine the relationship between the *T. gondii* IFAT titer and extent of sample hemolysis. In addition, *T. gondii* IFAT seroprevalence was compared across 3 geographically distinct populations of sea otters, i.e., live-sampled southern sea otters from California and northern sea otters (*E. lutris kenyoni*; Wilson et al., 1991) from Alaska and Washington.

MATERIALS AND METHODS

Postmortem examination

Between February 1997 and February 2001, 96 freshly dead (<72 hr postmortem) southern sea otters from central California were examined at the Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Game (CDFG), in Santa Cruz, California. A detailed necropsy was performed on each animal, and selected tissues were screened for pathogenic bacteria or fungi. Otter age was recorded on the basis of previously established length, body weight, and dentition criteria (Morejohn et al., 1975). Serum was separated from whole blood via centrifugation and stored at −70 C until tested. The extent of hemolysis (hemolysis score) was noted for each serum sample tested: 0 = no hemolysis (clear serum with no red discoloration), 1 = minimal hemolysis (red-tinged but clear samples), 2 = moderate hemolysis (deep red and opaque serum samples), or 3 = marked hemolysis (dark red to black and opaque, sometimes with a viscous consistency).

Tissues were immersion-fixed in 10% neutral buffered formalin, trimmed, dehydrated through a graded series of ethanol and xylol, and embedded in paraffin. Five-micrometer sections were cut using a rotary microtome, deparaffinized, stained with hematoxylin and eosin (H&E), and examined using a compound microscope. The primary and contributing causes of death were determined through gross necropsy, histopathology, bacterial culture, and other techniques, as required. From

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each otter, at least 7 5-μm-thick HE-stained sections of brain were examined, including frontal cortex, rostral hippocampus, temporal cortex, cerebellum, medulla, and brainstem. In most cases, multiple sections of spinal cord were also examined.

Immunohistochemistry

From each otter, 2 blocks of brain tissue containing at least 1 section each of cerebrum and cerebellum were selected for immunohistochemistry. The blocks that were selected were those with visible protozoan parasites or the greatest degree of nonsuppurative inflammation on histopathological examination or both. All immunohistochemical slides were prepared at the California Animal Health and Food Safety Laboratory, Davis, California, using standardized techniques, equipment, and reagents. Immunohistochemical stainings for *T. gondii*, *Neospora caninum*, and *S. neurona* were performed as described (Miller, Sverlow et al., 2001). Slides were evaluated by a pathologist with no prior knowledge of each otter's IFAT titer or parasite isolation status. An otter was considered positive on immunohistochemistry only if stained parasites were observed, and both the external and internal portions of the organisms were stained using the immunoperoxidase technique.

Parasite isolation

At necropsy, samples of cerebrum and cerebellum were collected aseptically into chilled, sterile antibiotic saline solution (0.85% saline with 100 IU/ml penicillin G and 100 μg/ml streptomycin), homogenized, and placed over stationary cultures of monkey kidney (MA104) cells (BioWhittaker, Walkersville, Maryland) as previously described (Miller, Crosbie et al., 2001; Miller, Sverlow et al., 2001). Both trypsinized and nontrypsinized brain homogenates were processed for cell culture inoculation, and cell cultures were maintained as described by Conrad et al. (1993). Cell cultures were considered positive when characteristic refractile intracytoplasmic protozoal cysts or motile extracellular zoite stages, or both, were first observed. The identity of each protozoan isolate was confirmed through parasite morphology in cell culture, antigenic characterization, molecular characterization, or electron microscopy or both as previously described (Miller, Crosbie et al. 2001; Miller, Sverlow et al., 2001). Unless visibly contaminated, cell cultures were maintained for at least 1 mo after brain tissue inoculation before being deemed negative and discarded. Cell cultures that were parasite-negative when discarded (because of fungal or bacterial contamination) within 21 days of inoculation were considered indeterminate with respect to protozoal infection status. These cases were excluded from the IFAT evaluation unless the *T. gondii* brain immunohistochemistry result was positive.

Determination of *T. gondii* infection status for necropsied sea otters

The positive gold standards for *T. gondii* infection in sea otters were direct isolation and characterization of *T. gondii* in cell culture from brain homogenate, or observation of positively stained parasites with morphology consistent with *T. gondii* on immunohistochemistry, or both. The negative gold standards for *T. gondii*-free status in brain tissue were absence of detectable protozoan parasite stages via protozoal isolation and via immunohistochemistry.

IFAT

The IFAT was evaluated as a screening test for *T. gondii* by comparing serum IFAT titers to each otter's *T. gondii* infection status, as determined above. The IFAT was performed as previously described (Miller, Sverlow et al., 2001), except that 10 μl of a 1:100 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-ferret IgG (Bethyl Laboratories, Montgomery, Texas) was substituted for the FITC-conjugated rabbit anti-canine IgG used for the seal IFAT. Endpoint titers were determined by serial dilution from 1:80 to 1:81,920. Wells were examined using fluorescence microscopy at ×200, and the last well with distinct parasite outline fluorescence was the reported titer. All completed IFAT slides were assessed by the same observer to ensure consistency of interpretation.

The overall accuracy of the IFAT and the optimal cutoff titer was assessed by receiver-operating characteristic (ROC) analysis (Greiner et al., 2000) using MedCalc Version 6.0 (Mariakerke, Belgium). Ninety-five percent confidence intervals [CI] for IFAT sensitivity and specificity at the selected cutoff value were calculated by exact binomial methods (Greiner and Gardner, 2000) using Epi Info (Version 6.04; Centers for Disease Control and Prevention, Atlanta, Georgia). For serum collected from both live and dead otters, a Kruskal–Wallis test was used to determine whether degree of sample hemolysis and resulting *T. gondii* IFAT titers were associated. To assess the repeatability of the IFAT, slides were screened in a blinded fashion by 2 readers, and results were compared. The between-reader repeatability of the IFAT was assessed using a weighted kappa value (Fleiss, 1981).

Serologic screening of otters from California, Washington, and Alaska

Between 1997 and 2000, serum was obtained from live-sampled, presumably healthy sea otters from central California (*E. lutris nereis*), coastal Washington (*E. lutris kenyoni*), and coastal Alaska (*E. lutris kenyoni*) (Anderson et al., 1996). Animals were captured using dip nets, tangle nets, or Wilson traps as part of population biology studies by personnel of the Biological Resources Division, United States Geological Survey (USGS-BRD), and the CDFG. Samples were obtained for both sexes and all age classes, during all seasons (California and Washington) or only summer (Alaska), and from diverse geographic areas and marine habitats.

Live-sampled California sea otters (n = 80) were sampled between March 1998 and March 2000 in the vicinity of Monterey Bay, California (36.5500°–36.9667°N, 121.0334°–121.7500°W). Most otters were sampled within 5 km of coastal cities such as Santa Cruz, Capitola, Monterey, and Pacific Grove. Twenty-one serum samples were obtained from live-sampled sea otters from coastal Washington between February 1997 and August 1998. Most serum samples (n = 20) were obtained from otters located within the coastal boundaries of Olympic National Park, extending from Cape Alava (48.1696°N, 124.7314°W) to the Chilean Memorial (47.9650°N, 124.6653°W). This is an isolated stretch of wilderness along the northwestern coast of Washington, reachable only by foot or boat. One additional otter was sampled at Waadah Island (48.3846°N, 124.6056°W). Sixty-five otter serum samples were collected from coastal Alaska during the summer of 1997. Otters were captured near Adak (52.0000°N, 176.5000°W; n = 33), Kiska (52.0000°N, 177.5000°W; n = 14), and Kanaga Islands (52.0000°N, 177.0000°W; n = 7) in the western Aleutian Island chain and at Elfin Cove (58.1667°N, 136.3334°W; n = 11) in southeastern Alaska. Small towns with less than 500 people are found on Adak Island and at Elfin Cove. All sea otters were captured ≥10 km from these towns.

A physical examination was performed on all sampled otters, during which animals were weighed to the nearest 0.1 kg and their age class and sex determined. The age of each captured otter was estimated on the basis of pelage, tooth wear, weight, length, and reproductive status characteristics (Morejohn et al., 1975). Age classes were defined as pup (0–6 mo), immature (>6 to 12 mo), subadult (>1 to 3 yr), and adult (>3 yr). Serum samples obtained from live otters were screened using the same IFAT technique that was used for samples obtained from dead sea otters. The extent of hemolysis of each sample was recorded, and all samples were held at −70 C before testing.

Chi-square tests were used to compare seroprevalence of *T. gondii* among live-sampled sea otters in California, Washington, and Alaska, as well as between the live-sampled otters and the freshly dead otters with known *T. gondii* infection status. Otters were considered seropositive if their IFAT titer was ≥1:320, the optimal cutoff titer as determined in the validation study.

RESULTS

Postmortem examination

From February 1997 to February 2001, 96 freshly dead southern sea otters were examined grossly and via histopathology. Serum was obtained for IFAT screening from 84 of 96 otters. For 7 of the 84 otters, IFAT testing was completed, and immunohistochemistry was negative for *T. gondii*, but parasite isolation from the otter brain tissue was not attempted. Parasite isolation was not done when zoonotic pathogens such as *Coc-*

cidiodes immitis were suspected, because of chain-of-custody restrictions or as a result of logistical problems with sample shipment and processing, e.g., lost samples. These 7 cases were classified as indeterminate for *T. gondii* infection and were excluded from IFAT evaluation. For the 7 indeterminate cases, the *T. gondii* IFAT titers were <1:80 (n = 4), 1:160 (n = 1), 1:640 (n = 1), and 1:1,280 (n = 1). Thus, 77 of the freshly dead otters were evaluated for *T. gondii* infection status by immunohistochemistry and parasite culture and also had sufficient serum for IFAT screening. Adults, subadults, immatures, and pups made up 61, 12, 18, and 9% of the sample, respectively. Forty-seven percent (36 out of 77) of the otters were females.

Immunohistochemistry

Thirteen of 77 freshly dead otters were positive for *T. gondii* on brain immunohistochemistry. Immunohistochemical staining in brain tissue encompassed entire parasites, and no unstained protozoa were observed in slides prepared using rabbit antisera to *T. gondii*. *Toxoplasma gondii*-stained protozoa did not show evidence of cross-reactivity with *S. neurona* or *N. caninum* polyclonal antiserum.

Parasite isolation

For 77 culture attempts, the median interval between gross necropsy with brain tissue collection and inoculation of monolayer cultures with brain homogenate was 2 days (range <1 to 21 days). Twenty-four separate isolates of *T. gondii* were obtained from 75 culture attempts with sea otter brain homogenate, an isolation ratio of 32%. Four additional otters for which parasite isolation was negative (n = 1) or the monolayer cultures were contaminated with bacteria (n = 1) or parasite isolation was not attempted (n = 2) were parasite-positive on immunohistochemistry. Thus, 36% of the otters (28 out of 77) were shown to be infected with *T. gondii* at the time of necropsy, and 49 otters were noninfected on the basis of negative results on both immunohistochemistry and parasite isolation.

Of 24 isolates of *T. gondii*, 14 were obtained only from trypsinized brain tissue, 3 were obtained from both trypsinized and nontrypsinized brain tissue, and 4 were obtained from nontrypsinized brain tissue only. For the remaining 3 animals, the tissue treatment protocol was not recorded. The median time from cell inoculation to initial detection of parasites in cell culture was 14 days (range 3–35 days). Brain homogenate-inoculated cells were maintained in culture for a median of 31 days before being considered negative for parasites and discarded. However, 7 cultures were discarded within 9–21 days of inoculation because of fungal or bacterial contamination.

Determination of *T. gondii* infection status for necropsied otters

Of 24 otters positive for *T. gondii* on parasite isolation, only 11 (42%) were also detected by brain immunohistochemistry. In contrast, only 2 otters for which parasite isolation was negative were *T. gondii*-positive on immunohistochemistry. For 1 of these 2 cases, the inoculated cells were discarded because of bacterial contamination, presumably resulting in a false-negative isolation attempt.

TABLE I. Distribution of *Toxoplasma gondii* indirect fluorescent antibody test (IFAT) titers by protozoal infection status, as determined by parasite isolation and immunohistochemistry.

T. gondii IFAT titer	*T. gondii* infected	Noninfected
≥20,480	5	0
10,240	1	0
5,120	3	1
2,560	4	1
1,280	4	10
640	6	4
320	4	4
160	1	5
80	0	5
<80	0	19
Total	28	49

IFAT

End point *T. gondii* titers of otters ranged from <1:80 to 1:81,920 (Table I), and the area under the ROC curve was 0.84 (95% CI = 0.74–0.92). The maximal combined values for test sensitivity and specificity occurred at a cutoff of 1:320. At this cutoff, the *T. gondii* IFAT was highly sensitive (96.4%; 95% CI = 81.7–99.9%), but only moderately specific (67.3%; 95% CI = 51.6–79.6%), resulting in 1 false-negative and 20 false-positive diagnoses of *T. gondii* infection. At a cutoff of 1:320, 47 of the 77 otters (61%) were seropositive for *T. gondii*. Increasing the cutoff to 1:640 resulted in a sensitivity of 82.1% (95% CI = 63.1–93.9%) and specificity of 75.0% (95% CI = 60.4–86.4%).

Between-reader comparisons of IFAT reproducibility were restricted to samples with at least 1 end-point titer falling within 2 dilutions of the selected test cutoff of 1:320, e.g., samples with end point titers ≥1:80, but not greater than 1:1,280. In addition, if the primary reader determined the final titer value to be 1:1,280, the other reader had to agree to within ±2 serum dilutions for ≥90% of the tested samples. The IFAT results for 61 samples met the above restrictions (at least 1 end point titer ≥1:80 but not greater than 1:1,280) and were read out by both primary and second readers. For 93% of these samples (57 out of 61), the end point titers of both readers were within 2 serum dilutions of each other. The weighted kappa value for between-reader comparisons was 0.41, which indicated moderate agreement beyond chance.

For serum from freshly dead and live otters, the median hemolysis scores were 1 (range 0–3) and 0 (range 0–2), respectively. The degree of serum hemolysis was not statistically associated with end point *T. gondii* IFAT titers. This finding was the same for serum collected from dead ($P = 0.846$, n = 66) and live ($P = 0.204$, n = 166) sea otters.

Serologic screening of otters from California, Washington, and Alaska

Summary demographic and serological data for otters from California, Washington, and Alaska are shown in Table II. The sample collection periods, sex, and age distribution were comparable for the 3 populations. Using the established cutoff of

TABLE II. Summary of demographic data and *Toxoplasma gondii* indirect fluorescent antibody test results for sea otters (*Enhydra lutris*) from California, Washington, and Alaska.

	Necropsied, California	Live sampled, California	Live sampled, Washington	Live sampled, Alaska
Number sampled	77	80	21	65
Interval	1997–2001	1998–2000	1997–1998	1997
Females (%)	47	65	76	72
Adults (%)	61	65	62	85
Subadults (%)	12	9	19	10
Immatures and pups (%)	27	26	19	5
Seropositive (≥1:320)	61	36	38	0

1:320, 36% of the serum samples obtained from live-sampled California sea otters (29 out of 80) were seropositive for *T. gondii* on IFAT. For the live-sampled California otters, 61% (49 out of 80) had *T. gondii* IFAT titers ≤1:80. Twelve of the 80 live-sampled California otters had *T. gondii* IFAT titers of 1:160 or 1:320, and 19 otters had IFAT titers ≥1:1,280. Eight of the 21 Washington otter serum samples (38%) were positive (≥1:320) for *T. gondii* on IFAT. Observed *T. gondii* IFAT titers were <1:80 (n = 11), 1:80 (n = 1), 1:160 (n = 1), 1:320 (n = 2), 1:640 (n = 4), and 1:1,280 (n = 2). None of the serum samples from Alaska sea otters (0 out of 65) was positive. Of the 65 samples screened, 58 (89%) had *T. gondii* IFAT titers <1:80, 3 (5%) had 1:80, and 4 (6%) had 1:160.

Comparisons of seroprevalence indicated no significant difference ($P = 0.880$) in *T. gondii* IFAT seroprevalence between live-sampled *E. lutris nereis* from coastal California (36%) and live-sampled *E. lutris kenyoni* from Washington (38%). However, the *T. gondii* seroprevalence for both California and Washington otters was significantly greater than that of *E. lutris kenyoni* from Alaska ($P = 0.0001$ for both comparisons). In addition, the *T. gondii* IFAT seroprevalence was lower ($P = 0.002$) in the live-sampled California otters (36%) than in the dead otters (61%) used in the validation study.

DISCUSSION

The IFAT was validated using a cross-sectional sample of 77 freshly dead sea otters whose true *T. gondii* infection status was determined by immunohistochemistry and parasite isolation in cell culture. All otters were screened for *T. gondii* regardless of the apparent cause of death, and serum samples collected at the time of necropsy were used to compare *T. gondii* antibody titers with the results of the postmortem screening tests. Because the southern sea otter is a federally listed threatened species (Anderson et al., 1996), invasive sampling of live otters to confirm *T. gondii* infection for test validation was neither permissible nor desirable.

In the present study, the combined gold standard for *T. gondii*-free status was absence of detectable protozoan parasites via both cell culture and brain immunohistochemistry. It is recognized that some otters might have been incorrectly classified as negative for *T. gondii* infection on parasite isolation or immunohistochemistry, in part because of the small volume of brain tissue used in these tests. Also, false-negative results might have occurred if an isolate was missed in cell culture or was not detected because of fungal or bacterial contamination of cultures. In addition, some otters might have had *T. gondii* present in tissues other than brain.

Pretreatment of brain homogenate with trypsin before cell inoculation resulted in more successful isolation of *T. gondii* isolates than nontrypsinized homogenates obtained from the same otters. The additional trypsinization step likely facilitated the release of infective parasite stages from tissue cysts present in brain homogenate and minimized the likelihood of missing infected animals that had few parasites present.

When the results of *T. gondii* immunohistochemistry and parasite isolation were compared for the same otters, immunohistochemistry was less sensitive for parasite detection than for parasite isolation. This result is not surprising, given the sparse distribution of *T. gondii* on brain histopathology and the large difference in the volume of brain tissue screened via the 2 tests; <0.25 g of brain tissue (7–10 5-μm-thick histological sections) was examined from each otter via immunohistochemistry, compared with 2–4 g of brain homogenate layered over cells in culture. Examination of additional brain sections by immunohistochemistry might increase the sensitivity of this method, but numerous tissue sections would need to be examined to equal the volume of brain tissue screened via parasite isolation. Although mouse bioassay has been shown to be more effective in some situations (Dubey et al., 1995) when used in combination with tissue histopathology and immunohistochemistry, as in the present study, isolation in cell culture is an effective means of parasite detection. In addition, it obviates the need and expense of laboratory animal care and animal sacrifice.

At a cutoff titer of 1:320, the IFAT had high sensitivity (96.4%) but only moderate specificity (67.3%). However, IFAT specificity was likely underestimated because of the inclusion of unrecognized *T. gondii*-infected otters as gold standard negatives for the reasons outlined above. Some otters in the sample that were negative for *T. gondii* on parasite isolation and immunohistochemistry had high *T. gondii* titers, e.g., ≥1:2,560, and were likely to have been falsely classified as noninfected. This phenomenon has been noted for serologic tests for *T. gondii* in pigs when an imperfectly sensitive but perfectly specific gold standard (mouse bioassay) was used (I. Gardner, unpubl.).

One potential disadvantage of IFAT tests is the subjective element of test interpretation. To minimize variation caused by differences in interpretation between different test readers, a single reader was employed to interpret all IFAT results for this study. The *T. gondii* IFAT performed adequately on hemolyzed serum samples, and IFAT titers were not associated with the

extent of serum hemolysis. Thus, the *T. gondii* IFAT should be appropriate for screening serum samples collected at necropsy as well as samples from live otters where hemolysis is a concern. Other serological tests for protozoa such as the modified agglutination test are more sensitive to serum hemolysis, potentially resulting in false-positive test results (Packham et al., 1998).

In addition to validation of the IFAT, the present study provided important data regarding *T. gondii* infections in California sea otters. On the basis of the results of immunohistochemistry and parasite isolation, 36% of the freshly dead otters in the California sample were infected with *T. gondii*. This is likely an underestimate of the true prevalence of infection because some infections were not detected. In spite of this possibility, our findings indicate that *T. gondii* is prevalent and highlights the need for studies on modes of transmission of the parasite in the marine environment.

The validated *T. gondii* IFAT has several potential applications. First, it will be valuable as a diagnostic test for hospitalized sea otters with neurological disease consistent with protozoal meningoencephalitis. Second, it will facilitate investigation of risk factors for *T. gondii* infection in live-sampled otters. The cutoff titers selected for *T. gondii* seropositivity in each case will depend on assessment of the ''cost'' of a false-positive and false-negative diagnosis and the true prevalence of *T. gondii* infection in the population. For example, the cost of a false-negative diagnosis of toxoplasmosis is high in an otter with severe neurological disease compared with that of a false-positive diagnosis. Although some *T. gondii*-infected otters do not exhibit neurological disease, no southern sea otter is reported to have survived clinical toxoplasmosis without antiprotozoal therapy. In contrast, the risk of adverse effects caused by short-term antiprotozoal therapy in sea otters, either infected or uninfected, appears to be low. Thus, an IFAT with optimal sensitivity is best for diagnostic screening for *T. gondii* infection in otters with neurological disease. However, a clinical diagnosis of toxoplasmosis should be based on demonstration of a rising *T. gondii* titer, or clinical response to antiprotozoal therapy, or both.

A key goal in screening serum from live-sampled sea otters is to identify differences in *T. gondii* infection or seroprevalence among geographically distinct otter groups and investigate risk factors for *T. gondii* infection. The cost of a false-positive or false-negative diagnosis in an epidemiological study is inclusion of animals in the wrong infection group for analysis; imperfect sensitivity and specificity usually result in nondifferential misclassification of odds ratio estimates for risk factors, e.g., bias toward finding no effect. Thus, consideration of both test sensitivity and specificity is important for interpreting IFAT data that are used in epidemiological studies. On the basis of the gold standards used in this study, the maximal combined values for IFAT sensitivity and specificity occurred at a cutoff value of 1:320 as *T. gondii*-positive. This value was selected for screening sera collected from live-sampled sea otters, recognizing that the *T. gondii* infection status of some otters could be misclassified.

Although the populations had similar age and sex distributions (Table II), IFAT seroprevalence of *T. gondii* in California sea otters was greater for freshly dead (61%) than for live-sampled otters (36%). The reasons for this difference are speculative. The higher proportion of seropositive dead otters might reflect spectrum-of-disease bias because otters with more severe *T. gondii* infections might be more likely to die than otters with less extensive infections. Alternatively, concurrent immunosuppressive disease (Kannan et al., 1998) might result in reactivation of chronic, inapparent *T. gondii* infection and a change in corresponding IFAT titers. Otters may also have an increased risk of mortality resulting from seemingly unrelated factors when infected by *T. gondii*. For example, some otters with gross evidence of mortality resulting from boat strike or shark predation had *T. gondii*-related meningoencephalitis on histopathology and immunohistochemistry (M. Miller, pers. obs.). Neurological abnormalities induced by protozoal brain infections might make otters more susceptible to predation or trauma and render them less able to forage effectively or avoid danger, thus indirectly resulting in mortality. Severe clinical deficits, including seizures, apparent blindness, and obtundation have been observed in stranded sea otters and harbor seals with *T. gondii*-associated meningoencephalitis (Lapointe et al., 1998; Lindsay et al., 2000; Miller, Crosbie et al., 2001; Miller, Sverlow et al., 2001). Finally, otters that die from other causes may have a lower probability of appearing as beach-cast carcasses.

The *T. gondii* seroprevalence in live-sampled sea otters from coastal California and Washington was similar, but was greater than the *T. gondii* seroprevalence observed in Alaskan otters. Geographical differences in *T. gondii* seroprevalence might be attributable to differential *T. gondii* exposure, unrecognized environmental factors affecting *T. gondii* survival in the nearshore environment, differences in otter susceptibility, differences in paratenic prey species abundance, or other, unrecognized, factors. Geographical comparisons of *T. gondii* seroprevalence between populations or individuals must be interpreted with caution because of the chronicity of *T. gondii* infection, the large distances traveled by some otters within their respective ranges, and the unknown mechanisms of *T. gondii* transmission in sea otters. All 3 otter groups (California, Washington, and Alaska) are widely separated geographically, and movement of animals between the groups is expected to be minimal. However, rare, long-distance movements of otters have been reported (Pederson and Stout, 1963; Leatherwood et al., 1978).

In conclusion, the sensitivity and specificity of an IFAT for diagnosis of *T. gondii* infection in sea otters were estimated, and the IFAT was used to compare seroprevalence of *T. gondii* infection in live-sampled sea otters from California, Alaska, and Washington. All sampled Alaskan otters were seronegative for *T. gondii,* but ≥36% of live-sampled otters from California and Washington were seropositive at ≥1:320 serum dilution. It is proposed the IFAT will be useful for screening sea otters with neurological disease for *T. gondii* infection, so that antiprotozoal therapy can be expedited. In addition, it will facilitate epidemiological studies of risk factors for *T. gondii* exposure in live-sampled otters. This is important for the conservation of the threatened southern sea otter: Elevated mortality is felt to be a key factor in the failure of this population to recover; infectious disease appears to be an important cause of the elevated mortality, and processes leading to *T. gondii* exposure and death in otters are largely unstudied.

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