

COCCIDIOIDOMYCOSIS AND OTHER SYSTEMIC MYCOSES OF MARINE MAMMALS STRANDING ALONG THE CENTRAL CALIFORNIA, USA COAST: 1998–2012

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ABSTRACT: A wide range of systemic mycoses have been reported from captive and wild marine mammals from North America. Examples include regionally endemic pathogens such as *Coccidioides* and *Blastomyces* spp., and novel pathogens like *Cryptococcus gattii*, which appear may have been introduced to North America by humans. Stranding and necropsy data were analyzed from three marine mammal stranding and response facilities on the central California coast to assess the prevalence, host demographics, and lesion distribution of systemic mycoses affecting locally endemic marine mammals. Between 1 January 1998 and 30 June 2012, >7,000 stranded marine mammals were necropsied at the three facilities. Necropsy and histopathology records were reviewed to identify cases of locally invasive or systemic mycoses and determine the nature and distribution of fungal lesions. Forty-one animals (0.6%) exhibited cytological, culture- or histologically confirmed locally invasive or systemic mycoses: 36 had coccidioidomycosis, two had zygomycosis, two had cryptococcosis, and one was systemically infected with *Scedosporium apiospermum* (an Ascomycota). Infected animals included 18 California sea lions (*Zalophus californianus*), 20 southern sea otters (*Enhydra lutris nereis*), two Pacific harbor seals (*Phoca vitulina richardsi*), one Dall's porpoise (*Phocoenoides dalli*), and one northern elephant seal (*Mirounga angustirostris*). Coccidioidomycosis was reported from 16 sea lions, 20 sea otters, and one harbor seal, confirming that *Coccidioides* spp. is the most common pathogen causing systemic mycosis in marine mammals stranding along the central California coast. We also report the first confirmation of *C. gattii* infection in a wild marine mammal from California and the first report of coccidioidomycosis in a wild harbor seal. Awareness of these pathogenic fungi during clinical care and postmortem examination is an important part of marine mammal population health surveillance and human health protection. Temporal–spatial overlap may be observed for pathogenic mycoses infecting coastal marine mammals and adjacent human populations.

Key words: California sea lion, *Coccidioides*, coccidioidomycosis, *Cryptococcus*, harbor seal, sea otter, systemic mycosis, zygomycetes.

INTRODUCTION

Superficial or systemic mycoses caused by 19 fungal groups have been reported in 24 species of captive and free-ranging North American marine mammals (Reiderson et al. 1999, 2001). Pulmonary aspergillosis has been reported in pinnipeds and dolphins (Joseph et al. 1986;

Higgins 2000). Opportunistic yeasts, including *Candida* spp., *Torulopsis* spp., *Trichosporon* spp., and *Malassezia* spp. cause superficial dermatitis, rarely disseminate, and disproportionately affect captive marine mammals (Higgins 2000). Zygomycetes, a diverse group of soil-dwelling opportunists, infect numerous marine mammals, typically causing respiratory

infections. However, vascular invasion and systemic spread have been documented (Higgins 2000; Haulena et al. 2002).

Cryptococcus spp. are basidiomycetous fungi transmitted through inhalation that have been described in Atlantic bottlenose dolphins (*Tursiops truncatus*) (Miller et al. 2002), a spinner dolphin (*Stenella longirostris*) (Rotstein et al. 2010), Dall's porpoises (*Phocoenoides dalli*), and a harbor porpoise (*Phocoena phocoena*) (Stephen et al. 2002). Prevalence and pathogenicity vary among *Cryptococcus* species: *Cryptococcus neoformans* typically infects immunocompromised hosts, and virulent strains of *Cryptococcus gattii* have caused illness and death in immunocompetent humans, domestic animals, and wildlife (Byrnes et al. 2010). Previously associated with regions of *Eucalyptus* spp. tree growth in tropical and subtropical climates, an outbreak of *C. gattii* infection caused by the virulent molecular type VGII was reported in humans, Dall's and harbor porpoises, cats (*Felis catus*), and dogs (*Canis lupus familiaris*) in British Columbia in 2000 and 2001, before being recorded further southward in humans and animals from Washington and Oregon (Stephen et al. 2002; Byrnes et al. 2010). Infection by molecular types VGII and VGIII has also been reported in cats and dogs from northern California (Singer et al. 2014), and a captive Atlantic bottlenose dolphin in San Diego, California (Miller et al. 2002). *Cryptococcus albidus*, considered less pathogenic than *C. neoformans* and *C. gattii*, was recently reported from a stranded California sea lion (*Zalophus californianus*) (McLeland et al. 2012).

The pathogenic fungi *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Coccidioides* spp. are endemic to the US, and have distinct geographic distributions. *Blastomyces* spp. and *Histoplasma* spp. are endemic to the Mississippi–Ohio river basin (Burek 2001), although disseminated histoplasmosis was recently reported from a wild sea otter in Alaska (Burek-Huntington et al. 2014). *Coccidioides* spp.

infections are commonly reported from the southwestern US, including California's San Joaquin Valley and southern counties along the Pacific coast (Pappagianis 1994). All three fungal genera produce microscopic infectious particles that easily aerosolize when dry, facilitating inhalation (Reidarson et al. 1999). Fatal coccidioidomycosis has been reported in wild California sea lions (Fauquier et al. 1996), a bottlenose dolphin (Reidarson et al. 1998), and southern sea otters (*Enhydra lutris nereis*) (Cornell et al. 1979; Thomas et al. 1996). Pulmonary infection by *Coccidioides* can result in chronic illness in humans, and systemic dissemination can be fatal (Kirkland and Fierer 1996). Two morphologically identical species cause human illness: *Coccidioides immitis* and *Coccidioides posadasii*. Although *C. immitis* is more prevalent in California's San Joaquin Valley, both species are endemic to southern California (Fisher et al. 2002).

We reviewed stranding data from central California for 1998–2012 to assess the prevalence, host demographics, and lesion distribution for locally invasive or systemic mycoses of wild endemic marine mammals. Molecular methods were used to identify *Coccidioides* spp. strains associated with disseminated infection in sea otters and a *Cryptococcus* spp. strain isolated from a porpoise. We assessed stranding patterns for marine mammal infection by locally invasive or disseminated fungal pathogens and compared observed patterns with prior reports.

MATERIALS AND METHODS

Data compilation

We conducted a retrospective study with the use of case files from The Marine Mammal Center (TMMC), Sausalito, California, the Marine Wildlife Veterinary Care and Research Center (MWVCRC), Santa Cruz, California, and the University of California, Santa Cruz Marine Mammal Stranding Program (UCSC), Santa Cruz, California for January 1998–June 2012. The MWVCRC examines dead sea otters recovered anywhere in California (typ-

ically San Mateo to Santa Barbara counties), the TMMC examines other marine mammals initially recovered alive in central California (typically Marin to San Luis Obispo counties), and UCSC examines other marine mammals recovered dead in Santa Cruz County. Criteria for case inclusion consisted of gross or microscopic lesions consistent with locally extensive or systemic mycosis at necropsy, and confirmation of fungal infection via histopathology, fungal culture, or cytology.

For all marine mammals, age, sex, nutritional condition, and reproductive status were assessed with the use of published criteria (Kreuder et al. 2003; Greig et al. 2005). Some categories were pooled to facilitate analyses (“adult” = adults and aged adults; “immature” = subadults, juveniles, and pups). Postmortem condition was recorded as fresh, fresh-frozen, and moderately decomposed. Stranding location was recorded by county and scored as either north or south of Big Sur, California (36°15'N, 121°48'W). Nutritional condition was assessed as emaciated/poor or average to excellent, based on subjective assessment of muscle atrophy and body adipose stores. Reports and photographs were reviewed to assess fungal dissemination patterns. In cases where necropsy was abbreviated to minimize human exposure to *Coccidioides* spp., infection was confirmed by veterinary pathologists through detection of characteristic gross lesions (Cornell et al. 1979; Fauquier et al. 1996; Thomas et al. 1996), plus microscopic identification of fungal spherules on impression smears (Dip Quick Stain Set, Jorgensen Laboratories, Inc., Loveland, Colorado, USA).

Description or illustration of fungal dissemination to a given organ was considered sufficient to confirm the tissue distribution of *Coccidioides* in animals where *Coccidioides* infection was confirmed via the above-listed methods. When fungal distribution was unknown, categories were left blank. Adult female reproductive status was determined as pregnant/recently pregnant or not pregnant, based on confirmation of a fetus, an open cervix, a large, minimally involuted uterus, corpus luteum, or fresh placentation site at necropsy. The probable cause of death was noted for each animal.

PCR and sequencing of *Coccidioides* spp. strains

To identify the *Coccidioides* species associated with marine mammal infections, cryoarchived lung, lymph node, or spleen from four known *Coccidioides*-infected sea otters were submitted to UC Davis for PCR. The *Coccid-*

oides species was inferred with the use of real-time quantitative PCR (qPCR), and confirmed following sequence analysis of nested endpoint PCR products, as described by Diab et al. (2013). DNA from cryoarchived lung, lymph node, or spleen was extracted with the use of the QIAextractor (Qiagen Inc., Valencia, California, USA) following manufacturer's instructions. We performed qPCR assays that amplify and detect *Coccidioides* spp. DNA (qCoccy), or that are species-specific (i.e., amplify either *C. immitis* or *C. posadasii* DNA, but not both) with the use of extracted DNA. Species-specific reactions (qSNP.Ci and qSNP.Cp) that differentiate based on single-nucleotide polymorphism (SNP) were considered positive if ≤ 40 amplification cycles were required to achieve the predetermined fluorescent threshold. Quality-control reactions amplified and detected the 18S ribosomal gene with the use of pan-eukaryote primers and probes. The PCR products from three-step nested PCR amplification of the *Coccidioides* ribosomal gene (Diab et al. 2013) were sequenced and compared with those in the National Center for Biotechnology Information GenBank database with the use of the basic local alignment search tool (BLAST). *Coccidioides* species determination was made with the use of phylogenetically informative sites within the ribosomal ITS1 and ITS2 regions (Tintelnot et al. 2007). Sequences were submitted to GenBank (accessions KJ783445–KJ783449).

Cryptococcus multilocus sequence typing (MLST)

Cryptococcus spp. yeasts were isolated from a Dall's porpoise as described by Singer et al. (2014). High-molecular-weight genomic DNA was extracted and purified as described by Meyer et al. (2003). The MLST was performed with the use of the International Society of Human and Animal Mycology MLST consensus scheme for the *C. neoformans/C. gattii* species complex (Meyer et al. 2009).

RESULTS

Overview

From 1998 through 2012, at least 7,000 stranded marine mammals were recovered along the central California coast and necropsied by collaborating institutions. Case review revealed 41 animals spanning five species with focally extensive or disseminated fungal infections: Southern sea otter, California sea lion, Dall's porpoise, northern elephant seal (*Mir-*

ounga angustirostris), and Pacific harbor seal (*Phoca vitulina richardsi*). *Coccidioides* spp. infection was confirmed in 36 animals, including a harbor seal, 15 sea lions, and 20 sea otters. Five additional animals died with focally extensive or disseminated infections by other fungi, including zygomycetes in a sea lion and a harbor seal, *C. albidus* infection in a sea lion, *C. gattii* infection in a Dall's porpoise, and *Scedosporium apiospermum* infection in an elephant seal (Table 1). Findings for the *S. apiospermum*-infected elephant seal and *C. albidus*-infected sea lion were summarized by Haulena et al. (2002) and McLeland et al. (2012), respectively.

The subadult female harbor seal that stranded in 2009 with zygomycete infection exhibited antemortem seizures. Brain histopathology revealed necrotizing encephalitis and vasculitis with intralesional 7.5- μ m-diameter, colorless, nonparallel walled and nonseptate, acute to right angle branching fungal hyphae. Infection was most severe in the thalamus and adjacent cerebral cortex, where fungal hyphae surrounded blood vessels and occasionally penetrated vascular walls. The portal of entry appeared to be the upper respiratory tract.

A juvenile male sea lion stranded in 2005 with clinical evidence of acute renal failure, possibly due to leptospirosis. Also noted was ulcerative rhinitis and pharyngitis associated with putative otarine herpesvirus-1 infection, and severe pyogranulomatous nasopharyngitis, tonsillitis, lymphadenitis, and mural tracheitis. Intralesional fungal hyphae consistent with zygomycete infection were observed in all the above tissues, along with possible involvement of the rhinencephalon. Ulcerated upper respiratory mucosa was the most likely portal of entry.

A moderately decomposed, frozen-thawed Dall's porpoise exhibited marked lymphadenopathy (especially the hilar lymph nodes), and diffuse, marked pulmonary consolidation. On cut surface, both tissues

were markedly expanded by light tan, viscous to gelatinous material (Fig. 1A). Numerous thick-walled yeasts were observed on histopathology (Fig. 1B), and fungal culture yielded pure growth of *C. gattii*. According to MLST, the isolate (JS79/WM11.30) belonged to molecular type VGIII. The sequence type (143) was identical to an isolate from a shelter cat in San Francisco, California (JS110/WM11.139) and a musk deer (*Moschus moschiferus moschiferus*) from San Diego, California (JS84/WM11.35) (allele types 18, 3, 1, 3, 6, 40, and 23 for loci *CAP59*, *GPD1*, *IGS1*, *LAC1*, *PLB1*, *SOD1*, and *URA5*, respectively).

***Coccidioides* spp. infection in pinnipeds**

Data for 15 sea lions and one harbor seal with disseminated coccidioidomycosis are summarized in Table 2. Yearly stranding patterns suggest an increasing trend for pinniped stranding with disseminated coccidioidomycosis: for 1998–2005, only one pinniped with coccidioidomycosis was reported, compared with 15 from 2006 through 2012. A slight predominance of cases was noted during the second half of the year, with five *Coccidioides*-infected pinnipeds stranding January–June, compared with 11 during July–December.

Equal numbers of pinnipeds with coccidioidomycosis stranded in the northern and southern portions of the central coast. Northern-area strandings included Monterey ($n=1$), Santa Cruz ($n=3$), San Mateo ($n=3$), and Marin ($n=1$) counties. Nearly all *Coccidioides*-infected pinnipeds from the south-central coast were recovered from San Luis Obispo County ($n=7$), with only one recovered from Santa Barbara County. Most (2/3) *Coccidioides*-infected sea lions were immature, and female (2/3). All pinnipeds were minimally decomposed when examined.

Primary and contributing cause(s) of death for *Coccidioides*-infected pinnipeds are summarized in Table 2. Coccidioidomycosis was the primary cause of death for 13 of 15 (87%) *Coccidioides*-infected pinnipeds, and two died of domoic acid

TABLE 1. Stranded marine mammals from central California, USA with noncoccidioidal systemic fungal infections (1998–2012).

Species	Latin name	County	Strand date	Sex ^a	Age ^b	Carcass condition ^c	Body condition score ^d	Fungus isolated	Main findings
Northern elephant seal	<i>Mirounga angustirostris</i>	Not reported	25 April 1999	M	J	F	P	<i>Scedosporium apiospermum</i>	Abscessation in brain, kidney, and subcutaneous tissue (Haulena et al. 2002)
California sea lion	<i>Zalophus californianus</i>	Monterey	14 October 2005	M	J	F	P	Zygomycete	Primary renal disease; invasive respiratory zygomycete; OthV-1 infection
California sea lion	<i>Zalophus californianus</i>	Santa Clara	27 May 2009	M	J	F	P	<i>Cryptococcus albidus</i>	Primary bacterial pneumonia; secondary <i>C. albidus</i> (McLe-land et al. 2012)
Pacific harbor seal	<i>Phoca vitulina richardsi</i>	Monterey	28 November 2009	F	J	F	P	Zygomycete	Zygomycete-associated encephalitis and vasculitis
Dall's porpoise	<i>Phocoenoides dalli</i>	Santa Cruz	24 September 2010	M	A	FF	G	<i>Cryptococcus gattii</i>	Pyogranulomatous pneumonia and lymphadenitis

^a M = male; F = female.

^b J = juvenile; A = adult.

^c F = fresh; FF = fresh frozen.

^d P = poor; G = good.

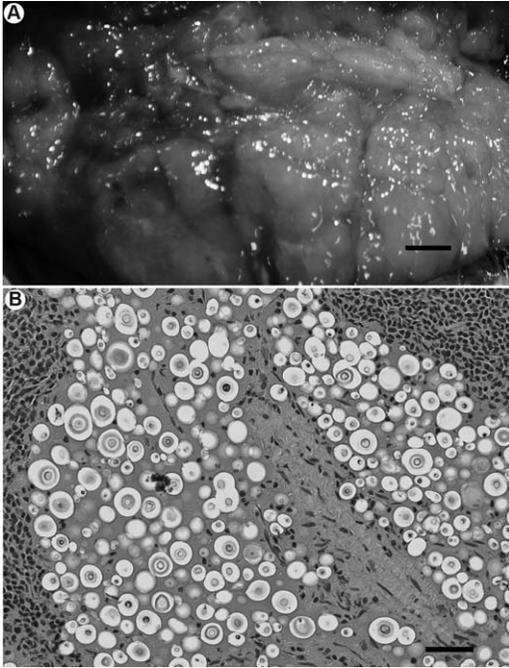


FIGURE 1. (A) Cross section of a massively enlarged hilar lymph node from a moderately decomposed, wild Dall's porpoise (*Phocoenoides dalli*), that died from disseminated *Cryptococcus gattii* infection. Note the wet, gelatinous appearance on cut surface due to the presence of myriad thick-walled, intralésional encapsulated yeasts. Bar = 0.5 cm. (B) Photomicrograph of hematoxylin and eosin-stained hilar lymph node from the same Dall's porpoise as above. Disseminated *Cryptococcus gattii* infection is indicated by infiltration and proliferation of large numbers of thick-walled, encapsulated yeasts throughout the cortex and medulla, effacing normal tissue. Bar = 30 μ m.

(DA) intoxication. Nearly all (14/15; 94%) *Coccidioides*-infected pinnipeds were thin or emaciated. A single *Coccidioides*-infected sea lion that stranded in average nutritional condition was diagnosed with DA intoxication as the primary cause of death.

The lesion distribution for *Coccidioides*-infected sea lions (indicated as percentages below) is provided as the number of animals with each specific condition, divided by the number of animals where the presence or absence of each condition could be accurately assessed, based on available data. Pulmonary granulomas were identified grossly or histologically in

10 of 13 sea lions (77%) with sufficient data for determination. *Coccidioides*-associated pleuritis was reported in five of nine sea lions (55%), whereas pleural effusion was noted in only two of seven (29%). In contrast, *Coccidioides*-associated peritonitis and peritoneal effusion were reported for 11 of 12 (98%), and four of seven sea lions (57%), respectively. Gross or histologic evidence of dissemination to the hilar (tracheobronchial) or mediastinal lymph nodes was apparent for nine of 10 sea lions (90%). Fungal-associated abdominal lymphadenopathy was present in 100% of nine cases with sufficient data for determination, and peripheral lymphadenopathy was also common (4/5 cases; 80%). Commonly affected abdominal nodes included the iliac, ileocecal, splenic, and gastric lymph nodes. Commonly affected peripheral lymph nodes included the inguinal, mandibular, axillary, and popliteal nodes.

Fungal dissemination was common in otariid liver (10/11; 91%) and spleen (5/12; 42%). *Coccidioides*-associated myocarditis or epicarditis were reported in six of 13 animals (46%). Importantly, surface-oriented fungal growth with development of hyphae and arthroconidia was observed on the epicardium of a freshly dead sea lion (Fig. 2B). Five of 11 sea lions (45%) exhibited thickened pericardial sacs or histologically confirmed fungal pericarditis. Less common sites for fungal dissemination included kidney (4/12; 33%) and brain (1/11; 9%). Eyes were examined microscopically in only two cases, but ocular coccidioidomycosis was confirmed in one. Of five adult females with coccidioidomycosis, one was pregnant at necropsy, and two had partially involuted placentas indicative of recent pregnancy. Examination of the fetus and placenta was not performed. Reproductive data were unavailable for two adult females.

The *Coccidioides*-infected harbor seal exhibited multifocal pulmonary granulomas and pulmonary emphysema, with fungal dissemination to the thoracic and abdominal lymph nodes, liver, spleen, and

TABLE 2. Stranding data for southern sea otters (*Enhydra lutris nereis*), California sea lions (*Zalophus californianus*), and a Pacific harbor seal (*Phoca vitulina richardsi*) from central California, USA with disseminated coccidioidomycosis (1998–2012).

	Date	Sex ^a	Age ^b	Carcass condition ^c	N or S of Big Sur ^d	Body condition score ^e	Primary cause of death	Contributing causes of death
Sea otter								
1	21 June 1998	M	A	F	S	G	Domoic acid	Blunt trauma, Coccidioidomycosis
2	9 May 2000	M	A	F	S	P	Coccidioidomycosis	Pneumothorax, starvation
3	10 April 2003	M	A	M	S	G	Coccidioidomycosis	
4	28 December 2003	F	A	F	S	P	Coccidioidomycosis	
5	6 February 2004	F	J	F	S	P	Coccidioidomycosis	Blindness, starvation
6	22 April 2004	M	J	F	S	P	Coccidioidomycosis	Protozoal encephalitis, gastric ulcers
7	27 July 2004	M	A	F	S	G	Coccidioidomycosis	Cerebral hemorrhage, cardiomyopathy
8	4 February 2005	M	A	M	S	P	Coccidioidomycosis	Starvation
9	3 February 2006	M	A	M	N	P	Coccidioidomycosis	Starvation
10	13 July 2006	M	A	M	N	P	Coccidioidomycosis	Starvation
11	14 April 2007	M	A	F	S	P	Bacterial septicemia	Ulcerative gastritis, coccidioidomycosis
12	21 July 2007	M	A	M	S	P	Coccidioidomycosis	
13	15 March 2008	M	A	F	S	P	Coccidioidomycosis	Pleuritis
14	27 January 2009	F	A	F	N	P	Coccidioidomycosis	Aspiration pneumonia, starvation
15	28 March 2009	F	A	F	S	P	Coccidioidomycosis	Pleuritis
16	25 March 2010	M	J	F	S	P	Coccidioidomycosis	Acanthocephalan peritonitis, starvation
17	14 March 2011	M	A	F	S	P	Coccidioidomycosis	
18	24 June 2011	M	A	F	N	P	Coccidioidomycosis	
19	7 March 2012	F	A	M	S	P	Coccidioidomycosis	
20	14 June 2012	M	J	FF	S	G	Coccidioidomycosis	Pneumothorax
Sea lion								
1	30 June 2002	F	J	F	N	P	Coccidioidomycosis	Streptococcal sepsis
2	30 April 2005	M	J	F	S	P	Coccidioidomycosis	
3	17 August 2005	F	A	F	S	G	Domoic acid	Coccidioidomycosis, urogenital carcinoma
4	15 November 2005	M	J	F	S	P	Domoic acid	Leptospirosis
5	5 June 2006	M	J	F	S	P	Coccidioidomycosis	
6	17 September 2006	F	J	F	S	P	Coccidioidomycosis	
7	22 November 2007	F	A	F	N	P	Coccidioidomycosis	
8	20 December 2007	F	J	F	N	P	Coccidioidomycosis	
9	13 August 2008	F	A	F	N	P	Coccidioidomycosis	Domoic acid, malnutrition
10	4 May 2009	F	A	F	N	P	Coccidioidomycosis	Domoic acid

TABLE 2. Continued.

	Date	Sex ^a	Age ^b	Carcass condition ^c	N or S of Big Sur ^d	Body condition score ^e	Primary cause of death	Contributing causes of death
11	2 July 2010	F	J	F	S	P	Coccidioidomycosis	
12	5 July 2010	M	J	F	N	P	Coccidioidomycosis	
13	4 July 2011	F	J	F	N	P	Coccidioidomycosis	
14	31 August 2011	F	A	F	S	P	Coccidioidomycosis	
15	6 March 2012	M	J	F	N	P	Coccidioidomycosis	Leptospirosis
Harbor seal								
1	31 December 2010	M	1	F	S	P	Coccidioidomycosis	

^a M = male; F = female.

^b J = juvenile; A = adult.

^c F = fresh; FF = fresh frozen; M = moderately decomposed.

^d N = north; S = south.

^e G = good; P = poor.

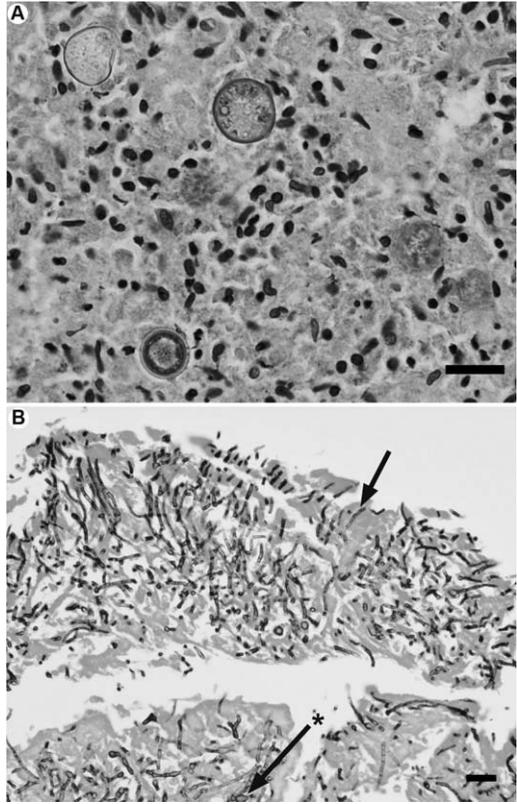


FIGURE 2. (A) Photomicrograph of hematoxylin and eosin-stained hilar lymph node from a moderately decomposed southern sea otter (*Enhydra lutris nereis*), showing an immature spherule (bottom left), a mature spherule containing endospores (top center), and a ruptured, partially empty spherule (upper left corner). Bar = 25 μm. (B) Photomicrograph of Gomori methenamine silver (GMS)-stained epicardial fibrin from a California sea lion (*Zalophus californianus*) that died from disseminated coccidioidomycosis. *Coccidioides* spp. mycelia are apparent as myriad 2–4-μm diameter branching, septate, linear, grey-black bands (arrow). Arthroconidia formation is also apparent, characterized by multifocal areas of oval, or barrel-shaped mycelial expansion (asterisk; bottom edge of image, below tip of arrow). Bar = 20 μm. Background fibrin matrix is stained pale green.

kidney. This is the first report of coccidioidomycosis in a wild harbor seal. No concurrent disease was identified.

Coccidioides spp. infection in sea otters

Twenty wild southern sea otters with coccidioidomycosis stranded between 1998 and 2012 (Table 2). Similar to pinnipeds, the number of *Coccidioides*-

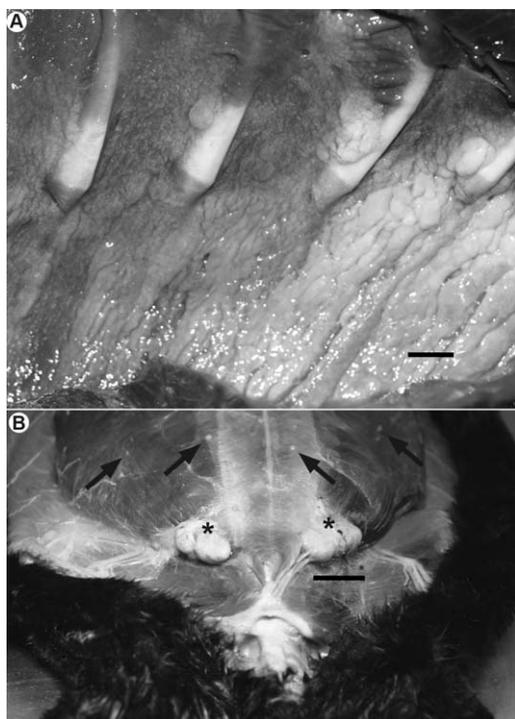


FIGURE 3. (A) Pyogranulomatous pleuritis in a southern sea otter (*Enhydra lutris nereis*) that died due to disseminated coccidioidomycosis. When observed, this lesion is highly suggestive of disseminated *Coccidioides* infection in sea otters. Bar = 1.5 cm. (B) Pyogranulomatous inguinal lymphadenitis in a southern sea otter that died because of systemic coccidioidomycosis. In addition to markedly enlarged (approximately 4× normal size), firm, pale tan inguinal lymph nodes (asterisks), numerous tiny tan, raised plaques are scattered randomly throughout the subcutis (arrows). When observed in association with diffuse, marked peripheral lymphadenopathy, this lesion is highly suggestive of disseminated *Coccidioides* spp. infection in sea otters, providing an alert prior to opening the body cavity and releasing fungal-contaminated body fluids into the environment. Bar = 6 cm.

infected sea otters presenting to marine mammal stranding facilities in central California appears to be increasing. From 1998 to 2005, eight sea otters with coccidioidomycosis were recovered (1.14 cases/yr), compared with 12 cases (1.85/yr) from 2006 to 2012. Most (80%) sea otters with coccidioidomycosis stranded between January and June and along the southern half of the central coast (80%). Fifteen

otters stranded in San Luis Obispo County, and one in Santa Barbara County. Four *Coccidioides*-infected otters (20%) were recovered on Monterey County beaches, representing the first detection of sea otters with coccidioidomycosis north of San Luis Obispo County over 45 yr of monitoring.

Among 16 otters stranding south of the Big Sur coast, Pismo Beach, and Morro Bay were overrepresented, with five *Coccidioides*-infected otters stranding at each site (50% of all cases). Sixteen otters with coccidioidomycosis (80%) were adults, and 75% were male. Three stranded alive and died or were euthanized; all remaining animals were found dead. Coccidioidomycosis was the primary cause of death for 90% of *Coccidioides*-infected otters, except two cases, where DA intoxication and bacterial septicemia were considered primary causes of death (Table 2). Sixteen *Coccidioides*-infected otters (80%) were thin or emaciated, and four (20%) were in good to excellent body condition. Thirteen carcasses were fresh, one was fresh-frozen, and six were moderately decomposed.

Respiratory system involvement was common, with pyogranulomatous pleuritis (Fig. 3A) reported for 12 of 13 (92%), pleural effusion for 11 of 12 (92%), and pulmonary nodules for 15 of 16 (94%) sea otters. Pneumothorax was detected in three of 12 *Coccidioides*-infected otters (25%). Fungal-associated peritoneal effusion was noted in five of nine sea otters (55%) and peritonitis was present in two of seven (29%). All otters with sufficient data for determination had gross or microscopic evidence of fungal dissemination to the hilar or mediastinal lymph nodes (15/15), and peripheral nodes (12/12), including the axillary, retropharyngeal, or inguinal lymph nodes (Fig. 3B). Many otters with coccidioidomycosis exhibited numerous tan, raised, subcutaneous plaques; a potential “early warning” sign during necropsy that has not previously been reported (Fig. 3B). On histopathology these plaques were composed of

TABLE 3. Results of *Coccidioides* spp. quantitative (q) and conventional (nested endpoint) PCR amplification and sequencing for four southern sea otters (*Enhydra lutris nereis*) with disseminated coccidioidomycosis.

Sea Otter	Tissue type	Real-time qPCR			Nested endpoint PCR	
		qCoccy ^a	qSNP.Ci ^b	qSNP.Cp ^c	Nest 3	Nest 2
A	Lung	Positive	Negative	Negative	No product	No product
	Lymph node	Positive	Negative	Negative	<i>Coccidioides</i>	Not done
	Spleen	Positive	Negative	Negative	<i>Coccidioides</i>	<i>Coccidioides immitis</i>
B	Lung	Positive	Not done	Not done	No product	No product
	Lymph node	Positive	Positive	Negative	Mixed sequence	Uncultured ascomycete
C	Lung	Positive	Negative	Negative	<i>Coccidioides</i>	Not done
	Lymph node	Positive	Positive	Negative	<i>Coccidioides</i>	<i>C. immitis</i>
	Spleen	Positive	Not done	Not done	<i>Coccidioides</i>	Not done
D	Lung	Positive	Positive	Negative	<i>Coccidioides</i>	<i>C. immitis</i>
	Spleen	Positive	Positive	Negative	<i>Coccidioides</i>	<i>C. immitis</i>

^a qCoccy=qPCR assay for *Coccidioides* spp.

^b qSNP.Ci=qPCR assay for *C. immitis* with the use of single-nucleotide polymorphism.

^c qSNP.Cp=qPCR assay for *Coccidioides posadasii* with the use of single-nucleotide polymorphism.

pyogranulomatous inflammation with rare intralesional fungal spherules. Ten of 11 otters (91%) had enlarged abdominal nodes, including the mesenteric, perigastric, and sublumbar nodes.

The spleen (14/15; 93%), meninges +/- brain (8/9; 89%), and liver (11/13; 85%) were common sites for fungal dissemination, whereas renal involvement was less common (6/12; 50%). A thickened pericardial sac (5/9; 55%) and fungal myocarditis or epicarditis (3/7; 43% were also noted. *Coccidioidal* ophthalmitis was confirmed for three of nine otters (33%) with eyes available for microscopic examination. Of four adult female *Coccidioides*-infected sea otters, one was pregnant with a minimally developed fetus, and one had recently been pregnant, indicated by an enlarged uterus, a fresh placental scar and an open cervix. No evidence of *Coccidioides* spp. infection was noted for the fetus and placenta. Reproductive status was unknown for two adult females.

All qPCR assays performed with the use of sea otter tissue were positive for *Coccidioides* spp. (Table 3). The qSNP.Ci assay was also positive when tested with DNA from at least one tissue from all but sea otter A, suggesting the presence of *C. immitis* DNA. The qSNP.Cp assay was

negative for all samples. Sequence analysis of nested endpoint PCR products confirmed the diagnosis of coccidioidomycosis and more specifically, *C. immitis* infection for sea otters A, C, and D. For otter B, the nest 3 PCR sequence product was mixed. Sequence was obtained from the nest 2 PCR product, but had greatest similarity to uncultured ascomycete when compared with other GenBank sequences. No *C. posadasii* infections were identified in sea otters.

DISCUSSION

Coccidioides spp. infection was the most common cause of systemic mycosis in necropsied marine mammals from central California, and limited PCR testing detected only *C. immitis*. *Coccidioides* spp. are thermally dimorphic fungi that grow as yeasts at mammalian body temperature and under elevated CO₂ conditions and form hyphae in soil at ambient temperature, leading to production of aerial mycelium and thick-walled arthroconidia. Arthroconidia are easily aerosolized and inhaled, especially during dry, windy weather or when soil is disturbed (Burek 2001). When *Coccidioides* spp. invade host tissue, 30–60- μ m diameter,

thick-walled spherules form (Fig. 2A), mature and ultimately rupture, culminating in release of endospores (Fig. 2A) that form the next generation of spherules (Hector et al. 2005). Spherules and endospores are resistant to leukocyte-mediated phagocytosis and cytolysis, stimulating development of pyogranulomatous inflammation. Although *Coccidioides* spp. are often described from warm, dry, alkaline soils, arthroconidia can also persist in saline soil and seawater (Burek 2001).

Coccidioides spp. are important human pathogens in California, the American southwest, and northern Mexico (Pappagianis 1994). The incidence of human coccidioidomycosis in California has increased over the past decade (Hector et al. 2011) and temporospatial correlations between human and marine mammal coccidioidomycosis have been reported (Pappagianis 1994).

Our findings suggest northward expansion of *Coccidioides* infections in California marine mammals, compared with prior studies (Cornell et al. 1979; Fauquier et al. 1996; Thomas et al. 1996). During 45 yr of surveillance, no *Coccidioides*-infected sea otters were reported from Monterey Bay until 2006. However, improved disease detection and other factors can complicate efforts to monitor disease prevalence and distribution over time. Future studies would be helpful to see if this potential northward expansion continues.

Variations in home range size (Estes et al. 2009; Heath and Perrin 2009) and in precision of mapping stranding patterns can complicate efforts to pinpoint fungal sources. Because sea lions can move hundreds of kilometers in a week, stranding locations may not reflect fungal exposure sites. However, most *Coccidioides*-infected otters and sea lions in previous studies (Cornell et al. 1979; Thomas et al. 1996), and 60% in this study, stranded along San Luis Obispo County beaches, suggesting that this region is a “hot spot” for *Coccidioides* exposure. Confirmation

of pulmonary infection in sea otters supports inhalation as the primary route of *Coccidioides* exposure for marine mammals (Thomas et al. 1996). The hydrophobic nature of arthroconidia (Pappagianis, 1988) could facilitate aerosolization by wave action. Marine mammal infections appear to occur purely as a consequence of land-to-sea pathogen transfer: No nidus for coccidioidal proliferation has been identified in the marine environment.

Although *Coccidioides*-infected sea lions stranded year-round, most infected sea otters stranded January–June. In humans, clinical signs develop 7–28 d postexposure, but disease can persist for months (Kirkland and Fierer 1996). We hypothesize that sea otters, like humans, are most commonly infected during the dry, windy late summer and fall, but exposed otters may not die for 4–6 mo.

Sea otters with systemic coccidioidomycosis were predominantly adult males, similar to previous reports (Cornell et al. 1979; Thomas et al. 1996). Some males make periodic excursions to the southern range (including San Luis Obispo County), leading to a higher risk of *Coccidioides* spp. exposure (Thomas et al. 1996). Sixty-five percent of human coccidioidomycosis cases in California (2001–2009) also occurred in males (Hector et al. 2011). Certain genes influence resistance to coccidioidomycosis in mice (Fierer et al. 1999). Thus, genetic, hormonal, and behavioral influences could all contribute to a higher prevalence of coccidioidomycosis in males. In contrast, most sea lion cases involved immature females.

Although female otters may be less commonly infected, exposure during pregnancy may enhance risk of dissemination (Thomas et al. 1996). Pregnancy is an important risk factor for fungal dissemination in humans, especially during the third trimester (Kirkland and Fierer 1996; Crum and Ballon-Landa 2006; Hooper et al. 2007). T_H1 -mediated immunity provides the primary response to *Coccidioides* infection. A switch toward T_H2 -mediated

immunity in late pregnancy may underlie increased susceptibility to disseminated *Coccidioides* during late pregnancy (Kirkland and Fierer 1996; Hooper et al. 2007).

Lesion patterns for *Coccidioides*-infected marine mammals were similar to previous reports (Cornell et al. 1979; Fauquier et al. 1996; Thomas et al. 1996). Although pulmonary pyogranulomas were common in sea lions and otters, significant pathology of the pleura and respiratory tract were more consistent findings in otters. Thomas et al. (1996) described diffuse pyogranulomatous pleuritis, especially along the inner chest wall (Fig. 3A) as a characteristic of *Coccidioides* infection in sea otters. We found pleuritis in 92% of otters, versus 55% of sea lions, and pleural effusion in 92% of otters, and 29% of sea lions. Our data support a prior report by Thomas et al. (1996) that pneumothorax can be a sequela of pulmonary coccidioidomycosis in sea otters. In contrast, significant peritoneal involvement was more common in sea lions, with peritoneal effusion and peritonitis affecting 92% of sea lions, versus 22% of sea otters (Fauquier et al. 1996).

Markedly enlarged, variably firm lymph nodes are a striking feature of disseminated coccidioidomycosis in sea otters and sea lions (Fig. 3B), providing early warning during clinical care and necropsy. The presence of numerous tiny tan, raised, subcutaneous plaques in association with marked lymphadenopathy is highly characteristic of disseminated coccidioidomycosis in sea otters (Fig. 3B). Thoracic and peritoneal effusion often contains abundant fungal spherules and endospores, so early recognition can minimize human exposure and facility contamination.

Although splenic and hepatic fungal dissemination was common, we found that ocular dissemination may also be common and antemortem ophthalmic examination might aid diagnosis. Although the frequency of systemic coccidioidomycosis was high when compared with other,

opportunistic fungal pathogens, the paucity of *Coccidioides* cases in cetaceans and phocids was striking, and could be due to species-specific differences in susceptibility, or sample bias.

Strains of *C. gattii* found in British Columbia, Washington, and Oregon (e.g., VGIIa and VGIIc) appear to be quite virulent, based on confirmation of illness and death of humans and animals with no discernable immune compromise (Byrnes et al. 2010). Molecular type VGIII has now emerged as a virulent pathogen of humans and companion animals in California (Byrnes et al. 2010; Singer et al. 2014; Thompson et al. 2014). Our detection of *C. gattii* VGIII in a wild Dall's porpoise from central California, and confirmation of identical strains in cats and a captive musk deer from California expands the host range of *C. gattii* VGIII and suggests terrestrial-to-marine pathogen transport.

Because of their pathogenicity and limited options for medical therapy, detection of lesions consistent with coccidioidomycosis or cryptococcosis, as described above, should alert personnel to take extra precautions during necropsy. *Coccidioides* is not generally considered to be zoonotic. However, detection of mycelia and arthroconidia on histopathology (Fig. 2B) and reports of coccidioidomycosis acquired during laboratory work (Pike 1978), clinical care (Gaidici and Saubolle 2009), or necropsy (Kohn et al. 1992) confirm that these fungi can pose health risks to laboratory personnel, including through fungal colonization of facilities.

In humans, enhanced susceptibility to disseminated coccidioidomycosis was noted for humans of Filipino and African American ancestry (Galgiani et al. 2005), pregnant women, and immunocompromised populations (Hooper et al. 2007, Hector et al. 2011). Individuals who are at greater risk should avoid contact with infected animals and tissues. Body fluids should be contained to prevent facility contamination. Personnel should be educated about these

potentially dangerous pathogens, and trained to recognize characteristic lesions. Based on limited testing and previously published reports (Friedman et al. 1956), freezing will not inactivate pathogenic fungi. Potentially infectious material should be frozen and incinerated, and samples should not be submitted for diagnostic testing without clear labeling and advance warning. Because of a high risk of aerosol exposure, fungal cultivation and identification should be performed only by microbiologists with adequate training and facilities.

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