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Emergence of a Zoonotic Pathogen in a Coastal Marine Sentinel: *Capillaria hepatica* (syn. *Calodium hepaticum*)-Associated Hepatitis in Southern Sea Otters (*Enhydra lutris nereis*)

Melissa A. Miller[1,2]*, Pádraig J. Duignan[3], Erin Dodd[1], Francesca Batac[1],
Michelle Staedler[4], Joseph A. Tomoleoni[5], Michael Murray[4], Heather Harris[3] and
Chris Gardiner[6]

[1] Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Wildlife, Santa Cruz, CA,
United States, [2] Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, Davis, CA,
United States, [3] The Marine Mammal Center, Sausalito, CA, United States, [4] Monterey Bay Aquarium, Monterey, CA,
United States, [5] Santa Cruz Field Station, Western Ecological Research Center, U. S. Geological Survey, Santa Cruz, CA,
United States, [6] JPC Veterinary Pathology Services, The Joint Pathology Center, Silver Spring, MD, United States

Capillaria hepatica is a globally distributed zoonotic nematode parasite that most commonly infects feral and native rats. Soil contact, pica, and living in close proximity to rat populations are risk factors for human infection. Larval nematodes and eggs that were morphologically consistent with *C. hepatica* were observed microscopically in livers of stranded southern sea otters (*Enhydra lutris nereis*) from California. Large (90–100 × 45–55 μm), barrel-shaped non-embryonated parasite eggs with large polar prominences and a roughened or striated surface, or 105–120 μm diameter larval aphasmid nematode profiles with a prominent stichosome and hypodermal bands were observed in the livers of three otters. The liver of a fourth animal exhibited serpiginous tracts of necrosis, micro-cavitation and pleocellular inflammation, with intralesional linear eosinophilic material that resembled cuticle from degenerating metazoan parasites. *Capillaria hepatica*-associated hepatitis and capsular adhesions were the cause of death for one otter, and parasite-associated liver lesions were observed in all cases. All infected otters were adult females that stranded from 2006 through 2016 at multiple sites along the central California coast. All cases stranded from December through May; during and soon after peak seasonal precipitation and land-sea runoff in California. This same seasonal pattern has been reported for other land-based parasites infecting southern sea otters. Neither *C. hepatica,* nor any similar nematodes have been reported from marine mammals, and southern sea otters are not typical hosts for *C. hepatica* or any other nematode parasites. The most likely route of exposure was via freshwater runoff containing embryonated eggs liberated from predated or decomposing terrestrial hosts, especially rats. Similar to the land-based parasites *Toxoplasma gondii* and *Sarcocystis neurona*, *C. hepatica* eggs may be concentrated and transmitted through filter-feeding

marine invertebrates that serve as southern sea otter prey, which may also pose an unrecognized public health risk for people who consume these species.

INTRODUCTION

Capillaria hepatica (syn. *Calodium hepaticum*) is a globally distributed nematode parasite of mammals, including humans. Wild and feral rodents of the genus *Rattus* serve as the main source of environmental contamination by parasite eggs in urban environments. Under crowded and unsanitary conditions >70% of rats can be infected, and cannibalism facilitates transmission between successive rodent generations (Fuehrer et al., 2014a). Ingestion of water or food contaminated by embryonated *C. hepatica* eggs can cause infection in humans and other mammals (Fuehrer et al., 2014b). In an early report of human infection, eggs compatible with *C. hepatica* were detected in material recovered from a 3rd to 4th century grave in France (Mowlavi et al., 2014).

Capillaria hepatica is the only vertebrate nematode known to require host death for completion of the parasite life cycle (Wright, 1961). Following 3 weeks of hepatic maturation, females begin laying eggs around portal tracts. Nematode migration within the parenchyma can cause necrosis (Redrobe and Patterson-Kane, 2005), and adults ultimately die and disintegrate. Eggs and dying parasites elicit an inflammatory response composed of macrophages, multinucleated giant cells, lymphocytes, plasma cells and eosinophils, often resulting in granuloma formation (Redrobe and Patterson-Kane, 2005). In live animals, eggs remain un-embryonated in the hepatic parenchyma, and are not released through the biliary tract.

Eggs are deposited in the liver in dense masses and may be most concentrated in the subcapsular parenchyma (Moreira et al., 2013). Due to localized egg deposition and exuberant perilesional fibroplasia, affected areas can appear grossly as serpiginous or multinodular yellow-white capsular spots (Moreira et al., 2013). On histopathology the distinctive eggs are barrel-shaped and un-embryonated, with two shallow polar prominences (i.e., polar plugs), and a characteristically rough, pitted shell (Joint Pathology Center [Joint Pathology Center [JPC], 2015]). Once deposited in the liver, eggs can remain viable for months to years (Wright, 1961).

Eggs are released into the environment following either predation, or host death and decomposition. When liver from freshly killed prey is consumed by predators, un-embryonated *C. hepatica* eggs simply pass via feces into the environment without causing infection. Predators and scavengers serve as paratenic hosts, facilitating egg dissemination and maturation (Redrobe and Patterson-Kane, 2005). Following host death, decomposition and exposure to air and damp soil, liberated eggs embryonate (i.e., become infective) over approximately 30 days, and can overwinter in cold environments (Wright, 1961).

The infection cycle continues when embryonated eggs are consumed by a new mammalian host.

The larvae hatch, penetrate the intestinal mucosa, travel via the portal veins to the liver, invade the parenchyma, and mature over 35–50 days. Adults lay hundreds of eggs, then die 40–59 days later and decompose (Gardiner and Poynton, 1999). Due to their short lifespan, nematodes are less commonly observed on liver histopathology than deposited eggs (Moreira et al., 2013). When present, long, slender *C. hepatica* nematodes and migration tracks are observed in the parenchyma, often just beneath the hepatic capsule. Infection of other organs is less common (Moreira et al., 2013).

The host range of *C. hepatica* has been reviewed (Fuehrer et al., 2011, 2014a, 2014b), with only two prior reports of mustelid infection (Solomon and Handley, 1971; Forrester, 1992), and no reports of *C. hepatica* in marine mammals. Here, we describe *C. hepatica* infection in a threatened marine mammal from California, the southern sea otter (*Enhydra lutris nereis*), in which hepatic lesions ranged from mild and incidental, to severe and fatal.

Invertebrate-feeding southern sea otters are not normally parasitized by nematodes, in contrast with their more piscivorous northern counterparts (Margolis et al., 1997). No morphologically similar parasites or eggs have been described from marine mammal livers. Otters are likely infected via ingestion of embryonated *C. hepatica* eggs reaching the ocean through runoff, or through consumption of eggs in filter-feeding invertebrates, as reported for oocysts and sporocysts of the terrestrial parasites *Toxoplasma gondii* and *Sarcocystis neurona* (Arkush et al., 2003; Miller et al., 2008; Michaels et al., 2016). Because *C. hepatica* can infect humans, our findings could suggest health risks for humans consuming raw or undercooked aquatic invertebrates from areas where rats are abundant, where watersheds are degraded, and where environmental contamination by *C. hepatica* eggs is high.

MATERIALS AND METHODS

Stranded southern sea otters from throughout Central California were necropsied by collaborating veterinary pathologists (MM and PD). Necropsy included gross photography and microscopic examination of all major tissues as described (Kreuder et al., 2003). Livers containing suspect *C. hepatica* eggs, nematodes, and putative parasite migration tracts were further assessed via microscopic examination of formalin-fixed, paraffin-embedded, hematoxylin and eosin (H&E)-stained, 5 μm thick serial sections. The size, location, and morphology of eggs and nematodes were compared with published reports from terrestrial and aquatic mammals, and humans. The stranding location and date, stranding season, sex, and age were assessed to provide insight on the origin and timing of *C. hepatica* infection. One

animal had been implanted with a very high frequency (VHF) radio transmitter and time-depth recorder (TDR) antemortem as part of life history research, so visual resight and electronic monitoring data were compiled by collaborating biologists.

RESULTS

Table 1 summarizes case findings for four sea otters with confirmed or suspected *C. hepatica* infection.

Case 1 was an emaciated adult female that stranded alive, but died within hours. This animal had been captured, and a VHF radio transmitter and TDR had been surgically implanted in the peritoneal cavity approximately 3.5 years prior to death to facilitate life history research. At the time of capture, the otter weighed 18.5 kg and was in good nutritional condition with no apparent clinical or physical abnormalities. Surgical implantation was uneventful, and parameters on a perioperative complete blood count and blood chemistry panel were unremarkable. Post-capture monitoring revealed that this animal foraged mainly in the vicinity of San Simeon California, and her diet consisted of 46% clams, 18% *Cancer* spp. crabs, 19% other crab species, and smaller amounts of other prey. Case 1's TDR data showed body temperature fluctuations suggestive of potential estrus events in 2013 and 2014, but copulation or pupping was not visually confirmed. Data collection by her TDR ceased approximately 18 months prior to death.

At necropsy, the otter's weight had decreased by 4.5 kg (24%) to 14 kg. Based on mammary, uterine and ovarian morphology, it appeared that abortion had occurred approximately 3 weeks prior to death during the first half of post-implantation pregnancy (Chinn et al., 2016). Although aerobic culture of endometrium grew non-hemolytic *Escherichia coli* and *Vibrio parahaemolyticus*, significant endometritis was not noted. Gastric erosions and melena were considered perimortem lesions, reflective of severe stress.

Multifocal pale yellow, raised, 0.5 to >8 cm diameter fibrinous to fibrous plaques were scattered across the hepatic capsule, causing marked contracture of individual hepatic lobes, and extensive adhesions between lobes (**Figure 1A**). A large adhesion encompassed the visceral surface of the right lateral hepatic lobe and 50% of the omentum, which was bunched into a dense (12 cm × 8 cm × 5 cm) mass (**Figure 1A**). Adjacent omentum and mesentery were severely congested, thick and opaque. The TDR was recovered from the omental mass, and severe necrosuppurative omentitis with intralesional mixed bacteria was noted adjacent to the TDR entrapment site on histopathology. Aerobic and anaerobic culture of peritoneal exudate yielded *E. coli*, while *V.* parahaemolyticus and *Clostridium perfringens* were identified on fecal culture.

On histopathology, multifocal clusters of large (90–100 by 45–55 μm), non-pigmented, un-embryonated, barrel-shaped parasite eggs with large polar prominences at each end, and a roughened, striated and moderately refractile shell were apparent in the subcapsular hepatic parenchyma beneath the omental adhesion (**Figure 1B**). The hepatic capsule overlying the egg masses was markedly expanded by fibrinosuppurative inflammation, granulation tissue, and fibroplasia (**Figure 1B**). The egg masses were surrounded by areas of coagulation necrosis and moderate infiltrates of neutrophils, monocytes, macrophages, lymphocytes, eosinophils, hemorrhage, granulation tissue, and fibroplasia (**Figure 1C**). Egg masses were encased in a fine fibrovascular stroma admixed with granulomatous inflammation, and interspersed with large expanses of unaffected parenchyma (**Figure 1C**). Low numbers of intramuscular *Sarcocystis* sp. were also noted on histopathology, but were considered incidental.

Serological testing of serum collected at necropsy showed a high IgG titer to the protozoan parasite *T. gondii* (10,240; the cutoff for seropositivity is ≥320) (Miller et al., 2002). Mild lymphoplasmacytic meningitis and perivasculitis was observed in the brain on histopathology, accompanied by a focal mild scar in the cerebrum; no parasites compatible with *T. gondii* were observed on histopathology in any tissue. Serology for the protozoan parasite *Sarcocystis neurona* was negative (IgG titer 40; the cutoff for seropositivity is presumed to be ≥320) and a single mature intramuscular sarcocyst was observed in the left atrial wall. Protozoan parasite culture, immunohistochemistry, or PCR was not performed. Based on these findings, incidental *T. gondii* and *S. neurona* infection was suspected.

Case 2 was an emaciated adult female that died from wound infection and septicemia approximately 2 weeks after a white shark (*Carcharodon carcharias*) attack. Aerobic bacterial culture of a laceration yielded *Streptococcus pyogenes*, *Streptococcus viridans*, *Morganella morganii*, *Vibrio parahaemolyticus,* and *Shewanella putrefaciens*, and heart blood was positive for *S. pyogenes* and *V. parahaemolyticus*. Significant health conditions that predated shark bite included severe cardiomyopathy (Kreuder et al., 2003), end-lactation syndrome (ELS) (Chinn et al., 2016), and mild lymphoplasmacytic meningoencephalitis associated with *T. gondii* infection. Perimortem lesions included dehydration and intestinal melena. Multifocal discrete, pale yellow, raised hepatic capsular plaques and severe necrotizing hepatitis were noted grossly. Several large (90–100 by 45–55 μm), non-pigmented, un-embryonated, barrel-shaped parasite eggs similar to those described for Case 1 were found in hepatic parenchyma subjacent to the capsular plaques (**Figure 2A**). Parasite eggs were arranged in discrete masses, and were surrounded by large expanses of coagulation necrosis, hemorrhage, inflammation and fibrous connective tissue similar to that described above (**Figure 2B**). Also present were tiny circular foci of hepatic cavitation with perilesional granulomatous inflammation (putative parasite migration scars) (**Figure 2C**).

Serological testing revealed a low positive IgG titer to *T. gondii* (1,280), and this parasite was isolated from brain tissue in cell culture. Brain histopathology showed mild lymphoplasmacytic meningitis, perivasculitis and encephalitis with a single mature *T. gondii* tissue cyst; the tissue cyst was not present in recuts and immunohistochemistry was negative. Serology found a high IgG titer (10,240) to *S. neurona*, but no parasites were observed on histopathology or immunohistochemistry. No PCR was attempted. Based on these findings, incidental *T. gondii* and *S. neurona* infection was suspected.

TABLE 1 | Summary of stranding data, gross findings and histopathology for southern sea otters (*Enhydra lutris nereis*) with putative or confirmed *Capillaria hepatica* infection.

Case number	Strand date	Stranding County	Sex	Age class (years)	Nutritional condition at stranding	Primary cause of death	*Capillaria hepatica*-associated gross lesions (liver)	*Capillaria hepatica*-associated microscopic lesions (liver)	Concurrent parasitism
1	3/21/2016	San Luis Obispo	F	A (4–5)	Emaciated	Chronic hepatitis due to *Capillaria hepatica* infection, with secondary intrahepatic and hepatic-omental adhesions, time-depth recorder (TDR) entrapment and bacterial omentitis and peritonitis.	Multifocal necrotizing hepatitis. Multifocal discrete, pale yellow, raised capsular fibrinous plaques with hepatic lobular adhesions. Focally extensive omental adhesion with TDR entrapment. Secondary severe necrosuppurative omentitis and peritonitis.	Chronic multifocal necrosuppurative to granulomatous hepatitis. Intrahepatic clusters of un-embryonated parasite eggs with prominent polar plugs. Chronic severe fibrinosuppurative hepatic capsulitis.	Based on findings from serology and histopathology, incidental *T. gondii* and *S. neurona* infection was suspected.
2	5/26/2006	Santa Cruz	F	A (8)	Emaciated	Subacute shark bite with secondary bacterial infection and septicemia.	Multifocal necrotizing hepatitis. Multifocal discrete, pale yellow, raised fibrinous capsular plaques.	Putative parasite migration tracks. Chronic multifocal necrosuppurative to granulomatous hepatitis with clusters of un-embryonated parasite eggs with prominent polar plugs. Chronic fibrinosuppurative hepatic capsulitis.	Based on findings from serology and histopathology incidental *T. gondii* and *S. neurona* infection was suspected.
3	4/25/2011	San Luis Obispo	F	A (4–6)	Excellent	Acute boat strike	Multifocal pale pink to yellow sessile capsular plaques.	Mild multifocal hepatitis. Clustered larval aphasmid nematode profiles.	Based on findings from serology and histopathology, incidental *S. neurona* infection was suspected. Intestinal trematodes.
4	12/27/2016	Santa Cruz	F	A (4–6)	Excellent	Domoic acid intoxication.	Multifocal hepatic necrosis.	Putative parasite migration tracks with probable intralesional degenerating metazoan cuticle. Chronic multifocal necrosuppurative to granulomatous hepatitis.	Based on findings from histopathology, toxoplasmosis was considered a contributing cause of death.

Case 3 was an adult female in excellent nutritional condition that died due to boat strike, resulting in multiple limb, rib, and sternal fractures, a lacerated aorta, hemothorax and diaphragmatic rupture, with herniation of portions of the liver and intestines into the thorax. All liver lobes were fractured and hemorrhagic, limiting assessment of lesions that could be attributable to *C. hepatica* infection, but multiple 1–3 cm diameter pale pink to yellow, sessile capsular foci were noted on histopathology. No bacterial culture was performed.

Serial liver sections contained multiple cross-sectional, oblique and partial longitudinal larval nematode profiles in the parenchyma (**Figure 3A**). The parasites were 105–120 μm diameter and coelomate with a cuticle, hypodermal bands, a prominent stichosome, and single round to oval intestinal and gonadal profiles (**Figures 3B,C**). The gonads were small and immature, with no visible ova or spermatozoa. Parasite profiles were clustered within a localized region of subcapsular parenchyma encompassing approximately 50 μm of serial sections. Although small foci of coagulation necrosis and mild
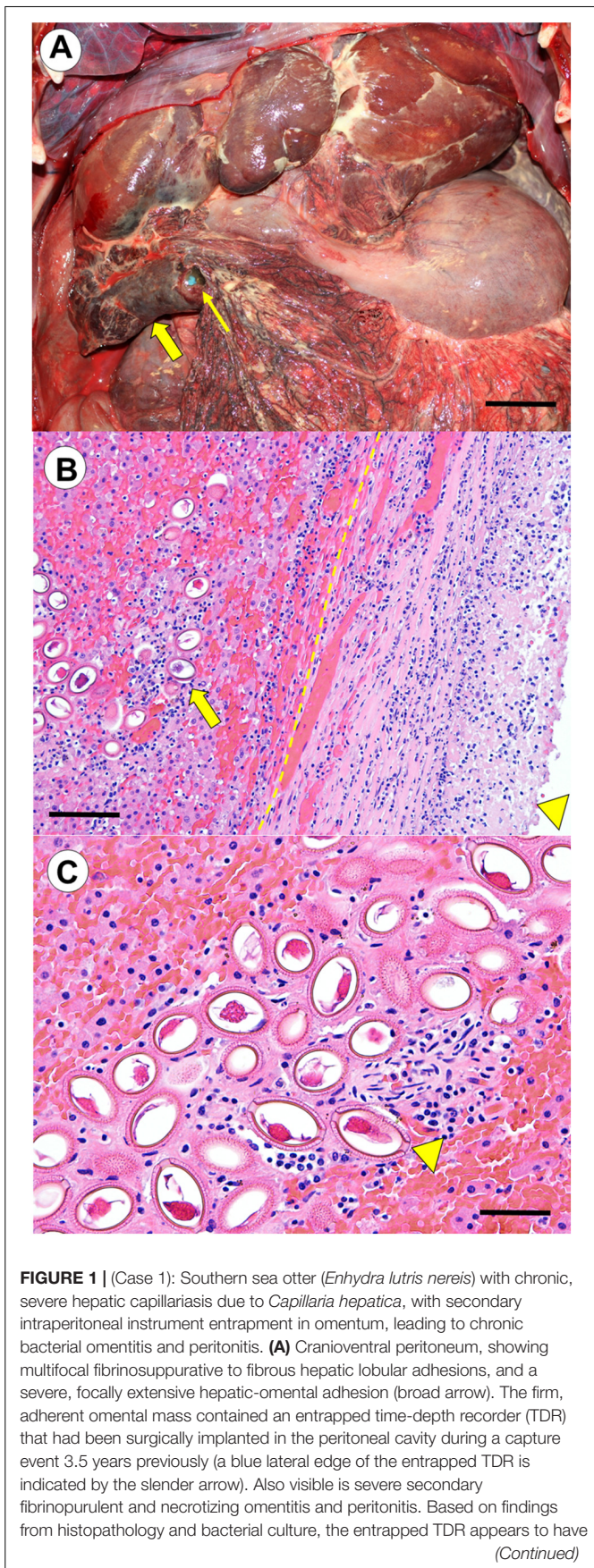
FIGURE 1 | (Case 1): Southern sea otter (*Enhydra lutris nereis*) with chronic, severe hepatic capillariasis due to *Capillaria hepatica*, with secondary intraperitoneal instrument entrapment in omentum, leading to chronic bacterial omentitis and peritonitis. **(A)** Cranioventral peritoneum, showing multifocal fibrinosuppurative to fibrous hepatic lobular adhesions, and a severe, focally extensive hepatic-omental adhesion (broad arrow). The firm, adherent omental mass contained an entrapped time-depth recorder (TDR) that had been surgically implanted in the peritoneal cavity during a capture event 3.5 years previously (a blue lateral edge of the entrapped TDR is indicated by the slender arrow). Also visible is severe secondary fibrinopurulent and necrotizing omentitis and peritonitis. Based on findings from histopathology and bacterial culture, the entrapped TDR appears to have

(Continued)

FIGURE 1 | Continued
served as a nidus for colonization by bacteria originating from the *C. hepatica*-associated hepatic lesions (Bar = approximately 6 cm). **(B)** Transverse histological view of the large hepatic-omental adhesion shown grossly in **(A)**. The hepatic-omental adhesion (arrowhead) is composed of a thick layer of fibrin, granulation tissue, and maturing fibrous connective tissue with admixed neutrophils and granulomatous inflammation. The former hepatic capsular surface (dashed line) lies well below this thick band of surface exudate. Subjacent hepatic parenchyma contains numerous large, elliptical to barrel-shaped nematode eggs (broad arrow), accompanied by mild to moderate pleocellular inflammation (hematoxylin and eosin stain. Bar = 200 μm). **(C)** Higher magnification view of the nematode eggs. These large, non-pigmented, un-embryonated, barrel-shaped nematode eggs had large polar prominences (arrowhead), and roughened, striated and moderately refractile shell that are characteristic of *C. hepatica*. Egg masses were encased in a fine fibrovascular stroma admixed with granulomatous inflammation. Inflamed tissue containing parasite eggs were interspersed with large expanses of unaffected hepatic parenchyma with no visible eggs. Linear bands and clusters of parasite eggs were often located immediately subjacent to the hepatic capsule, and were associated with capsular fibrin exudation, inflammation, granulation tissue and fibroplasia, facilitating formation of the severe intrahepatic and hepatic-omental adhesions observed at gross necropsy (hematoxylin and eosin stain. Bar = 100 μm).

suppurative hepatitis were apparent near the parasites, minimal necrosis, host inflammatory response or fibrosis were visible immediately adjacent to the nematodes, and no eggs were observed. Low numbers of intramuscular *Sarcocystis* sp. were noted on histopathology, but were considered incidental.

Serology was negative (IgG titers <40) for both *T. gondii* and *S. neurona*. Sparse mature sarcocysts were observed in skeletal myofibers without appreciable inflammation. No PCR or parasite isolation was attempted. Based on these findings, this otter appeared to have incidental *S. neurona* infection, and was not infected with *T. gondii*.

Case 4 was a lactating adult female in excellent nutritional condition that was euthanized due to severe neurological disease. At necropsy, a 778 gm, fully haired, meconium-smeared male fetus was recovered from the uterus. On histopathology the most significant lesion was hippocampal necrosis consistent with domoic acid intoxication; maternal and fetal urine were biochemically positive for domoic acid at 317 and 312 ppb, respectively. As with the previous cases, perimortem gastric erosions and melena were acute comorbidities. Multifocal subcapsular hepatic necrosis was randomly distributed within all lobes.

Serial sectioning of liver contained multifocal to coalescing serpiginous bands of necrosuppurative hepatitis, mild tissue cavitation and granulomatous inflammation, especially in the subcapsular hepatic parenchyma (putative parasite migration tracks) (**Figure 4A**). Multifocal discontinuous, linear to round fragments of eosinophilic hyaline and often striated material resembling partially degenerated nematode cuticle were observed at the center of some lesions (presumptive degenerating metazoan parasites) (**Figures 4B,C**). No parasite eggs were observed on multiple serial sections.

On histopathology, multifocal, mild to severe lymphoplasmacytic meningoencephalomyelitis and gliosis was associated with sparse mature *T. gondii* tissue cysts.
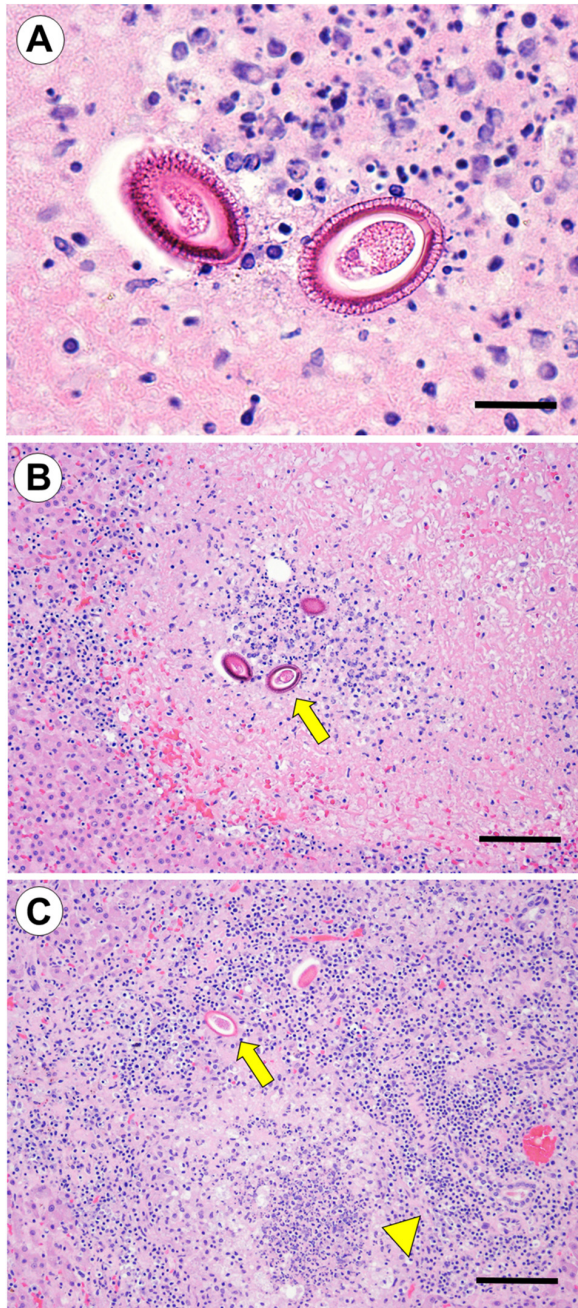
FIGURE 2 | (Case 2): Hepatic capillariasis in a southern sea otter (*Enhydra lutris nereis*) that died from shark bite with secondary bacterial infection. **(A)** Microscopic examination of hepatic parenchyma subjacent to grossly apparent hepatic fibrinous to fibrous capsular plaques revealed multifocal clusters of large (90–100 × 45–55 μm), non-pigmented, un-embryonated, barrel-shaped parasite eggs similar to those described for Case 1 above (hematoxylin and eosin stain. Bar = 50 μm). **(B)** Lower magnification view of the same parasite eggs shown in **(A)**: the parasite eggs were arranged in discrete masses, and were surrounded by areas of coagulation necrosis, hemorrhage, inflammation, and fibrous connective tissue (hematoxylin and eosin stain. Bar = 200 μm). **(C)** Small circular, linear, or serpiginous bands of hepatic cavitation with perilesional granulomatous inflammation were suggestive of parasite migration tracks (arrowhead). Parasite eggs were often located along the outer edge of the cavitated lesions (broad arrow) (hematoxylin and eosin stain. Bar = 200 μm).
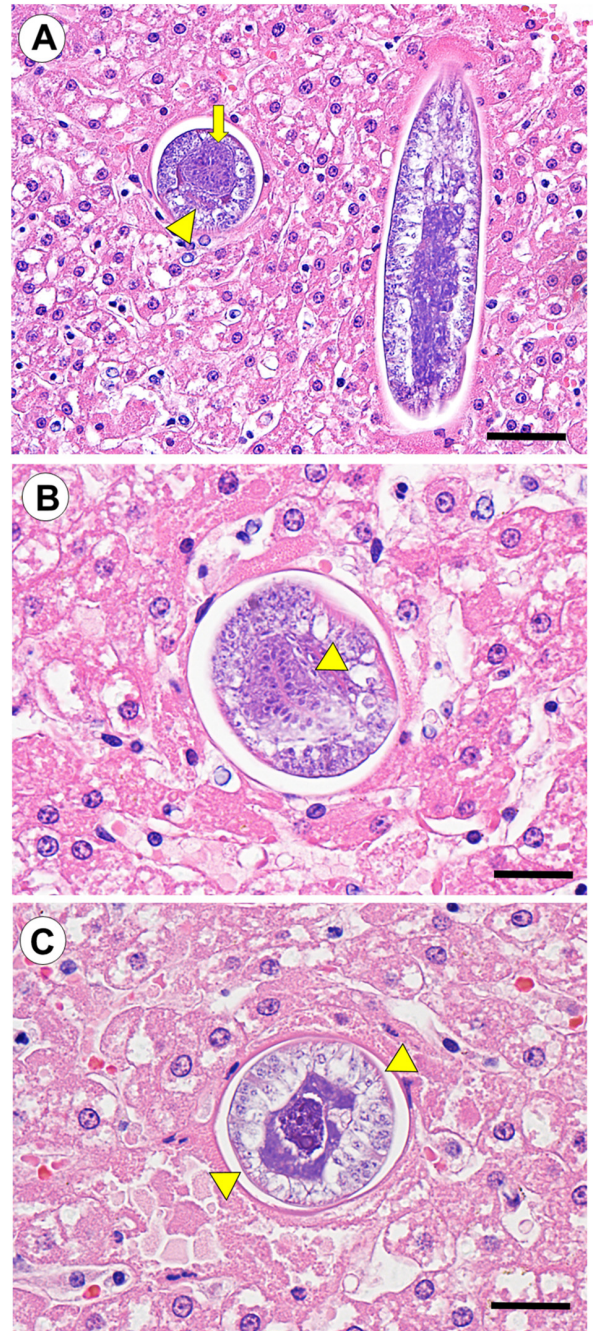

FIGURE 3 | (Case 3): Larval nematode profiles (putative *C. hepatica* larvae) in the hepatic parenchyma of a southern sea otter (*Enhydra lutris nereis*). **(A)** Serial 5 μm thick tissue sections revealed multiple cross-sectional, oblique, and longitudinal nematode profiles that were clustered in a small area of the hepatic parenchyma. Cross-sections of these parasites showed single round to oval intestinal (broad arrow) and gonadal (arrowhead) profiles (hematoxylin and eosin stain. Bar = 100 μm). **(B,C)** The nematodes were 105–120 μm diameter and coelomate with a cuticle, a prominent stichosome surrounding the esophagus (indicated by arrowhead in **B**), and paired hypodermal bands (indicated by arrowheads in **C**); these morphological characteristics are indicative of *C. hepatica*. The gonads were small with no visible ova or spermatozoa, and no eggs were visible in the adjacent hepatic parenchyma, suggesting that the parasites were immature (hematoxylin and eosin stain. Bar = 50 μm for both **B,C**).
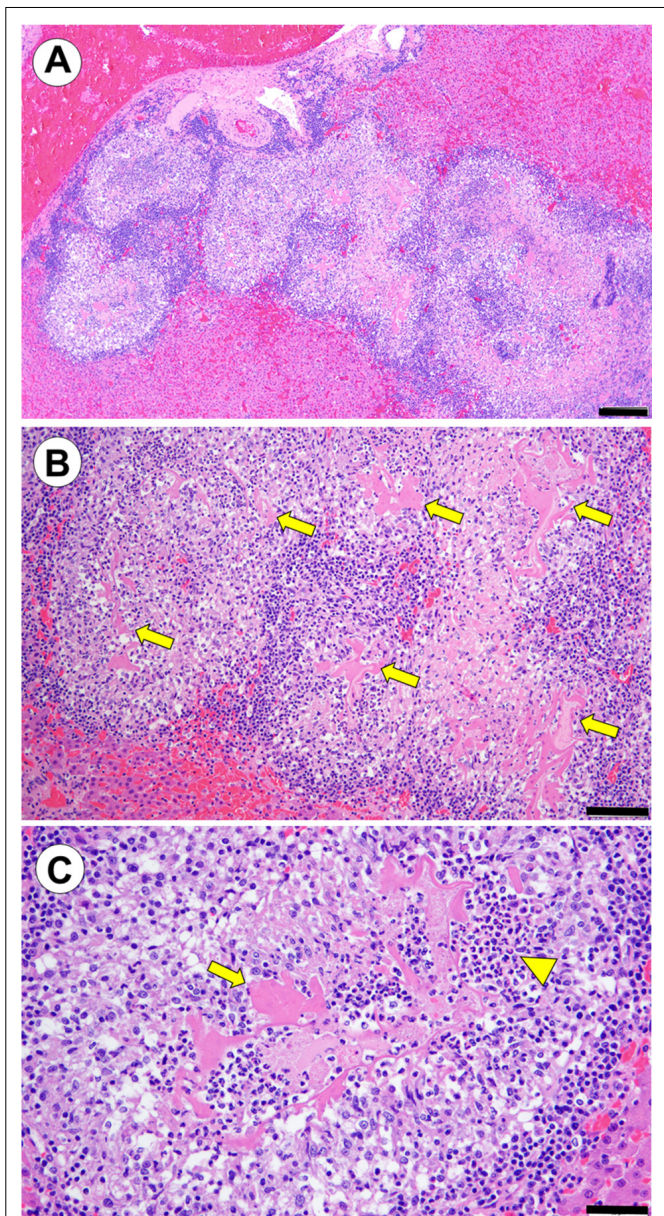
FIGURE 4 | (Case 4): Probable hepatic metazoan parasite migration tracks with intralesional degenerating metazoan cuticle. **(A)** Linear to serpiginous tracts of necrosuppurative hepatitis, mild tissue cavitation and granulomatous inflammation in the subcapsular hepatic parenchyma (putative parasite migration tracks) (hematoxylin and eosin stain. Bar = 200 μm). **(B)** Higher magnification view of affected liver. Multifocal discontinuous, linear to round fragments of eosinophilic hyaline, variably striated material (putative degenerating metazoan parasite cuticle) are visible at the center of some lesions (arrows) (hematoxylin and eosin stain. Bar = 100 μm). **(C)** In this high magnification view of a single inflammatory nodule, putative fragments of metazoan cuticle (arrow) are surrounded by dense clusters of eosinophils (arrowhead), and an outer rim of granulomatous inflammation (hematoxylin and eosin stain. Bar = 50 μm).

No sarcocysts were observed in any tissue and cell culture was negative for all protozoa. Protozoal serology, immunohistochemistry, and PCR were not performed.

Toxoplasma gondii-associated meningoencephalomyelitis was considered a contributing cause of death.

DISCUSSION

In the current study, three confirmed, and one suspect case of hepatic capillariasis were identified in wild sea otters stranding along the central California coast. Interestingly, all were adult females (**Table 1**). Given our small sample, this age and sex bias could represent random chance, or could suggest enhanced risk of *C. hepatica* infection for sexually mature females. Prior studies report distinct differences in sea otter behavior by sex; adult females have smaller home ranges and greater site fidelity than adult males (Loughlin, 1980; Jameson, 1989; Riedman and Estes, 1990). Females often feed at shallower depths when compared with males, and seek shorelines with calm water and higher kelp density when raising pups (Sandgren et al., 1973; Bodkin et al., 2006; Nicholson et al., 2018). These areas could correspond spatially to a higher density of introduced rats, a key source of *C. hepatica* infection (Fuehrer et al., 2014a), greater risk of exposure to parasite-contaminated freshwater runoff, and/or recurrent parasite introduction due to infected rodents escaping from moored ships. Infected sea otters stranded at several locations along the Central California coast spanning >250 km, suggesting multiple areas of *C. hepatica* exposure.

A possible seasonal bias was also noted, with all *C. hepatica*-infected sea otters stranding during or immediately following the period of peak annual precipitation and freshwater runoff into California coastal marine waters, and the period of highest prevalence and density of *C. hepatica* infection in *Rattus* sp. reservoir populations (Conlogue et al., 1979; Rothenburger et al., 2014). A late spring peak has also been reported for sea otter death due to other terrestrial parasites that are spread through dispersal of environmentally resistant stages, such as *S. neurona* sporocysts (Miller et al., 2008; Shapiro et al., 2012).

Differential diagnoses for gross, microscopic, and clinical pathology findings associated with *C. hepatica* include hepatic infection or aberrant migration by other nematodes (e.g., *Ascaris*, *Toxocara*, *Toxascaris,* or *Baylisascaris* spp.), cestodes (e.g., *Echinococcus* and *Mesocestoides* sp.), and flukes (e.g., *Fasciola hepatica* and *Fascioloides magna*). The range of hepatic macroparasites that could be confused with *C. hepatica* infection has been reported (Joint Pathology Center [JPC], 2015).

Localization of infection to the hepatic parenchyma, and the unique morphology of the parasites and eggs on histopathology helps distinguish *C. hepatica* from infection by other parasites. The nematodes are slender, with narrow anterior, and swollen posterior ends (Yadav et al., 2016). Adult *C. hepatica* average 100–150 μm diameter, and are pseudocoelomate with a cuticle, polymyarian-coelomyarian musculature, and single, round to oval gastrointestinal and gonadal profiles on cross-section (Gardiner and Poynton, 1999; Eberhard, 2014; Joint Pathology Center Joint Pathology Center [JPC], 2015). Other distinctive characteristics that can help distinguish *C. hepatica* from other nematodes include absence of lateral cords, presence of hypodermal bands (**Figure 3C**),

and presence of a unique glandular structure (stichosome) surrounding the esophagus that is composed of distinctive basophilic cells (stichocytes) (**Figure 3B**) (Gardiner and Poynton, 1999). The stichosome is diagnostic of *C. hepatica* (Gardiner and Poynton, 1999; Yadav et al., 2016). Other morphological features include a long esophagus that spans half of the female's body, and one third for males (Yadav et al., 2016) and a lack of sensory papillae (i.e., phasmids) on their caudal end. Although phasmids are not identifiable histologically, when observed in intact worms they can help differentiate *C. hepatica* from phasmid nematodes (e.g., strongyles, ascarids, oxyurids, rhabditoids, and spirurids) (Joint Pathology Center [JPC], 2015).

Capillaria hepatica eggs are also distinctive based on their distribution in hepatic parenchyma and morphology; eggs are approximately 66 μm × 36 μm, barrel-shaped, non-pigmented, and un-embryonated, with large polar prominences, and a roughened, striated and moderately refractile shell containing numerous minipores (Yadav et al., 2016) (**Figures 1C, 2A**). Although some other nematode eggs are elliptical and bioperculate, they differ in size, shape and morphological characteristics, may be embryonated or un-embryonated in tissue, and are unlikely to be observed in dense clusters in the hepatic parenchyma. *Trichuris trichiura* eggs resemble those of *C. hepatica*, but differ in size, morphology and tissue location (Cochrane et al., 1957; Li et al., 2010). Although aberrant larval migration is possible, *T. trichiura* and ascarid eggs are unlikely to be observed in hepatic parenchyma.

Finally, the peri-parasitic, egg-associated and hepatic capsular fibrin exudation and fibrosis that often accompanies *C. hepatica* infection is distinctive. The host response to *C. hepatica* can be severe and fatal, forming adhesions between liver lobes, or from the liver to other abdominal structures (Otto et al., 1954). This lesion was apparent for Case 1 (**Figure 1A**), and the resulting hepatic-omental adhesion with TDR entrapment was the underlying cause of death. The tightly adherent omental pocket containing the TDR appeared to serve as a nidus for chronic secondary bacterial peritonitis, with the bacteria likely originating from the adjacent necrotic hepatic lesions. A pattern of parenchymal fibrosis in association with discrete clusters of subcapsular, bioperculate nematode eggs in the hepatic parenchyma is characteristic of *C. hepatica* infection in mammals (Joint Pathology Center [JPC], 2015).

In addition to visualization of parasites and eggs, other microscopic lesions can suggest *C. hepatica* infection, including elongated, linear to serpiginous, tracks that are variably necrotic, cavitated and/or inflamed (putative parasite migration tracts) (**Figure 4A**), degenerating nematodes associated with these tracts (**Figures 4B,C**), and multifocal discrete areas of subcapsular hepatitis and fibrosis, with relative sparing of adjacent tissue. The pattern of hepatic fibrosis can also be distinctive: Rats infected with *C. hepatica* commonly exhibit bridging fibrosis, where hepatic portal tracts are connected to adjacent portal tracts (or rarely centrilobular spaces) by thin strands of connective tissue (Joint Pathology Center [JPC], 2015). Interestingly, *C. hepatica*-associated septal fibrosis is not necessarily preceded

by hepatic necrosis or chronic inflammation (Ferreira and Andrade, 1993; Gaban et al., 2010). In severely affected areas (especially in rats), the hepatic architecture can be reminiscent of porcine liver.

Lymphoplasmacytic, and to a lesser extent, eosinophilic infiltration can also be apparent in portal tracts and near centrilobular veins, and aggregates of eggs may be surrounded by granulomatous inflammation or fibrosis. Liver damage induced by *C. hepatica* in rodents has been used to study the extensive regenerative capacity of mammalian liver (Santos et al., 2007), and for testing antifibrotic drugs (De Souza et al., 2000). Yi et al. (2010) also noted associations between *C. hepatica*-induced hepatitis and pulmonary arteriolar hypertrophy in rats, and suggested this model to clarify mechanisms of porto-pulmonary hypertension in humans.

Rodents in the genus *Rattus* are considered primary *C. hepatica* hosts (Resendes et al., 2009), and the main source of parasite contamination in urban environments (Redrobe and Patterson-Kane, 2005). In Maryland, Norway rats were far more frequently infected (87.4%) than sympatric house mice (5.4%), and no lesions were observed in meadow voles or white-footed mice (Childs et al., 1988). Rats from urbanized locations had a higher prevalence and intensity of infection, when compared with parkland-associated rats, and higher rat population density and body mass were associated with increased *C. hepatica* prevalence and lesion severity. Risk factors for *C. hepatica* infection in feral Norway rats from Vancouver, Canada included older age, sampling during fall and winter, and presence of cutaneous bite wounds (Rothenburger et al., 2014). Higher prevalence of *C. hepatica* infection in rats sampled during winter was also reported in Connecticut, United States (Conlogue et al., 1979). In studies of *C. hepatica* transmission ecology, the mean egg count per rat liver was 457,783 (range 11,270–1,400,000) (Farhang-Azad, 1977a,b). Rapid population turnover and cannibalism are considered primary drivers of transmission within rat populations (Farhang-Azad, 1977a,b; Rothenburger et al., 2014).

Other communally living rodents with a high prevalence of *C. hepatica* infection include muskrats, eastern wood rats (Fuehrer et al., 2014a), and captive prairie dogs (Landolfi et al., 2003; Redrobe and Patterson-Kane, 2005). Along with a high prevalence of *C. hepatica* infection in native aquatic rodents (i.e., muskrats), cotton rats sampled from freshwater marshes in Florida had higher prevalence of *C. hepatica* infection (30%), compared with those from saltwater marshes (12%) and upland habitat (5%) (Kinsella, 1974). *Capillaria hepatica* has also been reported from <140 non-rodent mammals, including humans (Berentsen et al., 2015). The only prior reports of *C. hepatica* infection in North American mustelids were in a river otter (*Lontra canadensis*) in Florida (Forrester, 1992), and two long-tailed weasels (*Mustela frenata*) from Virginia (Solomon and Handley, 1971).

Through 2011, 72 confirmed cases of *C. hepatica*-associated human hepatic capillariasis were reported worldwide, with the majority affecting children (Fuehrer et al., 2011). Infection was

associated with pyrexia, chronic eosinophilia, hepatomegaly, eosinophilic granulomatous hepatitis, hepatic fibrosis, cirrhosis, and rarely, death (Otto et al., 1954; Cochrane et al., 1957; Li et al., 2010). Also reported were abdominal pain, anorexia, emaciation, ascites, cholelithiasis (Ferreira and Andrade, 1993), anemia, and hypergammaglobulinemia (Yadav et al., 2016). Humans can be infected following ingestion of embryonated eggs in food, water, or soil. Residing in areas with poor hygiene, close contact with rats or other rodents, pica, and extensive soil contact are risk factors for human infection (Cochrane et al., 1957; Yadav et al., 2016; Walker et al., 2017). Substandard housing conditions may facilitate rat infection rates of up to 85%, increasing risk of human infection (Walker et al., 2017). Common non-native rodents along the central California coast include roof rats (*Rattus rattus*), Norway rats (*Rattus norvegicus*) and Eastern fox squirrels (*Sciurus niger*). Common native rodents include California ground squirrels (*Spermophilus beecheyi*), Botta's pocket gophers (*Thomomys bottae*), California voles (*Microtus californicus*), western harvest mice (*Reithrodontomys megalotis*) and dusky-footed woodrats[1] (*Neotoma fuscipes*).

Hepatic capillariasis is often diagnosed in humans by finding *C. hepatica* nematodes and eggs in biopsy or autopsy specimens, but serodiagnostic tests are now available (Juncker-Voss et al., 2000; Assis et al., 2004; Huang et al., 2001). Because infections are non-patent, detection of *C. hepatica* eggs in feces indicates spurious passage of ingested eggs, not infection, and has been associated with consumption of wild game (Foster and Johnson, 1939). Options for treating clinical *C. hepatica* infection in humans include thiobendazole, albendazole, and ivermectin (Yadav et al., 2016).

The potential for parasite co-infection to facilitate *C. hepatica* infection, or cause more severe disease should be considered. Recent reports have described fatal co-infection of a Korean squirrel (*Tamias sibiricus*) (Carrasco et al., 2006) and a ring-tailed lemur (*Lemur catta*) (Siskos et al., 2015) with *C. hepatica* and *T. gondii*. In the current study, all four sea otters were concurrently infected with *T. gondii*, *Sarcocystis* sp., and/or intestinal trematodes (**Table 1**). Given the very high prevalence of *T. gondii* and *S. neurona* infection in southern sea otters (Miller et al., 2017), and the potential for polyparasitism to cause more severe disease in marine mammals (Gibson et al., 2011), the potential for parasite interactions should be considered as part of future assessment of *C. hepatica* infections.

Gross lesions suggestive of *C. hepatica* infection were noted for both cases where *C. hepatica* eggs were observed on histopathology, but were less clear for the cases where only larval or degenerating worms were present. Although comparatively uncommon, the prevalence of *C. hepatica* infection in southern sea otters is probably much higher than the four cases reported here. Because the hepatic lesions are often multifocal and are interspersed with areas of normal tissue, *C. hepatica* infection is very easy to miss unless suspect lesions are collected and examined microscopically. The capsular lesions

could also be easily confused with other common peritoneal diseases of sea otters, including acanthocephalan or bacterial peritonitis (Kreuder et al., 2003). Future efforts should include microscopic examination of hepatic parenchyma beneath areas of capsular fibrin deposition and fibrosis. Efforts to more accurately characterize the prevalence of infection could include digesting liver tissue to recover parasite eggs from patchy areas of deposition, and serological testing. It would also be interesting to screen coastal rodents, soil, and runoff for *C. hepatica*.

Similar to the land-based parasites *T. gondii* and *S. neurona* (Miller et al., 2008, 2010), *C. hepatica* eggs may be concentrated and transmitted through filter-feeding marine invertebrates that serve as southern sea otter prey, which may also pose an unrecognized public health risk for people who consume these species. Although *C. hepatica* can be transmitted to humans via ingestion of embryonated eggs, risks from handing infected sea otters are negligible unless the carcasses have significantly decomposed, allowing the eggs to become infective. However, because the most likely source of *C. hepatica* infection for sea otters is consuming water or prey contaminated with embryonated eggs originating from predated or dead, decomposing, land-based rats, sea otter parasitism could suggest un-recognized human health risks for acquiring *C. hepatica* infection from contaminated water, or raw/undercooked filter-feeding marine and estuarine invertebrates; this pathway for marine wildlife and human exposure to *C. hepatica* merits further investigation.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study was performed using dead southern sea otters submitted for necropsy to the California Department of Fish and Wildlife as per federal MOU.

AUTHOR CONTRIBUTIONS

MAM directed the case investigations, conducted gross necropsy and histopathology, completed a literature review on *C. hepatica* and wrote the manuscript. PD conducted necropsy and histopathology for case 4, contributing photomicrographs and a detailed case history for case 4, and helped substantially with manuscript editing and completion. ED and FB assisted with gross necropsies, case data compilation and review, coordinated completion of serial sections for livers of affected sea otters, and helped with manuscript preparation. MS, JT, MM, and HH contributed live capture and antemortem data for cases in the study and helped with manuscript editing. CG provided expertise to facilitate parasite and egg identification in tissue sections,

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[1]http://kennethadair.org/mammals.htm

optimized text related to histological identification of *C. hepatica*, ensured that key structural features in the figures were labeled correctly, and provided substantial input during manuscript editing and completion.

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