Aquatic Mammals 2021, *47*(4), 362-370, DOI 10.1578/AM.47.4.2021.362

Multimetastatic Hepatocellular Carcinoma in a South American Sea Lion (*Otaria flavescens*; Shaw, 1800)

Guillermo J. Sánchez Contreras,[1] Barbara Biancani,[2] Nicola Pussini,[3] Claudia Gili,[3,4] Livio Galosi,[5*] and Giacomo Rossi[5*]

[1]*Mediterraneo Marine Park, Costa Edutainment SPA, Bahar ic-Caghaq NXR9038, Malta*
E-mail: vet@mediterraneopark.com
[2]*Oltremare, Costa Edutainment SPA, Riccione 47838, Italy*
[3]*Acquario di Genova, Costa Edutainment SPA, Genova 16128, Italy*
[4]*Stazione Zoologica Anton Dohrn, Napoli 80121, Italy*
[5]*School of Biosciences and Veterinary Medicine, University of Camerino, Matelica 62024, Italy*
**Equal contributors*

Abstract

An 18-year-old captive born and neutered male South American sea lion (*Otaria flavescens*; Shaw, 1800) presented with intermittent gastrointestinal signs of disease (anorexia, reduced activity, vomit, and diarrhea). Over the course of 12 days, severe leukocytosis, signs of liver damage, and anemia developed, accompanied by rapid weight loss and physical deterioration. Ultrasonographic examination revealed hepatomegaly and reduction of liver vascularization with altered hepatic parenchyma along with ascites. Hemoculture results revealed no growth, and PCR tests from nasal, oral mucosa, and fecal swabs ruled out a *Mycobacterium* infection. Treatment consisted of antibiotics, anti-inflammatories, analgesics, probiotics, vitamin supplements, antitoxic and hepatoprotective medication, supportive fluid therapy, and nutritional support. Medical condition of the animal did not improve despite the treatments, presenting as severe apathy (almost comatose), jaundice, oral hemorrhage, and melena. Thus, humane euthanasia was performed. On necropsy, major lesions were seen in the liver, confirming the ultrasonographic findings. Histopathology revealed a poorly demarcated hepatic epithelial neoplasia, metastasized to the lungs, spleen, and lymph nodes. Also, the presence of another neoplastic nodule at the level of the parathyroid made it difficult to define the primary tumor site. An immunohistochemical examination using a panel of antibodies (pan cytokeratin AE1/AE3, cytokeratin 18 [CK18], and CK8, together with p-CEA, CD10, and vimentin) was used in an attempt to differentiate between hepatocellular carcinoma from cholangiocarcinoma and metastatic non-hepatic carcinoma. Results revealed that the tumor was of primary hepatic origin and metastasized to various organs, including the parathyroid which does not represent a usual site of metastasis. To our knowledge, this is the first case describing hepatocellular carcinoma with evidence of metastases to the lungs and parathyroid gland in a South American sea lion.

Key Words: hepatocellular carcinoma, lung metastasis, parathyroid gland metastasis, South American sea lion, *Otaria flavescens*

Introduction

South American sea lions (*Otaria flavescens*; Shaw, 1800; SASLs) are an abundant species found along most of the Pacific coast as well as on the southern portion of the Atlantic coast of South America (Cárdenas-Alayza et al., 2016). It is a heavy and robust species of Otariidae with marked sexual dimorphism (males up to 350 kg; females up to 144 kg) (Webber, 2014). *The IUCN Red List* catalogues the species as "Least Concern" with more than 222,000 mature individuals in the population, and notes that the major threats to these animals in the wild are the human use of biological resources (fishing and harvesting), as well as the pollution generated by agricultural and forestry effluents (Cárdenas-Alayza et al., 2016).

There are roughly 200 SASLs kept under human care in Europe according to the regional studbook (Walter & Kuzniar, 2018), which represents the major source of information on the most common causes of death of SASLs under human care. Infection-associated problems seem to be the most frequent cause of death. These statistics were highly influenced by the outbreaks of *Mycobacterium pinnipedii* that were historically

reported in European zoos (European Association for Aquatic Mammals [EAAM], 2009; Walter & Kuzniar, 2018) and resulted in a number of individuals being euthanized.

Neoplasms are not unknown to marine mammals and have been reported in several species of pinnipeds (Colegrove, 2018), especially in the California sea lion (*Zalophus californianus*; CASL), a species commonly maintained in zoological collections. However, at this point, only an ovarian interstitial cell tumor (Biancani et al., 2010), a multicentric hemangiosarcoma (You et al., 2008), and a gastric carcinoma (Yamazaki et al., 2016) have been reported in SASL in the literature (Colegrove, 2018). Mauroo et al. (2010) reported the antemortem diagnosis of a hepatocellular carcinoma, and Acevedo-Whitehouse et al. (1999) reported a hepatic carcinoma with spleen metastasis, both in CASL. This is the first report of a hepatocellular carcinoma with lung and parathyroid gland metastases in an SASL.

Methods

In December 2017, an 18-y-old captive born and neutered male South American sea lion held in a zoological institution in Malta (35.57° N, 14.25° E) presented with intermittent gastrointestinal signs of disease (anorexia, lethargy, vomit, and diarrhea). Over the course of 12 d, severe leukocytosis, signs of liver damage (acute increase in liver enzyme levels), and anemia developed (Table 1), accompanied by rapid weight loss and physical deterioration. Hepatomegaly and reduction of liver vascularization with alteration of the hepatic parenchyma was observed along with ascites on ultrasonographic examination (Figure 1). Hemoculture results revealed no growth, and PCR tests from nasal, oral mucosa, and fecal swabs ruled out a *Mycobacterium* infection. Treatment consisted of antibiotics, anti-inflammatories, analgesics, probiotics, vitamin supplements, and antitoxic and hepatoprotective medication, together with supportive fluid therapy and nutrition. The medical condition of the animal did not improve despite the treatments, and it developed

Table 1. Blood parameters during the 12-d treatment period

	Unit	Day 1	Day 3	Day 8	Day 10	Day 12
Leucocytes (WBC)	10^9/l	23.5	27.4	38.5	41.6	45.8
Eosinophils	%	1	0	0	1	0
Segmented neutrophils	%	82	84	92	95	95
Lymphocytes	%	15	12	4	1	2
Monocytes	%	2	4	4	3	3
Eosinophils	/ul	235	0	0	416	0
Segmented neutrophils	/ul	19,270	23,016	35,420	39,520	43,510
Lymphocytes	/ul	3,525	3,288	1,540	416	916
Monocytes	/ul	470	1,096	1,540	1,248	1,374
Erythrocytes (RBC)	10^12/l	4.5	3.9	4.1	2.9	
Hemoglobin	g/dl	15.9	14.9	15.3	11.2	
Hematocrit (HCT)	%	47	44	43	32	28
MCV	fl	113.2	111.2	119	108.4	
MCH	pg	38.1	37.9	38	37.6	
MCHC	g/dl	33.7	34.1	31	34.7	
Platelets	10^9/l	59	64	83	80	
Total bilirubin	mg/dl	0.7	1.18	1.7	9.45	
ALT (GPT)	UI/l	237.1	352.2	399	498.9	
Alkaline phosphatase (ALP)	UI/l	83	151.2	152	185.3	
GGT	UI/l	719.3	881.6	968	1152.1	
AST (GOT)	UI/l	236.2	377.7	384	501.7	
GLDH	UI/l	41		305		
Creatine kinase (CPK/CK)	UI/l	205	206.8	205	1,092.4	
LDH	UI/l		1,745.8	2,266	3,083.1	

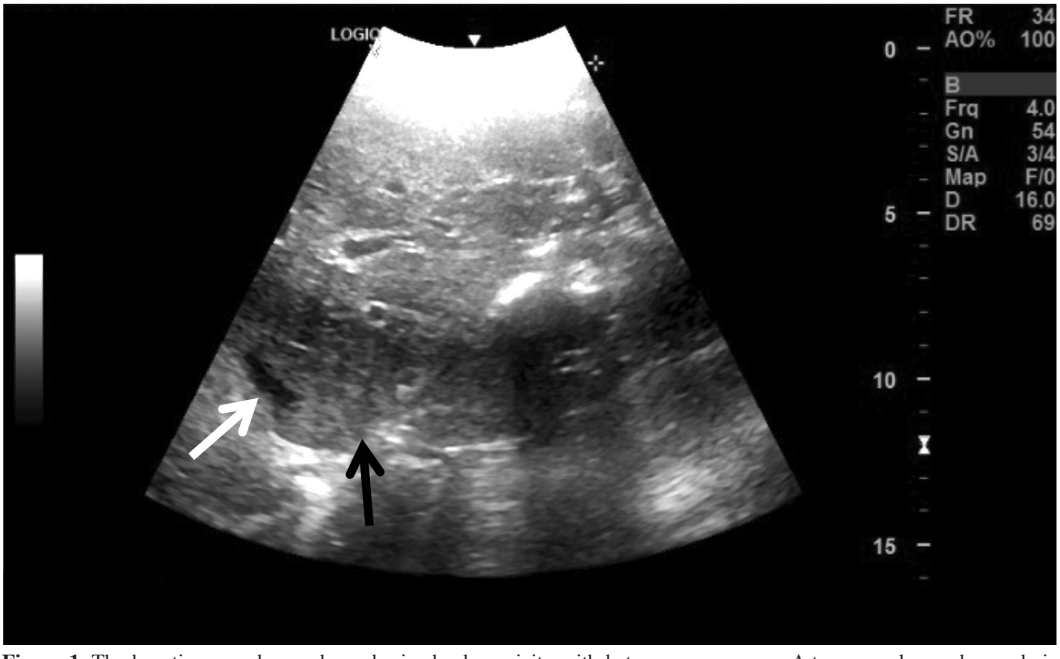

Figure 1. The hepatic parenchyma showed mixed echogenicity with heterogeneous areas. A transversal area, hypoechoic compared to the surrounding parenchyma, is present along the image. On the left side (white arrow), a cavernous lesion and spheroidal buttoned lesions (black arrow) with undefined margins and hypoechoic structure (approx. 0.5 to 1 cm) are observed.

further symptoms of severe apathy (almost comatose), jaundice, oral hemorrhage, and melena. Thus, humane euthanasia was performed.

Generalized jaundice was evident in the immediate postmortem examination. A large amount of icteric free fluid was present in the abdominal cavity. The liver was enlarged with white-yellow margins visible (Figure 2), and it had a consolidated consistency that did not bleed when cut (Figure 3). The spleen was normal in size and consistency but pale yellow in color; it had a few granular lesions (1 × 1 mm) that were only evident on the surface (Figure 4). Mucus and saliva were found in the trachea and, despite the generalized pale-yellow coloration, no evident lesions were found in the lungs. The pericardial sac was filled with approximately 15 ml of icteric fluid, but the heart presented no lesions macroscopically. The stomach contained some dark fluid, and the mucosa seemed pale, without ulcers. Dark, odorous ingesta was found within the intestinal tract. Only a gastric and a mesenteric lymph node presented obvious alterations macroscopically. The gastric lymph node was enlarged, edematous when cut, and presented an area compatible with a small infarction. The mesenteric lymph node, though normal in size, was edematous when cut, and the cortex had a dark coloration. The only alteration observed in the kidneys was the icteric coloration of the serosa. No abnormalities in the central nervous system (CNS) were detected at gross examination.

No significant bacterial or fungal pathogens were isolated from the samples collected during the necropsy, and no evidence of acid-fast bacteria was found in imprints of the liver and kidney. Small portions of each organ were sampled, fixed in 10% formalin buffered solution, and sent to the Laboratory of Animal Pathology of the University of Camerino, Italy, for histological and immunohistochemical (IHC) evaluations. Fixed tissues were dehydrated by a progressive alcohol-xylene mixture and then embedded in paraffin. To perform the histopathological examination, 3 µm sections from tissue blocks were mounted on electrostatic pre-treated slides (Histoline, St. Louis, MO, USA) and deparaffinized in xylene, rehydrated in graded alcohols, and rinsed in 0.05 m Tris-buffered saline (TBS).

IHC is a helpful tool to identify the distribution of an antigen of interest in health and disease (Duraiyan et al., 2012). IHC has an important application to resolve tumors of unknown origin (Selves et al., 2018) and to differentiate metastatic carcinomas with morphologic similarity (Chen & Lin, 2015). The use of IHC markers plays an

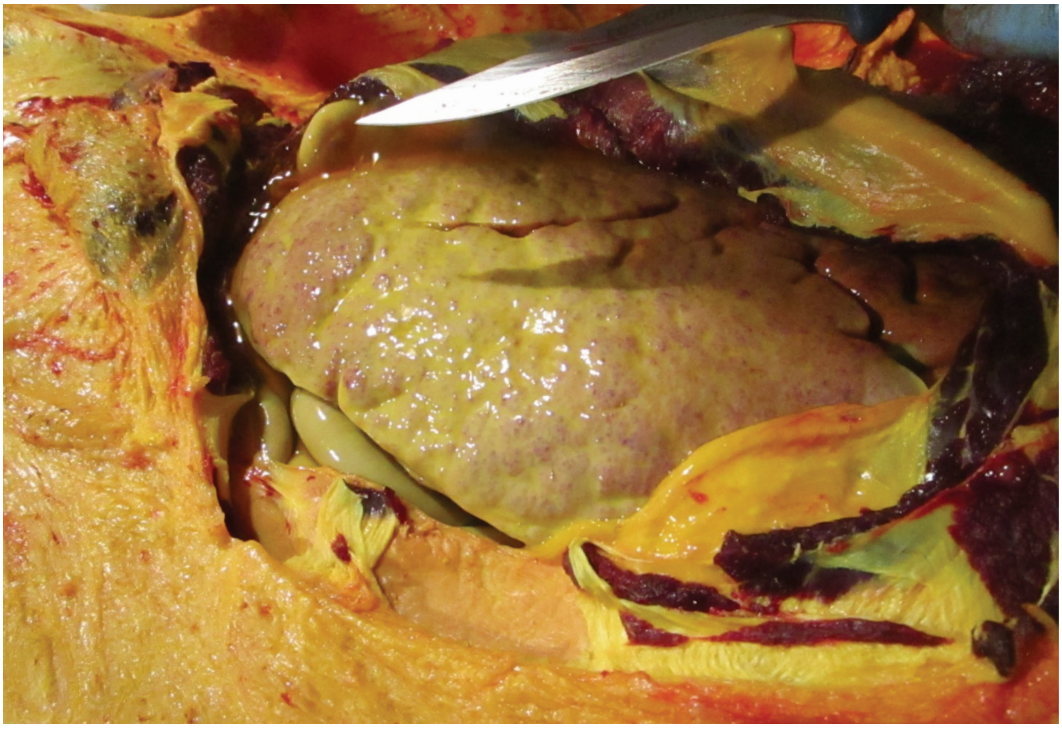

Figure 2. Liver of the necropsied South American sea lion (*Otaria flavescens*): enlarged, presenting rounded and white-yellow margins.

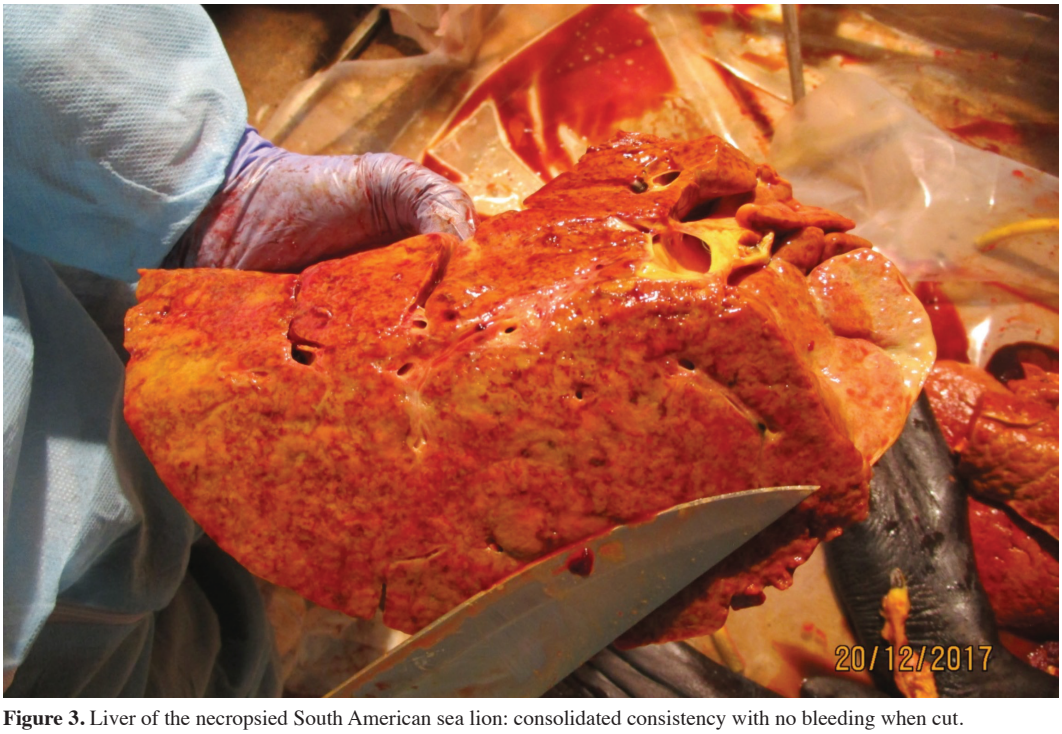

Figure 3. Liver of the necropsied South American sea lion: consolidated consistency with no bleeding when cut.

Figure 4. Spleen of the necropsied South American sea lion: normal in size and consistency, but pale yellow in color, and presenting few granular lesions (1 × 1 mm) on the surface.

important role in establishing an accurate diagnosis (Choi et al., 2017). To carry out an IHC, sections of the different tissues—liver, lung, spleen, lymph nodes, and parathyroid—were boiled in 10 mm citrate buffer for antigen retrieval at a pH of 6.0. Endogenous peroxidase was blocked with aqueous 0.3% H_2O_2 (hydrogen peroxide) for 15 min. An Avidin–Biotin complex kit was employed as described in the manual (Vector Laboratories, Burlingame, CA, USA). The sections were incubated in 5% normal goat serum (Merck Life Science S.r.l., Milano, Italy) followed by a one-night incubation in monoclonal pan cytokeratin (PCK, AE1/AE3 clone, 1:100; Dako, Carpinteria, CA, USA), cytokeratin 18 (CK18, 1:100, Dako), cytokeratin 8 (CK8, 1:100, Dako), vimentin (clone V9, 1:100, Dako), and CD10 (56C6 clone, 1:50; AbCam, Cambridge, UK) antibodies, and in a polyclonal rabbit-anti carcinoembryonic antigen (p-CEA, 1:50; Thermo Fisher Scientific, Waltham, MA, USA) antibody. As a negative control, the primary antibody was replaced with TBS, and the appropriate normal areas in the sections served as positive controls. Positive immunoreactivity was defined as more than 20% of cells staining with the proper pattern of reactivity. Immunopositivity to PCK AE1/AE3, CK18, CK8, and vimentin appeared as brown cytoplasmic staining of tumor cells, while positivity to p-CEA and CD10 appeared as a canalicular pattern staining of tumor cells. Cytokeratins and vimentin help to determine the nature of the tumor (carcinomatous or sarcomatous). PCK AE1/AE3, CK18, CK8, p-CEA, and CD10 are needed to understand which cell line is present at the origin of the neoplasm (Selves et al., 2018).

Results

The histopathology of the liver revealed proliferating epithelial cells that differed markedly from normal hepatic parenchyma cells in terms of morphology and nuclear-nucleolar atypia, as well as high mitotic rate (up to 7 mitotic figures per high-power field – 400× equivalents). The neoplasia appeared organized in a poorly demarcated, unencapsulated, epithelial neoplastic mass composed of islands varying in shape and diameter, separated by small amounts of collagen-rich, cell-poor, and variably vascularized stroma and multifocal to coalescing areas of central necrosis. The liver was strongly altered due to a combination of fibrosis, pseudo-regeneration phenomena, and the capillarization of sinusoids that eventually resulted in the development of a cirrhosis-like condition.

Presence of metastases were observed both in the spleen (many nodules diffusely disseminated

into the parenchyma) and in the gastric and mesenteric lymph nodes. A strong lipofuscinosis in the phage cells was present at the level of the spleen and lymph nodes, which indicated an excessive state of lipo-peroxidation, possibly secondary to the age of the animal and/or to the diet rich in polyunsaturated fatty acids but poor in antioxidant factors. At the level of the spleen, congested areas of red pulp were also observed, characterized by hyperplasia and extramedullary hematopoiesis due to severe blood stasis.

In the lungs, alveolar walls presented multifocal intravascular epithelial emboli that showed a high nuclear to cytoplasmic ratio, variably roundish hyperchromatic nuclei with one small nucleolus, and mild to moderate anisocytosis/anisokaryosis. Some micrometastases were seen in the cortical area of some mediastinal lymph nodes. The right parathyroid gland showed a medullary neoplastic nodule that, initially, was considered a possible primitive site of development of the adenocarcinoma of which metastases are observed in the liver, lungs, and lymph nodes. Kidneys appeared free of metastases. However, microscopically, a slight to moderate interstitial nephritis with slight interstitial fibrosis and lymphoplasmacytic infiltration between nephrons were documented. In many glomeruli, a slight glomerular jalinosis was also present.

No neoplastic lesions were observed in the CNS in which minimal lymphoplasmacytic infiltration in leptomeninges was associated with mild laminar oedema in the underlying cerebrum cortex. Multifocal mild satellitosis and mild perivascular oedema and haemorrhage were also observed in the absence of convincing evidence of acute neuronal degeneration.

The IHC exam at the hepatic level, where the most severe and extensive pathological picture was found, showed an intra-parenchymal proliferation, characterized by pseudo-cordonal or acinar structures, of vimentin-negative, PCK AE1/AE3, CK18, and CK8 positive cells. A characteristic canalicular positivity of these cells was also observed in CD10 and p-CEA stained sections (Figure 5). As summarized in Table 2, the neoplastic cells, consistently negative for vimentin, expressed a strong cellular positivity for CK18 and CK8, and a positivity for PCK AE1/AE3 in all the organs examined. Conversely, a canalicular positivity for p-CEA was observed only in the

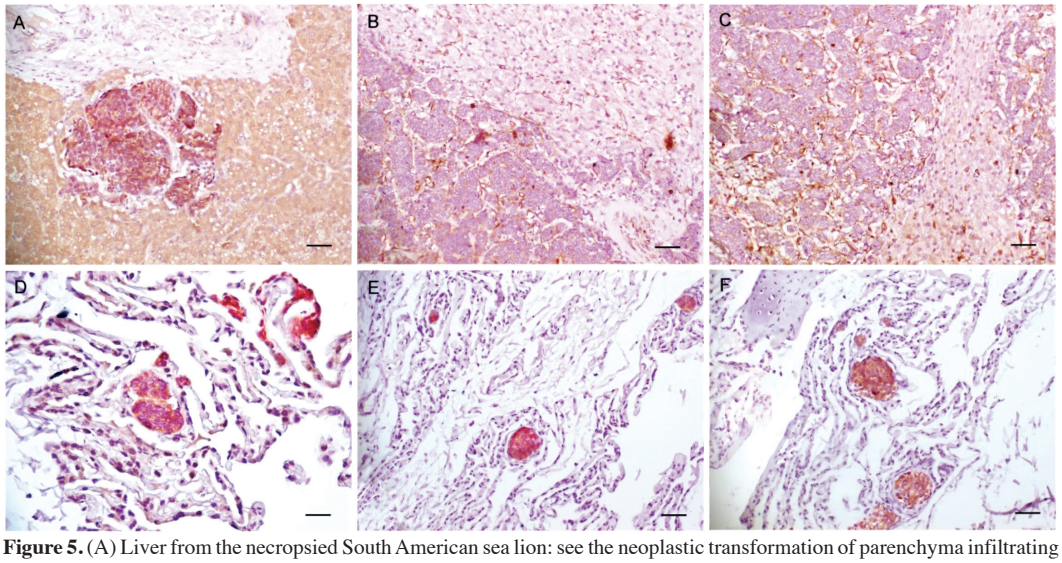

Figure 5. (A) Liver from the necropsied South American sea lion: see the neoplastic transformation of parenchyma infiltrating the dystrophic, poorly preserved remaining tissue; note the diffuse cytokeratin 18 (CK18) expression of neoplastic cells; a diffuse intracellular pattern of expression of this antigen is observed only in neoplastic cells; (B) in the same neoplastic mass, expression of the CD10 antigen is observed, organized in a typical intercellular/canalicular pattern; and (C) a similar intracanalicular expression of p-CEA antigen is observed in the same neoplastic tissue. (D) Lung from the necropsied South American sea lion: note the presence of some small metastatic neoplastic nodules expressing a diffuse intracytoplasmic positivity for pan cytokeratin (clone AE1/AE3), indicating the epithelial nature of the metastatic tissue; (E) panel with a similarly strong intracytoplasmic positivity for CK18 observed in intrapulmunar metastasis, indicating the same nature of the hepatic primary mass; and (F) metastatic masses were also positive for CK8, confirming the hepatocellular nature of the primary neoplasia (IHC stain, Meyer hematoxylin nuclear counterstain; Figures A, B, C & D: scale bar = 100 μm, and Figures E & F: scale bar = 250 μm).

Table 2. Results of the immunohistochemical study

Antigen Organs	Pan cytokeratin AE1/AE3	Cytokeratin 18/8	p-CEA	CD10	Vimentin
Liver mass	Focally positive	Strongly/diffusely positive	Canalicular positivity	Canalicular positivity	Negative
Lung metastasis	Positive	Positive	Negative	Negative	Negative
Spleen/lymph nodes	Positive	Positive	Negative	Negative	Negative
Parathyroid nodes	Positive	Positive	Canalicular positivity	Negative	Negative

liver and at the level of the parathyroid, while CD10 was expressed, in a similar canalicular pattern, only in hepatic neoplastic nodules.

Discussion

Hepatocellular carcinoma (HCC) is a malignant tumor not uncommon in domestic species (Cullen, 2016) and in pinnipeds (Acevedo-Whitehouse et al., 1999; Mauroo et al., 2010). HCC metastasizes throughout the hepatic parenchyma and can affect other tissues and organs such as the lymph nodes, lungs, or spleen (Mauroo et al., 2010; Cullen, 2016). HCC has been diagnosed both post- and antemortem in CSLs (Acevedo-Whitehouse et al., 1999; Mauroo et al., 2010). Acevedo-Whitehouse et al. (1999) also described metastases to the spleen.

However, the localization of the primary tumor at the time of initial clinical presentation of the metastatic disease is frequently unknown (Cullen, 2016). Occult primary tumors account for 5 to 10% of all neoplasms (Hainswort & Greco, 1993; van de Wouw et al., 2002), with the majority of them being adenocarcinoma (Levi et al., 2002). Chronic infections, viruses, chemical ingestion, and exposure to environmental carcinogens have been considered to be involved in the development of hepatic carcinomas (Brown et al., 1980; Acevedo-Whitehouse et al., 1999; Newman & Smith, 2006; Cullen, 2016). Different chemicals are known to cause HCC in domestic animals (Cullen, 2016), and the presence of pollutants, potentially carcinogenic in ocean waters, have been previously described (Britt & Howard, 1983; Procuraduría Federal de Protección al Ambiente [PROFEPA], 1995; Nunn et al., 1996).

Metastatic tumors with an unknown primary site tend to have an unfavorable prognosis, while identification of the site of origin has both prognostic and therapeutic significance (Ma et al., 1993; Abbruzzese et al., 1994). To determine the origin of metastatic epithelial neoplasms involving various organs is challenging. In the present case, it was evident that the primary tumor did not originate from the parathyroid. Parathyroid carcinomas (PTC) present an intensely positive reaction for cytokeratin AE1/AE3, including a focal positivity for the CK7 antibody, but total negativity for CK8 and CK18 antibodies (Piciu et al., 2013). In addition, carcinoembryonic antigen CEA is uniformly non-reactive in the neuroendocrine cells of PTC (Wilkins & Lewis, 2009). The diagnosis of PTC is usually difficult to establish on a simple histological examination in the absence of specific cytological, architectural, or IHC markers. Therefore, this diagnosis must be based on a combination of clinical signs, diagnostic imaging, and histological findings in a context of atypical presentation.

In hepatic epithelial neoplasms, if morphology of the tumor is not sufficient for a correct diagnosis (particularly with a poorly differentiated neoplasm), the first IHC panel must include (1) a few antibodies directed against epithelial antigens (broad spectrum anti PCK AE1/AE3, plus CK 18/8), (2) vimentin to exclude a sarcoma with the exception of alveolar soft part sarcoma, and (3) CD10 or pCEA staining to differentiate the hepatocellular component from the biliary component. CD10 and pCEA are also positive in normal liver tissue but with a differentiation in expression patterns (Selves et al., 2018); pCEA shows diffuse cytoplasmatic expression in most adenocarcinomas, but in HCC, there is a distinct "chicken-wire fence" pattern around the canaliculi. In HCC, CD10 shows a similar staining pattern to pCEA but is negative in adenocarcinomas (Melato et al., 1989; Kakar et al., 2003; Kojiro, 2009). The panel of primary antibodies used in the present study (vimentin and cytokeratins) was chosen based on the need to understand the nature of the hepatic tumor, whether it was a carcinomatous or a sarcomatous form. Testing the tumor for antigens PCK AE1/AE3, CK18, CK8, p-CEA, and CD10 appears to be the best approach for understanding which liver cell line (hepatocellular or cholangiocellular) is at the origin of the neoplasm as reported by Selves et al. (2018).

Conclusions

In the present study, the pathogenesis of neoplasia formation could not be established, but a correct clinical and histopathological algorithm permitted the final diagnosis of multimetastatic HCC, involving the lung, spleen, lymph nodes, and parathyroid gland. The IHC analysis confirmed that the tumor was of primary hepatocellular and not biliary origin and that it had metastasized to various organs, including the parathyroid which does not represent a usual site of metastasis. To our knowledge, this is the first case described of HCC with evidence of metastasis to the lungs and parathyroid gland in a male South American sea lion.

Acknowledgments

Authors thank Dr. Daniela Florio and Dr. Torsten Muller for their technical and logistical assistance, and Mediterraneo Marine Park Animal Care and Technical department staff for their continuous efforts to ensure the best care possible to the animals.

Literature Cited

Abbruzzese, J. L., Abbruzzese, M. C., Hess, K. R., Raber, M. N., Lenzi, R., & Frost, P. (1994). Unknown primary carcinoma: Natural history and prognostic factors in 657 consecutive patients. *Journal of Clinical Oncology*, *12*(6), 1272-1280. https://doi.org/10.1200/JCO.1994.12.6.1272

Acevedo-Whitehouse, K. A., Constantino-Casas, F., Aurioles-Gamboa, D., Rodríguez-Martínez, H. A., & Gordínez-Reyes, C. R. (1999). Hepatic carcinoma with spleen metastasis in a California sea lion from the Gulf of California. *Journal of Wildlife Diseases*, *35*(3), 565-568. https://doi.org/10.7589/0090-3558-35.3.565

Biancani, B., Lacave, G., Magi, G. E., & Rossi, G. (2010). Ovarian interstitial cell tumor in a South American sea lion (*Otaria flavescens*). *Journal of Wildlife Diseases*, *46*(3), 1012-1016. https://doi.org/10.7589/0090-3558-46.3.1012

Britt, J. O., Jr., & Howard, E. B. (1983). Tissue residues of selected environmental contaminants in marine mammals. In E. B. Howard (Ed.), *Pathobiology of marine mammal diseases* (Vol. 1, pp. 79-94). CRC Press.

Brown, R. J., Smith, A. W., Morejohn, G. V., & de Long, R. L. (1980). Metastatic adenocarcinoma in two California sea lions (*Zalophus californianus californianus*). *Journal of Wildlife Diseases*, *16*(2), 261-266. https://doi.org/10.7589/0090-3558-16.2.261

Cárdenas-Alayza, S., Crespo, E., & Oliveira, L. (2016). *Otaria byronia*. In International Union for Conservation of Nature (Ed.), *The IUCN red list of threatened species*. https://www.iucnredlist.org/species/41665/61948292

Chen, Z. E., & Lin, F. (2015). Application of immunohistochemistry in gastrointestinal and liver neoplasms: New markers and evolving practice. *Archives of Pathology and Laboratory Medicine*, *139*(1), 14-23. https://doi.org/10.5858/arpa.2014-0153-RA

Choi, W. T., Ramachandran, R., & Kakar, S. (2017). Immunohistochemical approach for the diagnosis of a liver mass on small biopsy specimens. *Human Pathology*, *63*, 1-13. https://doi.org/10.1016/j.humpath.2016.12.025

Colegrove, K. M. (2018). Noninfectious diseases. In F. M. D. Gulland, L. A. Dierauf, & K. L. Whitman (Eds.), *CRC handbook of marine mammal medicine* (3rd ed., pp. 267-296). CRC Press.

Cullen, J. M. (2016). Tumors of the liver and gallbladder. In D. J. Meuten (Ed.), *Tumors in domestic animals* (Chapter 14). Wiley Blackwell. https://doi.org/10.1002/9781119181200.ch14

Duraiyan, J., Govindarajan, R., Kaliyappan, K., & Palanisamy, M. (2012). Applications of immunohistochemistry. *Journal of Pharmacy & Bioallied Sciences*, *4*(2), S307-S309. https://doi.org/10.4103/0975-7406.100281

European Association for Aquatic Mammals (EAAM). (2009, September). *Proceedings of the EAAM Workshop on* Mycobacterium pinnipedii. Duisburg Zoo, Germany.

Hainswort, J. D., & Greco, F. A. (1993). Treatment of patients with cancer of unknown primary site. *New England Journal of Medicine*, *329*(4), 257-263. https://doi.org/10.1056/NEJM199307223290407

Kakar, S., Muir, T., Murphy, L. M., Lloyd, R. V., & Burgart, L. J. (2003). Immunoreactivity of Hep Par 1 in hepatic and extrahepatic tumors and its correlation with albumin in situ hybridization in hepatocellular carcinoma. *American Journal of Clinical Pathology*, *119*(3), 361-366. https://doi.org/10.1309/8L872RPHEJRKF5JJ

Kojiro, M. (Ed.). (2009). *Pathology of hepatocellular carcinoma*. Blackwell Publishing Ltd. 184 pp. https://doi.org/10.1002/9781444312775

Levi, F., Te, V. C., Erler, G., Randimbison, L., & La Vecchia, C. (2002). Epidemiology of unknown primary tumours. *European Journal of Cancer*, *38*(13), 1810-1812. https://doi.org/10.1016/S0959-8049(02)00135-1

Ma, C. K., Zarbo, R. J., Frierson, H. F., & Lee, M. W. (1993). Comparative immunohistochemical study of primary and metastatic carcinoma of the liver. *American Journal of Clinical Pathology*, *99*(5), 551-557. https://doi.org/10.1093/ajcp/99.5.551

Mauroo, N., Lee, F. K., Brook, F., & Bossart, G. (2010, May). *Antemortem diagnosis of hepatocellular carcinoma in a California sea lion (*Zalophus californianus*)*. Proceedings of the 41st International Association for Aquatic Animal Medicine (IAAAM) Annual Conference, Vancouver, Canada.

Melato, M., Laurino, L., Mucli, E., Valente, M., & Okuda, K. (1989). Relationship between cirrhosis, liver cancer, and hepatic metastases: An autopsy study. *Cancer*, *64*(2), 455-459. https://doi.org/10.1002/1097-0142(19890715)64:2<455::AID-CNCR2820640219>3.0.CO;2-C

Newman, S. J., & Smith, S. A. (2006). Marine mammal neoplasia: A review. *Veterinary Pathology*, *43*(6), 865-880. https://doi.org/10.1354/vp.43-6-865

Nunn, J. W., Livingstone, D. R., & Chipman, R. K. (1996). Effect of genetic toxicants in aquatic organisms. In E. W. Taylor (Ed.), *Toxicology of aquatic pollution, physiological, molecular and cellular approaches* (pp. 225-251). Cambridge University Press. https://doi.org/10.1017/CBO9780511735516.012

Piciu, D., Irimie, A., Kontogeorgos, G., Piciu, A., & Buiga, R. (2013). Highly aggressive pathology of non-functional parathyroid carcinoma. *Orphanet Journal of Rare Diseases*, *8*, Article 115. https://doi.org/10.1186/1750-1172-8-115

Procuraduría Federal de Protección al Ambiente (PROFEPA). (1995). *Mortalidad de mamíferos y aves marinas en el Golfo de California: Reporte preliminar de la Secretaría del Medio Ambiente, Recursos Naturales y Pesca* [Mortality of marine mammals and birds from the Gulf of California: Preliminary report of the Secretary of Environment, Natural Resources and Fisheries]. PROFEPA.

Selves, J., Long-Mira, E., Mathieu, M. C., Rochaix, P., & Ilié, M. (2018). Immunohistochemistry for diagnosis of metastatic carcinomas of unknown primary site. *Cancers*, *10*(4), 108. https://doi.org/10.3390/cancers10040108

van de Wouw, A. J., Janssen-Heijnen, M., Coebergh, J., & Hillen, H. (2002). Epidemiology of unknown primary tumours; incidence and population-based survival of 1285 patients in Southern Netherlands, 1984-1992. *European Journal of Cancer*, *38*(3), 409-413. https://doi.org/10.1016/S0959-8049(01)00378-1

Walter, O., & Kuzniar, B. (2018). *European regional South American sea lion studbook*. Twycross Zoo.

Webber, M. A. (2014). Family Otariidae (eared seals). In D. E. Wilson & R. A. Mittermeier (Eds.), *Handbook of the mammals of the world: Vol. 4. Sea mammals* (pp. 34-101). Lynx Edicions.

Wilkins, B. J., & Lewis, J. S., Jr. (2009). Non-functional parathyroid carcinoma: A review of the literature and report of a case requiring extensive surgery. *Head and Neck Pathology*, *3*(2), 140-149. https://doi.org/10.1007/s12105-009-0115-4

Yamazaki, M., Koutaka, M., & Une, Y. (2016). Gastric carcinoma in a South American sea lion (*Otaria flavescens*). *Journal of Veterinary Medical Science*, *78*(7), 1201-1204. https://doi.org/10.1292/jvms.16-0026

You, M. H., Bae, I. H., Jee, H., Yoo, M. J., Shin, N. S., & Kim, D. Y. (2008). Hemangiosarcoma in a South American sea lion (*Otaria byronia*). *Journal of Wildlife Diseases*, *39*(1), 118-120. https://doi.org/10.1638/2007-0019.1