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Prevalence of Urogenital Carcinoma in Stranded California Sea Lions (*Zalophus californianus*) from 2005–15

Alissa C. Deming,[1,2,5] Kathleen M. Colegrove,[3] Padraig J. Duignan,[2] Ailsa J. Hall,[4] James F. X. Wellehan,[1] and Frances M. D. Gulland[2] [1]University of Florida, College of Veterinary Medicine, Department of Comparative, Diagnostic, and Population Medicine, 2015 SW 16th Avenue, Gainesville, Florida 32608, USA; [2]The Marine Mammal Center, 2000 Bunker Road, Sausalito, California 94965, USA; [3]Zoological Pathology Program, College of Veterinary Medicine, University of Illinois at Urbana–Champaign, 3300 Golf Road, Brookfield, Illinois 60513, USA; [4]Sea Mammal Research Unit, Scottish Oceans Institute, University of St. Andrews, St. Andrews KY16 8LB, UK; [5]Corresponding author (email: ademing@ufl.edu)

ABSTRACT: Urogenital carcinoma is common in wild California sea lions (*Zalophus californianus*) along the west coast of the US. From 1979 to 1994, this cancer was observed in 18% (66/370) of necropsied subadult and adult sea lions at The Marine Mammal Center in Sausalito, California. A retrospective review of records from 1 January 2005 to 31 December 2015 was performed to characterize prevalence and characteristics of cancer over this decade. Fourteen percent (263/1917) of necropsied sea lions had cancer, of which 90% (237/263) were urogenital carcinoma. The prevalence of urogenital carcinoma was significantly higher in adults compared to juveniles and subadults. Advanced-stage disease with metastases was identified histologically in 78% (182/232) of cases and was the cause of death in 95% (172/182) of these cases. Metastases were most common in lung and lymph nodes, and hydronephrosis, secondary to ureter obstruction by metastases, was identified in 62% (114/185) of animals with advanced disease. No significant temporal change in prevalence was detected over the decade, and this highly aggressive, fatal cancer remains common in stranded California sea lions.

Key words: California sea lions, cancer, stranding, urogenital carcinoma, *Zalophus californianus*.

Cancer is rare in free-ranging marine mammals with the exception of two populations: beluga whales (*Delphinapterus leucas*) in the St. Lawrence River and California sea lions (*Zalophus californianus*) on the west coast of the US. Urogenital carcinomas (UGC) comprise the majority of cancers documented in California sea lions (Gulland et al. 1996) whereas a variety of cancers (papillomas, carcinomas, and adenocarcinomas) have been identified in belugas that have high levels of contaminants in their blubber (De Guise et al. 1995). In recent years, the annual prevalence of cancer reported in necropsied belugas from this population has declined (Lair et al. 2016); however, the current trend in prevalence of UGC in California sea lions is unknown. Environmental contaminants are also common in California sea lions, with cancer being eight- and six-times more likely in California sea lions with higher blubber levels of polychlorinated biphenyl (PCB) and DDT, respectively (Randhawa et al. 2015). Thus, contaminant exposure may play a role in initiation or promotion of UGC, which likely has a multifactorial etiology. Other cofactors associated with California sea lion carcinogenesis include otarine herpesvirus 1 (OtHV1) and genetics (King et al. 2002; Bowen et al. 2005; Browning et al. 2014). Urogenital carcinoma is most frequent in adults, and sexual transmission of OtHV1 has been suggested (Buckles et al. 2007). There is no evidence for direct transmission of a clonal cancer (Leathlobhair et al. 2017).

From 1979 to 1994, metastatic carcinoma was diagnosed at postmortem examination in 18% (66/370) of wild subadult and adult California sea lions (Gulland et al. 1996). Urogenital carcinoma originates in the genital epithelium, and local invasion and widespread metastasis are common (Lipscomb et al. 2000). From 1991 to 2000, 15% (88/568) of adult California sea lions, most of which were younger adults, at The Marine Mammal Center (TMMC) were diagnosed with UGC (Greig et al. 2005). Cancer continues to be observed in stranded California sea lions, but the current prevalence of cancer has not recently been evaluated. Our objective was to

TABLE 1. Prevalence of urogenital carcinoma in California sea lions (*Zalophus californianus*) of different age and sex classes necropsied at The Marine Mammal Center, Sausalito, California, USA, from 2005–15. Juvenile and subadult age classes were pooled because juvenile and subadult females cannot be easily distinguished and considered separately from adults.

Age class	Sex	No. of sea lions necropsied	No. of sea lions with carcinoma	Prevalence (%)
Adult	Females	821	174	21.2
	Males	111	37	33.3
	Total	932	211	22.6
Juvenile–subadult	Females	182	5	2.7
	Males	803	21	2.6
	Total	985	26	2.6
Total	Females	1003	179	17.8
	Males	914	58	6.3
	All	1917	237	12.4

assess the occurrence of UGC in California sea lions from 2005 to 2015 and to determine if temporal changes in prevalence have occurred.

The Marine Mammal Center is a rehabilitation facility that examines stranded marine mammals from 1,125 km of California coastline (Grieg et al. 2005). Necropsy records were reviewed from juvenile, subadult, and adult California sea lions examined at TMMC from January 1, 2005 to December 31, 2015. Records included gross findings, sex, weight, standard length, nutritional status (score 1–5), evidence of pregnancy (lactation, placental scarring, fetus), stage of sagittal crest development, and tooth development and wear. Standard length, tooth wear, and sagittal crest development were used to determine age class as defined in Grieg et al. (2005): pup (0–1 yr), yearling (1–2 yr), juvenile male (2–4 yr), subadult male (4–8 yr), juvenile or subadult female (2–5 yr), adult female (5+ yr), and adult male (8+ yr). Juvenile and subadult age classes were pooled and considered separately from adults in some data analyses, as the prevalence of cancer is known to be significantly higher in sexually mature animals (Grieg et al. 2005). Samples from all major organs were examined histologically from all fresh dead or euthanized animals. Any patient with a diagnosis of cancer was included in this study. Cases histologically characterized as carcinoma in situ or as locally invasive carcinoma with no metastasis (Colegrove et al. 2009) were defined as early stage disease. Advanced-stage disease was defined by gross and histologic evidence of metastasis. Prevalences were compared using chi-squared analysis, and a beta regression model was used to examine differences in proportions using the R package (R Development Core Team 2016).

Necropsies were performed on 985 juvenile and subadult and 932 adult California sea lions. There were 1,003 females and 914 males (Table 1). Fourteen percent (263/1917) of all California sea lions necropsied had cancers (Table 1), of which 90% (237/263) were grossly or histologically identified as UGC, while 10% (26/263) were other cancers including uterine leiomyomas, lymphomas, and adrenal cortical carcinomas (Table 2).

Of the juveniles and subadults, 2.6% (26/985) had UGC whereas adults had a significantly higher prevalence of 23% (211/932; $P<0.001$). In juveniles and subadults, metastasis was documented in 77% (20/26) of cases, and UGC was the cause of death in all but one of these animals. There were 13 to 30 (mean 19) cases of UGC in adult California sea lions each year (Fig. 1). Annual prevalence varied among years (range 11–51%), but when year was included in the model to test for a trend in the proportions (i.e., a decline or increase over time), there was no statistically significant

TABLE 2. Nonurogenital carcinoma cancer cases in California sea lions (*Zalophus californianus*) that were necropsied and evaluated histologically at The Marine Mammal Center, Sausalito, California, USA, from 2005–15. Age (A = adult; J = juvenile), sex (F = female; M = male), tumor type, location, and metastasis are listed for each case. The presence of urogenital carcinoma is also noted, as several animals had both urogenital carcinoma and an unrelated cancer concurrently.

Age class	Sex	Type of primary tumor and location	Metastasis location	Concurrent urogenital carcinoma
A	F	Adenoma, adrenal cortex	None	Cervix, carcinoma in situ
A	F	Adenoma, adrenal cortex	None	Cervix, carcinoma in situ
A	F	Carcinoma, adrenal cortex	Lymph node[a]	Vagina and cervix, carcinoma in situ
A	F	Leiomyoma, cervix	None	Vagina and cervix, carcinoma in situ
A	F	Leiomyoma, uterus; Papilloma, tongue	None	Vagina and cervix, carcinoma in situ
A	F	Leiomyoma, uterus	None	No
A	F	Leiomyoma, uterus	Oropharynx	Vagina and cervix, invasive carcinoma
A	F	Leiomyoma, uterus	None	No
A	F	Leiomyoma, uterus	None	No
A	F	Leiomyosarcoma, uterus	None	No
A	F	Lymphoma, esophagus	None	No
A	F	Lymphoma, multiple lymph nodes	Multiple lymph nodes	No
A	F	Lymphoma, T-cell	Duodenum, ileum, liver, lymph nodes	Vagina and cervix, invasive carcinoma
A	F	Papillary adenocarcinoma, ovary	Uterus and ovary[a]	Vagina and cervix, invasive carcinoma
A	F	Papillary adenoma, ovary	None	No
A	F	Adenoma, pancreas	None	No
A	F	Poorly differentiated sarcoma, eye; hibernoma, free abdominal mass	None	No
A	F	Poorly differentiated sarcoma, eye	Lungs	No
A	F	Hemangioma, uterus	None	Vagina and cervix, invasive carcinoma
A	F	Spindle cell sarcoma, abdominal mass	Unknown (patient released)	Unknown (patient released)
A	F	Squamous cell carcinoma, tonsil	Lymph nodes, lung, liver	No
A	F	Lipoma, vagina	None	No
A	M	Seminoma, testicle	None	No
J	M	Hemangioma, liver	None	No
J	M	Lymphoma, thoracic masses and gastric mass	Lungs, heart	No

[a] Metastasis associate with urogenital carcinoma.

change in prevalence over time (type II chi-squared test P=0.560).

Histology was performed on 232 of the 237 UGC cases identified on gross necropsy. Of these cases, 22% (50/232) were early stage and 78% (182/232) were advanced stage (Table 3). Carcinoma in situ of the cervix, vagina, penis, or prepuce with no evidence of metastasis was diagnosed in 19% (45/232) of UGC cases and locally invasive carcinoma with no evidence of metastasis in 2% (5/232). All early stage cases were considered incidental findings, and the primary cause of stranding was most-often associated with domoic acid intoxication, trauma, leptospirosis, or pneumonia. Carcinoma in situ of the cervix, vagina, penis, or

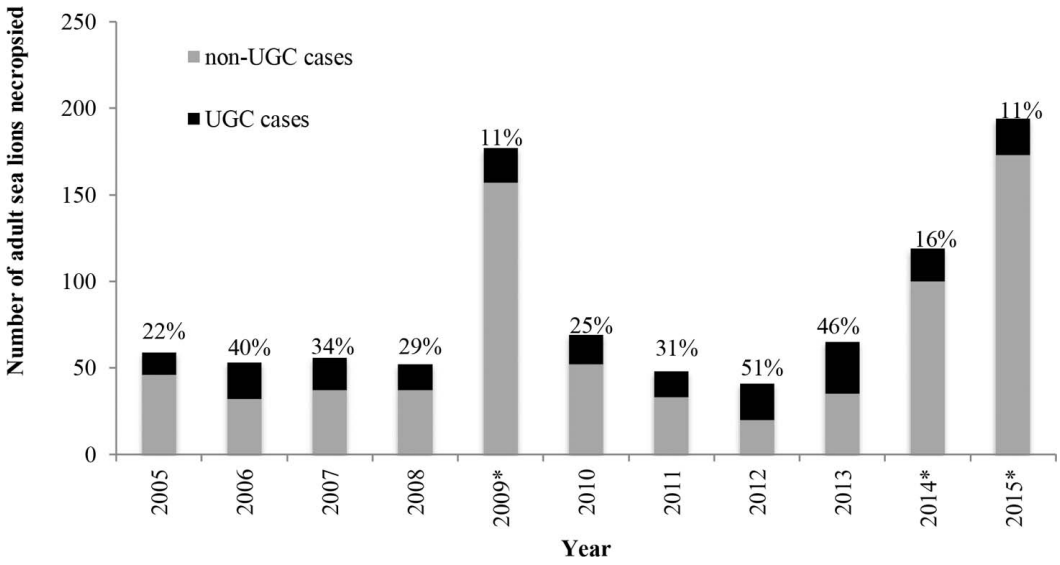

FIGURE 1. Total number of California sea lions (*Zalophus californianus*) necropsied (gray and black combined) and diagnosed histologically with urogenital carcinoma (black) and without urogenital carcinoma (gray) at The Marine Mammal Center, Sausalito, California, USA, by year, from 2005 to 2015. Each year's prevalence is indicated by percentage above bar. *=El Niño year.

prepuce with metastasis was diagnosed in 17% (39/232) of UGC cases; metastases, but no primary lesion, were seen in 12% (27/232) of UGC cases, and invasive carcinoma in the cervix or vagina or in the penis or prepuce with metastases was present in 50% (116/232) of UGC cases. Metastatic disease was considered the primary cause of death in 95% (172/182) of California sea lions with advanced-stage UGC.

Metastatic lesions were identified in a number of organs (Table 4), especially the sublumbar lymph node. Nodal enlargement secondary to metastases frequently resulted in obstruction of ureters with sequela of hydroureter and hydronephrosis. Hydronephrosis was present in 62% (114/185; male=19/42, female=95/143) of advanced-stage cases. Clinical signs in advanced-stage cases included hind limb swelling, paresis, or paralysis (49%, 90/185; male=21/42, female=69/143) and gen-

TABLE 3. Number (*n*) and prevalence (%) of various stages of urogenital carcinoma diagnosed histologically in juvenile, subadult, and adult California sea lions (*Zalophus californianus*, *n*=232) necropsied at The Marine Mammal Center, Sausalito, California, USA, from 2005–15. Early stage cases had no evidence of metastasis and included carcinoma in situ and locally invasive carcinoma; advanced-stage cases had metastatic lesions. Females and males are considered separately, as well as together, to show prevalence of each stage of urogenital carcinoma in all cases and between sexes.

Stage of urogenital carcinoma	Histologic description	No. of urogenital carcinoma cases (%)		
		Females	Males	All
Early stage	Carcinoma in situ	29/175 (17)	16/57 (28)	45/232 (19)
	Locally invasive carcinoma	5/175 (3)	0/57 (0)	5/232 (2)
Advanced stage	Carcinoma in situ with metastases	27/175 (15)	12/57 (21)	39/232 (17)
	Invasive carcinoma with metastases	100/175 (57)	16/57 (28)	116/232 (50)
	No primary identified but metastases present	14/175 (8)	13/57 (23)	27/232 (12)

TABLE 4. Distribution of metastases detected via histologic evaluation of California sea lions (*Zalophus californianus*) with advanced urogenital carcinoma (*n*=182).

Location of metastatic lesion	No. (%) of animals
Lymph node	171 (94)
Lung	127 (70)
Liver	87 (48)
Uterus	78 (55)
Ovary	66 (37)
Kidney	66 (36)
Urinary bladder	52 (29)
Adrenal gland	47 (26)
Mesentery	33 (18)
Diaphragm	26 (14)
Spleen	22 (12)
Urethra/ureter	11 (6)
Skeletal muscle	11 (6)
Small intestine	11 (6)
Aorta	8 (4)
Pericardium	6 (3)
Prostate	5 (12)
Vertebrae	5 (3)
Large intestine	5 (3)
Pancreas	4 (2)
Heart	4 (2)
Brain	4 (2)
Mediastinum	4 (2)
Renal capsule	2 (1)
Testicle	3 (8)
Tonsils	3 (2)
Pituitary	1 (1)

ital swelling or penile or vaginal prolapse (62%; 114/185; males=29/42, female=85/143). None of these findings was present in early stage cases. State of nutrition (SON) was significantly lower (two-tailed *t*-test, *P*=0.008) in animals with advanced-stage disease (SON mean=1.98, SD=0.68, *n*=182) compared to animals diagnosed with early stage disease (SON mean=2.34, SD=0.72, *n*=50).

A high prevalence of cancer in stranded California sea lions was confirmed, with 263 cancer cases observed over a decade and urogenital carcinoma accounting for 90% of cases. The number of UGC cases observed from 2005 to 2015 were more than triple the number described for this species between 1979 and 1994 (Gulland et al. 1996) and more than twice the number observed from 1990 to 2001 (Greig et al. 2005). Although the prevalence in necropsied animals was highly variable among years, with no significant temporal trend, the increase in numbers of cases is likely a reflection of the increasing CSL population, which has expanded from approximately 161,000 in the mid-90s to over 296,000 (Carretta et al. 2015).

Most cancers occurred in adult CSL in our study, with few cases in juveniles, a distribution common in humans and animals (Howlader et al. 2017). The prevalence of carcinoma was highest in adult males, which is of interest because they also have the highest levels of organochlorine contaminants (Randhawa et al. 2015) and a higher prevalence of OtHV1 infection (Buckles et al. 2007). We did not diagnose UGC in yearlings or pups; however, there are rare reports of UGC in yearling California sea lions (Greig et al 2005). The virus associated with UGC, OtHV1, has been identified in CSL pups (Buckles et al. 2007), although no known cases of UGC have been documented in this age class.

In wild California sea lions, diagnosis of UGC in live animals is difficult. The number of cases with advanced-stage metastatic disease, but with no observed primary genital lesion, identified the risk of false-negative genital biopsies when testing live animals for carcinoma. Unbiased sampling of free-ranging California sea lions is challenging, making its true prevalence hard to establish. There is an inherent sampling bias associated with assessing prevalence of any disease in a stranded group of animals, as the results of sampling those animals reflects the factors influencing their stranding. Reasons other than cancer, such as changes in prey distribution, toxic algal blooms, and disease outbreaks, can increase strandings, altering the prevalence of cancer in the sample (Greig et al. 2005). However, the long-term data (1997 to present) collected on sea lions at TMMC minimized the effect of short-term influences on the mortality patterns that we observed.

The high number of animals that we diagnosed with this aggressive cancer demonstrated that UGC continues to be common in

wild California sea lions. Thus, UGC in sea lions can serve as a spontaneous disease model to help elucidate the complex cellular derangements that accrue in cellular transformation, malignancy, and metastasis (Browning et al. 2015).

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