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# Ectopic pregnancy with associated gestational choriocarcinoma in a California sea lion (*Zalophus californianus*)

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**ABSTRACT:** A wild-born, captive-reared, 14 yr old, primiparous female California sea lion *Zalophus californianus* presented for anorexia of 14 d duration and abdominal distention. Routine complete blood cell count revealed leukocytosis with a neutrophilia, and serum chemistry revealed hypoalbuminemia and hyponatremia. Treatment with broad spectrum antibiotics and non-steroidal anti-inflammatories were started, but the animal continued to decline. Abdominal radiographs revealed a mature mineralized fetal skull and spine in the caudal abdomen and abdominal ultrasound revealed ascites but could not confirm the fetus. The patient was taken to surgery where a full term fetus was found outside of the uterus but within the fetal membranes, representing a secondary ectopic pregnancy. The patient passed away during surgery and was taken to necropsy. Gross necropsy revealed a diffuse peritonitis with yellow deposits over the serosal surfaces of the abdominal organs. The uterus appeared intact grossly and the ovaries appeared abnormal. The mesenteric, renal, and sub-lumbar nodes were enlarged and edematous. Histopathology revealed choriocarcinoma in the right uterine horn with evidence of chronic uterine rupture and protrusion of the placental tissue into the abdomen. The choriocarcinoma had metastasized locally as well as to the liver, spleen and lung. Choriocarcinoma is a highly malignant trophoblastic neoplasm that is rare in domestic animals. This case represents, to the authors' knowledge, the first report of gestational choriocarcinoma causing secondary ectopic pregnancy in a California sea lion and presents questions regarding pregnancy monitoring and management in a population of captive, minimally trained California sea lions.

**KEY WORDS:** Choriocarcinoma · Neoplasia · California sea lion · Marine mammal · Pinniped · Ectopic pregnancy

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## INTRODUCTION

Reports of neoplasia in California sea lions *Zalophus californianus* are increasing in number and encompass a wide range of neoplasms (Gulland et al. 2001, Newman & Smith 2006). Most neoplasia seen in the collection setting is presumed to develop spontaneously because, most of the time, no etiology has been found, with the exception of urogenital carci-

noma. An 18% prevalence of urogenital carcinoma has been reported in California sea lions that stranded live along the central California (USA) coast (Gulland et al. 1996, Ylitalo et al. 2005). The etiology has not been determined but is hypothesized to involve a gamma herpes virus, genetics and contaminants (Newman & Smith 2006). Other types of reproductive tract neoplasia reported in California sea lions include squamous cell carcinoma, leiomyoma,

adenocarcinoma, adenoma granulosa-cell tumor, papilloma and hemangioma (Brown et al. 1980, Howard et al. 1983, Joseph et al. 1986, Gulland et al. 1996, 2001, Lipscomb et al. 2000, King et al. 2002).

This case report discusses choriocarcinoma, which has never been reported in a California sea lion but has been reported in a rhesus monkey (*Macaca mulatta*), a patas monkey (*Erythrocebus patas*), a nine-banded armadillo (*Dasypus novemcinctus*), and a rabbit (*Oryctolagus cuniculi*) (Lindsey et al. 1969, Bagshawe 1977, Rice et al. 1989, Kaufmann-Bart & Fischer 2008, Cushing et al. 2013). Choriocarcinoma is a highly malignant trophoblastic neoplasm that can occur after a pregnancy, as a component of a germ cell tumor, or in association with a poorly differentiated somatic carcinoma (Monchek & Wiedaseck 2012). Choriocarcinoma is characterized by trophoblastic tissue invading the myometrium and potentially metastasizing through the uterine blood vessels to the rest of the body. Choriocarcinoma is rare in domestic animals and occurs in approximately 1 in 20 000 to 40 000 human pregnancies (Hoffner & Surti 2012, Monchek & Wiedaseck 2012). Gestational choriocarcinoma occurs when the neoplasm is derived from pregnancies, induced or spontaneous abortions, ectopic pregnancies, and term or preterm deliveries (Hoffner & Surti 2012). Latent choriocarcinoma has appeared years after the last known pregnancy (Dehner 1980). In humans, the clinical presentation of malignant gestational trophoblastic disease is important in determining treatment. Symptoms may include abnormal bleeding for more than 6 wk following a pregnancy or may be more subtle. Monitoring blood serum levels of human chorionic gonadotropin, which is produced by all gestational trophoblastic tumors, can aid in diagnosis of this type of neoplasm (Hoffner & Surti 2012). This hormone generally increases or plateaus at an abnormally high levels (Soper et al. 2004).

Ectopic pregnancies are rare in animals and generally occur due to traumatic rupture of the uterus which expels the fetus into the abdomen (Corpa 2006, Foster 2007). Ectopic pregnancies have been reported in rabbit, domestic dogs, and a Himalayan cat (Segura Gil et al. 2004, Nack 2000, Eddey 2012).

## MATERIALS AND METHODS

### Case history

A wild-born, captive-reared, 14 yr old, primiparous female California sea lion was housed since she was

a pup in a multi-species exhibit at Six Flags Discovery Kingdom, Vallejo, California, where there was minimal formal training. On August 19, 2010, she presented for anorexia of 14 d duration and abdominal distention. The decision was made to restrain her in a squeeze cage in order to perform an exam and obtain diagnostics. An injection of diazepam (Hospira), 0.07 mg kg<sup>-1</sup>, was given intramuscularly (i.m.) to facilitate restraint. A physical exam was performed which revealed epaxial muscle atrophy and abdominal distention with no evidence of vulvar discharge. Blood was collected using the caudal gluteal vein and a 22-gauge 1.5 inch (~3.8 cm) needle on a syringe. A brief ultrasound of the abdomen was performed which revealed ascites and the edge of a soft tissue mass with no evidence of a fetal heartbeat. The attempt to image the fetal heart with the ultrasound was unsuccessful due to the limitations of the squeeze cage. The sea lion's heart rate and respiratory rate were normal at 100–120 beats min<sup>-1</sup> and 7–12 breaths min<sup>-1</sup>. She was then treated with 6 l of 0.9% saline subcutaneously due to dehydration. Routine complete blood cell count and serum chemistry were performed and revealed a leukocytosis of 18 900 (normal range 3400–11 300) with a neutrophilia and a hypoalbuminemia of 2.4 g dl<sup>-1</sup> (normal is 2.7–3.6 g dl<sup>-1</sup>) and hyponatremia at 142 mEq l<sup>-1</sup> (normal 149–156 mEq l<sup>-1</sup>) (Bossart et al. 2001). Fecal culture was performed and was negative for *Salmonella* and *Campylobacter* and in-house fecal float was negative for ova. Tests including heartworm antigen by ELISA, toxoplasma titer (IgG) by indirect fluorescent antibody test (IFA) and leptospirosis antibody panel by microagglutination were performed at IDEXX reference laboratories and all were negative.

The next day the sea lion continued to decline and was not interested in eating. A urine sample was obtained from the pen floor and sent to IDEXX for culture and leptospirosis PCR. Antibiotic treatment was initiated and included injectable enrofloxacin (Enroflox™ 100, Norbrook Laboratories Ltd) 5 mg kg<sup>-1</sup>, penicillin G procaine injectable solution (Penject®, Butler Schein) 1 ml 10 kg<sup>-1</sup>, and flunixin meglumine (FlunixiJect™, Butler Schein) 1 mg kg<sup>-1</sup>. The following day a blood sample was taken and sent out for complete blood cell count and serum chemistry. Furosemide (Bayer) 50 mg i.m. twice a day (4 mg kg<sup>-1</sup>), cimetidine 600 mg i.m. and butorphanol (Dolorex®, Intervet/Merk Animal Health) 0.1 mg kg<sup>-1</sup> were added to the treatment plan. This plan was continued for 2 d, then the sea lion was sedated with midazolam (Akorn Inc.) 0.1 mg kg<sup>-1</sup> and medetomidine (Zoo Pharm) 0.1 mg kg<sup>-1</sup> for radiographs. The



Fig. 1. *Zalophus californianus* fetus after removal during exploratory surgery

radiographs revealed a mature ossified fetal skull and spine in the caudal abdomen. An ultrasound was not performed at this time. The differential diagnosis at this point included ectopic fetus and uterine fetal mummification.

On August 24, 2010, the decision to pursue abdominal exploratory surgery was made, and the patient was taken to surgery the next day. The patient was sedated with medetomidine and midazolam, as described above, and anesthesia was induced with 5% isoflurane (Henry Schein Animal Health) via face mask. A basic midline approach was used for the laparotomy. Once the abdomen was open, ~8 l of yellow, turbid fluid was removed and a full-term fetus was found outside of the uterus but within the fetal membranes (Fig. 1). The fetus had fur coming off the skin in large clumps and the vibrissae had fallen off, indicating moderate autolysis. The patient went into cardiac arrest once the fluid and the fetus were removed and could not be resuscitated. The sea lion was immediately taken to necropsy.

#### Pathological examination and histopathology

A complete post mortem examination was carried out at the animal's home institution. Samples were collected into 10% neutral buffered formalin and submitted to the Anatomic Pathology Service of the Willian R. Pritchard Veterinary Medical Teaching Hospital at the University of California, Davis. Sections of submitted tissues, including the entire reproductive tract, were processed routinely for wax embedment and sectioning at 5  $\mu$ m. Hematoxylin and eosin staining was applied to all sections. Additionally, selected sections were also stained by

immunohistochemistry for human chorionic gonadotrophin (hCG Clone CG04+CG05, at 1:1) (NeoMarkers, Thermo Scientific™) and pancytokeratin (AE1/AE3, at 1:300 dilution) (BioGeneX). Routine immunohistochemistry was performed after heated citrate buffer antigen retrieval as previously described (Colegrove et al. 2009b)

#### RESULTS

Gross necropsy revealed a diffuse peritonitis, with fibrin along the serosal surfaces of most of the abdominal organs. The peritoneal surfaces were erythematous and covered in 1–5 mm yellow to white plaque-like structures. The fetal membranes had adhesions to the bladder caudally, the cranial border of the left kidney, and the body wall. The fetal side of the fetal membranes had scattered plaque-like structures. The liver was enlarged with yellow to tan plaque-like structures. The spleen was enlarged and covered in thickened omentum. The intestines had adhesions along the serosal surfaces, but the mucosal surfaces appeared normal. The uterus appeared intact grossly, and there were multiple yellow plaque-like structures on the serosal surface. The ovaries were dark red, enlarged and appeared to have localized abscessation. The uterus and ovaries had patches of shaggy yellow-orange deposits on the serosal surfaces (Fig. 2). The mesen-



Fig. 2. Uterus and ovaries grossly. Black arrow points to the shaggy yellow-orange deposits on the serosal surface of the uterine horn

teric, renal, and sub-lumbar nodes were enlarged and edematous. Vagina and cervix were without lesions. The heart appeared normal and the lungs were purple and consolidated. The fetus was fully developed, measured 76 cm straight length from nose to tip of tail and weighed ~5 kg.

Histopathology revealed chronic uterine rupture of the right uterine horn and protrusion of the placental tissue into the abdomen, which was not appreciated grossly. Vacuolated cells resembling glycogen-rich trophoblasts with papillary growth pattern and syncytia thought to be syncytiotrophoblasts were implanted on the uterine serosa near the site of rupture (Fig. 3). The cellular morphology and pattern of growth recapitulated products of conception leading to a diagnosis of choriocarcinoma. The choriocarcinoma had metastasized to the liver, adjacent lymph nodes, peritoneum and the lymphatics of the lung, spleen and liver (Fig. 4). In addition, the mesothelium of the hepatic, splenic and intestinal serosa was hypertrophied, and there was histiocytic, lymphocytic and neutrophilic peritonitis. There was a region of the liver covered in sheets of neoplastic trophoblast cells that was collapsed, and there was abundant fibrosis around bile ducts and stasis of bile. The kidneys had mild chronic lymphoplasmacytic interstitial nephritis, mild acute tubular necrosis, and minimal lower nephron granular casts. Abdominal fluid analysis revealed a septic-appearing mixed cell exudate with clusters of exfoliating carcinoma cells. Though the cellular morphology and pattern of growth of the tumor was consistent with a trophoblastic origin, the cellular lineage could not be confirmed with immunohistochemistry because the hCG antibody did not react with either the tumor or the internal control of normal portions of the sea lion placenta. The neoplasm, however, was diffusely and strongly positive with pancytokeratin immunohistochemistry, which is consistent with the diagnosis of choriocarcinoma (Fig. 5).

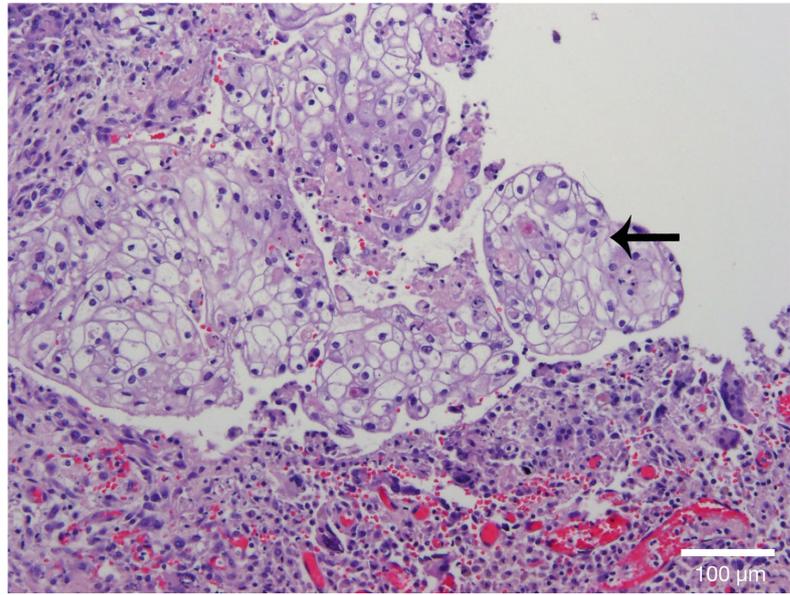


Fig. 3. Histopathology image showing neoplastic cells implanted on uterine serosa near the site of rupture. Black arrow points to one of the clusters of trophoblastic cells

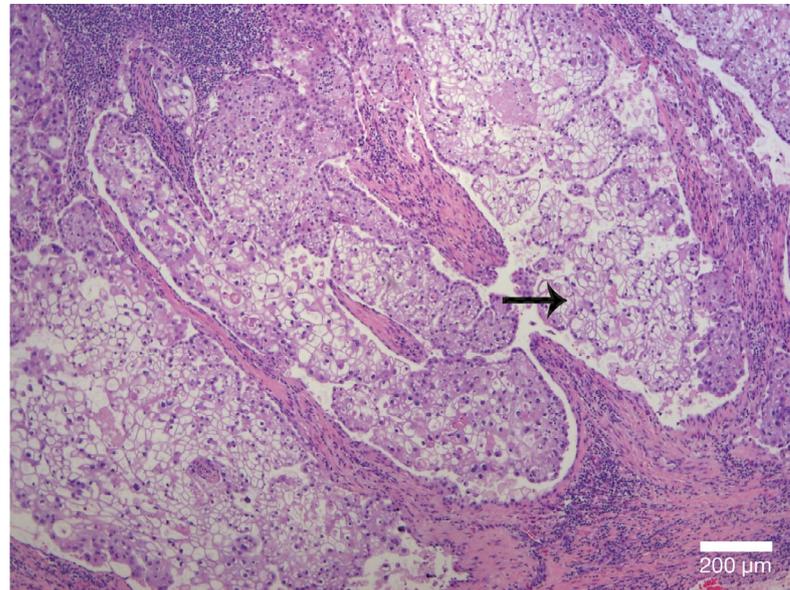


Fig. 4. Histopathology image of neoplastic metastases in the lymph node parenchyma. Black arrow points to one of the clusters of neoplastic cells

## DISCUSSION

The top differential at the time of the exploratory surgery was an ectopic pregnancy or uterine fetal mummification. Though the neoplastic lesions were seen grossly, histopathology was essential for the diagnosis of neoplasia rather than peritonitis.

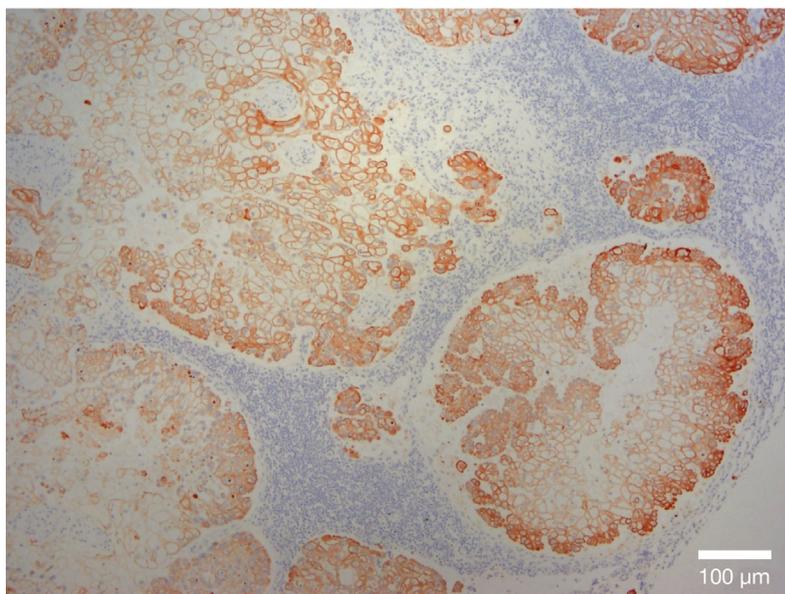


Fig. 5. Histopathology image of neoplastic metastases in the lymph node parenchyma with cytokeratin immunohistochemistry (AE1/AE3) staining, which shows the metastases in orange

The main differential diagnosis for this tumor was California sea lion urogenital carcinoma. However, the pattern of growth and cellular morphology were distinctly different from any of the patterns described for that well-characterized entity (Gulland et al. 1996, Colegrove et al. 2009a). Additionally, no neoplastic transformation was seen grossly or histologically in the lower urinary or genital tracts, which are the usual primary sites of the urogenital carcinoma. An additional differential was uterine adenocarcinoma; however, the biphasic nature of the neoplasm did not fit with that diagnosis. Immunohistochemistry analysis is useful for histological diagnosis of choriocarcinoma. Strong immunoreactivity to  $\beta$ -hCG can confirm the diagnosis. In this case there was no immunoreactivity to  $\beta$ -hCG, but this was likely lack of cross reactivity with sea lion tissues as the internal control of normal placental remnants was also negative. The antibody used had been validated only for humans and macaques. There was immunoreactivity to pancytokeratin, which supports the diagnosis, as cytokeratin is expressed on trophoblastic cells (Desai et al. 2010). However, cytokeratin staining is also expected in other epithelia for the urogenital tract.

In this case, the choriocarcinoma was most likely related to a term pregnancy the sea lion had in 2006 because choriocarcinoma in humans generally happen after, not during, a pregnancy (Dehner 1980). This case appears similar to latent choriocarcinoma in humans that can occur many years after a preg-

nancy and is hypothesized to be caused by activation of dormant trophoblastic cells in the uterus from the last pregnancy (Dehner 1980). The sea lion neoplasm metastasized primarily to the uterus, lymph nodes and peritoneum. This is in contrast to human cases of choriocarcinoma where 80% metastasize primarily to the lungs (Monchek & Wiedaseck 2012). The locations of metastasis in this case are most likely due to the rupture of the uterus and the subsequent extrusion of uterine contents into the peritoneum. Though the uterine rupture was not obvious on gross necropsy, as the uterus had already contracted down to a normal size, it was evident on histopathology.

This case can be considered a secondary ectopic pregnancy because the fetus was extruded from the uterus, most likely due to the integrity of the

uterus becoming compromised by the neoplasm, and there was no placental relationship with the peritoneal or omental surfaces (Corpa 2006). Most California sea lions are born on or around June 15 every year; therefore, this ectopic fetus was most likely in the abdomen for ~2 mo (The Marine Mammal Center pers. comm.). There has been a report of an abdominal ectopic fetus being in a dog for about a year, and there have been reports of long-existing abdominal fetuses in women which become calcified and are called lithopedions (Corpa 2006, Eddey 2012).

The sea lion in this case exhibited no symptoms until the disease had progressed to septic peritonitis. In domestic animals, clinical signs associated with an abdominal fetus are variable, and the only consistent clinical finding is an abdominal mass (Corpa 2006). In this case the clinical signs were most likely caused by the septic peritonitis rather than the abdominal ectopic pregnancy. The sea lion was kept in an exhibit that inhibited voluntary presentation for examinations by the veterinarian, thereby reducing the chances of catching a disease process early enough to treat. Had the sea lion's pregnancy been closely monitored via ultrasound, there would have been a chance of appreciating the initial abnormalities in the uterus and potentially either treating or retrieving the fetus and then treating the animal with chemotherapy. Abdominocentesis would have helped to diagnose the neoplasia and peritonitis prior to surgical intervention and could have led to treatment as well.

This was a unique case in that the fetus ruptured out of the uterus and into the peritoneum, thus spreading neoplastic cells to the peritoneal space. To the authors' knowledge there are no documented cases of this process in humans or animals. This case represents the first report of gestational choriocarcinoma causing secondary ectopic pregnancy in a California sea lion and helps increase the knowledge base of neoplasms in this species.

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