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Arcanobacterium phocae-associated Pathology in California Sea Lions Stranded Along the Southern California Coast

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Abstract

Arcanobacterium phocae can be isolated from mixed bacterial infections in a wide range of tissues. However, it is an under-reported pathogen in marine mammals. In the two cases reported here, pathology associated with several bacteria including *Arcanobacterium phocae* is described from two yearling California sea lions (*Zalophus californianus*) that were stranded along the southern California coast. The first case involved severe chronic pleuritis, secondary to a distal esophageal fishhook puncture. Such pathology is observed frequently in stranded California sea lions and thought to be the result of scrounging for food around 'fishing' wharfs. The second case involved a recrudescing cellulitis of the right foreflipper, thought to be the result of conspecific trauma.

Introduction

The Family *Actinomycetaceae* has been established on the basis of 16s ribosomal RNA gene signature nucleotides and includes the genera *Actinomyces, Arcanobacterium, Mobiluncus, Actinobaculum, and Varibaculum* (Stackebrandt et al., 1997). In the early 1980's, on the basis of phenetic, cell wall and lipid composition, as well as DNA analysis, *Corynebacterium haemolyticum* (MacLean et al., 1946) appeared as a taxon distinct from other coryneform and actinomycete species, supporting reclassification as *Arcanobacterium haemolyticum* in the new Genus *Arcanobacterium* (Collins et al., 1982). The Genus *Arcanobacterium* presently contains eight species with standing: *Arcanobacterium haemolyticum* (Collins et al., 1982), *Arcanobacterium bernardiae* (Ramos et al., 1982), *Arcanobacterium pyogenes* (Ramos et al., 1997), *Arcanobacterium hippocoleae (*Hoyles et al., 2002), *Arcanobacterium pluranimalium* (Lawson et al., 2001), *Arcanobacterium bialowiezense* (Lehnen et al., 2006), *Arcanobacterium bonsai* (Lehnen et al., 2006) and *Arcanobacterium phocae* (Ramos et al., 1997). *Arcanobacterium sp.* may be recovered from the respiratory and digestive tracts, abscesses, systemic infections, and other infections of healthy and diseased humans and animals (Funke et al., 1997; Mackenzie et al., 1995; Skov et al., 1998; Ramos et al., 1997).

In 1997, Ramos et al. conducted a phylogenetic analysis of the Genus *Actinomyces,* based on 16s rRNA gene sequencing and proposed the species *Arcanobacterium phocae* for bacteria of "uncertain pathological significance" isolated in 1994 from mixed cultures from the mouth, intestines, submaxillary and mesenteric lymph nodes, nasal sinuses, lungs and peritoneal fluid of Harbor seals (*Phocae vitulina*) and Gray seals (*Halichoerus grypus*) in Scottish coastal waters. *Arcanobacterium phocae* was characterized as a non-motile, non-spore-forming, non-acid resistant, gram positive cocco-bacillus, phylogenetically most closely related to *Arcanobacterium haemolyticum* Schaal et al., 2006).

In the first report (Johnson et al., 2003) of *Arcanobacterium phocae* from Pacific Ocean dwelling marine mammals, 141 isolates were made from 66 California sea lions (*Zalophus californianus*), 50 northern elephant seals (*Mirounga angustirostris*), 19 Harbor seals (*Phoca vitulina*), 5 sea otters (*Enhydra lutris*), and one Common dolphin (*Delphinus delphis*). All subjects were stranded along the central coast of California between 1994 and 2000. Isolates were obtained from sites of "abnormal discharge or evidence of inflammation", frequently in mixed cultures and confirmed by partial 16s rRNA gene sequence analysis. It was noted that *Arcanobacterium phocae* may have been under-reported between 1994 and 1998, as some *Arcanobacterium phocae* isolates may have been misidentified as *Listeria ivanovii,* because some tests used to differentiate between *Arcanobacterium phocae* and *Listeria ivanovii* were not conducted.

This was partly verified by a retrospective analysis of several *Listeria ivanovii* isolates that were obtained in 1998. Retesting showed that these were actually *Archanobacterium phocae* (Johnson et al., 2003). In 2006, a report was published detailing bacterial isolations from two wounds and an umbilical infection in Harbor seals (*Phoca vitulina*) along the coast of the state of Washington between 1992 and 2003 (Lockwood, et. al., 2006).

In a review of *Archanobacterium* infections of terrestrial mammals, *Archanobacterium pyogenes* was noted to be a facultative anaerobe and the causative agent of sporadic cases of suppurative infections in wild and captive ungulates, including cerebral abscesses in deer and a variety of infections in wild and captive deer, moose, pronghorn antelope and wild sheep. In summary, *Archanobacterium pyogenes* was noted to induce localized purulent abscesses 'almost anywhere in the body', but not uncommonly as diffuse inflammatory lesions within body cavities, joints or tendon sheaths (Wobeser, 2001).

Case Reports

California sea lion, Z-08-05-14-040, "DOA #2"

On May 14, 2008, a yearling, male California sea lion (*Zalophus californianus*) was found dead at Bolsa Chica State Beach, California (33° X 42' X 38.17" N and 118° X 02' X 50.17" W). The animal measured 1.04 m in length and weighed 24 kg, minimum age and weight metrics for yearling California sea lions. Approximately 30 cm of mono-filament fishing line was protruding from the mouth. The animal was impounded and transported to Pacific Marine Mammal Center (PMMC) for postmortem examination.

Postmortem blood samples were collected for CBC and serum chemistry evaluations (IDEXX Laboratories, Irvine, California). Blood analyses revealed relative polycythemia, elevated ALT, CK, and creatinine and moderate electrolyte imbalance, indicative of tissue damage and dehydration. Postmortem examination revealed a "fresh" carcass with no external lesions. Internally, there was marked depletion of visceral and subcutaneous adipose stores. A monofilament fishing line protruding from the mouth was followed distally into the esophagus, to a point ~5 cm anterior to the thoracic inlet, where it was found attached to a 25 mm single-barbed fishhook. The fishhook had penetrated the dorsal esophageal wall just past the level of its barb, which was associated with a ~5 cm area of moderate to marked caseating fasciitis and myositis involving the ventral aspect of the *longus colli* muscle and its fascia. The fasciitis continued distally along fascial planes into the thorax (Figures 1 and 2).

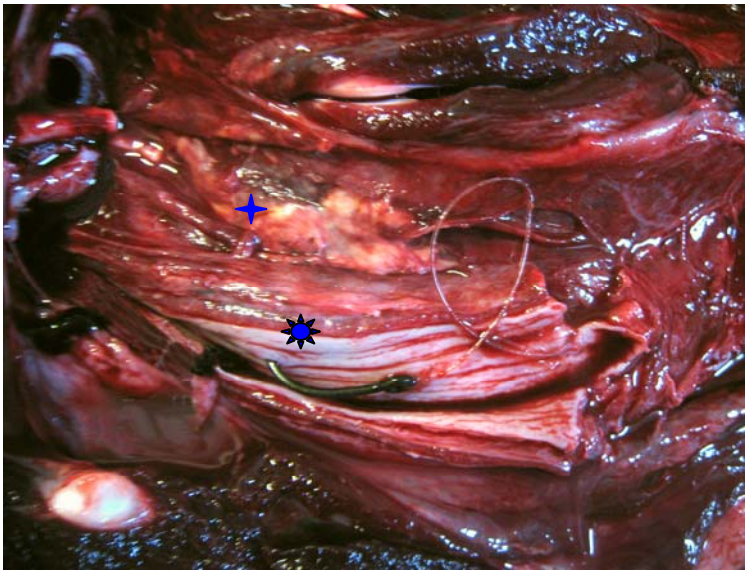

Figure 1. California sea lion, Z-08-05-14-040, "DOA #2", Esophageal Fishhook Wound
In the distal cervical esophagus, a 25 mm, barbed, half-curved fishhook (✹) has punctured through the dorsolateral aspect of the esophagus, embedding in the *longus colli* muscle and resulting in extensive moderate caseating fasciitis and myositis (✦). Note the very close proximity of this lesion to the thoracic inlet, i.e. on a plane with the transected first rib at the left bottom of the photo.

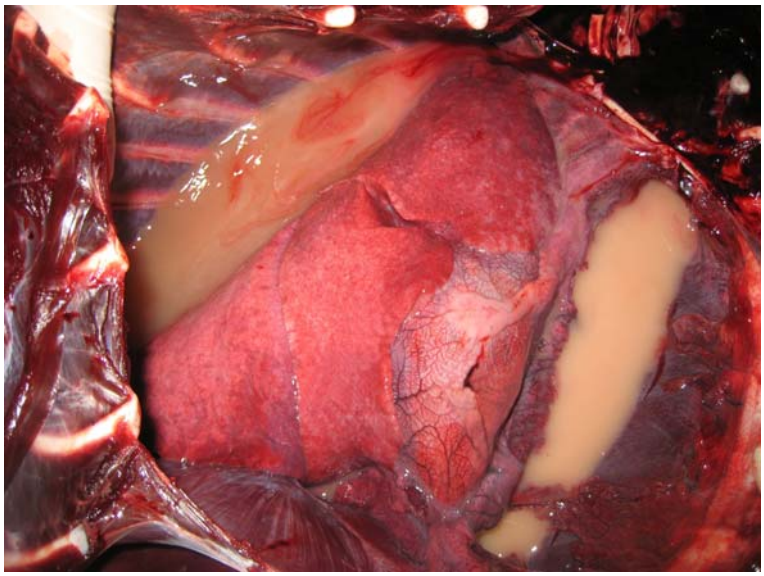

Figure 2. California sea lion, Z-08-05-14-040, "DOA #2", Bacterial Pleuritis
The thorax is filled with thick, cream-colored exudate. The lungs exhibit multifocal areas of moderate to marked consolidation and congestion in their ventral portions. The mediastinum and pericardium are opaque from marked hyperemia and edema and very prominent vasculature.

Both pleural cavities were filled with copious amounts (~ 640 ml) of a thick opaque tan-colored fluid (Figure 2). The visceral and parietal pleurae exhibited only mild reduction in translucence, but contained scattered multifocal areas of mild to occasionally moderate congestion and hyperemia (Figure 2). The pericardium was diffusely thickened and opaque (white) with an accentuated vascular pattern. The pericardial sac contained a moderate amount (~35 ml) of clear yellow fluid. The extra-pericardial mediastinum was mild-moderately opaque with an accentuated vascular pattern. The mediastinal and tracheobronchiolar lymph nodes were enlarged with visible cortical, follicular, lymphoid hyperplasia. The ventral aspects of the lungs, especially the caudal lobes, exhibited mild to moderate, multifocal consolidation and congestion. A section of the right ventricle, three pericardial samples, and a dorsal section of the right middle lung lobe, were taken for histological examination

Wright's Giemsa-stained (Volu-Sol, Inc., Salt Lake City, Utah, USA) smears of the pleural fluid (Figure 3) revealed cellular detritus, proliferating bacterial cocci, bacilli, and coccobacilli, moderate numbers of red blood cells, and marked leukocytosis (~60% polymorphonuclear and 40% mononuclear) in a basophilic matrix. Polymorphonuclear cells commonly exhibited degenerative morphology that included nuclear swelling, vacuolation, and sometimes karyorrhexis and karyolysis. The mononuclear cells were predominantly active macrophages, with small numbers of lymphoid and plasma cells. Macrophages usually were filled with vacuoles, detritus, and moderate to marked numbers of the bacterial morphotypes.

Histologic examination was conducted on sections of the left ventricle with associated pericardium and the right middle lung lobe. No significant lesions were noted in the left ventricle. The pericardium exhibited moderate to severe cuboidal epithelial hypertrophy, severe interstitial edema, vascular congestion and hyperemia, and moderate patchy infiltration by large mononuclear cells with vacuolated cytoplasm (macrophages) (Figure 4). Occasionally, macro-phages were found transiting the pleural epithelium. In the lungs, there was widespread postmortem congestion, a few moderate-sized foci of alveolar emphysema, and multifocal alveolar expansion with metastrongylid nematodes having morphometrics consistent with *Parafilaroides decorus.* Moderate to severe atelectasis prevented a complete appreciation of the distribution of inflammatory cells within the lung parenchyma. However, the pleura exhibited cuboidal epithelial hypertrophy, moderate edema, and infiltration by small numbers of lymphocytes, plasma cells, and macrophages.

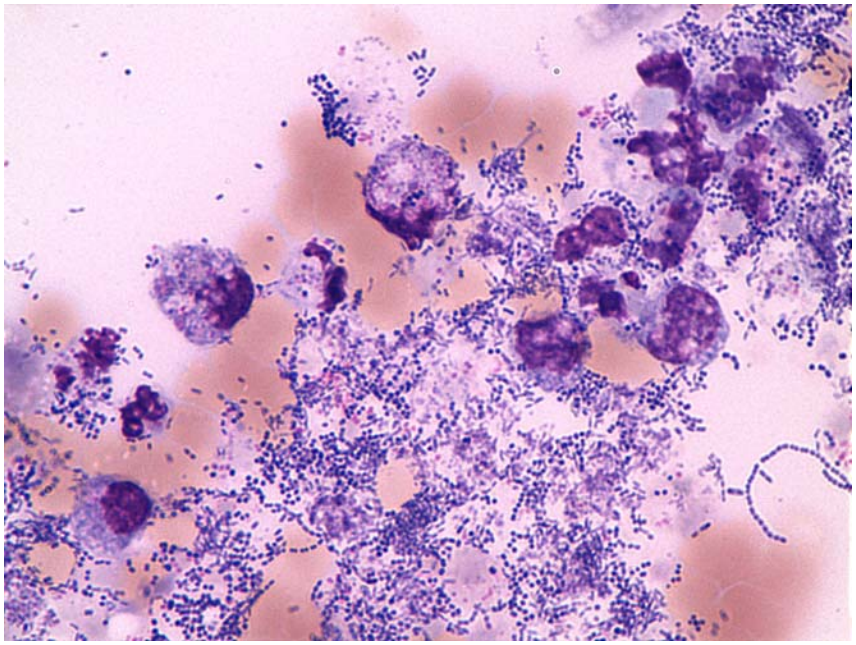

Figure 3. California sea lion, Z-08-05-14-040, "DOA #2", **Pleural Exudate Cytology (Wright's Giemsa, 10x).**
The exudate is chronic-active and highly cellular with approximately 60% polymorphonuclear, 40% mononuclear leukocytes, and multiple clumps of red blood cells. Most cells are undergoing degenerative changes. Intracellular bacteria are commonly seen in active macrophages and neutrophils and are of several different morphotypes. Additionally, tremendous numbers of coccal and bacillary bacterial morphotypes are found free in a dense proteinaceous matrix .

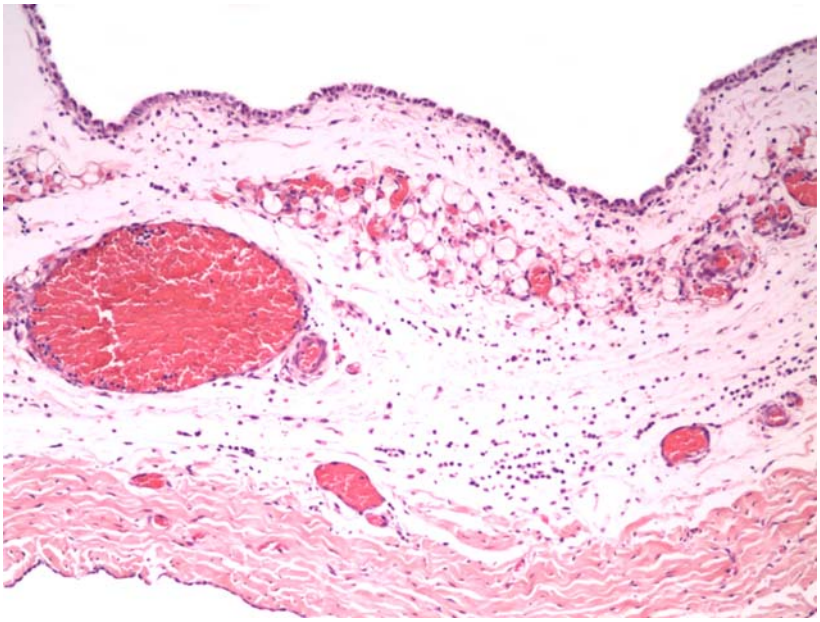

Figure 4. California sea lion, Z-08-05-14-040, "DOA #2", **Pericardium (Hematoxylin & Eosin, 40x).**
There is severe, diffuse interstitial edema, vascular congestion, scattered infiltration by macrophages and lymphoid cells (mid-lower area), and cuboidal to columnar pleural epithelial hypertrophy.

Swabs of pleural fluid were taken aseptically and submitted for bacterial culture (IDEXX Laboratories, Irvine, California). *Arcanobacterium haemolyticum, Pseudomonas aeruginosa, Pseudomonas sp.*, and *ß-Hemolytic Streptococcus* were isolated from the pleural fluid. In light of the close relationship between *Arcanobacterium phocae and Arcanobacterium haemolyticum,* culture samples were sent to the University of California, School of Veterinary Medicine Microbiology Laboratory (Davis, California), which confirmed the organism to be *Archanobacterium phocae.*

California sea lion, Z-08-01-19-005, "Tatum"

"Tatum", a yearling, male California sea lion (*Zalophus californianus*) was stranded on the beach in Corona Del Mar, California, (33° X 35' X 34.88" N and 117° X 52' X 21.88" W) on June 17, 2008. The animal was impounded and transported to Pacific Marine Mammal Center. Examination at the Center revealed "Tatum" to be emaciated at 0.965 m in length and weighing 17 kg. Several fishhooks were embedded in the seal's mouth. Moderate lameness was associated with a ~2.5 cm round, ulcerated, cutaneous wound on the dorsal aspect of the right fore-flipper at the level of the metacarpophalangeal articulations (Figure 5a). With palpation, significant amounts of a thick, tenacious, gray fluid could be expressed from the subcutaneous area along the margins of the wound.

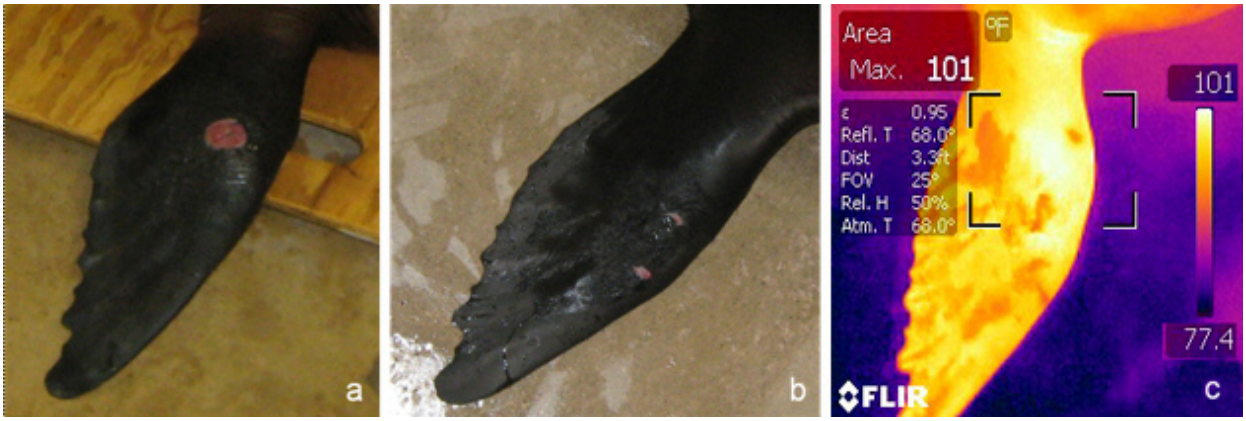

Figure 5. California sea lion, Z-08-01-19-005, "Tatum", Right Flipper Wound Healing Progression (see test for description).
a. Wound after initial cleaning and debridement,
b. Recurrence of infection 10-12 weeks later in relative same location as original lesion
c. FLIR photo of flipper revealing marked elevation in tissue temperature over area of original infection, highly suggestive of infection recurrence.

The fishhooks were removed from the mouth without complication. Because of "Tatum's" poor condition, wound treatment was undertaken following the judicious subcutaneous infiltration of Lidocaine (Med-Pharmex Inc., Pomona, California), rather than general anesthesia. A sterile, 18 gauge, Teflon IV catheter (Advantiv®, Medex Inc., Carlsbad, California) was used to probe the wound and collect exudate for bacterial culture and cytology. During this procedure, a moderate sized (~2 cm) exudate-filled area was found on the ventral aspect of the flipper, opposing the wound on the dorsum. The area was opened for drainage and a 0.25-inch drain (Kendall®, Tyco Healthcare Group, Mansfield, MA) was placed. Both ventral and dorsal wounds were debrided extensively to a "clean" granulation bed, surgically scrubbed (Technicare, Care-Tech®, Saint Louis, MO.), flushed with sterile saline (Water Pix®, Inc., Fort Collins, CO), disinfected (Clinical Care®, Care-Tech®, Saint Louis, MO) and covered with an anti-infective moisture barrier (Barre-Care®, Care-Tech®, Saint Louis, MO). Systemic antibiotic therapy was initiated with amoxicillin-clavulanic acid at 15mg/kg PO BID (Clavamox®, Pfizer, New York, New York) and amikacin at 6mg/kg BID IM (Amikacin C Injection, Phoenix Pharmaceuticals, St. Joseph, Missouri).

A Sample of the exudate was collected, placed in sterile transport media (Microorganism Collection & Transport System, Fisher*Finest*, Houston, Texas) and sent to IDEXX Laboratories for microbiologic culture and sensitivity. *Arcanobacterium phocae* was isolated in mixed culture with *ß-Hemolytic Streptococcus sp*. and Corynebacterium sp. and

found to be sensitive to amoxicillin-clavulanic acid, enrofloxacin (Baytril®, Bayer HealthCare, Shawnee Mission, Kansas) and Amikacin®. Cytologic examination of the exudate revealed large numbers of leukocytes, predominantly neutrophils (~90%) and small numbers (~10%) of macrophages and lymphocytes. Neutrophils invariably exhibited degenerative changes, characterized by nuclear swelling and vacuolation and occasionally contained bacilli (Figure 6). Additionally, occasional scattered, extracellular small foci of bacilli or varying-sized coccal chains were observed.

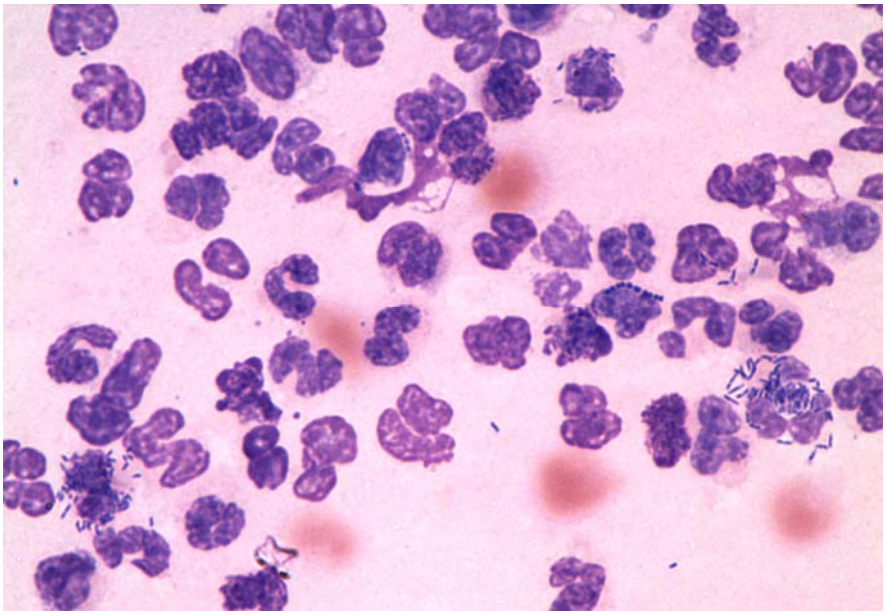

Figure 6. California sea lion, Z-08-01-19-005, "Tatum", Right Flipper Wound Exudate Cytology (Wright's Stain, 40x).
The exudate is highly cellular with ~90% polymorphonuclear and ~10% mononuclear leukocytes, indicating a chronic-active condition. The polymorphonuclear cells are undergoing degenerative changes and occasionally contain several bacillary morphotypes. Additionally, extracellular clusters of bacilli and varying-sized coccal chains are noted.

Each day the wound was flushed and cleaned under local anesthetic. When the wound failed to drain exudate, the drain was removed and warm hydrotherapy was initiated for 15 minutes every other day by hosing the wound intermittently with warm water under mild to moderate pressure. Progressive weight-bearing was noted on the injured flipper within 1-2 weeks and a month later, the wound had healed completely and the animal was walking and swimming normally. Since "Tatum" had lost considerable body weight in the form subcutaneous adipose stores, release was postponed to allow time to regain normal body condition.

Approximately 10-12 weeks later, weight-bearing lameness again was noted on the affected limb. A FLIR T200 infra-red camera (Forward Looking Infra-Red, FLIR Systems, USA Thermography Center, North Bellerica, MA, USA) was used to examined two small (~8-10 mm), open, draining wounds on the right flipper in the vicinity of the original wound (Figure 5b). The FLIR digital images (Figure 5c) clearly demonstrated marked temperature increases in these areas, suggesting a relapse of inflammation. Exudate was aspirated from the lesions under local anesthesia and examine cytologically. Again, the sample contained large numbers of leukocytes with a differential of ~85% polymorphonuclear cells and ~15% mononuclear cells, indicating a more subacute condition, consistent with recrudescence (Figure 7). The wound was flushed thoroughly and irrigated every other day for 1 week. Based on the work cited above on *Arcanobacterium phocae* in pacific marine mammals (Johnson et al., 2003), antibiotic therapy was initiated with enrofloxacin (Baytril®) at 3mg/kg PO BID for 10 days. Two weeks later, repeated physical, cytological, and FLIR imaging examinations over a 14-day period failed to reveal evidence of recrudescence. In January of 2009, "Tatum" was released back into the Pacific Ocean off Orange County, and to date he has been sighted several times.

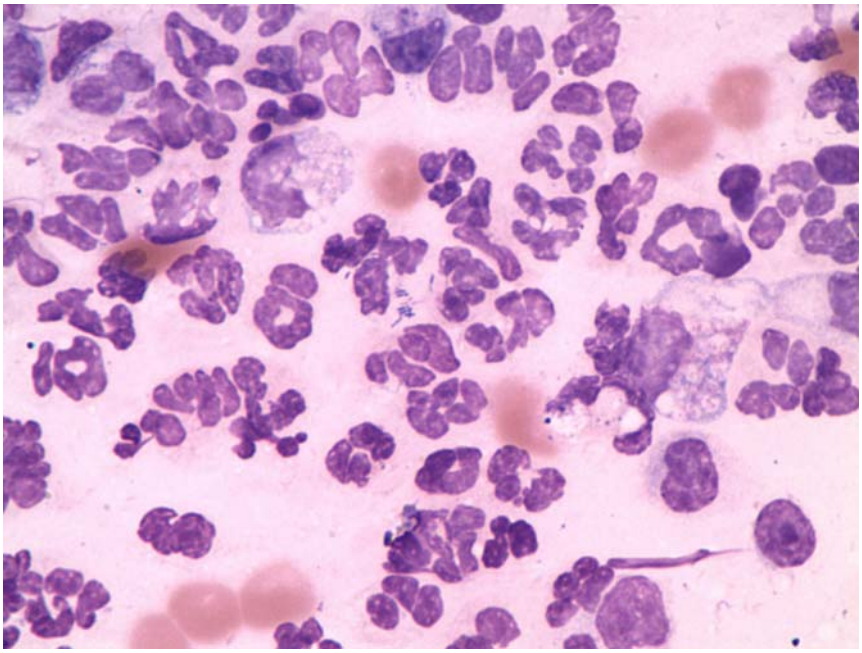

Figure 7. California sea lion, Z-08-01-19-005, "Tatum", Right Flipper Wound Recrudescence Cytology (Wright's Stain, 40x). The sample is highly cellular with a differential of ~85% polymorphonuclear cells and ~15% mononuclear cells. The former were neutrophils exhibiting at least a moderate degree of degenerative nuclear changes, while the mononuclear cells included monocytes, large active macrophages and lymphoid cells. Bacterial bacilli were rarely seen within neutrophils.

Discussion

This report graphically illustrates the severe, morbid, mediastinal and pleural pathology that a single small hook wound in the distal esophagus may cause. Injuries from fishing gear are an important problem for California marine wildlife, including pinnipeds, cetaceans, birds, and turtles (Stewart et al., 1989; Goldstein et al., 1999 and Dau et al., 2009). These injuries have been categorized as (1) entanglement with line only, (2) entanglement with line and associated hook injury, (3) hook injury only, or (4) ingestion (Dau et al., 2009). Ingestion may lead to perforation of the gastrointestinal tract and secondary peritonitis and/or pleuritis. Such injuries are especially common in pups and yearlings whose foraging habits are frequently less selective than mature animals, commonly leading to encounters with lost or discarded fishing gear (Dau et al., 2009). Similarly, we have noted that entanglement injuries are more frequent during certain ocean climatic fluctuations, such as El Niño events. By their nature, these climatic fluctuations can cause dramatic changes in prey distribution, which in turn result in altered "ranging" behaviors, thus increasing the opportunity for contact with "ocean trash".

Arcanobacterium phocae is a non-motile, non-spore-forming, non-acid resistant, gram-positive coccobacillus, displaying *β*-hemolysis on sheep blood. It is a facultative anaerobe with fermentative metabolism. Information on the natural habitat of *Arcanobacterium spp.* is scarce, although it is assumed that these organisms are present as part of the normal flora on the mucous membranes of terrestrial and marine mammals (including humans) and probably as opportunistic pathogens (Reddy et al. 1982 & Funke et. al., 1997). In humans, *Arcanobacterium haemolyticum* has been well documented as a 'distinct' cause of upper respiratory disease and "when specifically sought", was found in 0.5 – 2.5% of adolescent pharynghitis cases. The organism may produce lipid hydrolyzing enzymes, including a neuraminidase and phospholipase that are toxic and damaging to cellular membranes in both humans and animals (Linder, 1997 and Soucek, et. al., 1997). Specifically, the phospholipase was "found responsible for the dermonecrotic, as well as the synergistic hemolytic, activity…" (Soucek et. al., 1997).

Johnson et al., (2003) noted that *Arcanobacterium phocae* is under-reported as a pathogen in marine mammals; it is commonly present as part of mixed bacterial infections with *Escherichia coli* or *β-Hemolytic Streptococcus spp.* In both of the cases that we have described, *Archanobacterium phocae* was isolated together with *β-Hemolytic Streptococcus*. *Arcanobacterium phocae* and *β-Hemolytic Streptococcus* produce the biologically active toxin *β*-hemolysin. Thus, one might hypothesize a synergistic pathogenic effect similar to that reported between *Arcanobacterium haemolyticum* and other microorganisms (Dobinsky et al., 1999).

Since heat is one of the major signs of inflammation, infrared thermography can be used to detect and evaluate inflammatory processes. Infrared thermal imaging has recognized clinical applications that include evaluation of breast malignancy in humans, as well as cardiovascular and inflammatory disease and sports trauma in humans and animals. In this report, the use of a FLIR® (Forward Looking Infra-Red) camera, a non-invasive and non-contact diagnostic technique, was instrumental in recognizing the dermal inflammatory recrudescence in "Tatum" and monitoring resolution of the inflammatory process, without unnecessary restraint and/or sedation.

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