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Source: Journal of Wildlife Diseases, 42(1) : 40-45

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-42.1.40

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Journal of Wildlife Diseases, 42(1), 2006, pp. 40–45
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LESIONS ASSOCIATED WITH A NOVEL *MYCOPLASMA* SP. IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*) UNDERGOING REHABILITATION

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ABSTRACT: From July 1999 to November 2001, *Mycoplasma* sp. was cultured from lesions in 16 California sea lions (*Zalophus californianus*) undergoing rehabilitation. The *Mycoplasma* sp. was the likely cause of death of four animals in which it was associated with either pneumonia or polyarthritis. The most common lesion associated with this bacterium was subdermal abscessation, found in 12 animals. Other lesions included intramuscular abscesses, septic arthritis, and lymphadenopathy. Infection was associated with a leukocytosis and left shift in 12 animals. Animals with abscesses improved clinically after surgical lancing, irrigation, and systemic antibiotic therapy. The mycoplasma isolates had a consistent 16S rRNA sequence dissimilar from other *Mycoplasma* spp. and represent a novel species, *Mycoplasma zalophi* proposed sp. nov.

Key words: Abscess, California sea lion, marine mammal, mycoplasma, otariid, pinniped.

INTRODUCTION

Mycoplasmas are a diverse group of bacteria without a cell wall that infect a wide range of mammalian hosts worldwide (Whithear, 2001). Although recognized as pathogens of domestic livestock and laboratory animals, their role in wildlife disease is less well documented, probably due to the lack of readily available diagnostic tests for mycoplasmas and the difficulty in culturing the organisms, compounded by the difficulty in determining their pathogenicity in wildlife (Simecka et al., 1992; Razin et al., 1998; Johansson and Pettersson, 2002). Mycoplasmas are commonly isolated from mucosal surfaces of apparently healthy animals, yet are also associated with inflammation, especially of the respiratory tract and joints.

Lesions associated with mycoplasmas are increasingly recognized in a variety of wild host species (Ley et al., 1996; Grattarola et al., 1999). In pinnipeds, isolation of mycoplasmas is most common from phocid seals. The three species of mycoplasmas reported from phocids are *Mycoplasma phocicerebrale*, *Mycoplasma phocae*, and *Mycoplasma phocirhinis* (Königsson et al., 2001). Mycoplasmas were thought to have exacerbated pneumonia caused by an influenza virus in harbor seals (*Phoca vitulina*) (Geraci et al., 1982; Madoff et al., 1982; Ruhnke and Madoff, 1992) and to have secondarily invaded the lungs of seals that died during an epizootic attributed to morbillivirus (Giebel et al., 1991). Other pathogens were involved in both of these disease outbreaks and the role of mycoplasmas in the pathogenesis of the pneumonias is not clear. A single report of an unspeciated mycoplasma is associated with pneumonia in a California

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sea lion (*Zalophus californianus*) (Howard et al., 1983). However, mycoplasmas have not previously been considered important pathogens of marine mammals in general and otariids in particular (Higgins, 2000).

Mycoplasma spp. are the most likely causative agents of "seal finger," which is a potentially serious infection that may develop in people that have been bitten by pinnipeds or in people whose broken skin comes into contact with infected pinniped tissue (Stadtlander and Madoff, 1994; Baker et al., 1998). Culture of oral swabs taken from a variety of pinnipeds including California sea lions has demonstrated that *Mycoplasma* spp. are likely to be part of the normal oral flora of pinnipeds (Measures, pers. comm.). Mycoplasmas of pinniped origin, therefore, may be important zoonotic pathogens (Brown et al., 2005).

This study describes lesions associated with a novel *Mycoplasma* sp. in a wild otariid species, the California sea lion, undergoing rehabilitation.

MATERIALS AND METHODS

From July 1999 to November 2001, *Mycoplasma* sp. was cultured from lesions found in 16 (12 males; four females) California sea lions that were brought to a rehabilitation center (The Marine Mammal Center) in Sausalito, California, USA. The animals were originally found along the central California coast between 37°42′N, 123°05′W and 35°59′N, 121°59′W, and weighed between 19.5 and 174.5 kg.

Blood samples were collected from each animal for a complete blood count (CBC; Vet ABC® hematology analyzer, Heska Corporation, Fort Collins, Colorado, USA), a manual 200-cell differential count, and a clinical chemistry profile (AU5200®, Olympus America Inc., Melville, New York, USA) within 48 hr of admission. Blood was drawn from the caudal gluteal vein (Bossart et al., 2001) using 0.9 by 40 mm multiple sample blood collection needles (Monoject®, Sherwood Medical) directly into Vacutainers®. Animals requiring general anesthesia for diagnosis or treatment were anesthetized by using methods as described by Haulena and Gulland (2001).

Gross postmortem examination was performed on all animals that died and representative samples of tissues were preserved in 10% neutral buffered formalin. Fixed tissues were later embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histologic examination.

Samples for culture from live animals were collected aseptically by fine needle aspiration using 6 or 12 ml syringes and 1.2 by 40 mm needles (Monoject®, Sherwood Medical, St. Louis, Missouri, USA). Fluid samples from dead animals were swabbed directly and placed into transport media (Venturi Transystem [Patent Pending], Copan Diagnostics Inc., Corona, California, USA). Tissue samples from dead animals were cultured with aseptic technique. All samples were cultured on tryptic soy agar with 5% sheep blood, chocolate, and MacConkey agar plates (Hardy Diagnostics, Santa Maria, California, USA). The blood and chocolate plates were incubated at 35 C in 5% CO_2. The MacConkey plates were cultured in air at 35 C.

Mycoplasma sp. cultures were transferred overnight on chocolate agar slants (Hardy Diagnostics) to the University of Florida Mollicutes Collection Laboratory. SP4 broth and agar were used for identification and characterization of the mycoplasmas (Tully, 1995; Waites et al., 2004). Isolates were tested by growth inhibition assay (Clyde, 1983) against a standard battery of antisera to 80 of the 121 nonrare mycoplasma species (Garrity et al., 2004) and included antisera to all species isolated from any marine or freshwater animal and mycoplasmas closely related by 16S analysis. Polymerase chain reaction (PCR) was used to amplify a section of the 16S ribosomal RNA gene and the University of Florida Sequencing Core Laboratory did partial sequencing of this gene. The partial 16S rRNA gene sequence was determined as described in Brown et al. (2001) and matched to the small subunit rRNA sequences in the Ribosomal Database Project release 9.22 (Cole et al., 2003) by using SEQUENCE_MATCH version 2.7. The Ribosomal Database Project provides a database of 16S rRNA gene sequences and sequence analysis tools, including classification software that searches for nearest neighbors to a query sequence, and assigns query sequences to a taxonomical hierarchy.

RESULTS

A moderate to severe leukocytosis characterized by a neutrophilia with a left shift was found in 12 of 15 animals in which a CBC was performed. Lesions,

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TABLE 1. Summary of lesions, culture results, and disposition of California sea lions (*Zalophus californianus*) undergoing rehabilitation from which *Mycoplasma* sp. was cultured.

Identification	Sites or lesions cultured	Additional organisms cultured	Disposition
CSL 4296	left carpal joint	pure culture	released
CSL 4698	subdermal neck abscess, retropharyngeal abscess, and occipital condyles (joint fluid)	pure culture, but *Pseudomonas aeruginosa*, *Proteus vulgaris*, *E. coli*, *Klebsiella* sp. grown on postmortem culture of occipital condyles	died
CSL 4779	subdermal hip abscess	pure culture	died
CSL 4801	lung and pleural fluid	pure culture	died
CSL 4859	pleural fluid and subdermal axillary abscess	pure culture	died
CSL 4945	subdermal flipper and subdermal mandible abscess	pure culture	released
CSL 4949	subdermal neck abscess	pure culture	released
CSL 4956	subdermal neck abscess	pure culture	released
CSL 4957	subdermal flipper abscess, prescapular lymph node, and lung	unidentified Gram-negative rod	died
CSL 4998	subdermal hip abscess	unidentified Gram-negative rod	released
CSL 5025	subdermal hip abscess	*Gemella* sp., *E. coli*	euthanized
CSL 5026	axillary muscle abscess	unidentified Gram-negative rod	died
CSL 5027	sublumbar lymph node effaced by carcinoma	pure culture	died
CSL 5059	subdermal neck abscess	pure culture	released
CSL 5070	subdermal neck abscess	unidentified Gram-negative rod	released
CSL 5152	subdermal hip abscess	pure culture	released

culture results, and animal disposition are summarized in Table 1. Eight of the sea lions were eventually released and the remaining eight either died or were euthanized during treatment. Mean (±SD) length of rehabilitation for released animals was 48 (±16) days. *Mycoplasma* sp. was thought to have contributed to the death of four of eight animals that died. It was associated with severe pleuritis and necrotizing pneumonia in three animals and septic polyarthritis in one animal. In the animal with septic polyarthritis, *Mycoplasma* sp. was grown as a pure culture from a cervical subdermal abscess and a retropharyngeal abscess antemortem and grown on postmortem culture of the atlanto-occipital joint along with *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, and *Klebsiella* sp. In these four animals, *Mycoplasma* sp. was thought to have been the primary cause of stranding. Of the four animals that died of causes unrelated to their *Mycoplasma* sp. infection, two animals died of carcinoma as described by Gulland et al. (1996), one animal died of peritonitis resulting from a perforating duodenal ulcer, and one animal died of a severe pleuritis from which *Streptococcus viridans* and *E. coli* were cultured. *Mycoplasma* sp. was cultured from subdermal abscesses from three of these animals and from a sublumbar lymph node effaced by carcinoma in one sea lion. In summary, *Mycoplasma* sp. was cultured from subdermal abscesses in 12 animals, pleural fluid or lung tissue in three animals, arthritic joints in two animals, lymph nodes in two animals, and one muscle abscess.

A localized arthritis from which *Mycoplasma* sp. was cultured in one animal

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resulted in a non-weight-bearing lameness of the affected limb. The carpal joint was moderately swollen and warm to the touch. Joint aspiration revealed increased synovial fluid that was serosanguinous and slightly cloudy and had decreased viscosity. Radiographs showed marked osteolysis of the joint surfaces of most of the carpal bones and severe periosteal reaction surrounding the carpal joint. The sea lion was treated with three through-and-through joint flushes performed at 1-wk intervals with sterile 0.9% saline solution (Baxter Healthcare Corporation, Deerfield, Illinois, USA) while under general anesthesia. The sea lion was also given oral erythromycin stearate (Mylan Pharmaceuticals Inc., Morgantown, West Virginia, USA) at a dosage of approximately 15 mg/kg per os twice a day for 25 days and oral buffered aspirin (Bufferin®, Bristol-Myers Squibb Co., New York, New York, USA) at a dosage of approximately 5 mg/kg per os twice a day for 5 days. Clinical improvement was noted after approximately 15 days of treatment and the animal was eventually released.

Abscesses were treated by surgical lancing and irrigation with chlorhexidine solution (Vet Solutions, Inc., Fort Worth, Texas, USA) under general anesthesia. In addition, Penrose drains (C. R. Bard, Inc., Cranston, Rhode Island, USA) were surgically inserted into deep tissue abscesses or those that were thought to be too large for adequate drainage to be accomplished by lancing alone. Sea lions with abscesses were given oral doxycycline hyclate (Mutual Pharmaceutical Co., Inc., Philadelphia, Pennsylvania, USA) at a dosage of approximately 10–15 mg/kg per os twice a day for 10 days. Clinical improvement was usually seen approximately 5 days after therapy was initiated.

The mycoplasma was cultured on blood and chocolate agars and appeared as small, pinpoint colonies embedded in the agar approximately 3–6 days after swabs were plated. In general, most mycoplasmas are extremely fastidious in their culture requirements and very few grow on blood or chocolate agars (Tully, 1983). However, a pure culture of *Mycoplasma* sp. was obtained from 11 of the animals. An unidentified Gram-negative bacillus was cultured with the *Mycoplasma* sp. in four samples from abscesses. The significance of this organism is unknown. A single animal had *E. coli* and *Gemella* sp. cultured in addition to the *Mycoplasma* sp. from a subdermal abscess over the hip.

The *Mycoplasma* sp. fermented glucose and grew within 24 hr on SP4 mycoplasma medium incubated in ambient air at 37 C. On SP4 agar, the colonies ranged in size from barely visible to approximately 2 mm in diameter. These morphologic subgroups were tested independently in subsequent tests. The growth inhibition testing was negative for all 80 mycoplasma species tested, strongly suggesting that the organism was a new species. This mycoplasma is thus provisionally named *Mycoplasma zalophi* (sp. nov.). The partial 16S rRNA gene sequence of *M. zalophi* (GenBank accession number AF493543) was compared with other mycoplasmas. The best match (score=0.779) was to *Mycoplasma gypis*, positioning *M. zalophi* in the *Mycoplasma hominis* phylogenetic clade (Johansson and Pettersson, 2002). A Kimura 2-parameter distance matrix (Cole et al., 2003) was then calculated to identify the closely elated type species within that clade (*M. gypis*, *Mycoplasma anseris*, *Mycoplasma spumans*, *Mycoplasma falconis*, *Mycoplasma faucium*, and *Mycoplasma subdolum* of the *M. hominis* subcluster [matrix similarity scores=0.953, 0.928, 0.928, 0.926, 0.919, and 0.917, respectively] and *Mycoplasma auris*, *Mycoplasma arginini*, and *Mycoplasma gatae* of the *Mycoplasma alkalescens* subcluster [matrix similarity scores=0.925, 0.920, and 0.920, respectively]).

DISCUSSION

This is the first report of lesions, including abscessation and arthritis, associated

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with *Mycoplasma* sp. in an otariid species. The organism cultured represents a novel mycoplasma. It is likely that this organism in California sea lions is newly recognized due to improved diagnostic techniques rather than being newly introduced to this species.

The association of superficial abscesses in these animals suggests a transdermal route of introduction for the organism. The culture of *Mycoplasma* sp. from the oral cavity of other sea lions undergoing rehabilitation at The Marine Mammal Center suggests that transmission may occur by conspecific bites. It is also possible that *Mycoplasma* sp. is an opportunist found on the skin that may be introduced through a break in the epithelial barrier. The association of this new mycoplasma species with lesions that were likely to have contributed to the death of several animals, and isolations from pleural fluid, lung tissue, arthritic joints, muscle abscesses, and lymph nodes indicate that the infection has a blood phase in which the organism spreads systemically, at least in some animals. This is consistent with mycoplasmal diseases of other animals and humans and highlights the importance of this organism as a potential pathogen in California sea lions. Furthermore, this new mycoplasma may be of zoonotic significance as are other mycoplasmas of pinniped origin. Although associated with lesions in 16 animals, the tissue distribution and significance of mycoplasmas in healthy California sea lions is unknown. Further studies are required to characterize the epizootiology and pathogenesis of this organism.

Animals responded well clinically to surgical lancing, flushing, and insertion of drains to treat abscesses and to through-and-through sterile saline joint flushes to treat septic arthritis. Clinical improvement was noted following treatment with doxycycline and erythromycin.

Successful treatment of bacterial infections depends on correct identification of the pathogen and use of the appropriate antibiotic therapy. However, mycoplasma colonies may be easily missed on routine bacterial culture plates because of their small size and fastidious nature, and because it may take longer for mycoplasma colonies to grow than for some other bacteria. It is important, therefore, that clinicians be aware of their pathogenic potential in California sea lions.

ACKNOWLEDGMENTS

The authors wish to thank the veterinary technicians and husbandry staff of The Marine Mammal Center including D. Wickham, L. Phoenix, T. Padilla, and D. Greig for their constant assistance and advice. The care of stranded marine mammals and the opportunity for research would not be possible without the many dedicated volunteers at The Marine Mammal Center. Veterinary medicine and research at The Marine Mammal Center are supported by the Page Evans Veterinary Fellowship and the Arthur and Elena Court Nature Watch Conservancy and by the John H. Prescott Grant Program of the National Marine Fisheries Service.

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Received for publication 12 December 2004.

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