



***Klebsiella rhinoscleromatis*-associated Pleuritis and Pneumonia in a California Sea Lion (*Zalophus californianus*)**

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Abstract

Klebsiella rhinoscleromatis is a gram-negative coccobacillus in the Family Enterobacteriaceae. It is commonly found in the environment and is considered to be part of the normal flora of the skin, respiratory and gastrointestinal tracts of humans and animals. This case report describes *Klebsiella rhinoscleromatis* associated pneumonia and pleuritis in a California sea lion (*Zalophus californianus*) and compares it with 'rhinoscleroma', a *Klebsiella rhinoscleromatis* associated upper respiratory granulomatous disease in humans from lower socioeconomic areas of Africa, Europe, Asia, and Central and South America.

Introduction

Klebsiella rhinoscleromatis is a gram-negative, non-motile, intracellular coccobacillus belonging to the Family Enterobacteriaceae (Chen and Spiele 2007, Brisse et al 2006 and Sedano et al 1996). It is still uncertain whether *Klebsiella rhinoscleromatis* is a subspecies of *Klebsiella pneumoniae* or a separate species in its own right (Brisse et al 2006, Sedano et al 1996). All species of *Klebsiella* are found in the environment and frequently are part of the normal flora of the skin, respiratory and gastrointestinal tracts of humans and animals (Vincent 2004, Brisse et al 2006).

Klebsiella spp. infections in humans are rare in the United States but are endemic in lower socioeconomic areas of Africa, Eastern Europe, Southern Asia, Central and South America and China, where they frequently are responsible for a 'rhinoscleroma' or localized, chronic, granulomatous inflammatory disease of the respiratory tract (Kim et al 2003, Chan & Spiele 2007, Vincent 2004). Their mucopolysaccharide capsule protects them from opsonization and phagocytosis (Kim et al 2003, Gaafar et al 2002, Vincent 2004), and therefore *Klebsiella spp.* are thought to evade immune surveillance.

Clinical History

On June 13th, 2008, "Jasmine", a yearling, female California sea lion (*Zalophus californianus*) stranded at Seal Beach, California (33°43'71" X 118°05'19"), USA. A rescue team from Pacific Marine Mammal Center (PMMC) was dispatched and impounded "Jasmine" because of a moderate-sized, right jaw lesion, obvious wasting and marked lethargy.

"Jasmine" (Z-08-06-013-053) was transported to PPMC where a physical examination was conducted. She was found to be moderately underweight (40 lbs.) with a ruptured, 3cm, cutaneous, apparent abscess on the right submandibular area and a bilateral, 4-5cm, fluid-filled subcutaneous mass in the anterior cervical area. The masses were opened and drained under local anesthesia and a 7-day course of Clavamox® was prescribed. Over the next 10 days "Jasmine" made an uneventful recovery.

On July 7th, 2008, "Jasmine" exhibited abdominal-press breathing. Auscultation revealed markedly diminished pulmonary parenchymal sounds. Pneumonia and/or pleuritis were suspected and antibiotics were reinstated, but within six hours, she became apneic and died shortly afterward. Shortly after death, blood was taken for a CBC and serum chemistries, and a necropsy was performed.

The carcass was depleted of subcutaneous and visceral adipose stores. The pleural cavity was filled with large amounts of thick, opaque, foul-smelling exudate (Figure 1) and there was marked, bilateral atelectasis. The pericardium and visceral pleura were markedly thickened and opaque with a diffuse fine-granular texture. In the right, lateral, diaphragmatic lung lobe, a 2 x 3cm, irregular-shaped, tan-colored, granulomatous-appearing lesion, was noted in juxtaposition to a side-by-side, healed fracture of the distal portion of right rib #9 (Figure 2), suggesting conspecific trauma as an etiology. The cranial mediastinal and tracheobronchiolar lymph nodes were moderately enlarged.

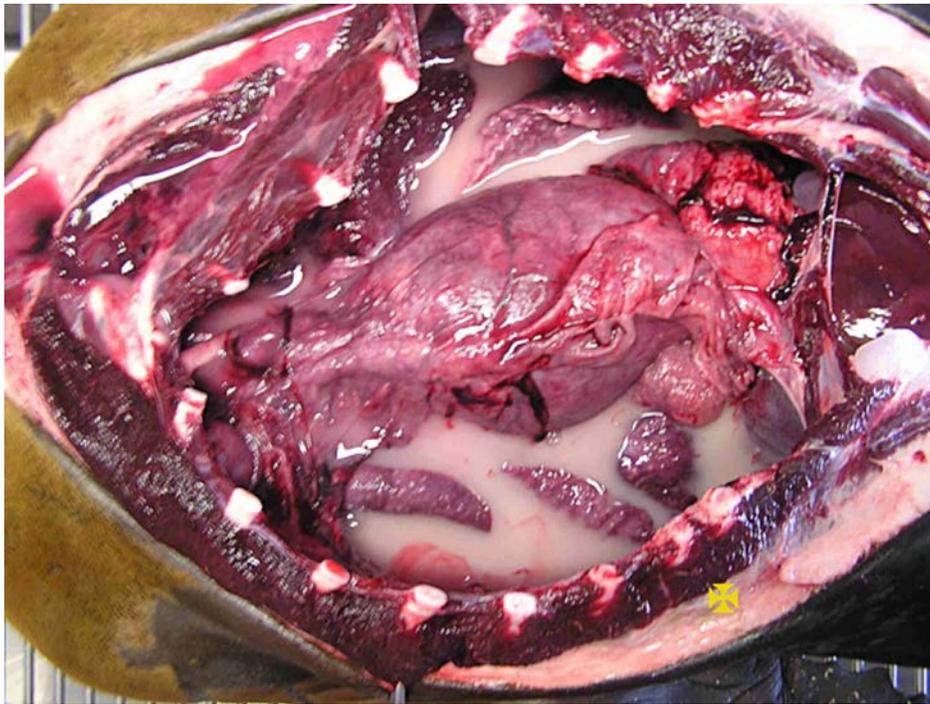


Figure 1. Severe Bilateral Pleural Effusion

The pleural cavity is filled with large amounts of a foul-smelling, thick opaque exudate and the lungs are atelectatic. The pericardium and visceral pleura are markedly thickened and opaque with a diffuse fine-granular texture. Note the side to side, healed fracture of the distal portion of right rib #9 (yellow Maltese Cross).

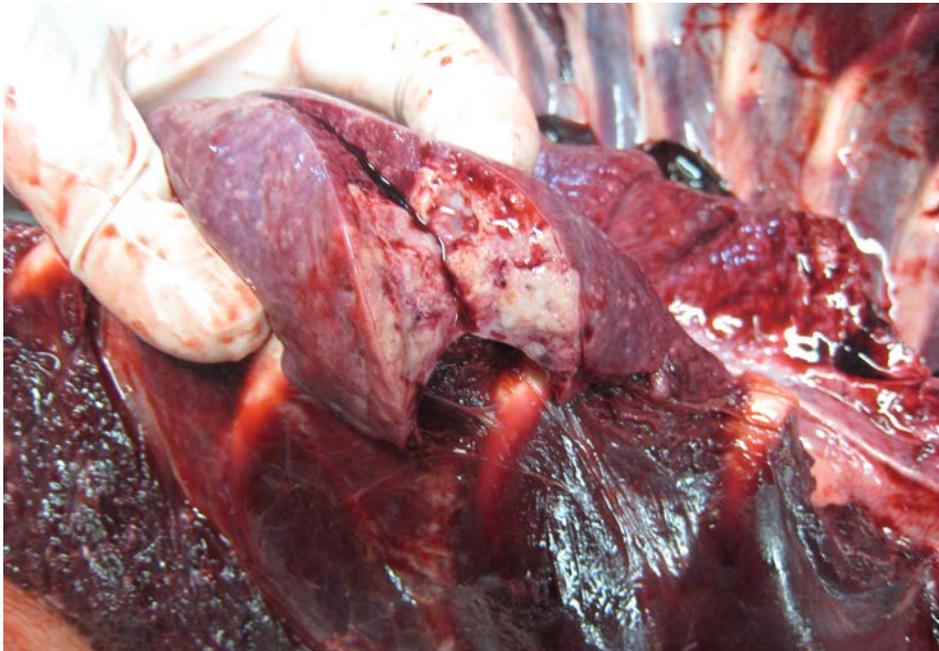
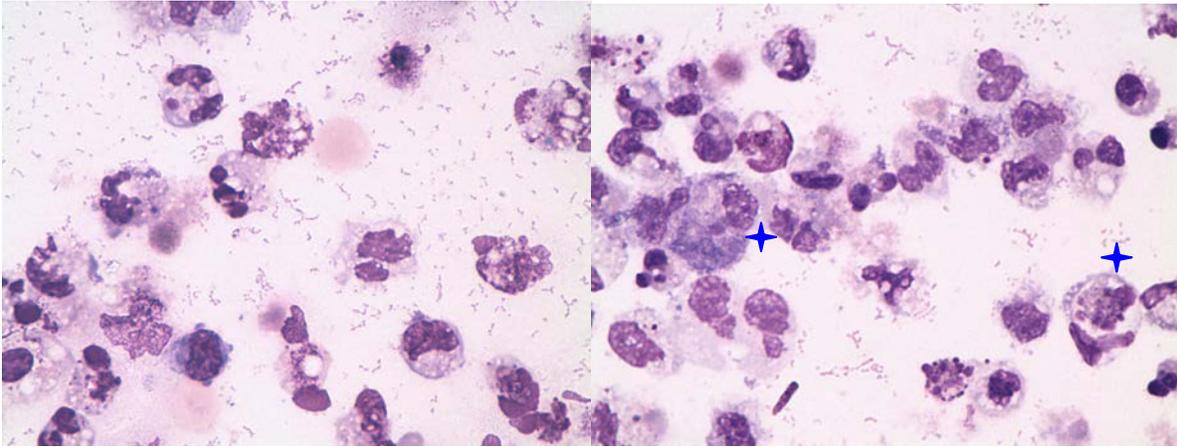


Figure 2b. Focal Granulomatous Pneumonia

A 2 x 3cm, irregular-shaped, tan, granulomatous-appearing lesion was noted in the right, lateral, diaphragmatic lung lobe, juxtaponed to a side to side, healed fracture of the distal portion of right rib #9.

The CBC was within normal limits. Serum chemistry irregularities included mild hyperphosphatemia (10.2), marked hyperkalemia (9), mild elevation of creatinine phosphokinase (CK) (556) and moderate hypoalbuminemia (2.2).

A sample of pleural exudate was taken for cytologic examination (Figure 3 and 4). The sample was highly cellular with a differential of 70% neutrophils and 30% mononuclear cells, that were predominantly active macrophages with appreciable numbers of lymphocytes and plasma cells. The cells exhibited at least a mild degree of degenerative changes such as anisokaryorrhexis, pyknosis and cytoplasmic vacuolization.



Figures 3 & 4. Pleural Exudate Cytology (Wright's Stain , 40x)

Slides are highly cellular with a differential of 70% neutrophils and 30% mononuclear cells. Most cells are undergoing at least mild to moderate degenerative changes. Macrophages frequently contain necrotic cellular debris or even intact leukocytes (★). The background is moderately proteinaceous with small 'tangles' of precipitate.

Mononuclear phagocytes were occasionally found to have engulfed cellular remnants as well as relatively intact cells (Figure 4). Clusters of epitheloid, mononuclear phagocytes were scattered within the exudate. The latter had eccentrically placed, round to ovoid nuclei with fine-stippled chromatin, conspicuous nucleoli and cytoplasm "bulging" with very large numbers of bacilli (Figure 5). Plasma cells and reactive lymphocytes, without degenerative changes were also present in small numbers.

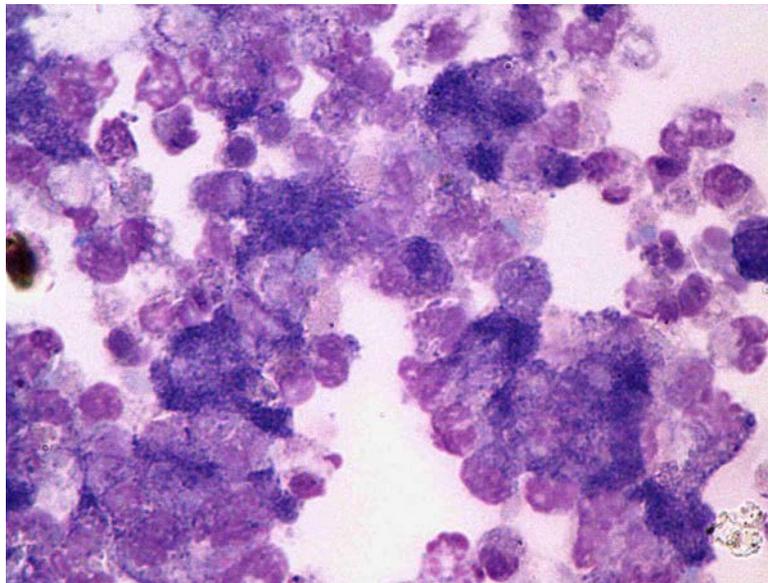


Figure 5. Pleural Exudate Cytology (Wright's Giemsa Stain, 40x)

Note mononuclear cells in center pack with 'wispy' bacilli

Klebsiella rhinoscleromatis, *Acinetobacter sp.* and few *Enterobacter sp.*, were isolated from samples of the pleural exudate and granulomatous appearing lung lesions.

Tissue samples were collected in 10% neutral-buffered formalin for histopathologic evaluation, from the spleen, tracheobronchiolar and cervical lymph nodes, kidney, liver, lung (area adjacent to the fractured ribs), and pleura. No significant lesions were noted in the kidney and liver. In the lymph nodes, a characteristic reactive immunologic response was noted and included moderate, follicular proliferation with a “starry-sky” pattern from the presence of large dendritic cells, common mitotic figures and active macrophages containing cellular detritus.

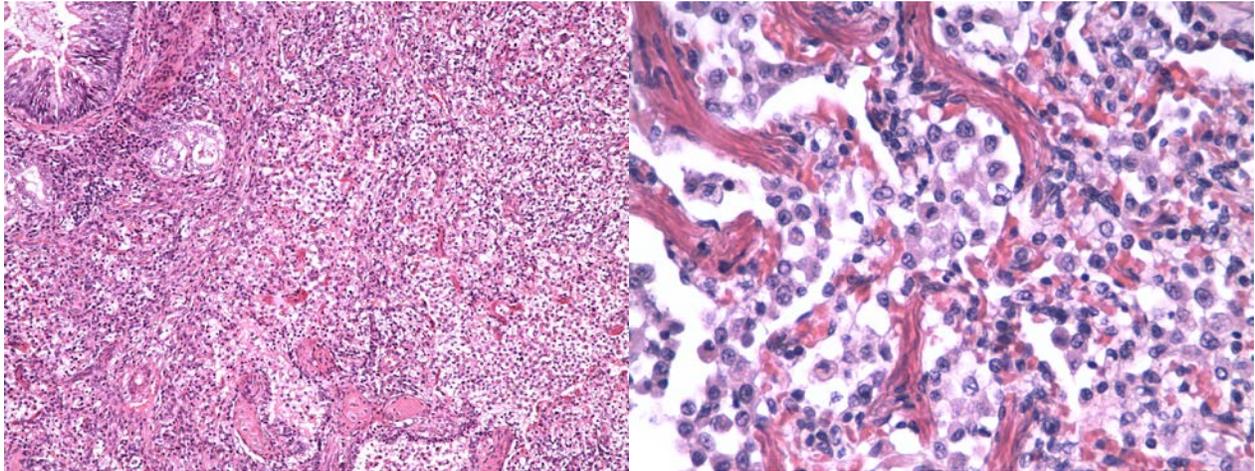


Figure 6 and 7. Right Diaphragmatic Lung Lobe (see text for description) (Hematoxylin & Eosin, 10x and 40x).

In the right diaphragmatic lung lesion, there was a generalized, marked alveolar-interstitial infiltration of leukocytes, resulting in the effacement of normal alveolar and interstitial spaces. Infiltrates were ~90% mononuclear and 10% polymorphonuclear cells (predominantly neutrophils) (Figure 6). Among the mononuclear population, ~90% contained large, ovoid to irregularly shaped, central to concentrically placed, nuclei with lacy, pale basophilic cytoplasm consistent with epithelioid mononuclear cells. The remaining ~10% are characterized by round, dark, marginalized nuclei and very abundant deeply basophilic cytoplasm consistent with plasma cells (Figure 7). Moderate numbers of *Parafilaroides decorus* nematodes were found multifocally disseminated within alveoli and occasionally bronchioles. The visceral pleura and pericardium were markedly thickened by moderate to marked interstitial edema, neo-vascularization and infiltration of mononuclear phagocytes, lymphocytes and occasionally neutrophils (Figure 8).

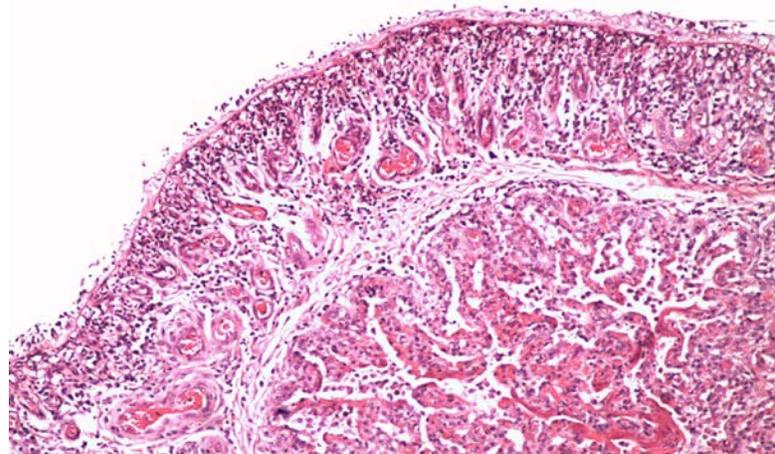


Figure 8. Visceral Pleura (see text for description) (Hematoxylin & Eosin, 10x).

Discussion

Klebsiella rhinoscleromatis-associated rhinoscleroma usually occurs in the oral cavity, nasal passages, larynx, trachea, and bronchi of humans in rural areas of developing countries in Africa, South America, Central and Eastern Europe, Mexico, Korea and China (Vincent 2004, Hill and March 1990 and Chan and Spiele 2007). Sporadic cases have been reported in the United States principally among immigrants from these areas. To date, humans are the only known natural host for *Klebsiella rhinoscleromatis* (Vincent 2004, Chan and Spiele 2007). This report appears to be the first naturally occurring case in a nonhuman mammal. The exact mechanism and pathophysiology of infection by *Klebsiella rhinoscleromatis* is poorly understood, although recent reports speculate that it begins with the organism invading the epithelium of the respiratory tract².

Gaafar et al (2003) conducted *Klebsiella rhinoscleromatis* pathogenicity studies in rats. Following intravenous injection of an inoculum of *Klebsiella rhinoscleromatis*, the bacterium was recovered only from the nose, larynx, and lungs. Immunohistochemistry and special stains confirmed the presence of intracellular *Klebsiella rhinoscleromatis* in tissues of the respiratory tract. Additionally, to investigate the possibility of an oral route of infection, five rats were inoculated intragastrically with *Klebsiella rhinoscleromatis*. Bacterial culture and histologic analysis of nasal and laryngeal tissue, lung, liver, kidney, spleen, and brain, failed to reveal evidence of *Klebsiella rhinoscleromatis* infection, suggesting 'per os' to be an unlikely route of infection.

Transmission has been thought to occur following prolonged exposure to infectious respiratory secretions, through inhalation, or by direct inoculation (Gaafar et al 2003). It is suspected that bacterial cell wall mucopolysaccharides enable the bacteria to be resist ingestion and killing by macrophages (Chan and Spiele 2007). However, in this case the gross-pathology and histology of the 'scleroma-like lesion' very closely resembles those described in rhinoscleroma in humans (Kim et al 2003, Chan and Spiele 2007).

Following infection, the formation of a 'rhinoscleroma' is thought to begin with replication of *Klebsiella pneumonia* in rhinal sub mucosal tissues, which results in inflammation characterized by capillary proliferation, polymorphonuclear cell infiltration into the subepithelial spaces and phagocytosis of the bacteria (Kim et al 2003, Chan and Spiele 2007). However, *Klebsiella* resist destruction by the polymorphonuclear cell phagosome, the polymorphonuclear cell dies and macrophages phagocytize the ingested bacteria and the polymorphonuclear cell remains. The ingested *Klebsiella*, cause the phagosome of the macrophage "to undergo massive dilation" forming the characteristic Mikulicz Cell, which eventually ruptures and releases bacteria into the surrounding tissues, continuing the pathogenic cycle and enlarging the 'rhinoscleroma' (Kim et al 2003, Chan and Spiele 2007, Gaafar et al 2000). In the case reported here, both gross and histological 'rhinoscleroma-like' lesions can be clearly identified (Figures 2-8).

Rhinoscleromas tend to occur in areas where epithelial pathology results in clinical signs that vary in severity and include nasal obstruction, rhinorrhea, epistaxis, dysphagia, nasal deformities, or dysphonia. Diagnosis and treatment can be difficult, but include surgical debridement, surgical removal, and long term antibiotic treatment. Immediate treatment with flouroquinolones or rifampin has been shown to help, but the infection has a high reoccurrence rate (Kim et al 2003, Chan and Spiele 2007, Sedano et al 1996).

Acknowledgments

I would like to thank the staff and especially Richard H. Evans, DVM, MS, Medical Director and Dennis Lawler, DVM Research Associate of Pacific Marine Mammal Center, for their help in completing this paper.

* This paper was in partial fulfillment of a 4th year veterinary student preceptorship (Western University School of Veterinary Medicine, Pomona, California) at Pacific Marine Mammal Center.

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