



Aerobic bacterial flora of the vagina and prepuce of California sea lions (*Zalophus californianus*) and investigation of associations with urogenital carcinoma

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Received 20 November 2004; received in revised form 3 November 2005; accepted 4 November 2005

Abstract

To investigate the association between genital bacterial infection and urogenital carcinoma in California sea lions (*Zalophus californianus*), vaginal and preputial swabs for bacterial isolation were taken from 148 free-ranging and 51 stranded California sea lions including 16 animals with urogenital carcinoma. Cytological examination of vaginal or preputial smears showed a majority (65.5%, 57/87) of animals examined had mild or no inflammation. Aerobic bacteria were isolated from 116 (78.4%) wild sea lions and 100% of stranded animals. A total of 403 isolates were identified representing 51 unique bacterial species. The median number of isolates per animal increased with age in the wild group, but there was no difference in the number of isolates per animal between wild and stranded adults. The most common bacteria isolated from the wild sea lions were *Psychrobacter phenylpyruvicus* (39 isolates), non-hemolytic *Streptococcus* (35 isolates), *Corynebacterium* spp. (30 isolates), and *Escherichia coli* (20 isolates). More bacterial species were isolated from stranded animals than wild animals (33 versus 26) and there was significantly less growth of *P. phenylpyruvicus*, *Corynebacterium* spp., and *Moraxella*-like spp. in the stranded animals. Beta-hemolytic *Streptococcus* was the only bacterium significantly associated with urogenital carcinomas in California sea lions, but only in females.

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Keywords: Sea lion; Bacteria; Microbiology; *Streptococcus phocae*; Urogenital carcinoma

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1. Introduction

A high prevalence of urogenital carcinomas has been observed in California sea lions (*Zalophus californianus*) (Gulland et al., 1996). Although the etiology of these carcinomas is unknown, a herpesvirus infection has been detected in all cases examined (Lipscomb et al., 2000; King et al., 2002, Lowenstine, unpublished data). As the etiology of many carcinomas is multifactorial, urogenital bacterial infections and associated inflammation may play a role in the development of these aggressive metastatic carcinomas in California sea lions. In humans, epidemiologic studies of cervical carcinomas suggest that human papillomavirus (HPV) in conjunction with *Chlamydia trachomatis* (Tamim et al., 2002) and other vaginal bacteria are primary agents in the development of these tumors (Guijon et al., 1985). Bacterial infections appear to serve as cofactors in tumor development by reducing local immunity and inducing inflammation thus rendering vaginal epithelial cells more susceptible to other agents (Guijon et al., 1992). To determine the significance of bacterial infections in urogenital carcinogenesis in California sea lions, the normal microbial flora of the urogenital tract must be identified, taking into account variations in the host and environment. However, little is known about the normal urogenital flora of California sea lions or the presence of infectious and sexually transmitted diseases in the species. Johnston and Fung (1971) cultured the vulva and prepuce of only seven wild caught California sea lions isolating *Escherichia coli*, *Aerobacter* spp., *Staphylococcus aureus*, and *Streptococcus fecalis*, all of which are considered potential pathogens. Reproductive disorders such as abortion and neonatal death have been associated with *Leptospira pomona* infection on San Miguel Island, CA (Smith et al., 1974). *Brucella* antibodies are present in marine mammals of the Pacific Ocean (Nielsen et al., 2001) and the organism has been isolated from an adult female California sea lion in rehabilitation (Gulland, personal communication); however, its significance remains unclear. Studies of Steller sea lions (*Eumetopias jubatus*), a species closely related to the California sea lion with overlapping habitat, revealed 49% of animals tested had serologic evidence of *Chlamydia* exposure (Calkins and Goodwin, 1988) and *Chlamydia* (*Chlamydia*) *psittaci* was cultured from an aborted fetus (Bradley et al., 1994). Unfortunately, no

studies have adequately characterized the normal urogenital bacterial flora of different age classes California sea lions.

Without knowledge of the endemic microbial flora of free-ranging animals, understanding the role of bacteria in marine mammal strandings, reproductive disorders, and morbidity and mortality is difficult. In an attempt to understand the pathogenesis of urogenital carcinomas in California sea lions, this study aimed to characterize vaginal and preputial aerobic bacterial flora in different age classes of wild and stranded California sea lions and investigate associations among vaginal and preputial bacterial flora, inflammation, and urogenital carcinoma in this species.

2. Materials and methods

2.1. Study populations

Vaginal and preputial swab specimens were obtained from two groups of California sea lions from January 2001 to March 2003. The first group consisted of apparently healthy free-ranging adult females (>4 years), juveniles (1–3 years) and pups (<1 year) captured during the spring and fall of both years from rookeries on San Miguel Island, CA, and adult males (>4 years) inhabiting Puget Sound, WA in May 2001 and June 2002. The second (stranded) group consisted of debilitated adult California sea lions that stranded along the central and northern California coast due to acute diseases such as trauma and neurotoxin (domoic acid) intoxication and were taken to a rehabilitation center (The Marine Mammal Center, Sausalito, CA). Vaginal and preputial swabs for bacterial culture were obtained from the live or fresh dead (<12 h since death) stranded animals that had not been administered antibiotics prior to sampling. The presence of urogenital carcinoma was diagnosed post mortem in stranded animals by gross and histological examination of tissues.

2.2. Specimen collection

Swabs for bacterial culture were obtained by either the use of Accu-culshure™ guarded collection device (Accu-Med, Pleasantville, NY), a double-guarded swab (Jorgensen Laboratories, Loveland, CO) with

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