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BACTERIAL AND FUNGAL FLORA OF WILD NORTHERN FUR SEALS *(CALLORHINUS URSINUS)*

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Abstract: Tissues from healthy subadult and moribund newborn northern fur seals *(Callorhinus ursinus)* on St. Paul Island, Pribilof Islands, Alaska, and from healthy pups and yearlings on San Miguel Island, California, were sampled for bacteria and fungi. *Corynebacterium* spp. and *Staphylococcus* spp. were more frequently present in tissues from animals on St. Paul Island whereas *Pseudomonas* spp. were frequently isolated on San Miguel Island. Approximately half of the blood samples were positive for bacteria. *Salmonella* spp. were isolated from rectal swabs of animals only on San Miguel Island. Fungi were isolated from the hair and skin of subadult males.

INTRODUCTION

Bacterial and mycotic diseases have been proposed as major causes of death in marine mammals (Medway, 1980). The recognizable pathogens that have received the most attention are *Erysipelothrix rhusiopathiae* (Geraci et al., 1966; Gilmartin et al., 1971), *Leptospira interrogans* var. *pomona* (Smith et al., 1974; Vedros et al., 1971), *Clostridium* spp. (Greenwood and Taylor, 1978), *Pseudomonas pseudomallei* (Dodin and Galimand, 1978), *Pasturella multocida* (Keyes et al., 1968), *Salmonella* spp. (Gilmartin et al., 1971), and *Candida albicans* (Ridgway, 1979). Several other pathogens have been isolated from marine mammals (Medway, 1980), particularly those larger parasites responsible for parasitic pneumonia (Schroeder et al., 1973). The majority of pathogens noted above were isolated from captive animals. Our knowledge of infectious agents in marine mammals in the wild relies mainly on sampling occasional trapped animals, stranded animals, or animals held for tagging.

Base line data on the microbial flora of marine mammals in the wild are important if we are to understand the role of infectious diseases in the survival of these animals, the potential zoonotic implications for man, and implement effective husbandry.

The present report describes the bacterial and fungal flora of 131 northern fur seals *(Callorhinus ursinus)* randomly selected during the fur seal harvest on St. Paul, Pribilof Islands, Alaska, during the years 1972-1979, and during the tagging operation on San Miguel Island, California, in 1977.

MATERIALS AND METHODS

Tissues sampled. The number of animals and kinds of tissues and anatomical sites sampled are shown in Table 1. Immediately after the adult animal was killed by a blow on the head, blood was obtained with a 50 ml sterile syringe and 20 gauge needle from a cutdown of the carotid artery. Blood from moribund pups was obtained by heart puncture. Blood from yearlings being tagged on San Miguel Island was collected by syringe from the hind flipper after the skin was first cleaned with 70% ethyl alcohol. In males, the external surface of the kidney, liver, spleen, gallbladder, prostate, testicle, trachea, and colon were first seared with a hot spatula before incision with a sterile scalpel and insertion of sterile cotton swabs. Similar procedures were followed with the

TABLE 1. Tissues of northern fur seals cultured for bacteria and/or fungi.

Animals	Tissues sampled	No. sampled
Males	Kidney	74
(2-4 yr)	Liver	61
	Spleen	15
	Gallbladder	46
	Prostate	6
	Testicles	6
	Rectum	32
	Colon	74
	Trachea	25
	Nose	13
	Oropharynx	23
	Blood	52
	Skin	15
Females	Vagina	5
(3-5 yr)	Ovaries	5
	Fallopian tubes	5
Pups	Blood	15
(1-3 wk)	Liver	5
	Kidney	5
	Oropharynx	5
	Rectum	8
Yearlings	Blood	5
(Approx. 1 yr)	Oropharynx	5
	Rectum	26

ovaries and fallopian tubes of females. Other anatomical sites were sampled directly with sterile cotton swabs.

Isolation of bacteria/fungi. All specimens were cultured within 1 hr after collection on non-selective Trypticase Soy Agar medium containing 3% defibrinated fur seal blood (TSBA)[1] (on St. Paul Island) or 3% defibrinated sheep blood (on San Miguel Island). The cultures were incubated for a minimum of 7 days at 35-37 C (5% CO_2). Brain Heart Infusion Agar (BHI)[2] containing 3% defibrinated fur seal blood was also tested as a non-selective medium on St. Paul Island, Alaska.

Selected specimens also were cultured as follows: blood was used to inoculate biphasic Tryptose Agar Casteneda bottles and *Brucella* agar;[2] rectal swabs were cultured on Xylose-Lysine-Desoxycholate Agar (XLD) and MacConkey Agar;[2] hair and skin samples were cultured on Sabouraud's Dextrose Agar (SAB)[2] and Sparrow's marine fungi medium (Johnson and Sparrow, 1961). All cultures were incubated as noted above except for *Brucella* agar cultures which were observed for a minimum of 20 days.

Swabs of the colon for anaerobic culture were immediately placed in pre-reduced fluid thioglycollate broth for transport to the laboratory. An inoculum from the thioglycollate broth was cultured on TSBA under a blanket of Argon gas using the VPI anaerobic culture system inoculators.[3] The cultures were incubated at 35-37 C in a Gas-Pak system with holding jar[1] for 5-7 days.

[1] Baltimore Biological Laboratories, Cockeyville, Maryland 21030, USA.

[2] DIFCO, Detroit, Michigan 48232, USA.

[3] Bellco Glass Inc., Vineland, New Jersey 08360, USA.

Identification of bacteria/fungi. Aerobic bacteria were identified as described by Cowan and Steel (1974), with supplemental standard techniques (Lennette et al., 1980). Gram negative isolates were further characterized using the API-20E.[4] Anaerobes were identified according to the methods of VPI (Holdeman et al., 1972) and detection of volatile and non-volatile fatty acids by gas chromatography.[5] (Larsson et al., 1978). Fungi were identified by morphological criteria (Bessey, 1950).

RESULTS

Isolation media. In the initial phase of this study attempts were made to determine the most suitable non-selective medium for optimum, primary isolation of bacteria. Both TSBA and BHI were reconstituted with 3× distilled water or artificial sea water (Lyman and Fleming, 1940) and supplemented with either 3% defibrinated fur seal blood or sheep blood. The cultures were incubated at 35-37 C with and without 5% CO_2. There were no statistically significant differences as determined by Chi square tests among the number of dissimilar colonies observed under any of the test conditions. Occasionally a particular isolate, e.g., *Staphylococcus aureus*, would be recovered on TSBA reconstituted with distilled water but not with sea water. Similarly, an occasional isolate, e.g. *Moraxella* sp., would be recovered on BHI containing 3% defibrinated sheep blood but not 3% defibrinated seal blood. Examination of the data from all the field trips indicated however, that all bacteria could be isolated on either of the two test media and that 5% CO_2 enhanced growth. Because of the difficulty of obtaining fresh seal blood on San Miguel Island, California, it was decided to use TSBA containing 3% defibrinated sheep blood as the non-selective medium for primary isolation.

No recognizable marine fungi were isolated on Sparrow's medium from the hair or skin of the animals but other common fungi could readily be isolated on SAB.

Microbial Profile.

The aerobic bacterial isolates from various tissues of male and female fur seals (ages 2-5 yr) are shown in Table 2. The majority of animals had only a single bacterial species in their blood and few in number (avg. 4-5 colonies per 0.2 ml blood). However, in one animal (male) approx. 10^2-10^3 *Staphylococcus aureus* per ml/blood was noted. Bacteria were isolated from 12% of the kidneys (9/74) and 10% of livers (6/61). No gross lesions were visible in any of these organs. No isolates were recovered from the vagina, ovaries, and fallopian tubes of females or the prostate gland and testicles of the males. In the gallbladder of the male one strain of *Escherichia coli* was recovered from each of three animals.

The lack of bacterial isolations from the small intestines prompted us to examine the hookworms *(Uncinaria lucasi)* in that area of the gut. Two pups were heavily infected with hookworms. Approximately 25 of the parasites were removed from each animal and thoroughly washed (4×) in minimal essential tissue culture medium (MEM) containing penicillin (200 units/ml), streptomycin (50μg/ml) and fungizone (1 μg/ml). This MEM was being used in a concurrent virology project with gut specimens of the same animal. Bacterial growth was inhibited in the tissue culture preparations. When the hookworms were macerated, *E. coli* could be recovered from each preparation.

Although many pups were sampled that had died the night before in the

[4] Analytab Products, Plainview, New York 11893, USA.

[5] CAPPO Inc., Sunnyvale, California 94065, USA.

TABLE 2. Aerobic bacterial isolates from northern fur seals on St. Paul Island, Alaska.

Tissue examined	No. of animals examined	No. of animals positive	Isolates	No. of animals positive
Blood	52	29	*Moraxella* sp.	1
			Streptococcus sp.	8
			Lactobacillus subgenus *streptobacterium*	3
			Klebsiella sp.	2
			Aerococcus sp.	1
			Staphylococcus aureus	6
			Escherichia coli	4
			Corynebacterium sp.	4
			Proteus vulgaris	2
Kidney	75	9	*Micrococcus* sp.	2
			Staphylococcus epidermidis	3
			Corynebacterium sp.	3
			Bacillus circulans	1
			Neisseria caviae	1
			Alcaligines-Pseudomonas gp.	1
			Staphylococcus aureus	2
			Enterobacter sp.	1
Liver	61	6	*Staphylococcus epidermidis*	1
			Acinetobacter sp.	1
			Corynebacterium sp.	2
			Micrococcus sp.	2
Spleen	15	6	*Corynebacterium* sp.	3
			Citrobacter freundii	1
			Enterobacter sakazaki	1
			Lactobacillus sp.	1
			Proteus vulgaris	1
			Moraxella sp.	1
			Streptococcus sp.	1
Oropharynx	23	18	*Moraxella* sp.	2
			Streptococcus sp.	3
			Neisseria cuniculi	14
			Escherichia coli	3
			Proteus mirabilis	2
			Listeria sp.	1
			Staphylococcus epidermidis	2
			Corynebacterium sp.	1
			Bacillus sp.	2
Rectum	32	32	*Pseudomonas* sp.	3
			Escherichia coli	23
			Escherichia coli (Alkalescens-Dispar gp.)	1
			Enterobacter hafnia	2
			Acinetobacter calcoaceticus	1
			Alcaligines faecalis	1
			Corynebacterium sp.	4
			Enterobacter sp.	1
			Klebsiella sp.	3
				2

TABLE 2. (continued)

Tissue examined	No. of animals examined	No. of animals positive	Isolates	No. of animals positive
Rectum (continued)			*Actinobacillus* sp.	2
			Staphylococcus epidermidis	3
			Moraxella sp.	4
			Streptococcus sp.	1
			Pseudomonas fluorescens	3
			Aeromas punctata	2
Nose	1	13	*Corynebacterium* sp.	5
			Corynebacterium bovis	1
			Moraxella sp.	6
			Escherichia coli	4
Colon	14	14	*Staphylococcus epidermidis*	10
			Aerococcus sp.	4
			Corynebacterium sp.	2
			Escherichia coli	14
			Enterobacter sp.	4
			Streptococcus sp.	5
			Enterobacter cloacae	1
			Acinetobacter calcoaceticus var. *lwoffi*	2

TABLE 3. Aerobic bacterial isolates from northern fur seal pups on St. Paul Island, Alaska.

Tissue examined	No. of animals examined	No. of animals positive	Isolates	No. animals positive
Blood	10	9	Streptococcus sp.	5
			Straphylococcus aureus	3
			Corynebacterium sp.	4
			Proteus sp.	1
			Proteus vulgaris	1
Liver	5	2	*Micrococcus* sp.	2
Kidney	5	3	*Micrococcus* sp.	1
			Corynebacterium sp.	1
			Bacillus circulans	1
Rectum	3	3	*Escherichia coli*	1
			Alcaligines sp.	1
			Alcaligines fecalis	1
			Acinetobacter calcoaceticus	1
			Moraxella sp.	1

rookeries, only a few were available as moribund. The isolates from 13 of these animals are shown in Table 3. The numbers of animals are too few to compare with adults, but similar genera of bacteria were observed in the pups as in adults. Because of other studies in progress it was not possible to sample all tissues from every animal. The entire intestinal tract from four animals was

removed, sectioned every 200 cm for histology, and sampled for bacteria. No bacteria were isolated in any portion of the intestine up to the ileocecal junction. In the large intestines, the isolates were similar to those obtained from rectal samples (Table 2).

Samples of the colon of 74 fur seals were examined for bacterial anaerobes. The 49 isolates recovered are listed in Table 4. A variety of *Clostridium* species and other anaerobes are represented including 17 *Clostridium* species which could not be speciated by the techniques used in this study. The two potential pathogens, *C. novyi* (two isolates) and *C. perfringens* (one isolate), were isolated from one animal.

Only the oropharynx, rectum, and blood could be sampled from tagged pups and yearlings on San Miguel Island, California. Only gram negative bacteria and *Staphylococcus aureus* isolates were identified as noted in Table 5. The *Pseudomonas* species appeared to be the most common bacteria isolated. Three species of *Salmonella* were isolated; *S. heidelberg, S. newport,* and *S. adelaide.* These known human pathogens were not isolated from animals on St. Paul Island, Alaska (Tables 2, 3).

Fungi were isolated from the hair and skin of northern fur seals both on St. Paul Island, Alaska, and San Miguel Island, California. The genera represented were *Trichophyton* sp., *Acremonium* sp., *Penicillium* sp., *Cephalosporium* sp., *Scopulariopsis* sp., *Trichoderma* sp., and *Streptomyces* sp.

DISCUSSION

Conclusions concerning the significance of any microbial isolate involves understanding complex interactions between the host and environment. *Acinetobacter* sp., *Alcaligines* sp., *Pseudomonas* sp., *Staphylococcus aureus, Bifidobacterium* sp., and *Clostridium* sp. are only a few of the bacteria which have been implicated as causes of clinical disease in humans and other animals, particularly when the hosts have been compromised. Many of the isolates reported in this study can be found in soil and water and may be transient inhabitants of anatomical sites sampled. However, as noted by Rosebury (1961), the persistent finding of numerous microorganisms in areas that normally are sterile or transiently inhabited is as reliable a marker as can be

TABLE 4. Obligate anaerobe isolates from the large intestines of 74 northern fur seals.

Isolates	No. of animals positive
Clostridium felsineum	1
Clostridium mangenotii	1
Clostridium scatologenes	1
Clostridium novyi	2
Clostridium paraputrificum	2
Clostridium sordellii	3
Clostridium perfringens	1
Clostridium spp.	17
Bacteroides hypermegas	2
Bacteroides sp.	5
Propionibacterium sp.	2
Propionibacterium acnes	1
Eubacterium sp.	3
Eubacterium lentum	2
Fusobacterium sp.	3
Bifidobacterium sp.	1

TABLE 5. Aerobic bacterial isolates from northern fur seals on San Miguel Island, California.[a]

Animals examined	Number of animals sampled	Isolates	Number of animals positive
PUPS			
Oropharynx	5		
		Moraxella sp.	5
		Alcaligenes sp.	5
		Pseudomonas stutzeri	1
		Staphylococcus aureus	2
		Proteus sp.	5
		Pseudomonas sp.	5
Blood	5		
		Pseudomonas stutzeri	5
		Pseudomonas paucimobilis	5
		Alcaligenes sp.	1
		Staphylococcus aureus	1
Rectum	5		
		Acinetobacter calcoaceticus var. *lwoffi*	3
		Salmonella adelaide	1
		Pseudomonas putrefaciens	3
		Escherichia coli	3
		Proteus rettgeri	1
		Salmonella heidelberg	3
ADULTS			
Oropharynx	5		
		Staphylococcus aureus	4
		Pseudomonas maltiphilia	1
		Proteus sp.	5
		Pseudomonas stutzeri	3
		Pseudomonas putrifaciens	5
Blood	5		
		Acinetobacter calcoaceticus var. *lwoffi*	1
		Pseudomonas putrifaciens	1
		Staphylococcus aureus	1
Rectum	26		
		Proteus sp.	20
		Staphylococcus aureus	16
		Salmonella newport	5
		Acinetobacter calcoaceticus var. *lwoffi*	15
		Alcaligenes sp.	10
		Pseudomonas sp.	10
		Pseudomonas stutzeri	3
		Pseudomonas putrifaciens	3
		Pseudomonas paucimobilis	1

[a]Only Gram negative and *S. aureus* isolates identified.

found for the operational distinction between health and disease.

The isolation of bacteria from blood must be interpreted with caution. Sampling of the blood and organs of the portal system within 1 min of killing would probably rule out growth of opportunists. Positive blood cultures may have resulted from trauma during the killing of the animals and this was evident in the very few organisms isolated per sample and the occasional positive cultures

from kidney, liver, and spleen. Similar findings have been made by others. Rand (1975) isolated *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas aeruginosa*, and beta-hemolytic streptococci from a Galapagos sea lion *(Zalophus californianus wollebaeki)*. The animal was killed by a rifle shot and blood collected spurting from a wound in the left parietal region of the head. Possible contamination of the cultures by other tissues and difficulties in proper handling of the specimens was emphasized by the author. A more recent study (Adeduji and Boness, 1981) in which blood was collected from 20 grey seals *(Halichoerus grypus)* by heart puncture, 90% of the blood samples were positive for gram negative bacteria. Four similar animals in captivity also yielded positive blood cultures.

A wide variety of genera were represented among isolates from animals on St. Paul Island, Alaska (Tables 2-4). *Corynebacterium* spp. and *Staphylococcus* spp. were most frequently isolated. Of particular interest was the isolation of *Neisseria caviae* and *Neisseria cuniculi*. The latter has only been found as part of the normal flora in the oropharynx of rabbits (Berger, 1962). Although the young adult males (2-4 yr) sampled in this study have had a pelagic existence since birth to the time of killing, pinnipeds have close contact with land. The isolation of such bacteria as *N. cuniculi* may indicate a broader host range for these saprophytes as recently noted for *N. canis* (Hoke and Vedros, 1982). The manner in which these saprophytes become established in the normal flora of marine mammals requires further study.

The known human pathogens *Salmonella heidelberg*, *S. newport*, and *S. adelaide*, were isolated from animals on San Miguel Island, California, similar to the findings of others (Gilmartin et al., 1979). No *Salmonella* spp. were isolated from animals on St. Paul Island, Alaska. Enrichment techniques were not used at either location, but selective media (e.g. XLD) was satisfactory for primary isolation of *Salmonella* from rectal swabs of animals on San Miguel Island, California. The transmission vehicle for these *Salmonella sp.* (e.g., birds, rodents) is not known.

It was not unexpected to routinely isolate fungi from the fur and skin of animals on St. Paul Island, Alaska. Previous studies have indicated that fur seals easily acquire dermatophytes on their skin and fur but that they have the physiological and anatomical barriers to serious dermatophytosis (Waldorf and Vedros, 1979, 1980).

Since marine mammals are constantly under stress during capture and often in captivity, the basic microbial background presented in this study should aid in a better understanding of potential disease agents of the animals in the wild and diagnosis when in captivity.

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