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# A PRELIMINARY REPORT ON POTENTIALLY PATHOGENIC MICROBIOLOGICAL AGENTS RECENTLY ISOLATED FROM PINNIPEDS[1,2]

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*Abstract:* Sea lions aborting on San Miguel Island, California, and fur seals on St. Paul Island, Alaska, were studied for the presence of infectious disease agents. *Leptospira* were isolated from both groups and may have been one cause of reproductive failure in both species. From a total of seven virus isolations made, one isolate from fur seals and two isolates from sea lions appear antigenically related by serum neutralization tests. In their host range, morphology, and physicochemical properties, the virus isolates are indistinguishable from Vesicular Exanthema of Swine Virus. Six mycoplasma isolations have been made but have not been fully characterized. A fungus, *Scopulariopsis* sp., isolated from three different sea lions, is the same genus that was repeatedly isolated from Navy divers during prolonged submergence studies.

## INTRODUCTION

Recent investigations have shown that sea lions *(Zalophus c. californianus)* harbored *Leptospira*[16] and other bacterial isolates from marine mammals were taxonomically comparable with organisms isolated from land mammals[4]. This relationship was reinforced by a report that *Neisseria mucosa* var *Heidelbergensis,* isolated from cetaceans, was identical to isolates causing pneumonia in children.[15]

Commencing in March 1972, two pinniped populations were studied, sea lions on San Miguel Island, California, where a high incidence of abortion had been reported[9] and fur seals *(Callorhinus ursinus)* on St. Paul Island, Alaska. The purpose of this paper is to report preliminary findings on some potentially pathogenic microbiological agents which have been isolated from these pinniped groups.

## MATERIALS AND METHODS

### Animals

Sea lions on San Miguel Island were studied between 27 March and 17 June 1972. Of the 20 adult females examined, 10 had aborted approximately 30-60 days before full term, and 10 had de-

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[2] Presented in part at the 4th Annual Conference of the International Association of Aquatic Animal Medicine, Victoria, British Columbia, Canada, May 1973.

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livered normal full term offspring. Specimens were obtained from 29 fur seals between 8 and 22 July 1972. Of these, one adult female, two pups and two bachelor bulls were presumably normal. The remainder were either found dead on the rookeries or were exhibiting signs of terminal illness.

#### Leptospira isolation

Inocula for isolating *Leptospira* were prepared by searing the surface of kidneys, livers and placentas with a hot spatula, aseptically removing 1-2 g of tissue from beneath the organ surface and grinding this with 5 ml of phosphate buffered saline pH 7.2 in a Tenbroeck grinder. One or 2 drops of this homogenate were inoculated into 7.5 ml of Fletcher's semisolid medium with and without 5 $\mu$g of 5 fluorouacil. Alternatively, specimens were inoculated within a 25 mm paraffin ring applied to the surface of a 47 mm millipore filter (0.22 $\mu$ pore size)[6] placed on Fletcher's semisolid medium in petri dishes. Wet mounts of all cultures were periodically examined by darkfield microscopy for the presence of typical *Leptospira* forms. After 10 weeks negative cultures were discarded.

#### Virus isolation

Dacron swabbings[7] of the nose, throat, rectum and pharyngeal tonsil of each animal were introduced immediately into tubes containing monolayers of Vero, PK-15, sea lion skin and dolphin skin cell cultures maintained in Eagles Minimum Essential Medium (MEM) supplemented with 5% fetal bovine serum, 200 units of penicillin, and 100 $\mu$g of streptomycin per ml. Cultures from sea lions were held at an ambient temperature of about 20 C for up to 5 days during the collection period and were then transported to the laboratory and incubated at 37 C. Cultures from fur seals were incubated at 37 C immediately after inoculation. Each culture was examined daily for cytopathic effect (CPE) and positives were immediately frozen and stored at —70 C. After 10 days tubes showing no CPE were stored at —70 C. All negative samples were blind passaged at least three times in each cell line.

#### Fungus isolation

Samples of fluid from cell culture tubes showing visual signs of fungal growth were transfered onto malt agar, mycosel agar, Sabouraud agar and GYE (2% glucose 1% yeast extract) agar. The cultures were examined microscopically over a 2 week period and positive cultures classified.

#### Mycoplasma isolation

Primary fur seal cell lines were established using kidney, lung and skin from two neonates. These cell lines were examined for mycoplasma using DIFCO[8] PPLO broth and PPLO agar. The media were inoculated with 0.2 ml of tissue culture fluid containing fur seal cells lysed by freezing and thawing. Broth cultures were incubated at 37 C in air and the agar cultures were incubated at 37 C in 95% N and 5% $CO_2$. Both were examined for growth over a 14 day period.[9]

#### Pathology

All animals in both study groups were examined for gross lesions at the time of collection. Selected tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 7 $\mu$, and stained with hematoxylin and eosin for histopathologic examination. Sections of liver, kidney and placenta were stained with Warthin-Starry stain to visualize *Leptospira* in the tissues.

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[6] Millipore Corporation, Bedford, Massachusetts 01730.
[7] Culpack Products, Inc., Evanston, Illinois.
[8] DIFCO Laboratories, Detroit, Michigan, 48201.
[9] Thabiso M'Timkulu. 1973. University of California School of Public Health. Culture of fur seal mycoplasma. Personal communication.

**RESULTS**

Leptospires were isolated from the placenta of a sea lion which aborted about 30 days before full term. Gross lesions of the aborted fetus included subcapsular hemorrhage of the kidney, free blood in the peritoneal cavity and a friable liver with extensive subcapsular hemorrhage. Two additional leptospiral isolations were made from fur seals. The first positive culture was from the liver of a dead newborn pup. Histologic examination of that organ showed numerous argyrophilic forms resembling *Leptospira*. Gross lesions were identical to those of the sea lion pup just discussed. This condition has been described in the past as a multiple hemorrhagic perinatal complex,[5] but the association of *Leptospira* is a new finding. The second isolate was from the urine of a 13 year old bull that had abandoned the breeding area. All three isolates were *Leptospira* Serogroup Pomona.

TABLE 1. Comparisons of VESV and SMSV*

| | VESV | SMSV[11] |
|---|---|---|
| **PHYSICO-CHEMICAL PROPERTIES** | | |
| pH stability | pH3-Labile[9] | pH2.7-Labile |
| Ether sensitivity | Not sensitive[9] | Not sensitive |
| Heat sensitivity 50 C | Sensitive | Sensitive |
| Stabilization by 1M $MgCl_2$ at 50 C | Not stabilized[15] | Not stabilized |
| Sedimentation coefficient | 207 S[9]<br>160-170 S[14] | 180 S |
| Buoyant density (g/ml) in $C_2Cl$ | 136-1.38 g/ml[14] | 1.37 g/ml |
| **MORPHOLOGY** | | |
| Size (nanometers) | 35-40 | 32 |
| Shape of particle | Spherical with projections[7,16] | Spherical with projections |
| In host cell | Crystalin lattice and tubular configuration Type $H_{34}$[16] | Crystalin lattice and tubular configuration |
| Plaque forms in cell culture | Variable size[9] | Variable size |
| **ANIMAL INFECTIVITY** | | |
| Mice | No[6] | No |
| Hamsters | No[6] | No |
| Guinea pigs | No[6] | No |
| Swine** | Yes[6] | Yes |

*San Miguel Sea Lion Virus (SMSV) has been declared indistinguishable from Vesicular Exanthema of Swine Virus (VESV) on the basis of these comparisons.

**SMSV injected intradermally into the lips of 2 swine produced erosions with raised, moist coverings which spread by extension. These were not typical vesicles but were compatible with lesions caused by less virulent strains of VESV such as $F_{55}$, $G_{55}$ and $J_{56}$.[6]

Seven virus isolations were made, five from sea lions and two from fur seals. One isolate, from the rectum of an adult female sea lion which aborted about 60 days prior to full term, was indistinguishable from Vesicular Exanthema of Swine Virus (VESV)[13] (Table 1). This isolate was not neutralized by antisera against serotypes A-K of VESV. Although VESV does cause abortion in swine[7] we have insufficient data to establish a casual relationship between sea lion abortion and the San Miguel sea lion virus (SMSV).

Based on physico-chemical testing, all seven of the isolates appear to be the same virus, however, serum neutralization tests show at least two serotypes (Table 2). The virus isolated from the nose of an emaciated fur seal pup is neutralized by antiserum against one of the sea lion virus isolates, indicating the likelihood of common infection between the two species.

A fungus of the genus *Scopulariopsis* was repeatedly isolated from sea lions.[10] Six mycoplasma isolations have been made but the significance of these agents as pathogens is not yet known.

TABLE 2. Serum Neutralization Tests*.

| Virus Isolates** | Rabbit Antiserum | | | |
|---|---|---|---|---|
| | 2MT | 2MR | 1FN | 1MR |
| 2MT | +*** | + | + | (—)**** |
| 2MR | + | + | + | (—) |
| 1FN | ± | (—) | + | (—) |
| 1MR | (—) | (—) | (—) | + |
| 7MR | (—) | (—) | (—) | + |
| 1233 | (—) | (—) | (—) | + |
| 1126 | — | — | — | + |

*Tests were carried out using Vero cells, 100 $TCID_{50}$ virus vs. 20 units of antibody and read at 24, 48, 72 hrs.

**Isolates 2 MT and 2 MR are from the throat and rectum of a sea lion aborting 30-60 days before full term. Isolate 1 MR and 7 MR are from the rectums of two additional aborting females. Isolate 1 FN is from the nose of the fetus aborted by the mother from whom 1 MR was isolated. Isolates 1233 and 1126 are from the nose of an emaciated fur seal pup.

***Virus neutralized (+)

****Virus not neutralized (—)

## DISCUSSION

*Leptospira* has been shown to cause abortion and neonatal deaths among cattle and swine.[3,11] It has been postulated by Smith that *Leptospira* play a role in abortions among California sea lions[16] and though our present data are limited, the finding of *Leptospira* in an aborted sea lion pup and in a dead neonatal fur seal supports this hypothesis. Furthermore, both of these newborn animals died with hemorrhages suggesting that *Leptospira* causes this condition in newborn pinnipeds.

Although the pathogenicity of all the leptospires isolated from pinnipeds have not been fully tested in other mammalian species, a recent *Leptospira pomona* isolate from a sea lion did not

[10] Cobb, J. 1973. Naval Biomedical Research Laboratory. Culture and identification of marine mammal fungi. Personal communication.

cause observable disease in weanling Dunkin Hartley (Fort Detrick) guinea pigs.[16] It did, however, have a marked cytotoxic effect on monolayers of human embryonic lung (HEL) cells when compared to another *Leptospira* of the serogroup Pomona isolated from a terrestrial mammal (Smith, A. W., 1972. Unpublished data). The zoonotic potential of pinniped leptospirosis has not yet been explored. However, it could be of considerable public health significance, especially on St. Paul Island where the fur seal harvest places a large percentage of the human population in intimate contact with fur seals which may be carrying *Leptospira.*

The fungus, *Scopulariopsis* sp., isolated from pinnipeds, did not cause observable infection in these animals, although fungi of this genus have been repeatedly isolated from the superficial and deep mycotic lesions of man.[1] Fungi of the genus *Scopulariopsis* were isolated from the normal skin of aquanauts during the TEKTITE-I 60-day submergence study in the Virgin Islands.[6] Disease was not associated with these isolates.

The finding that marine mammals may be a reservoir for a virus disease such as VES which infects terrestrial mammals is new in environmental interrelations.[18] VES was first described in California in 1932, where the initial outbreaks were thought to be Foot and Mouth Disease.[7] Repeated and costly outbreaks of VES continued to occur but were confined to California until 1952, at which time the disease spread to all the major swine producing areas of the United States. Since 1956, when federal regulations prohibited the feeding of raw garbage to swine, there have been no reported outbreaks of VES in the United States. We now believe that the pinnipeds inhabiting the coastal waters of California were a reservoir for VESV and were the source of virus for the unexplained outbreaks of this disease in California swine herds.

The virus reported here has broad economic implications as a possible threat to the food animal industry, and several of the other agents, such as the *Leptospira, Neisseria* and *Scopulariopsis* may possess zoonotic potential. Such evidence emphasizes the importance of in-depth studies to define the disease interrelationships in our total environment.

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