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Abstract: The light microscopic demonstration of eosinophilic intracytoplasmic inclusion bodies in epithelial cells and the electron microscopic demonstration of poxvirus in these inclusions has shown that sealpox occurs in free-living California sea lions, *Zalophus californianus.* This is the first evidence of a poxvirus skin infection in free-living California sea lions.

INTRODUCTION

Information obtained from a questionnaire survey[1] indicated that sealpox probably occurs in free-living California sea lions. This field survey was planned to test this supposition.

MATERIALS AND METHODS

This field survey was conducted on *Zalophus californianus californianus,* one of three geographically separate populations of *Z. californianus,* which breed on islands in the Gulf of California and along the west coast of Mexico south to Mazatlan and off Baja California and California coasts northward to San Miguel, the most northwesterly of the California channel islands.[1] Bureaucratic difficulties prevented field surveys on most islands but with the co-operation of the U.S. Navy a 1-week survey during May, 1970, was conducted on the west coast rookeries of San Nicolas Island. This island, the outermost of the California channel islands, is about 130 km (66 nautical miles) southwest of Los Angeles Harbor. Other animals captured in nets off the coast of San Miguel Island, a northern island in the channel island groups, were examined in Santa Barbara Harbor.

Because drug immobilization techniques have not been perfected for field use on California sea lions[2] and because the field team was small (2 men), a capture method employing a hoop net was used. The hoop net consisted of a heavy circular plastic ring about 1 meter in diameter to which was attached a nylon net sleeve about 2 meters in length. A short handle was attached to the hoop.

A man in full view and walking toward California sea lions on San Nicolas Island can alarm them at a distance of 75 meters or more during daylight.[1] Because this alarm can cause a wild stampede that will clear the beach of seals, a rush technique was employed during capture.

This method consisted of a crouched or crawled approach behind rocks or on the sand to within a short distance of a group of seals. As the seals became wary of our presence, as noted by their alert behavior and slow movements to the sea, a simultaneous rush by both field workers was made. This rush was directed along the water's edge in an attempt to cut off the escape route of the seals. The seal groups were usually of a mixed age and therefore size distribution and as the animals made their attempt to reach open water, the smaller, slower ones fell behind and were available for capture. They were captured by approaching them from the rear, grasping one or both hind flippers and dragging them up on the beach to be placed in the hoop net. The hoop net could also be placed over the seal as it rushed toward the open water.

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One worker, wearing thick leather gloves, restrained the seal by grasping it about the neck and straddling the back while the other worker made an examination of the entire body for skin nodules. A subcutaneous injection of 1-2 cc of Xylocaine was made at the base of a lesion and it was excised with a scalpel. Local hemorrhage was controlled by the application of silver nitrate sticks.

Half of each epithelial biopsy was placed in 10% formalin; the epithelium from the other half was cut into 1 mm cubes, placed immediately into 2.5% glutaraldehyde for 4-8 hours and later stored in sucrose phosphate buffer.

Specimens were post-fixed in 1% osmium tetroxide for 1 hour, dehydrated through a graded series of alcohol solutions and embedded in Epon.

Selection of tissue blocks containing intracytoplasmic inclusions was made after examination of 1 micron thick sections stained with toluidine blue. The sections, stained with lead citrate,[3] were examined with a Philips 200 electron microscope at 60 kv.

Formalin - fixed tissue was routinely prepared, sectioned and stained with hematoxylin and eosin. Light microscopic examination for the presence of eosinophilic intracytoplasmic inclusions in proliferative stratum spinosum cells and electron microscopic study of these cells for the presence of poxvirus were the criteria used for the diagnosis of sealpox.

RESULTS

Twenty-nine sea lions, each weighing less than about 220 kg (100 pounds), were captured and examined on San Nicolas Island. Two females had solitary skin nodules about 1-2 cm in size on the dorsal thorax and ventral cervical areas (Fig. 1).

Sealpox was diagnosed in these two cases upon demonstrations of eosinophilic intracytoplasmic inclusion bodies in epithelial cells with the light microscope and electron microscopic evidence of pox virions in these intracytoplasmic inclusions.

Of fifty sea lions captured off the coast of San Miguel Island, two yearlings, a male and a female, had solitary skin nodules. Both lesions were about 1-2 cm in diameter and located on the dorsal thorax. Microscopic examination as above confirmed a diagnosis of sealpox.

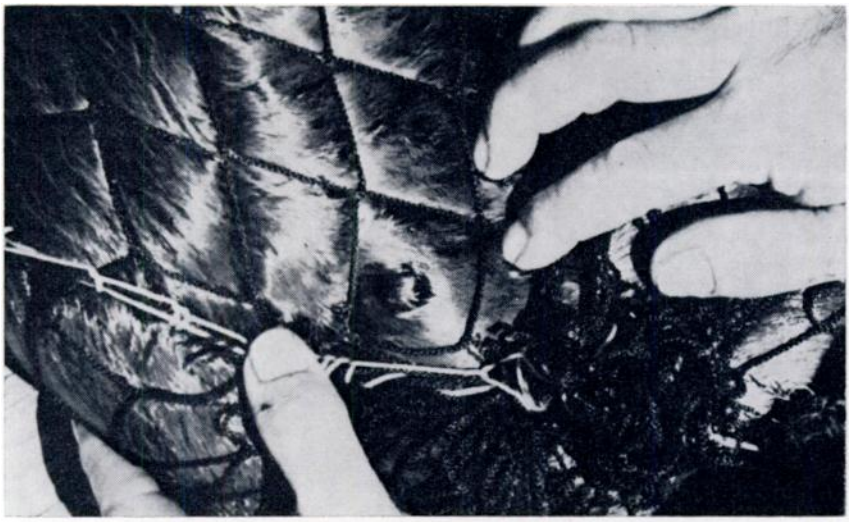

FIG. 1. Sealpox skin nodule, California sea lion, field case.

DISCUSSION

The rush method, a reliable method for sea lion capture, was used to confirm that sealpox occurs in free-living California sea lions. Although this capture technique is rudimentary, it appeared at the time and under the circumstances, to be the only reliable method available.

The data from this field survey on San Nicolas and off the coast of San Miguel Islands indicate that sealpox does occur in California sea lions from these locales and is the first evidence to our knowledge of pox in free-living California sea lions. The low numbers sampled does not permit conclusions on the frequency or significance of sealpox in these populations, however, these poxvirus-infected seals are a potential source of infection for zoos and aquaria.

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