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GENITAL PAPILOMATOSIS IN SPERM WHALE BULLS

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ABSTRACT: Examination of 31 male sperm whales (*Physeter catodon*) caught off the western coast of Iceland revealed three cases of genital papillomatosis involving the unsheathed penis. One subadult and two sexually mature bulls were affected. Gross lesions resembled papillomas common in terrestrial mammalian species. Transmission electron microscopy of these lesions revealed nonenveloped intranuclear virus particles 28–40 nm in diameter and round to hexagonal in shape. In two cases immunoperoxidase staining was negative for group-specific papillomavirus antigen. These findings indicate that the spectrum of animal species affected with virus-associated genital papillomatosis includes at least one globally distributed species of the order Cetacea (whales, dolphins, and porpoises).

Key words: Sperm whale, papillomavirus, papillomatosis, histopathology, Cetacea, *Physeter catodon*, venereal disease.

INTRODUCTION

Papillomatosis are generally classed with neoplastic diseases, and affect epidermal or mucosal surfaces. The lesions which occur in these diseases have a number of forms, ranging from the small plaques seen in human epidermodysplasia to large nodular warts, or condylomas. Intranuclear virus particles or virus-specific antigens have been found in epidermal cells in papillomatous lesions in humans (Murphy et al., 1983; Okagaki et al., 1984; Penneys et al., 1984; Ferenczy et al., 1985), non-human primates (Ragan et al., 1980), rabbits (Noyes and Mellors, 1957; Sundberg et al., 1984), cattle (Brobst and Hinsman, 1966; Fujimoto and Olson, 1966; Jarrett et al., 1984; Sundberg et al., 1984), dogs (Watrach, 1969; Sundberg et al., 1984), deer (Tajima et al., 1968; Sundberg et al., 1984), avian species (Jacobson et al., 1983; Sundberg et al., 1984), and reptiles (Jacobson et al., 1982). In domestic species, papillomaviruses are the cause of the neoplasia (Bruner and Gillespie, 1973). The lesions are usually benign, although they may be locally invasive. Malignant transformation of certain of the genital papillomatosis has been linked to infection with specific pap-

illomaviruses (Gissmann, 1984; Pfister, 1984).

Here we report the finding of genital papillomatosis in sperm whale bulls taken from the North Atlantic off the western coast of Iceland (64–67°N latitude, 24–29°W longitude) by commercial whalers.

MATERIALS AND METHODS

Thirty-one male sperm whales (*Physeter catodon*) taken late in the summer or early fall of 1981 and 1982 were examined postmortem at the Icelandic Whales Research Laboratory, Hvalfjördur, Iceland. Necropsies were conducted in conjunction with routine flensing operations. Only male animals were available for examination, since female sperm whales do not migrate into these latitudes.

Representative penile lesions were sampled and fixed in neutral buffered 10% formalin, dehydrated in a graded series of ethanol, and embedded in paraffin. A selection of these were placed in the International Registry of Reproductive Pathology (Department of Veterinary Pathobiology, University of Illinois, Urbana, Illinois 61801, USA; accession no. RS 20515). Tissue sections 5–7 μ m thick were stained with hematoxylin and eosin and Masson's trichrome stain for routine histologic examination, and Lillie Nile blue, 1% borax buffered toluidine blue, and Feulgen stains for special studies (Humason, 1979). Descriptive terminology used for the cel-

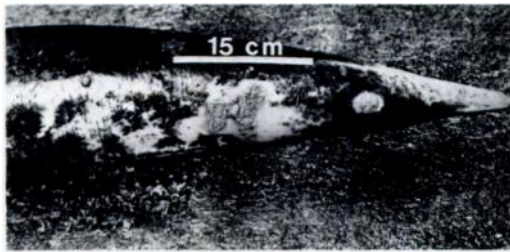


FIGURE 1. Papillomas on penis of sperm whale 278-82.

lular strata of the cetacean epidermis follow Britt and Howard (1983).

Samples for transmission electron microscopy studies were preserved in 2.5% glutaraldehyde in filtered seawater, postfixed with 1% aqueous osmium tetroxide for 4 hr, and embedded in Epon Araldite (Polysciences, Inc., Warrington, Pennsylvania 18976, USA). Thin sections were prepared on a Porter Blum MT-2 ultramicrotome with glass knives, and stained with saturated aqueous uranyl acetate and lead citrate (Reynolds, 1963). Ultrastructural examination and measurements were made with a Zeiss 10 transmission electron microscope.

Immunohistochemical staining to detect common (bovine papillomavirus) structural antigens was performed on formalin fixed tissues. The peroxidase-antiperoxidase (PAP) technique was used to detect group-specific antigens as previously described by Sundberg et al. (1984). Duplicate paraffin sections, cut at 5 μ m, of 11 sections of papillomatous lesions from two whales were examined. Normal penile mucosa from one whale was similarly tested as a negative control. A bovine cutaneous fibropapilloma and a canine oral papilloma were used as positive controls. Lesion material from one affected whale was not studied immunohistochemically since the tissue samples obtained from this animal were consumed in the initial histologic studies.

RESULTS

Multiple genital papillomas on the unsheathed penis occurred in three of the 31 (10%) male sperm whales examined. The first case, 237-81, was a 14.3 m sexually immature male (testes weight 1.2 and 1.8 kg). Twelve 1–4 cm diameter circumscribed nodules were observed on the ventral surface of the penis of this whale, along with a larger warty mass affecting an area 5 \times 8 cm. Sperm whale 258-82, a 15.8 m

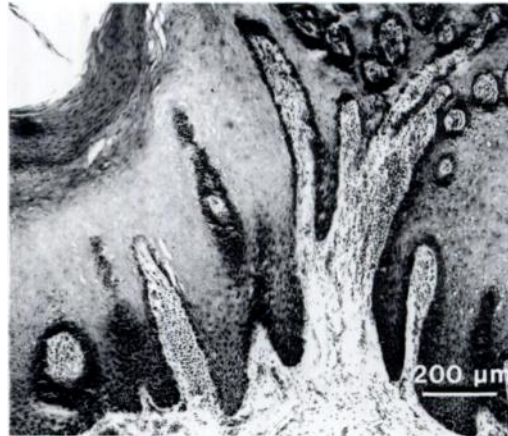


FIGURE 2. Photomicrograph showing epidermal hyperplasia, hyperkeratosis, abnormal dermal papillae and cellular infiltrate within a papilloma from penis of sperm whale 278-82. H&E.

sexually mature male, had an array of 17 smaller nodules (1–2 cm diameter) in approximately the same location. Sperm whale 278-82, a 15.2 m sexually mature male, was the most extensively affected. The papillomatous lesions in this animal varied in shape and size from 1 cm nodules to a 4 \times 6 cm plaque 3–4 cm in thickness. Together, these affected the ventral, lateral and dorsal surfaces of the penis. In the surrounding areas there was extensive depigmentation and scarring of the penile epidermis (Fig. 1).

Sperm whale 307-82, a 12.5 m sexually immature male, had no papillomas. However, in this whale several 1–2 cm diameter unpigmented scars were observed on the surface of the unsheathed penis. These were similar in appearance to the scars seen on the penis of whale 278-82.

The histological structure of unaffected penile epidermal tissue taken 30 mm from a papillomatous plaque consisted of stratified squamous epithelium. The dermal papillae were slender and uniform in size and structure, and extended into less than one-half the depth of epidermis. The stratum basale was one to two cells thick and consisted of cuboidal to short columnar cells

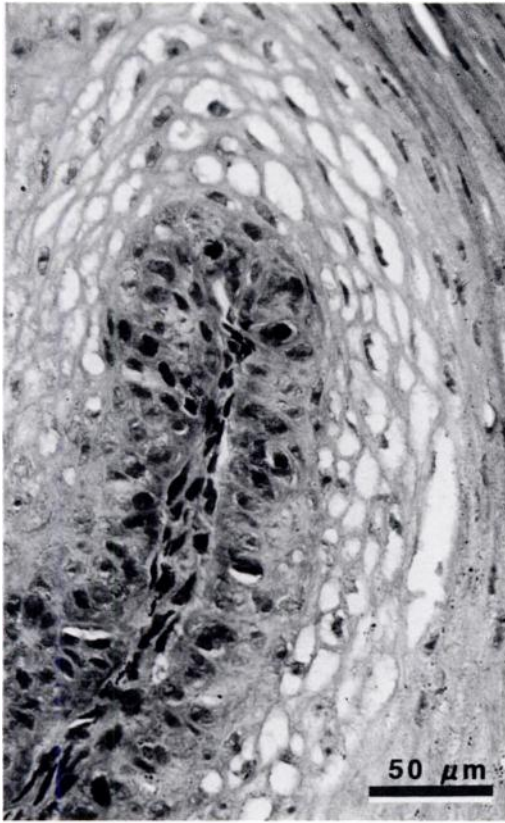


FIGURE 3. Photomicrograph showing hydropic vacuolation within a papilloma from penis of sperm whale 278-82. H&E.

with small, round, strong basophilic nuclei. The adjacent, thicker portion of the epidermis was the stratum spinosum, which blended indistinctly with the more superficial stratum intermedium. The combined stratum spinosum and stratum intermedium consisted of 15 to 20 layers of melanin-containing cells. The outermost layer, the stratum externum, was five to 10 cells thick and parakeratotic. As in normal cetacean skin, there was no anucleate stratum corneum.

The tissue immediately adjacent to the papillomas, which sometimes was unpigmented and scarred, showed abnormal histopathologic features. The dermal pegs were irregular and in some cases elongated. A sparse and diffuse lymphoplasma-

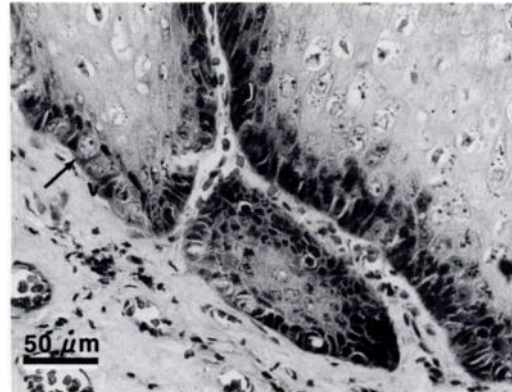


FIGURE 4. Photomicrograph showing euchromatic (arrow) and normochromatic nuclei of two distinct cell populations in the stratum basale of a papilloma from penis of sperm whale 278-82. H&E.

cytic infiltrate was present along the dermal-epidermal border. The epidermis was slightly thickened and contained few melanin granules. These abnormalities became more prominent toward the papillomatous growth.

Epidermal abnormalities within the papillomas (Fig. 2, 3) included ballooning degeneration, spongiosis, karyopyknosis, and dysplasia. An abnormally thickened stratum externum with swollen nuclei covered a stratum intermedium and stratum spinosum which contained cells with vacuolated cytoplasm. Nuclei in the stratum intermedium and stratum spinosum were commonly pyknotic. Feulgen staining revealed occasional vacuolation of epidermal cell nuclei with peripheral margination of the chromatin. Dyskeratosis was evident in both the stratum intermedium and stratum externum, which were hyperplastic. Pigment granules, confirmed to be melanin with Lillie Nile blue staining, were sparse. Dermal papillae were reduced in number, elongated and fused. Routine stains and Masson's trichrome showed no abnormal increase in fibrous connective tissue.

The stratum basale in most lesions appeared to be two cells thick and contained two distinctive cell populations (Fig. 4).

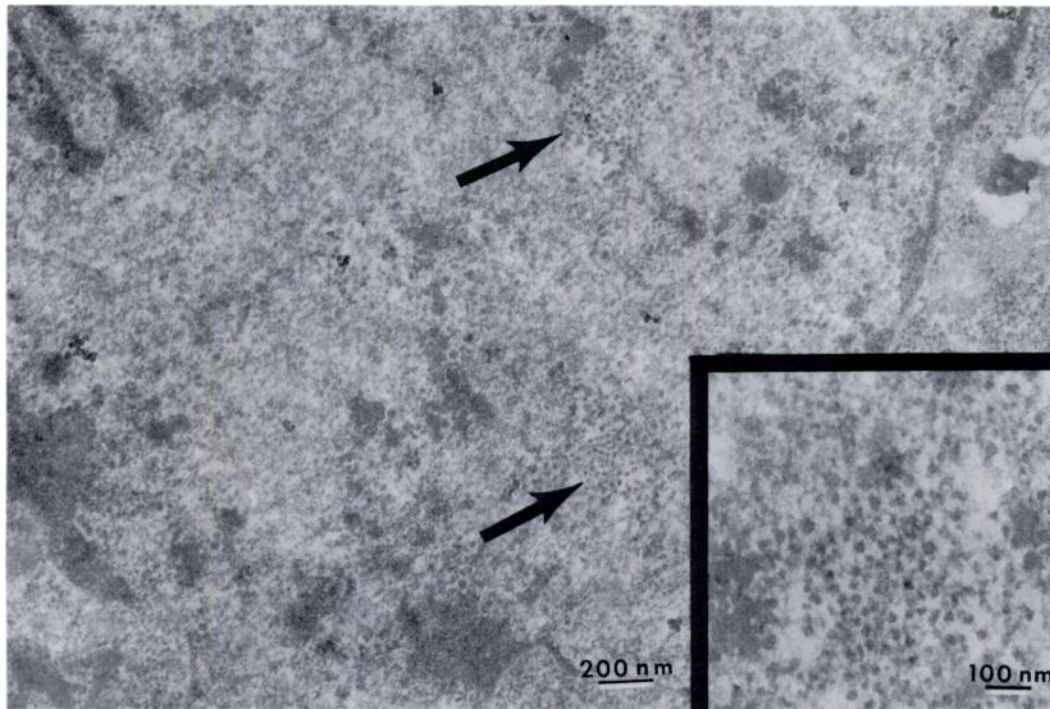


FIGURE 5. Electron micrographs showing intranuclear virus particles (arrows) in cell of papilloma from penis of sperm whale 278-82; inset shows higher magnification of virus particles.

The predominant and type in both normal and affected tissue consisted of small basophilic cells with deeply stained nuclei. The second cell type, occurring only in affected tissue, was abnormally large and contained swollen euchromatic nuclei with prominent nucleoli.

The inflammatory cell component associated with the aforementioned epidermal lesions was variable. In some sections from whale 278-82, scattered foci of dense lymphoplasmacytic infiltrates occurred at the dermal-epidermal junction. Toluidine blue staining revealed no admixed mast cells. Peripheral to the papilloma, there was mild perivascular cuffing with mononuclear cells. Lesions from other whales showed a more diffuse and usually less intense mononuclear cell reaction.

Transmission electron microscopy of the cells in the stratum intermedium and stratum externum in the lesions from sperm

whale 278-82 indicated the presence of intranuclear virus-like particles (Fig. 5). These particles were hexagonal, unenveloped, and ranged in diameter from 28 to 40 nm ($\bar{x} = 34$, $SD = 4$ nm; $n = 30$). They occurred in small, dense clusters widely scattered throughout the nucleoplasm.

Papillomavirus group specific antigens were not detected by immunohistochemical studies of 11 sections of proliferative lesions from two whales. However, electron microscopy did reveal intranuclear virus particles in the same lesions.

DISCUSSION

This study describes the first known occurrence of virus-associated genital papillomatosis in a species of the order Cetacea (whales, dolphins, and porpoises). Papillomas have been described on the penis of a killer whale (*Orcinus orca*), but light and electron microscopic examinations of the

lesions failed to identify inclusions or virus particles (Taylor and Greenwood, 1974). Unpublished gross observations of papillomas on the penis of a common porpoise (*Phocoena phocoena*) have also been cited, but not described, in a recent review of neoplasia in marine mammals (Landy, 1980). Other virus-associated diseases in Cetacea include dolphin pox (Geraci et al., 1979) and calicivirus-induced vesicular disease (Smith et al., 1983). Both of these have been reported in bottlenosed dolphins (*Tursiops truncatus*), a small cetacean commonly kept in captivity. Also, serologic studies (Smith and Latham, 1978) suggest that natural populations of gray whales (*Eschrichtius robustus*), fin whales (*Balaenoptera physalus*), sei whales (*B. borealis*), and sperm whales in the North Pacific are infected with caliciviruses, but there is no direct evidence in these species that such infections cause pathogenic effects. Two influenza A virus subtypes have been isolated from the lung and a hilar lymph node of a sick pilot whale (*Globicephala melaena*) (Hinshaw et al., 1986), and there is one report of influenza virus infection of a member of the family Balaeopteridae (rorqual) (Lvov et al., 1978).

Cockrill (1960), who was in charge of meat inspection on British whaling factory ships in the Southern Hemisphere during the 1948–1951 period, reported that Antarctic whalers believed sperm whales carried a venereal disease. Yet on the basis of his own inspections of the same species he refuted this claim. Our results now support the earlier conclusion of the whalers by establishing the diagnosis of genital papillomatosis in sperm whale bulls on the basis of the anatomic site affected, the morphologic features of the lesions, and the finding of an associated virus. As in domestic animals and humans, this disease in sperm whales presumably can be transmitted by coitus. Inasmuch as one of the cases we describe was reproductively immature, transmission by precocial sexual

contact or other mechanisms may also be involved.

Many morphologic features of papillomavirus-induced lesions are similar between affected species. In the virus-induced papillomatosis of humans (Strauss et al., 1950; Chapman et al., 1963), non-human primates (Shope, 1933; Ragan et al., 1980), rabbits (Moore et al., 1959; Richter et al., 1964), ox (McEntee, 1950), and dogs (Watrach, 1969), as in virus-associated genital papillomatosis in sperm whales, there is an increased number and enlargement of the epithelial cells in the outermost layers of the affected epithelium. Cytoplasmic vacuolation and nuclear vesiculation with margination of chromatin are other shared characteristics. The latter nuclear abnormalities may be associated with maturation of the papillomavirus. Genital papillomatosis in the sperm whale appears to have a peculiar similarity to bovine fibropapillomatosis, as in the latter condition a population of cells in the stratum basale also becomes hypertrophic and shows poor affinity for basic stains (Fujimoto and Olson, 1966). However, bovine fibropapillomatosis naturally differs from sperm whale genital papillomatosis in having a substantial connective tissue component in the core of the lesions.

Papillomaviruses are round to hexagonal, intranuclear particles with reported diameters of 35 nm (TEM; Brobst and Hinsman, 1966) to 50–55 nm (negative staining, Tajima et al., 1968) in bovine papillomatosis, 40–49 nm in oral and cutaneous papillomas in rabbits (Richter et al., 1964), 49 nm in cutaneous canine papillomas (Watrach, 1969), and 38 nm in genital (Strauss et al., 1950) and 40–55 nm in cutaneous (Okagaki et al., 1984) papillomas in humans. These characteristics are comparable and in general agree with the small size (28–40 nm) of the virus particles reported here, their round to hexagonal shape, and their intranuclear location.

The etiological significance of these virus particles remains open to question. Due to the difficulties inherent in conducting experimental studies on large whales, it was not possible to demonstrate a cause-effect relationship between the virus infection and papilloma formation. However, since it is well established that papillomaviruses can induce the formation of this type of neoplasia in other animals (Strauss et al., 1950; Kurman et al., 1983; Gissmann, 1984; Pfister, 1984) a similar oncogenic mechanism seems likely in the sperm whale.

Our negative immunohistochemical results are compatible with the notion that the papillomavirus-like virus particles revealed here by transmission electron microscopy are antigenically distinct from the papillomaviruses reported in terrestrial species. Caution is necessary in accepting this interpretation, however, for the sensitivity of the PAP technique is not unlimited. Given the comparatively low number of virus particles per nucleus, and their tendency to occur in small widely scattered clusters, it is possible that low virus density prevented our detection of antigen. Therefore, further work will be necessary to clarify antigenic characteristics of the virus. Aside from these observations, our finding of a virus-associated disease in 10% of a population sample of free-ranging sperm whales emphasizes the potential importance that microbial pathogens may have in governing the health and dynamics of populations of large whales.

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