AN EPIDERMAL PAPILLOMA OF THE ATLANTIC SALMON II: ULTRASTRUCTURE AND ETIOLOGY 1

Author: CARLISLE, J. C.

Source: Journal of Wildlife Diseases, 13(3) : 235-239

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-13.3.235

AN EPIDERMAL PAPILLOMA OF THE ATLANTIC SALMON II: ULTRASTRUCTURE AND ETIOLOGY[1]

J. C. CARLISLE,[2] Aquatic Pathobiology Unit, University of Stirling, FK9 4LA, Scotland[3]

Abstract: Ultrastructure of the Atlantic salmon papilloma was studied, and virus isolation was attempted. The papilloma cells were similar to normal epidermis in having interdigitating cell membranes with desmosomes. The nuclei, however, were more regular in shape than normal epidermal nuclei and the chromatin tended to be marginated and clumped. No cytopathic viral agents were isolated.

INTRODUCTION

The epizootiology, gross and microscopic pathology, and immunology of a papilloma of juvenile Atlantic salmon (*Salmo salar*) have been reported previously.[1]

An infectious etiology has been suspected, and the observation of virus-like particles under the electron microscope has led to the suggestion that the agent may be a transmissible virus.[14] Epidermal lesions somewhat similar to the lesion on salmon have been reported affecting a number of other teleost species and, in several, virus-like particles have been observed: Atlantic eel (*Anguilla anguilla*),[5,15] Walleye (*Stizostedion vitreum*),[8,9] Northern pike (*Esox lucius*),[13] Carp (*Cyprinus carpio*),[7] and the flathead sole (*Hippoglossoides elassodon*).[11] The relationship of these particles to the associated skin tumors is yet to be proven. There are, however, several papillomata of mammals which are of proven viral etiology, e.g. bovine papillomatosis, the Shope papilloma, and the human wart (*Verruca vulgaris*).

The purpose of this paper is to report observations on the ultrastructure and possible etiology of the salmon papilloma.

MATERIALS AND METHODS

The source of the affected salmon was previously reported.[1] Specimens from papillomata and normal skin were removed from freshly killed salmon and fixed in 1.5% glutaraldehyde for one h. This was followed by one h. in 2% osmium tetraoxide, dehydration in graded alcohols, and embedding in Lufts epon 812. One micron sections were cut for light microscopy and 60-90 nm. sections for electron microscopy. The former were stained with hot 1% toluidine blue in 1% borax and examined microscopically to locate areas for ultrastructural study and to correlate ultrastructural findings with histological observations. The latter were stained with saturated uranyl acetate for 15 min. and followed by 2.66% lead citrate for 15 min. and viewed under EMI Corinth and Joel 100C electron microscopes at 60-80 kv. Particle sizes were obtained by measuring the image size on the negative and dividing by the corrected magnification of the instrument.

Papillomata for primary culture were removed from fish anesthetized with methane tricaine sulfonate (MS222 Sandoz). The cells were dispersed in 5 ml. Eagle's minimum essential medium, Glasgow modification (MEM)[1] in a sterile plastic bag by kneading. The cell suspension

[1] This paper was presented at the International Meeting of the Wildlife Disease Association, Munich, Germany, 1976. An abstract appears in *Wildlife Diseases*, 1976, ed. by L. A. Page, Plenum Press.

[2] Present address: Department of Avian & Aquatic Animal Medicine, New York State Veterinary College, Cornell University, Ithaca, New York 14853, USA.

[3] The Aquatic Pathology Unit is supported in part by the Nuffield Foundation.

was then incubated at 12 C in 25 ml. plastic flasks.

Tissues for virus isolation were either stored at —20 C prior to inoculation or taken directly from freshly killed salmon. Papillomata, kidneys, and spleens were minced in a sterile grinder in MEM. The cell debris was removed by centrifugation at 3000 rpm for 10 min. The inoculum, at a final dilution of 1:20 to 1:50, was filtered through a 0.22 μm. Millipore membrane filter and layered on to confluent monolayers of Atlantic salmon cells.[6] After adsorption for one h., the filtrate was replaced with fresh growth medium. Flasks were incubated at 12 C, observed daily on an inverted microscope for cytopathologic changes, and compared to uninoculated control flasks. The medium was filtered and passed to a new cell sheet every week, until the fourth passage, which was left for 5 weeks.

RESULTS

At the ultrastructural level, interdigitating cell membranes, desmosomal attachments and cytoplasmic filaments were characteristic features which the papillomata shared with normal epidermis (Fig. 1-2). The outer surface of the superficial cells usually bore microridges.[2]

The nuclei of tumor cells were typically different from those of normal epidermal cells. They were generally circular in sections, not assuming the more irregular shapes often seen in normal epidermal cells. They had conspicuously clumped and marginated chromatin (Fig. 1) compared with the finely dispersed chromatin of the normal nuclei.[3]

Virus-like particles were seen in five blocks of tissue from three fish (Fig. 2-3). They were characterized by an outer, electron dense coat 125-150 nm in diameter, separated by an electron lucent

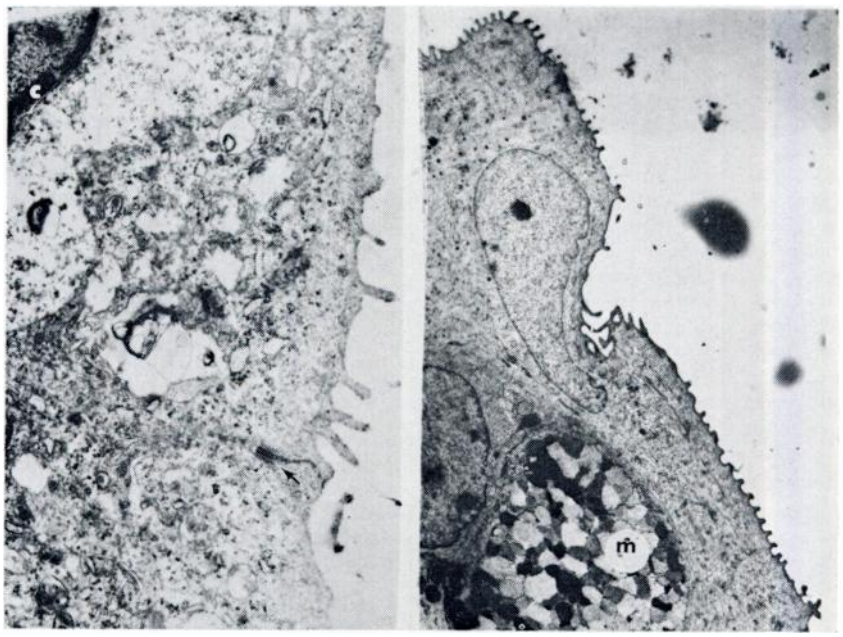

FIGURE 1. Composite electron micrograph with two epidermal papilloma cells on the left with a desmosomal attachment (arrow), irregular microridges on the surface, and clumped nuclear chromatin (c). 15000 X. On the right is normal epidermis with regular microridges of the surface. A goblet cell with mucous (m) packets is at the bottom. The nuclei have finely dispersed chromatin. 3750 X

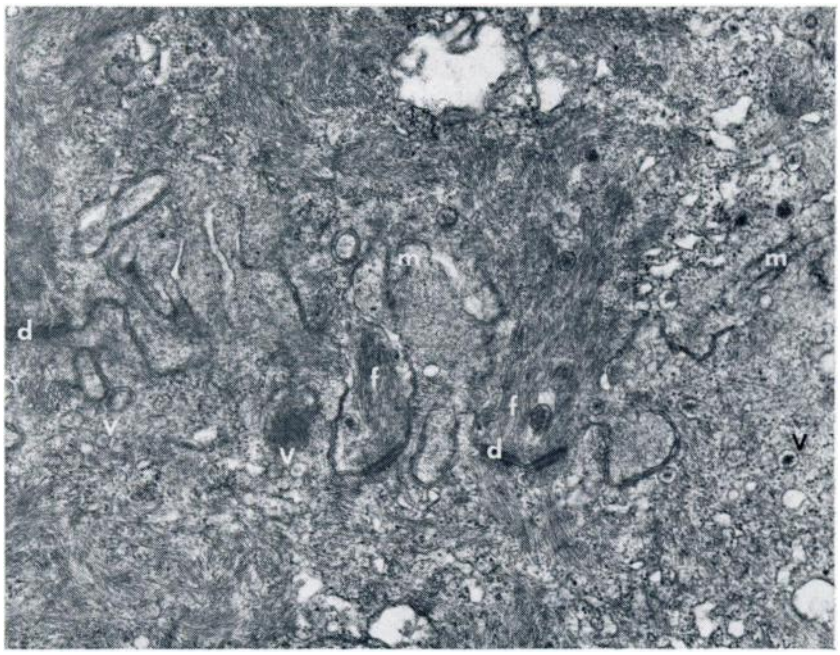

FIGURE 2. Electron micrograph of the junction between two epidermal papilloma cells showing interdigitating cell membranes (m) with desmosomal attachments (d) and cytoplasmic filaments (f). Virus-like particles (v) were frequently found near cell membranes. 25,000 X

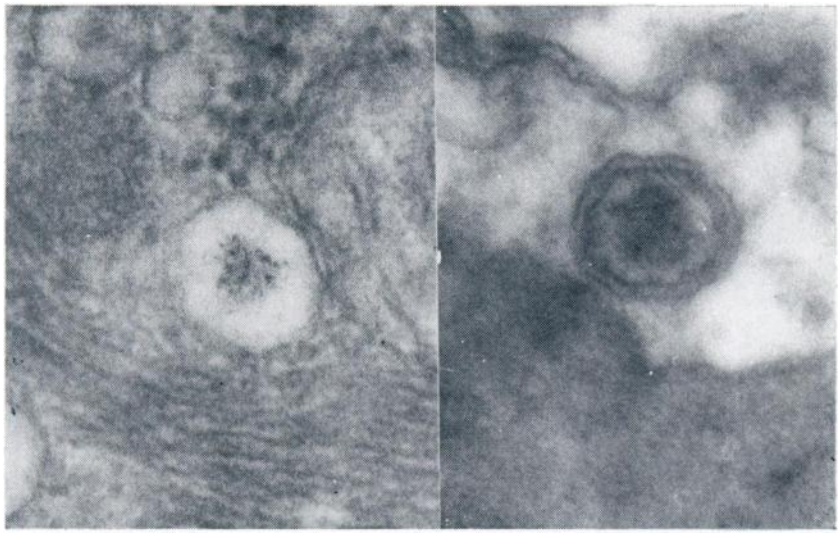

FIGURE 3. Composite electron micrograph comparing an intracellular particle on the left and an extracellular, double-coated particle on the right. Note the hexagonal shape and the suggestion of capsomeres. 150,000 X

area from a central electron dense "nucleoid" 70-95 nm. in diameter. Particles typically were seen in degenerating epidermal cells or associated with the plasma membrane of intact cells. A single extracellular, double coated particle was observed (Fig. 3). Extensive searching of the other 47 blocks of papillomata failed to reveal similar particles.

No evidence of viral cytopathologic effects was seen after four passages of papilloma material in Atlantic salmon cells.

In primary culture, the papilloma cells grew as clumps, attached or floating, and survived for over a month but they did not achieve sufficient cell density to allow serial passage.

DISCUSSION

The etiology of the salmon papilloma remains unknown. In one hatchery, all affected parr were offspring from a single mating, leading to suggestions of a possible genetic basis. Not all progeny of that particular mating were affected but that does not rule out the hypothesis. Similar information was not available for the other farms, since progeny of various matings are not kept separate.

The tendency for the condition to occur in epizootics suggests an infectious or chemical agent, which either spreads through a population or simultaneously exposes all fish in a tank. Since a common water supply was used for all salmon tanks or cages on four of the five establishments studied, and not all tanks contained affected fish, a water-borne agent seems unlikely unless other factors are necessary for expression of the clinical condition. Likewise, an agent present in a common feed supply seems unlikely. If feed were the source, exposure dose would depend on feed consumption, and there was no noticeable tendency for the lesion to occur on the largest fish. Whatever the etiologic agent, it would have to be rather widespread to account for the occurrence of an apparently identical condition in several countries, in farms and in free living populations, and in fresh and salt water.

The failure to demonstrate a virus in cell culture does not rule out a viral etiology. The cell line used (a fibroblast-like cell type) may not have been susceptible to infection by the virus, or may have been infected without producing cytopathologic effects.

Walker[9] observed extracellular virus-like particles of 80 nm. diameter in material from proliferative lesions on Walleye. They were similar in morphology, but smaller than the particles reported herein. The viral particles reported by Windqvist *et al.*[13] from epidermal proliferations in Northern pike were similar in size and morphology to those from the salmon in this study. However, the majority of their particles were extracellular as opposed to the intra-cytoplasmic location of the majority of virus-like particles in the salmon papilloma.

A plausible explanation for the seasonal occurrence, and the restricted age group affected,[1] would be the hormonal status of the fish. Profound endocrinologic changes occur in the fish before and during smoltification, which occurs in late summer through autumn, and these have direct physiologic effects on the skin.

Further attempts to isolate a viral agent from the salmon papilloma are needed. These should employ additional cell lines, particularly cells of epithelial origin. Careful epizootiologic studies also are needed, since hormonal and/or environmental factors may be necessary for clinical expression of the disease. A multifactoral etiology has been proposed for similar conditions in pike,[4] flathead sole,[10] and other fish.[12]

Acknowledgements

The author gratefully acknowledges the advice and assistance of Drs. Hugh Ferguson and Barry Hill in the electron microscopy and virus isolation procedures, respectively.

LITERATURE CITED

1. CARLISLE, J. C. and R. J. ROBERTS. 1976. An Epidermal Papilloma of the Atlantic Salmon (*S. salar*) I: Epizootiology, Pathology, and Immunology. J. Wildl. Dis. 230-234.
2. HAWKES, J. W. 1974. The S-ructure of Fish Skin I: General. Cell. Tiss. Res. 149: 147-158.
3. HENRIKSON, R. C. and A. G. MATOLSTY. 1968. The Fine Structure of Teleost Epidermis I: Introduction and Filament-Containing Cells. J. Ultrastructure Research. 21: 194-212.
4. LJUNGBERG, O. 1974. Epizootiological and Experimental Studies of Skin Tumors in Northern Pike (*Essox lucius L.*) in the Baltic Sea. International Conference on Tumors in Lower Animals. Cork, Ireland. Sept. 1974.
5. MAWDESLEY-THOMAS, L. E. 1962. Some Tumors of Fish. Symp. Zool. Soc. Lond. No. 30: 191-283.
6. NICHOLSON, B. L. and C. BRYNE. 1973. An Established Cell Line from the Atlantic Salmon, (*Salmo salar*). J. Fish. Res. Bd. Canada 30: 913-916.
7. SCHUBERT, G. H. 1966. The Infective Agent in Carp Pox. Bull. Off. Int. Epiz. 65: 1011-1022.
8. WALKER, R. 1971. Fine Structure of a Virus Tumor of Fish. Am. Soc. Zool. 1: 395-396.
9. ————. 1969. Virus Associated with Epidermal Hyperplasia in Fish. Nat. Cancer Inst. Monogr. 31: 195-207.
10. WELLINGS, S. R., R. G. CHUINARD, R. T. GOURLEY and R. A. COOPER. 1964. Epidermal Papillomas in the Flathead Sole (*Hippoglossoides elassodon*), with Notes on the Occurrence of Similar Neoplasms in Other Pleuronectids. J. Nat. Cancer Inst. 33: 991-1004.
11. ————, ———— and R. A. COOPER. 1967. Ultrastructural Studies of Normal Skin and Epidermal Papillomas in the Flathead Sole (*Hippoglossoides elassodon*), with Notes on the Occurrence of Similar Neoplasms in Other Pleuronectids. J. Nat. Cancer Inst. 33: 991-1004.
12. ————. 1969. Environmental Aspects of Neoplasia in Fishes. In: *Fish in Research*. O. W. Neuhaus and J. E. Halver eds. Academic Press, New York.
13. WINDQVIST, G., O. LJUNGBERG and B. HELLSTROEM. 1968. Skin Tumors of Northern Pike (*Esox lucius L.*) II. Viral Particles in Epidermal Proliferations. Bull. Off. Int. Epiz. 69: 1023-1031.
14. WIREN, B. 1971. Vartzjuka Hos Lax (*Salmo salar L.*). Swedish Salmon Research Institute—Report LFI Medd.
15. WOLF, K. 1972. Advances in Fish Virology: A Review 1966-1971. In: *Diseases of Fish*, ed. by Mawdesley-Thomas, L. E. Symp. Zool. Soc. Lond. 30: 305-331.

Received for publication 28 September 1976