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VIRAL DISEASES OF FISH: FIRST REPORT OF CARP POX IN GOLDEN IDE (*LEUCISCUS IDUS*) IN NORTH AMERICA

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ABSTRACT: Carp pox, a putative viral disease exotic to North America, occurred in golden ide 1 yr after the fish were imported into the United States from the Federal Republic of Germany. The raised, white, plaque-like lesions, which occurred on about 5% of the fish, healed spontaneously and caused no mortality. Electron micrographs showed herpesvirus-like particles associated with lesion specimens; however, no infectious viruses were detected in tests with seven warmwater fish cell lines.

INTRODUCTION

Carp pox or epithelioma papillosum is a benign epidermal hyperplasia of putative viral etiology. The disease is widely distributed in Europe, Asia, Russia, and Israel and affects primarily cyprinids, in particular the common carp, *Cyprinus carpio*. The superficial proliferative lesions that characterize carp pox are milky-white to gray and project 1 to several millimeters above the normal skin. The disease is benign; lesions eventually slough and the epithelium heals.

Eurasian cyprinids were successfully introduced into North America in the 1870's (Bartlett, 1905; Cole, 1905). Despite assertions that epithelioma papillosum is widespread among common carp in North America, only two reports of the disease have been published (Nigrelli, 1948; Sonstegard and Sonstegard, 1978).

The present report describes an outbreak of carp pox in golden ide, *Leuciscus idus*. The disease appeared 1 yr after the fish were imported into the United States from the Federal Republic of Germany.

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MATERIALS AND METHODS

In spring 1981, 500 ostensibly healthy fingerling golden ide were obtained from a West German supplier. The fish, judged to be about 6 mo old on the basis of their length (5–8 cm), were reared under quarantine in earthen ponds and grew to 20–25 cm by autumn. No unusual physical or behavioral signs were noted. When examined in March 1982, however, about 5% of the fish showed nodular lesions, tentatively diagnosed as carp pox (Fig. 1).

The lesions were distinct, raised, white, plaque-like areas on the dorsum. Infected fish healed spontaneously during the next 2 mo, and no mortality occurred. The fish did not appear stressed, and when the lesions healed there was no evidence of pigment deposition. The fish were grown to maturation (30–35 cm) through 1982, and no lesions were evident throughout the summer and autumn. In March 1983, postwintering examination showed a recurrence of carp pox, again at the 5% prevalence observed the previous year. The lesions were more extensive. Affected fish were often totally covered by an opaque whitish film, as though the lesions had coalesced. Because of the recurrence of the disease, the fish were killed.

Lesion bearing scales and fins were processed for electron microscopic examination using a modification of the method of Hoyer et al. (1979). The specimens were fixed in 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3), washed twice in buffer, and fixed with 1% OsO_4 in buffer. The preparations were stained with 1% aqueous uranyl acetate, dehydrated with a graded series of ethanol, and infiltrated and embedded in Spurr's low viscosity medium. Thin sections were cut with an LKB Ultratome III, stained with Reynold's lead citrate, and examined in a Hitachi HU-12A electron microscope at an accelerating voltage of 75 kV. Particle dimensions

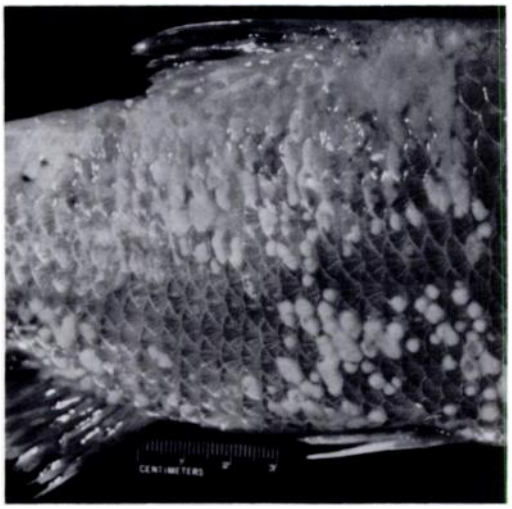

FIGURE 1. Golden ide, showing nodular carp pox lesions.

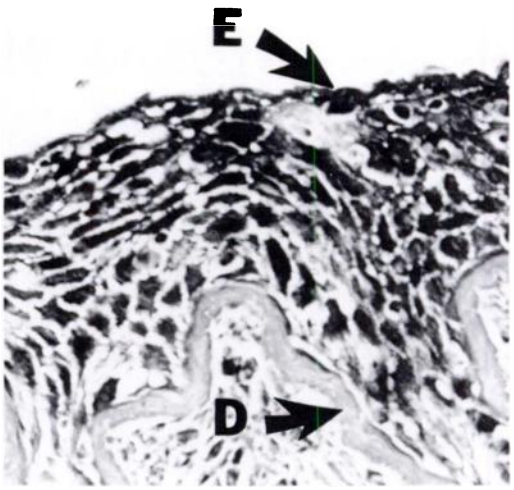

FIGURE 2. Histologic section of carp pox lesion from fin tissue of a golden ide showing hyperplastic epidermis (E) and thickened compact layer of the dermis (D). Giemsa, ×550.

were determined from measurements of 100 virions.

Portions of specimens fixed in glutaraldehyde plus paraformaldehyde, as described above, were retrieved after months of storage, dehydrated in alcohol, cleared with xylene, and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin, Masson's trichrome, or May-Grünwald Giemsa, and examined with a Leitz Laborlux 12 microscope.

Lesion material was removed from affected scales and fins and immediately assayed for infectious virus (McDaniel, 1979). Tissues were homogenized with chilled mortars and pestles. The homogenate was suspended at 1:10 dilution in phosphate buffered saline (PBS, pH 7.4) containing penicillin (100 IU/ml) and streptomycin (100 μg/ml). Tissue debris was removed by centrifugation at 1,500 *g* for 30 min at 4 C. The supernatant fluids were recovered and brought to a final 1:25 dilution in PBS. Drained, preformed monolayers of cells of American shad (AMSH), bluegill fry (BF-2), brown bullhead (BB), epithelioma papillosum of carp (EPC), fathead minnow (FHM), gold shiner fin (GSF), and goldfish fin (CAR) in 25-cm² plastic flasks were inoculated with 0.3 ml of the diluted lesion homogenate. Flasks were inoculated with PBS as a negative control; all assays were performed in duplicate. The inoculum was allowed to adsorb for 2 hr at 20 C. Cells were overlaid with 3.0 ml of Eagle's minimum essential medium containing 5% fetal bovine serum and antibiotics indicated above. Incubation was continued at 25 C. An additional set of cell cultures was pretreated with 50 μg/ml 5-iodo-2′-deoxyuridine (IUDR), according to the procedure of St. Jeor and Rapp (1973). Cultures were inoculated and incubated as described above and were observed daily for developing cytopathic effect. After 10 days, overlay medium and cells were removed for use as blind passage inoculum. Fresh cell cultures were inoculated as described above using undiluted and 1:10 and 1:100 dilutions of the overlay medium and cells recovered from the original assay cultures. The blind passage cultures were incubated at 25 C and observed daily for developing cytopathic effects.

RESULTS

Histological examinations were made of lesions taken from both scales and fins. The scales showed hyperplastic epidermal cells with possible hyperplasia of dermal elements. The fin lesions (Fig. 2) showed a similar hyperplastic epidermis, but no mucus or alarm substance cells. The compact layer of the dermis appeared somewhat thickened. Tissue eosinophils and lymphocytes were evident in the loose connective tissue or hyperdermis, thus suggesting an inflammatory response. Intranuclear inclusions could not be distinguished in either scale or fin lesions.

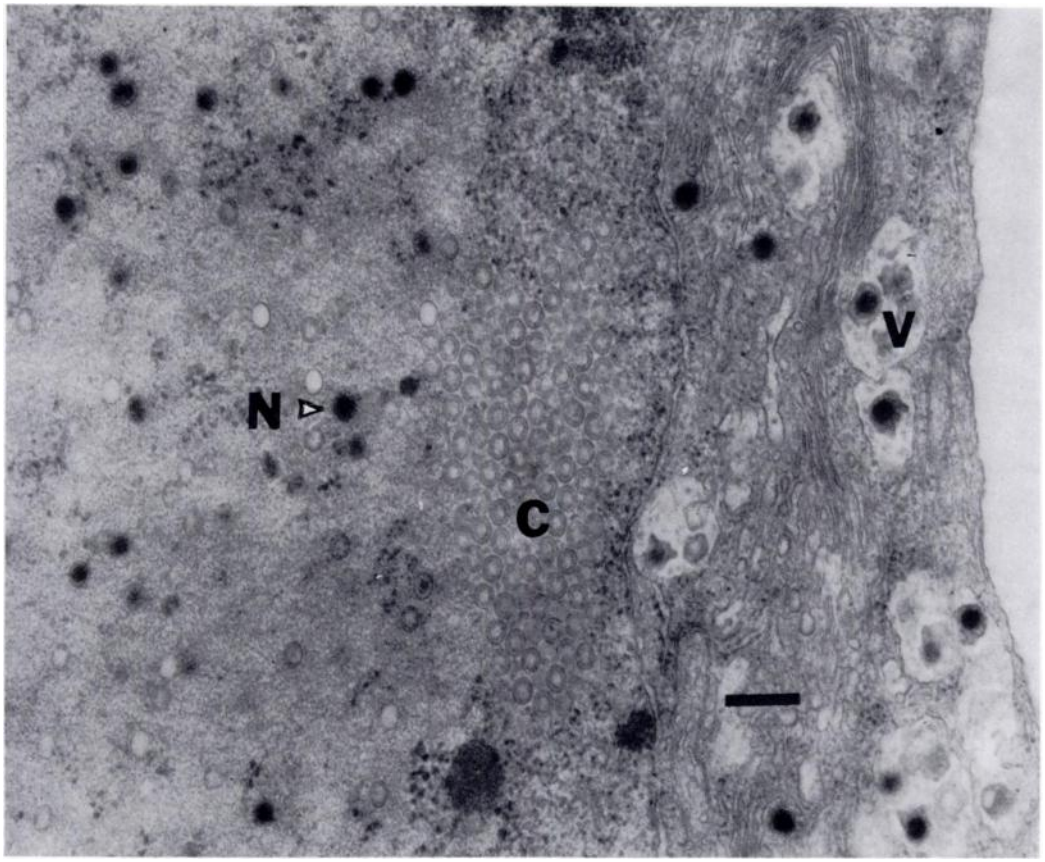

FIGURE 3. Electron micrograph of carp pox lesion from a golden ide showing intranuclear accumulations of electron dense encapsidated nucleoids (N), and translucent capsids (C), and intracytoplasmic enveloped virus (V). Bar = 200 nm.

No evidence of cytopathogenic virus was found in inoculated cell cultures or in blind passaged culture medium. All cultures maintained normal morphological characteristics throughout incubation. Pretreatment of cells with IUDR did not stimulate virus replication and release.

Electron microscopy of thin-sectioned lesions revealed intracellular virus-like particles with the morphology and apparent mode of replication of a herpesvirus. The virions measured ~115 nm in diameter and were composed of an envelope, a capsid ~60 nm in diameter, and an internal nucleoid ~53 nm in diameter. Viral replication was evident in the nucleus, where encapsidated, electron dense nucleoids had accumulated (Fig. 3). Intranuclear accumulations of electron translucent capsids, ostensibly incomplete or defective particles, were occasionally observed. Capsid envelopment occurred at nuclear and cytoplasmic membranes, and presumably mature virions appeared in membrane bound aggregates in the cytoplasm (Fig. 4). The aggregates remained cell-associated.

DISCUSSION

The benign epidermal hyperplasia commonly known as carp pox or epithelioma papillosum affects primarily the common carp but also occurs in other cyprinid species. Intensive trafficking of fish

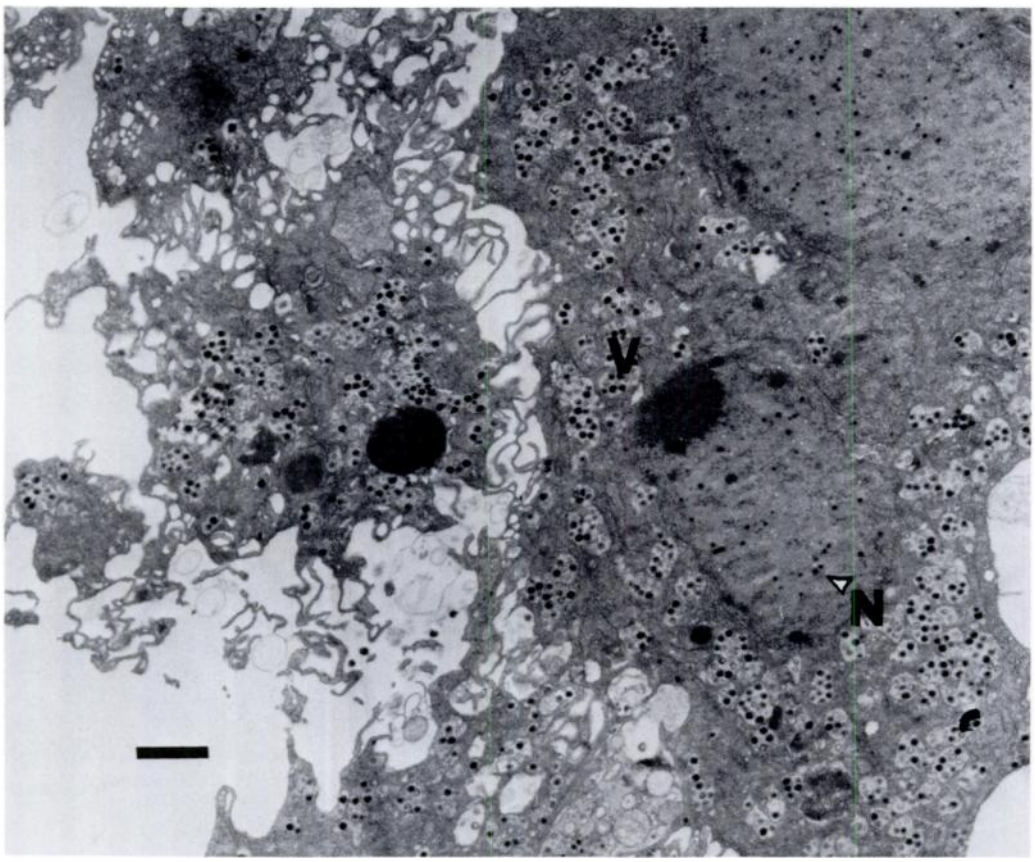

FIGURE 4. Electron micrograph of carp pox lesion from a golden ide showing intranuclear encapsidated nucleoids (N) and intracytoplasmic membrane bound aggregates of enveloped virus particles (V). Bar = 1 μm.

has no doubt contributed to the spread of the disease, which is now enzootic in eastern and western Europe, Asia, and the Middle East. Eurasian cyprinids were introduced into North America in the 1870's, but the appearance of carp pox has been rare. Only two papers document the appearance of the disease. Nigrelli (1948) reported carp pox-like lesions in bluegills (*Lepomis macrochirus*), and Sonstegard and Sonstegard (1978) described carp pox in common carp and hybrids of common carp and goldfish (*Carassius auratus*).

The disease incident we report involved golden ide imported to the United States from West Germany. This was the first introduction of golden ide at Hunting Creek Hatchery since the original ide fishery was established in the early 1930's. Carp pox had never been observed at the facility. These circumstances suggest that the fish became infected before importation. The mode of transmission of carp pox has not been extensively investigated. Bauer et al. (1969) stated that young fish are infected by brood stock. Empirical evidence indicates that the disease can be transmitted by cohabitation. Carp pox commonly occurs in fish 1–2 yr old or older, and rarely in yearling or younger fish. Whether this age distribution is a host-associated characteristic or a requisite of the infectious process is not known. In the present instance, the infection was not

readily transmitted, since the prevalence in successive years was about the same though lesions on individual fish became more extensive.

The clinical manifestations and histological and electron microscopic observations parallel those of Hines et al. (1974), Nigrelli (1954), Schlumberger and Lucke (1948), and Schubert (1964, 1966). The pathological changes associated with carp pox were confined essentially to the epidermis. The squamous epithelial cells became hyperplastic and their proliferation disrupted the normal strata of the epidermis and reduced the number of mucus and alarm substance cells. In our case we also observed accumulations of tissue eosinophils and lymphocytes, suggesting a host inflammatory response. Schubert (1964, 1966) reported that intranuclear and intracytoplasmic inclusions were clearly evident by light and electron microscopy. Inclusions were not evident in our preparations, perhaps due to reduced staining quality. Staining was less than optimal probably because specimens were stored in fixative for several months before paraffin embedding.

Defining the etiology of the disease has proved elusive. Electron micrographs showed herpesvirus-like particles associated with lesion specimens—observations consistent with those reported by Schubert (1964, 1966). The particle dimensions reported here (enveloped particle ~115 nm, capsid ~60 nm, and nucleoid ~53 nm) differ somewhat from those reported by Schubert (~140 nm, ~110 nm, and ~50 nm, respectively), but may merely reflect differences in specimen preparation. The morphology and intranuclear site of replication support assignment of the virion to the herpesvirus group. We were unable to detect infectious virus, using seven warmwater fish cell lines. Nor were we able to stimulate virus replication and release by using IUDR pretreated cells as described for other herpesviruses (St. Jeor and Rapp, 1973).

Recently, Sano et al. (1984) reported isolating a herpesvirus from carp pox tissue taken from Asagi carp (*Cyprinus carpio*). The virus was isolated using EPC and FHM cells, and cytopathic effects were evident in 2–3 wk at 20 C. Attempts to transmit the disease gave equivocal results—both the control and inoculated fish developed the papilloma.

Although we were unable to isolate virus, our electron microscopic observations and those of others, and the recent virus isolation reported by Sano et al. (1984), suggest that carp pox is most probably caused by a virus and that the agent is a herpesvirus. Although carp pox is a chronic fish health problem in many parts of the world, the disease is exotic to North America. Therefore, to avoid further introductions, imported cyprinids should be taken only from stocks specifically free of the disease.

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