

## NOTE

## Systemic granuloma in goldfish caused by a *Dermocystidium*-like aetiological agent

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**ABSTRACT:** Systemic infection by a *Dermocystidium*-like parasitic organism caused visceral granuloma and mortality in farmed goldfish *Carassius auratus*. Granulomatous nodules occurred mainly in the kidney and spleen and later spread to other visceral organs. Macrophages within the granulomata contained 1 to 20 uninuclear or 1 plurinuclear parasite, sometimes located within a parasitophorous vacuole. The granulomata gradually became necrotic. Parasites were gradually displaced to the periphery and disappeared within 1 to 2 mo. The granulomata however, persisted and continued to expand after the elimination of the presumed aetiological agent. Granulomata regressed into fibrotic capsules 6 to 9 mo after disease onset.

Over the past few years, outbreaks of systemic granuloma in pond-reared goldfish have occurred in several commercial farms in Israel. Such outbreaks have resulted in sporadic mortality, and in several instances mass mortality, of affected fish. In this research note we introduce and describe a systemic granulomatous disease of goldfish and its aetiological agent.

**Material and methods.** Since the recognition of a systemic granulomatous condition in goldfish in 1982 ('Goldfish Kidney Granuloma'; Landsberg & Paperna 1985), farm-reared goldfish, as well as common and koi carp, have been periodically checked for the presence of nodules in the viscera. At 3 affected fish

farms, comet (n = 26, 6 to 21 cm total length, TL), fantail (n = 31, 6 to 8 cm TL) and calico fantail (n = 19, 4 to 8 cm TL) varieties of goldfish were examined in late spring 1984, along with common carp (n = 40, 5 to 17 cm TL) and koi carp (n = 31, 6 to 14 cm TL). Samples were taken from the head and trunk kidneys, liver, spleen and heart for the following analyses: (1) fresh squashed tissue was directly examined by Nomarski interference microscopy; (2) smears were air-dried and methanol-fixed for Giemsa stain; (3) samples were fixed in 10 % neutral buffered formalin for histology. Fixed tissue was embedded in glycol methacrylate (Lulham 1979); 3 to 4 µm sections were made using a JB 4 Sorval glass knife microtome and stained in Meyer's Haemalum eosin (MH-E) or PAS.

**Results.** Since 1982, systemic granuloma has been reported in comet, fantail and calico fantail varieties of goldfish, but has not been found in carp or koi carp in any farms in Israel.

**External gross signs:** Exophthalmia, raised scales and swollen abdomen, occurring only in advanced cases.

**Gross pathology:** Grey-white nodules appear initially in the trunk kidney and then spread to the head kidney and spleen. In late infections 0.5 to 3.0 mm nodules also occur in other visceral organs, notably the liver, mesenteries and heart. Both kidneys and spleen swell; the trunk kidney sometimes swells so extensively that it presses against the side of the abdominal body wall, resulting in haemorrhagic ulceration, or even in the wall's perforation.

**Microscopy:** Lesions were primarily characterized by well-circumscribed granulomata of hypertrophic

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macrophages (Figs. 1 & 2) infected with 1 to 10 (exceptionally up to 20) round to oval, 2.0 to 3.5 × 2.5 to 5.0 μm (n = 15 in a fresh preparation) uninuclear *Dermocystidium*-like organisms (Figs. 3, 4 & 5), or with one, 5 to 10 × 4 to 7 μm (n = 5) plurinuclear parasite (Fig. 6). Some uninuclear forms contained a vacuole (Fig. 6, v). In part of the infected cell, parasites were located within a parasitophorous vacuole. Parasite nuclei multiplied by asynchronous successive binary divisions (Fig. 6, b). Larger nodules contained a necrotic core of PAS-positive ground substance which was fringed by infected cells and an outer layer of uninfected fixed macrophages (epithelioid), and circumscribed by infiltrating macrophages (monocytes), lymphocytes and a layer of fibroblasts (Fig. 2). Later-stage granulomata were free of parasitized cells (Figs. 7 & 8), eventually becoming fibroblast-encapsulated necrotic nodules (Fig. 9).

**Pathogenesis:** Lesions containing infected macrophages (Figs. 1 & 2) were found in fish (in both 0+ and 1+ age classes) from May through July. Lesions in fish examined after July/August were parasite-free. Nonetheless, granuloma formation progressed and led to morbidity throughout autumn (Fig. 7). Fibroblast-encapsulated necrotic nodules were detected in fish viscera throughout the winter and following spring (Fig. 9).

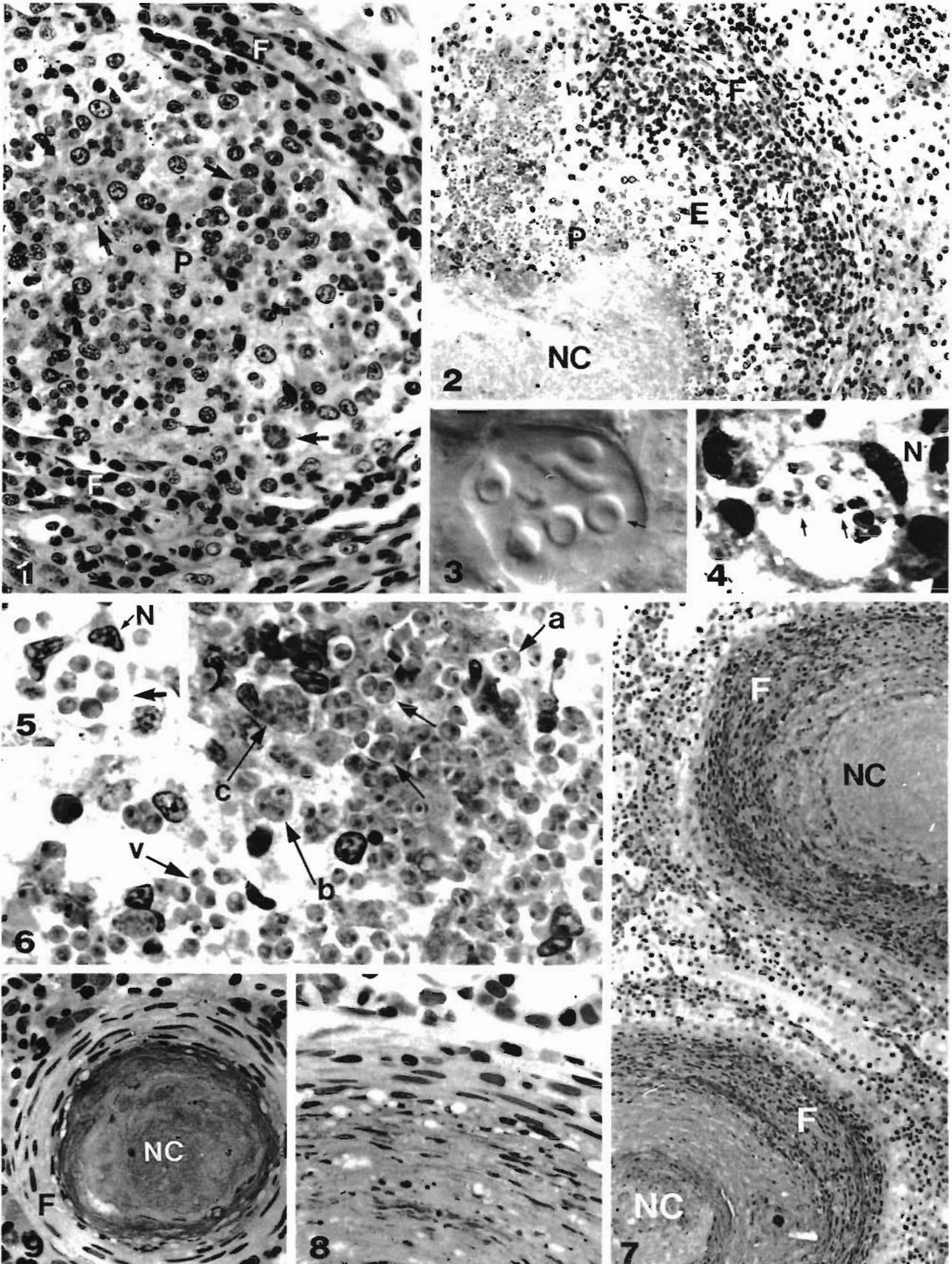
**Discussion.** The presumed aetiological agent detected in goldfish affected by visceral granuloma was very similar to *Dermocystidium macrophagi* or the *Dermocystidium*-like organisms which cause a similar systemic granulomatous disease in salmonids (McVicar & Wootten 1980, Van de Moer et al. 1986, Hedrick et al. 1989) and in carp (Kovacs-Gayer et al. 1986). Organisms similar to those presently reported from goldfish were detected in granuloma-affected

goldfish in the USA, and following light and electron microscopic study were regarded as hartmanellid amoebae (Voelker et al. 1977). However, no conclusive evidence has as yet been provided regarding the classification of these organisms. Their taxonomic affinity remains controversial as does their relationship with other organisms termed *Dermocystidium* causing integumentary infections in fish (Cervinka et al. 1974, Wootten & McVicar 1982) and oysters (*D. marinum* = *Perkinsus* spp.; Perkins 1974). Ultrastructural affinities of the integumental *D. salmonis* as reported recently by Olson et al. (1991) are convincingly different from the causative agent, also named *Dermocystidium*, of the salmonid systemic granulomatosis as described by Van de Moer et al. (1986) and Hedrick et al. (1989).

The absence of infection in common and koi carp in Israel, even when reared in the same pond, suggests that the organisms described by Kovacs-Gayer et al. (1986) from carp are a different species from that found in goldfish. The pathogenesis of the disease in goldfish is characterized by restriction of the aetiological agent to the early stage of the disease (the acute stage?) while granuloma formation persists (the chronic phase?). This type of pathogenesis has not to date been reported in salmonid and carp systemic infections.

There are several reports of multiple granulomata with unknown aetiology in goldfish viscera (Schlumberger 1950, Stolk 1956, Wakabayashi et al. 1969, Munkittrick et al. 1985). Although a wide range of causative agents (including bacteria and fungi) are known to produce granulomatous changes in fish kidneys (Bendele & Klontz 1975), some of these reported conditions could have initially been induced by *Dermocystidium*-like organisms.

Figs. 1 to 9. *Carassius auratus*. Fig. 1. Granuloma in goldfish trunk kidney containing hypertrophic macrophages (P) infected with *Dermocystidium*-like organisms (arrow) and circumscribed by fibroblasts (F). Histology, MH-E, ×800. Fig. 2. Granuloma in trunk kidney with necrotic core (NC), fringed by infected macrophages (P) and an outer layer of epithelioid (E), and circumscribed by infiltrating macrophages (monocytes) and lymphocytes (M) and a layer of fibroblasts (F). Histology, MH-E, ×400. Figs. 3 to 5. Enlarged views of infected macrophage from the trunk kidney. N: Macrophage nucleus; arrows, uninuclear parasites. Fig. 3, live, in Nomarski interference illumination, ×3000; Fig. 4, Giemsa stained, ×1500; Fig. 5, histology, MH-E, ×1000. Fig. 6. Macrophages from the periphery of granuloma with necrotic core containing uninuclear, binuclear (a), dividing, trinuclear (b) and plurinuclear (c) parasites. Some of these are contained within a parasitophorous vacuole (arrow). Some uninuclear forms contain a vacuole (v). Histology, MH-E, ×1275. Fig. 7. Parasite-free granulomata from goldfish trunk kidney with a necrotic core (NC) and a fibroblast-infiltrated superficial layer (F). Histology, MH-E, ×214. Fig. 8. Enlarged view of the periphery of a parasite-free nodule. Histology, MH-E, ×1000. Fig. 9. Residual fibroblast-encapsulated (F) necrotic nodule (NC). Histology, MH-E, ×800



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