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SUSPECTED VIRAL ERYTHROCYTIC NECROSIS (VEN) IN THE INTERTIDAL FISH *MAULIGOBIUS MADERENSIS* FROM MADEIRA, PORTUGAL

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Suspected viral erythrocytic necrosis (VEN) was detected in two specimens of the intertidal fish *Mauligobius maderensis* (Teleostei: Gobiidae) from Madeira, Portugal. While one host was lightly infected, the other showed intraerythrocytic cytoplasmic inclusions within all mature erythrocytes examined. The inclusions were round to oval, 0·8–2·0 μm in diameter, and most were associated with dense eosinophilic granular areas of various sizes and shapes. Up to three of these granular regions accompanied each inclusion body, but they were sometimes widely separated from it. In a number of infected erythrocytes, a granular halo was observed surrounding the nucleus. The cytoplasm enclosed by the halo often had a different refringence from that outside. None of the 120 other fishes examined from Madeira, representing 43 species of intertidal, pelagic, and deep-sea origin, had detectable infections.

Viral erythrocytic necrosis (VEN) is a term that was suggested by Evelyn & Traxler (1978) for infections of fishes caused by erythrocytic necrosis viruses (ENVs) (see Smail & Munro, 1989). First described as piscine erythrocytic necrosis (PEN) by Laird & Bullock (1969) from three species of fishes from eastern Canada and north-eastern America, VEN had by 1989 been recorded from 21 species of teleosts with a wide geographical distribution (Smail & Munro, 1989).

By light microscopy, VEN is characterized by intraerythrocytic, eosinophilic, cytoplasmic inclusions ~1–4 μm across, which may be accompanied by a cloud of particles and nuclear degeneration (Smail & Munro, 1989). Transmission electron microscopy (TEM) has shown that these changes are associated with virus particles. These viruses are small, medium, or large icosahedral structures, with a bilaminar capsid and often a toroidal core, and they are located in an electron-lucent area of cytoplasm, the viroplasmic matrix (Smail & Munro, 1989).

Pathological effects attributed to ENVs are variable, but they have included alterations in erythrocyte metabolism, increased red cell lysis, erythroblastosis with macrocytic hypochromic anaemia, reduced haematocrit, increased blood clotting times, and possible osmoregulatory difficulty (Smail & Munro, 1989).

In a recent study of the blood parasites of 43 species of intertidal, pelagic, and deep sea fishes (Table 1) from Madeira, Portugal, suspected VEN was observed in the intertidal species *Mauligobius maderensis* (Valenciennes, 1837) Miller, 1981. Some previously unreported observations of this infection from a new host are described here.

Offshore specimens were caught in either experimental or commercial trawls. Intertidal species were captured with benzocaine from pools at low tide on the south coast of Madeira, using anaesthetic procedures similar to those for quinaldine (Davies, 1982). Blood smears taken from the caudal vein or heart were stained with May-Grunwald Giemsa (Langeron, 1942), and examined under oil-immersion. At least 80 fields per blood film were examined under oil immersion.

No haematozoa were detected in any of the fishes examined but blood smears from two of 13 specimens of *M. maderensis* showed changes typical of VEN infection. Only mature erythrocytes

Table 1. *Numbers of fishes with blood parasites of those captured off Madeira, Portugal.*

Fishes examined	No. with blood parasites	Fishes examined	No. with blood parasites
Aphanopus carbo	0/2	*Pagrus pagrus*	0/3
Apogon imberbis	0/8	*Parablennius parvicornis*	0/3
Balistes carolinensis	0/1	*Phycis phycis*	0/1
Beryx decadactylus	0/4	*Polymixia nobilis*	0/2
Boops boops	0/1	*Polyprion americanus*	0/3
Brama brama	0/1	*Pontinus kuhlii*	0/2
Conger conger	0/1	*Promethichthys prometheus*	0/2
Coryphaena hippurus	0/2	*Pseudocaranx dentex*	0/2
Deania profundorum	0/12	*Ruvettus pretiosus*	0/1
Dentex gibbosus	0/2	*Sarpa salpa*	0/1
Diplodus sargus	0/1	*Scomber japonicus*	0/2
D. vulgaris	0/3	*Scorpaena scrofa*	0/1
Helicolenus dactylopterus	0/3	*Seriola* sp.	0/1
Katsuwonus pelamis	0/5	*Serranus atricauda*	0/4
Lepadogaster sp.	0/3	*Sparisoma cretense*	0/1
Mauligobius maderensis	2/13	*Sphyraena viridens*	0/2
Mora moro	0/1	*Synodus* sp.	0/2
Mullus surmuletus	0/5	*Thalassoma pavo*	0/3
Mycteroperca fusca	0/1	*Trachurus picturatus*	0/2
Pagellus acarne	0/1	*Trigloporus lastoviza*	0/1
P. bogaraveo	0/10	*Zeus faber*	0/1
P. erithrinus	0/2	**Total**	2/122

were affected. In one fish 1% of these were infected with inclusion bodies no bigger than 1 μm in diameter, while in the other specimen 100% of mature red cells contained these bodies (Figure 1A–I). In the heavily infected fish inclusion bodies were round to oval, pink staining, 0·8–2·0 μm in diameter, and they lay at the cell periphery, near the nucleus, or at varying distances in between. Near most inclusion bodies in the heavily infected fish, granular material was often arranged in streaks, comma or comet-tail-like shapes (Figure 1A–I), sometimes exceeding the size of the inclusion body.

Occasionally, up to three areas of granular material occurred near, or at some distance from, the inclusion body (Figure 1D). In a number of erythrocytes, a halo bordered by pink staining granules surrounded the nucleus, almost dividing the cytoplasm into two portions (Figure 1A,B,F & H). It was also evident that the cytoplasm enclosed by the halo had a slightly different refringence from that outside it (Figure 1B & H). In both fish, little nuclear degeneration was observed apart from occasional irregular nuclear shape and vesiculation, and nuclear displacement was uncommon. Similar nuclear changes were seen in the erythrocytes of other species of apparently uninfected fishes examined, and sometimes 100% of nuclei were affected.

Unlike our material, Smail & Egglestone (1980) found granules associated with inclusion bodies only when the slow Giemsa staining method was used. Our infections also had more granules than is usually reported for VEN in fishes, and they were arranged in extraordinary shapes. Although unstained haloes have been recorded by light microscopy around the inclusions bodies of VEN from *Lipophrys pholis* (Johnston & Davies, 1973; Smail & Egglestone, 1980), a well stained granular halo around erythrocyte nuclei has not been reported. As a consequence of this feature in *M. maderensis*, the cytoplasm is divided into two areas of different refringence, which may interfere with the oxygen transport capability of the erythrocyte.

As in this study, viral erythrocytic necrosis inclusion bodies have been described only in mature erythrocytes by some authors (Johnston & Davies, 1973; Walker & Sherburne, 1977; Reno & Nicholson, 1980, 1981; Gutierrez et al., 1985). Others have observed inclusion bodies in immature red cells (Meyers et al., 1986). In *Oncorhynchus keta*, MacMillan et al. (1980) observed these structures throughout erythrocyte development by light microscopy, and within early erythroblasts by TEM. Degeneration of erythrocyte nuclei is characteristic of VEN-infected fishes. Sherburne & Bean (1979) considered this feature alone sufficient to diagnose the infection in *Osmerus mordax*. However, nuclear changes can occur in freshwater and marine fishes apparently

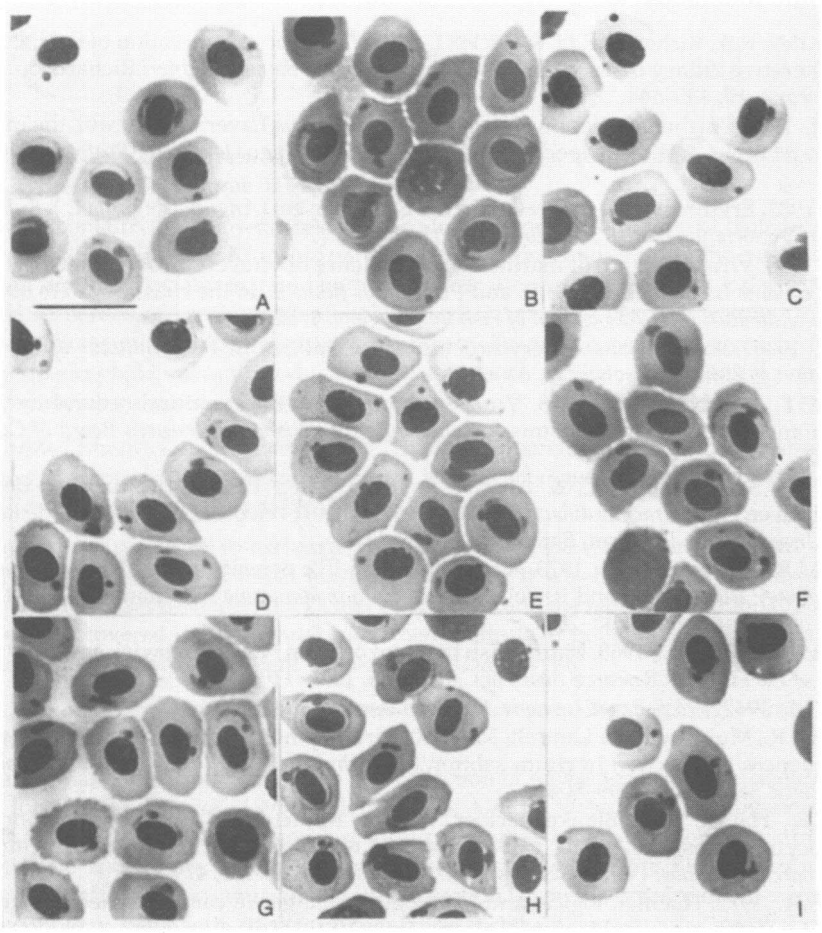

Figure 1. May-Grunwald Giemsa stained blood smears from *Mauligobius maderensis* with suspected viral erythrocytic necrosis (VEN). All mature erythrocytes contain inclusion bodies, and many have one or more granular areas arranged in streaks, commas or comet-tails. A,B,F & H show haloes of pink-staining granules, apparently separating the cytoplasm into areas of different refringence (B & H). Scale bar: 10 μm.

in the absence of VEN (this study, Eiras, 1983, 1990). Such degeneration can be linked to folic acid deficiency, diets containing oxidized oil, exposure to sublethal levels of residual chlorine, cadmium and lead (see Eiras, 1983, 1990), necrotic gill disease (Pilarczyck, 1977), or proliferative kidney disease (Clifton-Hadley et al., 1987). These reports suggest that detection of erythrocyte nuclear abnormalities alone is not enough to diagnose VEN.

Our observations by light microscopy strongly suggest VEN infection of *M. maderensis*, which would extend the geographical distribution and host range for VEN. The condition has previously been reported from North America, the Irish Sea, the North Sea, the Mediterranean coast of Spain, and the west coast of Portugal (Eiras, 1984; Pinto et al., 1989; Smail & Munro, 1989). It is likely that ENVs are widely distributed and probably infect far more host fishes than are currently known.

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