MASS MORTALITIES IN KOI CARP, CYPRINUS CARPIO, ASSOCIATED WITH GILL AND SKIN DISEASE

ACHIM BRETZINGER¹, THERESIA FISCHER-SCHERL¹, MUSTAPHA OUMOUNA¹, RUDOLF HOFFMANN¹ AND UWE TRUYEN²

¹Institute of Zoology, Fish Biology and Fish Diseases, University of Munich, Kaulbachstr. 37, D-80539 Munich, Germany. ²Institute of Medical Microbiology, University of Munich, Germany.

Abstract

In 1997 two and in 1998 six cases of mass mortality in Koi carp (Cyprinus carpio L.) with similar symptoms were examined. Gross examination revealed in each of the cases moderate to extensive epidermal lesions, gill necrosis and enopthalmus. Different ectoparasites were found on the skin only in low numbers, on the gills slight to heavy infestations mainly with protozoan parasites encountered. Pathological anatomy revealed pale internal organs and an enlarged anterior kidney. Different opportunistic bacteria were isolated from inner organs, with Aeromonas sobria in 7/8 cases. Cohabitation trials confirm the infectious nature of the disease to different breeds of Cyprinus carpio. Transmission electron microscopy (TEM) revealed the presence of virus-like particles in the nuclei and cytoplasm of respiratory epithelial cells of gills.

Introduction

Reports from veterinarians, Koi keepers and Koi dealers in Germany (Hannen 1998, pers. comm.), the United Kingdom (Walster 1999), and the Netherlands (Blom 1998) indicate, that in 1998 hobbyist Koi keepers and Koi dealers in different European countries, Israel and the USA suffered from enormous losses with fish dying with similar clinical symptoms. Some reports confirm the eradication of complete pond populations within a few days. The disease is characterised mainly by epidermal lesions, gill necrosis and an acute or peracute course in most cases. Water quality problems could be excluded. Infectious agents such as parasites or bacteria were found, but these organisms were mostly opportunistic, common in Koi carp and could not explain such a tremendous course of disease.

Clinical history

In September 1997 two and from June to December 1998 another six cases of mass mortality in Koi carp with similar symptoms could be observed. The fish were held in garden ponds (4 units with 18 - 56 fish) or Koi dealer units (4 dealers' sales units with approx. 100 - 400 fish each) at temperatures between 17 and 23 °C. Water quality (Oxygen, pH, Ammonia, Nitrite, Nitrate) was in most cases immaculate, only in one case pH, Ammonia and Nitrite were slightly raised. In 7 out of 8 cases new fish had been introduced without proper quarantine between 8 and 21 days before outbreak of the disease in the old population, the latest outbreak occurred in fish that had been kept at 8 - 12°C for months and were then heated up to 21°C. Diseased fish were showing unspecific symptoms such as lethargic swimming, frequent ventilation and anorexia. In typical cases at water temperatures above 20 °C fish behaved completely normal one day, became lethargic the other morning and died a few hours later. At lower temperatures the course was protracted. All sizes of Koi were affected (8 - 67 cm), best feeders usually dying first. Morbidity was in all cases near 100%, mortality ranged between 78% in one pond at 16 – 17 °C water temperature and 100% in ponds with temperature above 20 °C.

In this study Cyprinus carpio was the only species affected, regardless of the origin of the fish (Japanese or Israeli Koi, European "colour carp", European common mirror carp) with certain indications for reduced losses in the European breeds. In some cases goldfish (Carassius auratus auratus) or sturgeon (Acipenser sp.) were held together with Koi but none of these fishes were affected by the disease.
Fig. 1 Common mirror carp, Fig. 2 Koi (Ogon): Circumscribed pale discoloration of the skin (arrows). Fig. 3: Lesions of the extremities of the left pectoral fin of a Koi. Fig. 4: Severe gill necrosis and hemorrhagic inflammation in a Koi (Asagi) with the protracted course of the disease. Fig. 5: Excessive mucus production and swelling of the tips of gill lamellae in a Koi with the peracute course of the disease. Fig. 6: Extensive erosions of the primary lamellae of one complete gill arch (arrow), besides a gill arch with only little swelling of the epithelium. Fig. 7: Respiratory epithelial cell of a Koi. Nucleus (n), cytoplasm (c). Intranuclear and intracytoplasmic virus-like particles (arrows). Fig. 8: Nucleocapsids (arrows). Scale bar 200 nm

Materials and Methods
From each case one or two moribund Koi were examined. The fish were anaesthetised in 1.5% Chlorbutanol and subjected to a standard necropsy including histology and bacteriology. Transmission electron microscopy (TEM) was done from one, virology from two cases so far.
In addition, cohabitation trials were carried out:
In trial 1 two live but apparently sick Koi from one case were held in cohabitation with two Japanese and two European Koi as well as two Mirror Carp at a temperature of 23 °C in a 200-l-tank with water flow-through and aeration. These fish were 1+ to 2+ years old, sized 18 - 27 cm and had been held at the institute for at least 6 months. In trial 2 should be tested, if the infection was transmitted successfully with material from frozen fish. The same two sick fish that had died in trial 1 were frozen at -18 °C for 14 days and then, were cut in slices (max. 2 cm thick) and added in a gauze net (0.5 mm mesh size) to 5 Koi in a tank described above. The gauze net was removed after four days.

Results
The main pathological-anatomical findings of the disease were disorders of the external epithelia. In all the cases a focal or complete loss of the epidermis with pale discoloration and only little reddening of the lesion’s margins was seen (Figs. 1 & 2), giving the skin a sandpaper-like texture where underlying scales were present. Erosions on the extremities of the fins (Fig. 3) were as common as Enophthalmus. The gills showed an increased production of mucus, and in the peracute -acute course swelling of the tips of primary and secondary lamellae (Fig. 5). In the more protracted course of the disease gill necrosis and inflammation developed (Fig. 4), leading sometimes to extensive erosions of the primary lamellae of one complete gill arch (Fig. 6), in other cases gill necrosis showed a more focal pattern. The inner organs were mostly pale. A more or less enlarged anterior kidney was commonly seen.

Light microscopical examination
Microscopic examination of haematoxylin and eosin stained tissue sections revealed different histopathological findings depending on the time fish were suffering from the disease. The gills which are predominantly affected showed a strong infiltration of lymphocytes in the primary lamellae. In more severe cases eosinophilic granulocytes emerging mostly in the epithelium of the primary lamellae were seen, but also in the respiratory epithelium of the secondary lamellae. The tips of the primary lamellae were totally necrotised with masses of bacteria. Respiratory epithelial cells lifted from the basement membrane and necrotic areas were common. In a few degenerating respiratory epithelial cells, eosinophilic intranuclear inclusions were detectable. Necrotic foci were also present in the gut mucosa accompanied with lymphocytic infiltration in the liver and in the pancreatic tissue. Overlying epidermis of the skin was separated from the basement membrane with loss of the specialised cells such as mucus and club cells giving a ballooning impression. Musculature seems to be unaffected.

So far electron microscopy has been done from samples of gills from one case. Examination of ultra-thin sections from gill lamellae revealed the presence of virus-like particles both intranuclearly and in the cytoplasm of respiratory epithelial cells (Fig. 7). Nucleocapsids are either full or empty (Fig. 8). The particles measure 80 - 100 nm in diameter. Particles in the cytoplasm are often located within a membrane-bound vacuole. Capsids are also found enveloped in the perinuclear cisternae.

In the parasitological examination different parasite species without clear pattern were seen. Parasites on the skin were either missing or sometimes found in high numbers, whereas opportunistic protozoan ectoparasites on the gills were very common with high infestation rates. On the skin, *Ichtyobodo necator*, *Trichodina sp.*, *Chilodonella cyprini* and *Ichthyophthirius multifiliis* were found. The gills were parasitised by the same species and in one case by *Dactylogyrus sp.* Bacteriological examination of liver, spleen and kidney resulted in the isolation of mainly opportunistic bacteria with a non homogeneous pattern. *Aeromonas sobria* dominated the isolates with 7 isolates out of 8 cases. The other bacteria (number of cases in...
brackets) were: Gram positive rods and cocci (4), *Citrobacter freundii* (2), *Acinetobacter sp.* (1), *Aeromonas hydrophila* (1), *Shewanella putrefaciens* (1), *Pasteurella sp.* (1), *Flavobacterium sp.* (1).

The virological examination of organ material from gills, liver, spleen, kidney and anterior kidney of Koi from two cases via FHM-, BF2-, EPC- and RTG-cells was negative.

Cohabitation trial 1 resulted in the potentially infective, diseased fish dying after one day. 7 to 10 days after the start of the experiment, the typical symptoms of the disease could be observed in all fish with the exception of 1 „Euro-Koi“. All these fish died or were euthanised with heavy signs of the disease.

Trial 2 with the frozen material could not produce infection within 6 weeks.

**Discussion**

There is no doubt that in 1998 severe mortalities of Koi carp occurred in different European countries, Israel and the USA. The symptoms are similar but unspecific and as the etiological agent could not be identified until now, diagnosis must be based upon clinical findings and exclusion of other causes. The infectious nature was suspected after following the reports from other countries (Walster 1999) and the case histories in this study, and later proven by our own cohabitation trials. The fact, that German Mirror Carp were infected, is considered as being most important, as the European aquaculture industry might be affected by an epidemic of this new disease.

The clinical signs differ from every other disease with an infectious aetiology known in Koi and common carp so far. In the typical course, epidermal lesions are completely different from erythrodermatitis or skin ulceration, as haemorrhages in the skin, common in bacterial skin disease in fish, are missing. Furthermore, *Aeromonas salmonicida* could not be isolated in any of the cases. The loss of epidermis with the sandpaper-like texture of the skin is very typical for the disease and can otherwise be seen in Koi only in extremely deteriorating water conditions (pH, ammonia) or associated with the acute infestation of a high number of ectoparasites.

The peracute course of the disease, its infectivity and the high mortality indicate that a viral background of the disease must be taken into account. The viral particles detected in the respiratory epithelial cells of destroyed gill lamellae share features of members of Herpes viruses (intranuclear location, structure and size). Studies are done to select a cell line for virus isolation and characterisation. For more detailed morphological studies further organ samples are on their way to being examined by means of transmission electron microscopy.

**Acknowledgements**

The authors wish to thank Christine Vogt for preparing the ultrathin sections for TEM.

**References**

