Short Communication

Syncytial hepatitis of farmed tilapia, *Oreochromis niloticus* (L.): a case report

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Compared to other fish, tilapia are considered to be relatively disease-resistant animals, especially to many of the common pathogens that target intensively reared fish. They are still susceptible to protozoan parasites and to some bacteria, notably streptococcal infections, with both Streptococcus iniae and Streptococcus agalactiae frequently incriminated. Viral diseases, however, are not common, and there are only a few reports in the literature (McGrogan, Ostland & Ferguson 1998; Bigarré et al. 2009; Shlapobersky et al. 2010). Hepatic involvement in systemic infections in fish tends to be less than in mammals, possibly partly due to the lack of Kupffer cells (Wolf & Wolfe 2005). Certainly, this is reflected experimentally when fish are injected intravenously with radiolabelled bacteria, and their distribution subsequently tracked. The great majority end up in kidney and then spleen, and not in the liver as would be seen in mammals (Ferguson et al. 1982). Nevertheless, hepatic involvement is routinely seen, especially in viral infections. The increasing vagaries and longterm changes in global climate have been mirrored by a greater incidence of algal blooms, some of which have resulted in toxic hepatopathies. Investigations into major disease outbreaks in fish where the liver is a major target should, therefore, consider both infectious and toxic aetiologies. This study describes an outbreak of severe mortality in young farmed tilapia, restricted largely to one strain of the same species, *Oreochromis niloticus* (L.). To try and provide a case definition for the syndrome, particular attention is paid to the pathological changes which are characterized by necrosis of the gastro-intestinal tract and by distinctive hepatocellular syncytial cell formation.

The present case involves intensively reared O. niloticus fingerlings that were collected for diagnostic work-up following several months of above-normal mortality. The involved farm has a genetic improvement programme, and only its own farm-bred tilapia 'Chitralada' were affected to a significant and worrying degree. Another strain of tilapia, genetically all male (GMT - also O. niloticus), bought in from another producer remained largely unaffected. Typically, fish were transferred as fry/fingerlings from small hatchery ponds into larger on-growing ponds with the same recirculated water supply. Basic water quality parameters such as oxygen levels, temperature and total ammonia were all well within limits acceptable for this species. Mortality would be observed to start 4-7 days post-transfer. Fish at this time were each approximately 3 cm long and weighed roughly 3 g. Survivors from affected ponds would often total less than 20% of the number stocked, compared to 80-90% survival in the GMT. Gross lesions in moribund fish typically included

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darkening, abdominal distension, scale protrusion and exophthalmia in most (Fig. 1), although a few developed a progressively emaciated appearance. The fluid in the abdominal cavity was watery and colourless. Gill pallor was also seen.

Samples for diagnostic work-up included moribund Chitralada, apparently normal fish from affected ponds and clinically normal GMT of approximately the same age. They were sampled on 4 separate occasions, May, July and November 2011, and May 2012; temperatures in these months were 27, 25, 25 and 27 °C, respectively, while salinity levels were 3, 3, 4.5 and 3ppt, respectively. A total of approximately 250 fish were sampled overall. Samples comprised blood smears, packed cell volume measurements and whole fish with their abdomen opened up and fixed in 10% buffered formalin (for light microscopy - LM). Alternatively, the liver was dissected out, one half fixed in 10% buffered formalin and the other half fixed in 2% glutaraldehyde in a cacodylate buffer (for transmission electron microscopy - TEM). For light microscopy, routine paraffin wax sections were stained with haematoxylin and eosin (H&E). If sections from the dissected-out livers looked on LM to be worthy of further investigation, the other half was processed for TEM. Glutaraldehyde-fixed liver samples were kept at 4 °C prior to standard processing through 1% osmium tetroxide and resin embedding for ultrathin sectioning. Blood smears were stained by Diff-Quik (Protocol, Hema 3 Stain set, Fisher Diagnostics, Fisher Scientific Co.).

Histopathological examination showed that major lesions were restricted largely to the liver and gastro-intestinal tract (GIT). Hepatic changes



Figure 1 Two tilapia showing typical gross lesions of distended abdomen and exophthalmos.

were variable but were often dominated by ongoing necrosis of hepatocytes and syncytial cell for-There was limited accompanying mation. inflammation. Changes were characterized by the presence of multiple foci and focally extensive areas of necrosis of hepatocytes and all other cell types within the areas of necrosis, including fat-storing cells (Fig. 2). Less severe lesions seemed to start around sinusoids, the location of fatstoring (cells of Ito) and other cells (Fig. 2). Brightly eosinophilic or brown lipoproteinaceous material (probably ceroid) was present within the cytoplasm of hepatocytes and some of the syncytial cells. These inclusions varied in size from 1-2 mm up to 5-6 mm. Many surviving hepatocytes had a foamy cytoplasm, and in severely affected fish, little normal cellular architecture remained. Syncytia contained from 3-4 up to sometimes 15-20 nuclei (Figs 3 & 4). Bile ducts appeared to be largely uninvolved, although if adjacent to areas of hepatocellular necrosis, pancreatic exocrine cells were sometimes also affected (bystander necrosis?). Extrahepatic pancreatic acini were mostly unaffected, although in a few severely diseased fish, there was evidence of acinar necrosis with mild accompanying inflammation. Changes in the GIT comprised focally widespread necrosis of gastric glands, extending deep into the crypts; eosinophilic material was present at varying levels within the glands, presumably necrotic debris (Fig. 5). In some cases, an influx of inflammatory cells into the submucosa accompanied the necrotic changes. In addition, many fish had proteinaceous casts within the lumen of the intestine, sometimes with bacteria throughout the pale eosinophilic material. Minor lesions included evidence of mild activation of



Figure 2 Liver from moribund tilapia showing focally extensive necrosis of hepatocytes, (H&E, ×110).



Figure 3 Liver from moribund tilapia showing syncytial giant cells (arrow), (H&E, ×400).



Figure 4 Liver from moribund tilapia showing dissociation of hepatocytes often containing lipoprotein-like droplets, degeneration and single cell necrosis, plus early hepatocellular syncytial cell formation. Moderate diffuse inflammation is also seen, (H&E, ×220).

serosal mesothelial cells, no doubt a response to the fluid in the abdomen. There was diffuse congestion of many tissues, necrosis of renal portal macrophages and the accumulation of eosinophilic debris–laden macrophages within the renal interstitium and multifocal acute necrosis of skeletal muscle fibres. Debris–laden macrophages were easily seen within splenic ellipsoids and sinusoids, as well as within the vessels of the gills, but in addition, the latter tissue had necrosis in the progenitor zone at the base of lamellae, possibly including chloride cells. Some fish had scattered digenean metacercariae, typically in the gills, but occasionally in or around the bulbus arteriosus – these parasites were considered to be incidental, and not



Figure 5 Stomach from moribund tilapia showing extensive loss of gastric glands – arrow. Compare with relatively normal area above, (H&E, ×110).

contributing to the mortality. Clinically apparently normal fish had lesions similar to those seen in the moribund fish, but they were much less severe, and most of them still had food in their GIT. GMT fish had no significant lesions except for a few metacercarial digenean parasites in gills.

Packed cell volumes were variable but in the most obvious cases were roughly one-third of normal (16% v. a normal of 48–50%). Blood smears showed variable changes but overall, there were increased numbers of immature erythrocytes (indicating a responsive anaemia) and moderate to large numbers of what appeared to be large blasttype lymphocytes. In some smears, leucocytes were very vacuolated and showed further evidence of degenerative changes such as blebbing of the cell membrane. There was some evidence of erythrophagocytosis.

Ultrastructurally, it was easy to identify multinucleated cells (Fig. 6). The dense intracytoplasmic inclusions were also easy to see, and their appearance suggested that in some cases they represented the necrotic remains of cells, while in others, their lamellated appearance suggested lipofuscin. Nuclei often had dispersed chromatin, and mitochondria appeared enlarged and in some cases to have lost cristae (Fig. 7). Numerous examples of virus-like particles were present within the cytoplasm of hepatocytes and the space of Disse, where disruption of hepatocyte processes could be seen. These particles (Fig. 8) were consistently round to oval, 60-70 nm in diameter with a central variably electron-dense core, delimited by a capsid-like trilaminar structure composed of three



Figure 6 Electron micrograph of liver from moribund tilapia showing multinucleated hepatocyte.



Figure 7 Electron micrograph of hepatocyte from moribund tilapia showing swelling of mitochondria and loss of cristae (arrows).

thin bands (dense, lucid and dense) and an approximately 10–15 nm wide electron dense, poorly defined area from which projected denser structures at regular intervals. These virus-like particles were usually seen singly or in small groups and to be associated with rough-surfaced endoplasmic reticulum, but large arrays or assemblages of virions were not seen, either in the cytoplasm or nucleus.

The cause of this unusually high mortality is unknown. The findings allow for the possibility that this is a viral disease, but they are far from conclusive, and much more work needs to be done to unravel the aetiology. The pathogenesis of the disease and cause of death are also unknown, but the gross lesions of anasarca combined with the histopathological findings support the concept that there is a protein-losing enteropathy. The severity of the liver changes would probably lead to compromised plasma protein production overall, and the lesions in the gastro-intestinal tract



Figure 8 Electron micrograph of hepatocyte from moribund tilapia showing typical appearance of virus-like particle within cytoplasm (arrow).

would mean that there could be a compounding reduction in efficiency of nutrient uptake combined with protein loss into the lumen of the intestine. To complicate things yet further, the bacterial colonization of the consequent casts could result in some uptake of endotoxin. While it is common for fish with acute systemic disease, especially those with a viral aetiology, to go off feed, the severity of the lesions in the gastro-intestinal tract would doubtless contribute to any loss of appetite. The small size of the fish (roughly 3 g each) precluded blood chemistry, which would have been critically useful in investigating hypoproteinaemia as a cause of death.

Severe hepatocellular degeneration and necrosis can also be the result of toxins, and in a largely recirculation system such as this farm, algal toxins seem a logical possibility. Bizarre changes to hepatocytes and their nuclei are seen with algal toxins such as microcystin, especially as part of the repair/regeneration process, but in fish, syncytial cell formation is not described (Fisher et al. 2000; Wolf & Wolfe 2005; Evenson 2006). In humans, giant-cell hepatopathy is seen in children in the first 3 months of life. Agents associated with this disease in infants include the viruses of hepatitis A, B and C, as well as those of human immunodeficiency virus (HIV), Epstein-Barr virus (EBV) and paramyxoviruses. Drug-induced causes of similar lesions in humans include p-aminosalicylic acid, chlorpromazine and vinyl chloride (Estradas et al. 2009). In the present case, many of the eosinophilic lipoproteinaceous inclusions within the hepatocytes were considered to be most likely necrotic hepatocytes that had been endocytosed by adjacent viable cells (apoptotic or Councilman-like bodies). In some livers, however, the sometimes extensive nature of these accumulations suggested the possibility that they were ceroid and a consequence of toxic levels of oxidized (rancid) lipids in the diet. In some electron micrographs, lamellated bodies typical of lipofuscin were also seen. The presence of rancidity in the feed was tested for but shown to be negative (results not shown). Once again, however, the formation of hepatocellular syncytia is not described for this condition (Tacon 1996). While it is hard to explain why only one strain of the same species of fish would be so susceptible to a toxin, the same cannot also be said for an infectious-based actiology, as there is precedent. Different strains of the same species of Pacific salmon, sockeye, Oncorhynchus nerka (Walbaum), are known to have similarly widely differing susceptibilities to infectious haematopoietic necrosis virus (Amend & Nelson 1977).

Syncytial hepatocellular disease has been described in farmed juvenile halibut, Hippoglossus hippoglossus (L.), in Canada and Scotland, in both cases associated with a reovirus-like infection (Cusack et al. 2001; Ferguson, Millar & Kibenge 2003). In the Scottish fish, syncytial changes to the gastro-intestinal mucosa were also described. No syncytial changes were seen in the mucosa of the GIT in the present case; instead, it was necrosis of gastric glands. Hepatic syncytia are described in turbot, Scophthalmus maximus (L.), and rainbow trout, Oncorhynchus mykiss (Walbaum), in both species associated with herpesviruses (Wolf 1988). Hepatic syncytial disease has also been seen in a wild red-striped rockfish, Sebastes prorigor (Jordan & Gilbert). In that case, severe hepatic necrosis was also present, and herpesvirus was again suggested as an aetiology, partly on the basis of the presence of intranuclear Cowdray type A inclusions, as well as distinctive capsid-like structures within hepatocytes, as seen on electron microscopy (Kent & Meyers 2000). In the present case, it seemed as though early lesions originated round the sinusoids, and in that respect, there is a similarity to the sequential pathological changes seen in Atlantic salmon, Salmo salar L., with the viral disease infectious salmon anaemia (ISA). In ISA, early lesions in perisinusoidal cells preceded those in hepatocytes (Speilberg, Evenson & Dannevig 1995). Hepatic syncytia are not, however, seen in ISA.

Two recently described virus diseases of tilapia targeted the central nervous system, one of them a

nodavirus and the other a herpesvirus (Bigarré et al. 2009; Shlapobersky et al. 2010). Aside from mild congestion, the central nervous system in the fish in the present case had no significant lesions. There is a marked similarity, however, between the gross pathological changes seen in this case and those described for an earlier disease outbreak in tilapia (McGrogan et al. 1998), namely anaemia, ascites and exophthalmia. In that case, viral involvement (iridovirus) was also strongly implicated based on ultrastructural evidence of typical icosahedral virus particles but a syncytial cell hepatopathy was not seen.

During the early expression of the disease in the present case, there was no evidence of typical mortality or lesions in fish in the hatchery ponds. The rapid onset of mortality that followed transfer of fry/fingerlings from hatchery ponds to on-growing ponds, typically 4-7 days later, suggested initially, therefore, that this might be primarily a water quality-based problem, even though basic parameters (temperature, oxygen, total nitrogen) were well within normal limits for this species. Although the farm has roughly 1500 hectares of ponds, the water in the on-growing ponds is mostly recirculated, with approximately 5% new water pumped daily from the adjacent river to make up for evaporative losses. The hatchery and associated fry ponds have their own recirculation system, so there is a degree of separation. Nevertheless, aside from the hatchery building itself, and the standard practice of emptying and liming ponds post-harvest, little effort is presently expended on biosecurity procedures. It is, therefore, hard to imagine that the fish in the hatchery ponds, prior to stocking out, would not have been exposed to the same pathogens as those seen elsewhere on the farm. So what is the explanation for the sudden mortality post-transfer? Is it merely a consequence of transferring already infected fish that became stressed due to the subsequent massive reductions in stocking densities? Are the fish being faced with overwhelming levels of infection in the larger ponds? Is there some, as yet unknown, interaction between the fish and the environment of the larger ponds? Or is it a combination of some, or all of the above?

If the fish survived the outbreak, and were amongst the 20% or so that were subsequently transferred to the grow-out ponds, they seemed to perform very well, exceeding the growth and feed conversion data of the GMT fish that had an 80–90% survival. As yet we cannot say whether this is an age-related disease or whether larger fish are seemingly resistant due to the fact that they have recovered from prior infection and are now solidly immune. If the latter, it bodes well for success with prevention strategies such as vaccination. Age resistance to infectious disease has been well documented for tilapia, although in the case of *Streptococcus agalactiae* it was the young fish that seemed resistant to infection and disease (Hernández, Figueroa & Iregui 2009; Jiménez *et al.* 2011). Much more work needs to be done in this area of age susceptibility.

Of great interest is the fact that the GMT fish seemed to be largely refractory to the disease. Is this simply a case of genetic resistance in a different strain of the same species of fish? Possibly, it is the result of natural selection and breeding from survivors to this viral disease, in which case the suppliers need to be tested. Possibly, it is a consequence of not having been subjected to feeding with ethyl testosterone in early life. Aside from the genetics, this possibility would seem to be the only difference between the two groups of fish; diet, stocking density, water quality, etc. were overall very similar if not identical. Interestingly, even though they do not need it, preliminary trials that simply fed GMT fish with ethyl testosterone to mimic the treatment of the Chitralada had no effect on their subsequent resistance, that is, they did not become more susceptible and develop typical disease (data not shown). Similarly, if Chitralada were not fed their normal ethyl testosterone-laced diet for the usual period of time, their susceptibility did not lessen (again, data not shown). If it is simply a case of genetic resistance, as seems the most likely explanation, then until such time as a vaccine becomes available or the cause is identified, close attention should be paid to breeding programmes or to selecting and testing a range of different strains of tilapia with a view to finding those least susceptible.

The fact that the high mortality was seen in relatively small fish means that it was not the economic disaster it could otherwise have been, as high costs had not been already incurred on feeding and growing the fish to the fingerling stage. Nevertheless, the farm cannot keep on absorbing such high losses and trying to compensate by stocking more fish than normal in anticipation of subsequent mortality. If this does prove to be an infectious disease, as seems most likely, then until such time as a vaccine becomes available, much effort will need to be expended on genetic selection, and on biosecurity and stocking practices; indeed, consideration may have to be given to an all-in, all-out policy for a period of time, at least for the grow-out part of the farm. We believe this to be a new disease which we are calling syncytial hepatitis of tilapia.

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