Histopathology associated with experimental ranavirus infections in frog and toad tadpoles

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Introduction

Ranavirus (family *Iridoviridae*) infections have been associated with severe mortalities in cultivated and wild populations of fish and amphibians. The potential for these pathogens to cross species barriers has been recognised and is the subject of current research. There is a lack of data on the pathogenesis of these viruses in amphibian hosts. Preliminary data on the histopathology of common frog (*Rana temporaria*) and common toad (*Bufo bufo*) tadpoles challenged with two ranaviruses originally isolated from amphibian hosts, frog virus 3 (FV3) from the northern leopard frog (*Rana pipiens*) in the USA, and Rana esculenta iridovirus – like virus (REV-like virus) from the green frog (*Rana esculenta*) in Italy are reported here.

Materials and methods

Tadpoles of each species were challenged by bath immersion with approximately 10^4 $TCID_{50}$/ml FV3 or REV-like virus for one hour, or mock-challenged with tissue culture media only (negative control), at a temperature of 20°C. Challenge and mock-challenge were carried out in duplicate. One tank was used to produce cumulative mortality figures (see abstract O-028 Bayley & Hill this conference). Animals from the duplicate tanks were sampled sequentially over a period of 8 days for histopathological investigations (reported here). Three animals were sampled daily from each tank, beginning on day 1 post exposure for the frog tadpoles and day 5 for the toad tadpoles and ending at days 8 and 12 respectively. Tadpoles were sacrificed by terminal anaesthesia, fixed whole in neutral buffered formalin and Karnovsky's fixatives, processed using standard wax embedding, sectioned and stained with haematoxylin and eosin. Immunohistochemistry was also performed using an avidin-biotin method and polyclonal primary antibody to the ranavirus European catfish virus.

Figure 1: Virus, diluted in tissue culture media (GMEM with 2%FCS), was added to each challenged group to a final concentration of 10^4 $TCID_{50}$/ml.

Figure 2: During the one-hour challenge period plastic tubs containing the challenged animals were suspended in tank water at 20°C (±1°C) to maintain water temperature. Air was also provided.

Results

Hepatocellular necrosis (HCN) was observed in frog tadpoles exposed to REV-like virus observed at day 5 post exposure (pe), which appeared lethargic or were consistently observed at the water surface. Specimens from day 6 pe displayed similar hepatic pathology and focal necrosis of the gastric glands. Tadpoles sampled at days 7 and 8 did not show significant pathology. A single animal sampled at day 9 exhibited renal haematopoietic cell necrosis. Tadpoles challenged with FV3 virus became lethargic at day 6 pe with one tadpole exhibiting mild renal haematopoietic cell necrosis, which was also seen in single tadpoles sampled at days 7 and 9. At day 7 pe, HCN was observed in both tadpoles sampled. A bacterial infection was detected in one tadpole sampled at day 8. Moribund toad tadpoles exposed to REV-like virus exhibited HCN from day 6 pe with severe changes observed by day 9 pe with renal necrosis also evident in some tadpoles. Epithelial cell necrosis was also evident in the lining of the branchial cavity and digestive tract. Exposure to FV3 also resulted in HCN in single tadpoles at days 6 to 10 pe, but pathology was mild and absent in tadpoles sampled on day 7 pe to experiment termination at day 12 pe. Necrosis of renal tissue and branchial cavity epithelia was also observed up to day 10 pe. No pathology was seen in non-challenged controls of frog and toad tadpoles.

Figure 3: Metamorphosing toads

Figure 4: Metamorphosing frog

Acknowledgements

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Figures 5-9 are of frog tadpoles, 10-12 are of toad tadpoles.

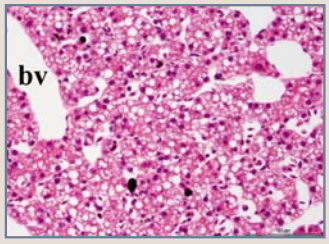

Figure 5: Normal liver. Trabecular arrangement of hepatocytes, many of which contain conspicuous vacuoles. Blood vessels (bv) and melanomacrophages are evident. Bar = 100µm. H&E.

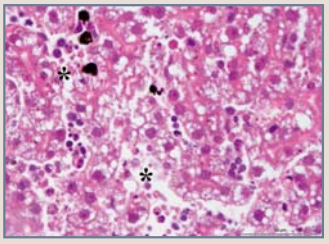

Figure 6: Hepatocellular necrosis (*) associated with infection with FV3. Note the disruption to the normal hepatic architecture. Bar = 50µm. H&E.

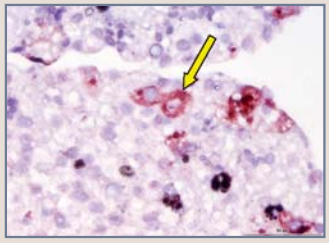

Figure 7: The same animal depicted in Figure 6, showing labelling of hepatocytes infected with FV3 (arrow). In this section, hepatocellular necrosis is not as evident as in the H&E section. Bar = 50µm. Immunohistochemistry (IHC).

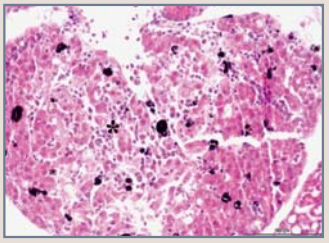

Figure 8: Hepatocellular necrosis (*) associated with infection with REV-like virus. Bar = 100µm. H&E.

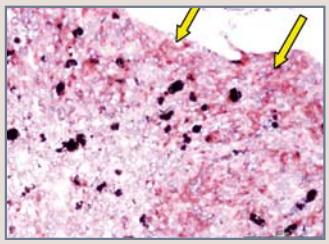

Figure 9: The same animal as depicted in Figure 8, showing labelling of hepatocytes for the presence of REV. Note the relatively intense staining towards the periphery of the organ (arrow). Bar = 50µm. IHC.

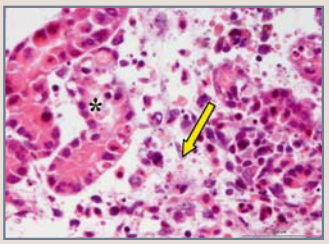

Figure 10: Renal tubule (*) and haematopoietic cell necrosis (arrow) in toad tadpole challenged with REV-like virus. Bar = 50µm. H&E.

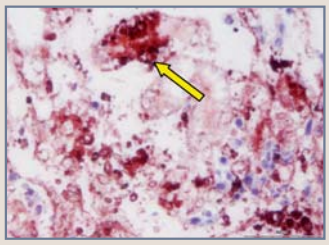

Figure 11: The same animal as depicted in Figure 10. Intense labelling can be seen in affected tubule epithelia (arrow) and amongst the necrotic haematopoietic tissues. Bar = 50µm. IHC.

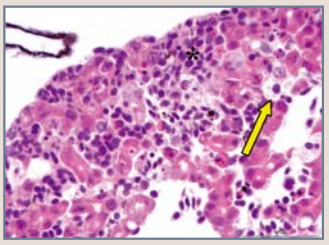

Figure 12: Focal hepatitis in toad tadpole infected with REV-like virus. Influx of lymphocytes (*) and hepatocellular necrosis are evident (arrow). Bar = 50µm. H&E.

Conclusions

- Both viruses (FV3 and REV-like virus) induced mortalities and histopathological changes in frog and toad tadpoles.
- Liver and kidney showed the most significant pathology in both species.
- The presence of virus in these organs and other tissues was demonstrated using immunohistochemistry.
- Further investigations including ultrastructural analysis of the material presented here and from experiments with metamorphosed frogs and toads are in progress.

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