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Guest Editorial

Amphibian decline and mass mortality: The value of visualizing ranavirus in tissue sections

Ranaviruses negatively impact amphibian populations throughout the world and have been associated with population fluctuations and mass mortality events (Collins and Storfer, 2003; Daszak et al., 2003). Amphibian ranaviruses infect multiple species, with host susceptibility differing among species and developmental stages (Brunner et al., 2005; Duffus et al., 2008; Gray et al., 2007; Robert et al., 2007; Schock et al., 2008). One of the dilemmas that researchers, diagnosticians and pathologists face is determining what cell types are targeted by the virus. Existing reports suggest that multiple cell types are targeted by ranaviruses (Bollinger et al., 1999; Burton et al., 2008; Cunningham et al., 2008; Docherty et al., 2003; Gantress et al., 2003; Jancovich et al., 1997). However, published studies identifying target tissues are limited, because the testing required to document the presence of virus can be costly or requires specialized equipment (such as electron microscopy) and virions can be easily missed if only small tissue sections are available for examination.

The article by Dr Ana Balseiro and colleagues published in this issue of *The Veterinary Journal* demonstrates the use of a common diagnostic tool (immunohistochemistry, IHC) to visualize ranavirus within cells (Balseiro et al., 2010). This approach allows researchers and diagnosticians to link the presence of virus with histological changes. The authors compare the cell tropism of the virus in two different species, the common midwife toad (*Alytes obstetricans*) and the alpine newt (*Mesotriton alpestris cyreni*). Their work provides insight into host responses to ranavirus and provides a useful approach for linking viral infection with pathological changes.

Identifying the cell types targeted by ranavirus is important for several reasons. First, it can lead to a better understanding of the possible routes of viral transmission. For example, is the virus shed into the intestinal lumen or are germ cells affected? Swab specimens collected from the cloaca of subclinically-infected hosts have revealed positive test results for ranavirus suggesting that environmental shedding is possible by hosts displaying no apparent signs of illness (Driskell et al., 2009; Gray et al., 2009). Identifying the source of the shed virions (e.g., renal vs. intestinal vs. cutaneous) can help manage this pathogen in captive environments. Similarly, it is unknown if infection of eggs or sperm during gametogenesis is possible (Duffus et al., 2008). Rather, post-gametogenesis exposure (e.g., in the cloaca or in the environment) of gametes or embryos has been theorized. The ability to visualize ranavirus within an egg or spermatid would provide insight into the possibility of vertical transmission.

One of the most useful applications of identifying the cell types infected by ranavirus is relating the presence of the virus with the tissue changes (such as cellular degeneration or necrosis), or determining where the virus is replicating in clinically normal ranavirus-positive animals. Subclinical infection with either no gross or histological changes or only minimal non-specific histological changes have been reported in ranavirus surveillance studies (Gray et al., 2009; Miller et al., 2009). Thus, IHC (or similar techniques such as in situ hybridization) may be useful in identifying possible reservoir species. It also may allow identification of the virus when virus isolation is unsuccessful or viral inclusions are not observed. Ultimately, identifying the cell types infected by ranavirus will improve our understanding of pathological changes from early infection through morbidity and mortality.

Finally, visualizing ranavirus within tissue sections will add to our knowledge regarding how infection varies by viral species or strain, host species, and among host developmental stages. This will be especially helpful in animals with concurrent infection by multiple pathogens, because it can be difficult to match a lesion with a pathogen (Miller et al., 2007, 2008). Techniques such as IHC will allow researchers and diagnosticians to identify the histological changes associated with a particular pathogen in concurrent infection and differentiate between a non-specific or secondary change and a primary pathological change due to the virus.

As Balseiro et al. (2010) point out, the impact of ranaviruses on vulnerable species may be devastating. Our ability to identify reservoirs and understand the interaction of ranaviruses within a community of hosts (multiple amphibian, reptile and fishes) will most likely be the key to preventing local population declines. This task will require teams of researchers composed of ecologists, pathologists, virologists, epidemiologists, and others, each using their expertise to ensure the successful application of conservation strategies.

Debra L. Miller
Veterinary Diagnostic and Investigational Laboratory,
University of Georgia,
Tifton, GA 31793, USA
E-mail address: millerdl@uga.edu

Matthew J. Gray
Center for Wildlife Health,
Department of Forestry, Wildlife and Fisheries,
University of Tennessee,
Knoxville, TN 37996, USA

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