

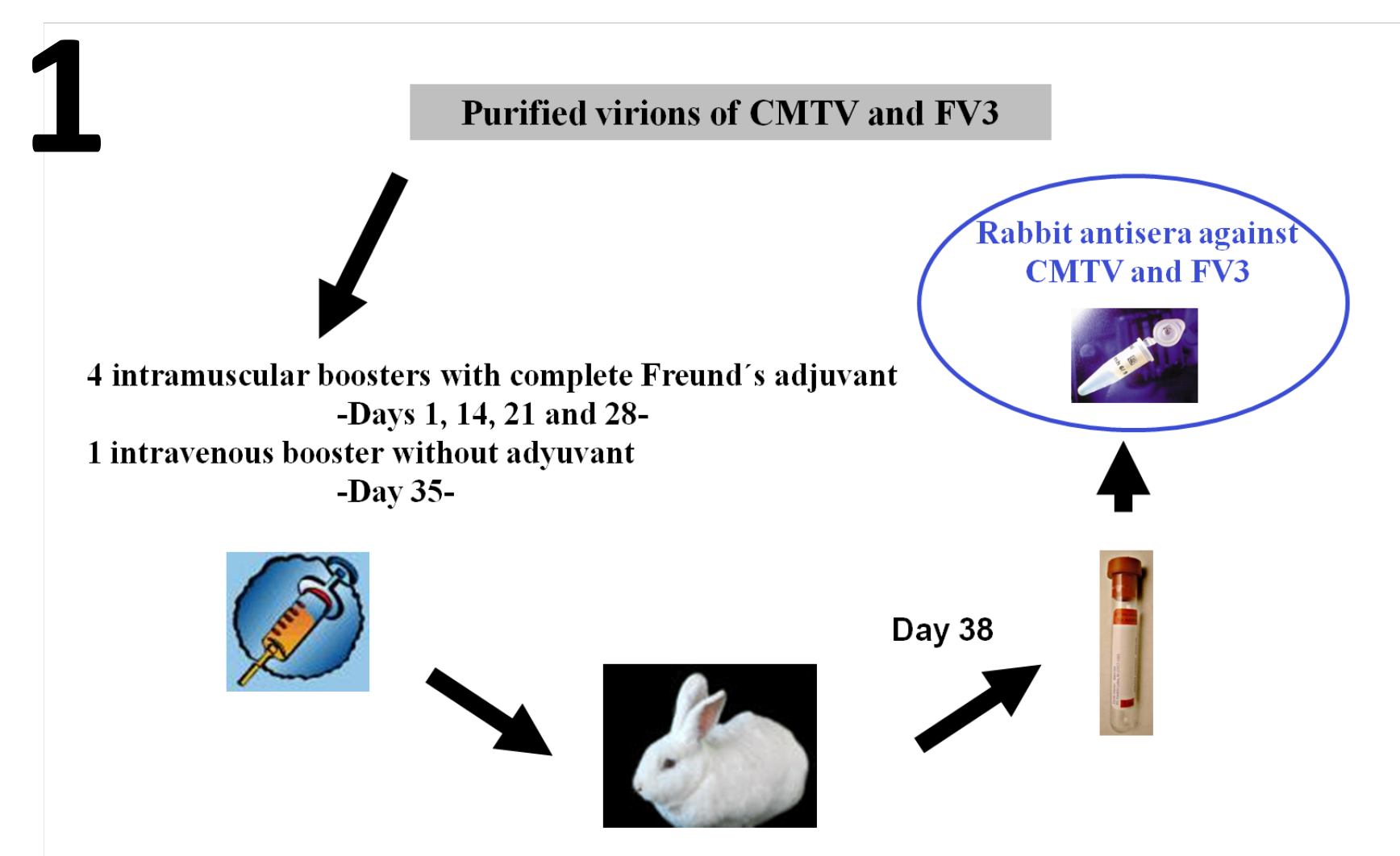
**Summary:** The ability to detect and confirm ranaviruses in tissue sections is necessary to link pathological changes to the virus. We have begun a study to investigate the feasibility of using immunohistochemistry to identify cell populations targeted by ranaviruses and the cell damage caused by the viruses. Rabbit antisera was prepared against Common midwife toad virus (CMTV) and Frog Virus 3 (FV3), respectively. In brief, two New Zealand white rabbits were immunized with purified virions emulsified with complete Freund's adjuvant at days 1, 14, 21 and 28. A final booster was administered intravenously at day 35 and serum collected on day 38. Pre and post-immunization sera were used for immunohistochemical studies. Initial staining was performed on tissues from 3 species of North American anurans and one species of North American turtle that were challenged with 4 different FV3-like ranavirus isolates. Positive staining was not achieved using the CMTV antibody; however, positive staining was achieved using the FV3 antibody. Positive staining was observed in various cell types including hepatocytes, renal tubular epithelium and intestinal epithelium; cytoplasmic inclusions also displayed positive staining. Initial evidence suggests that immunohistochemistry may be useful tool to aid diagnostic investigations of morbidity and mortality events that are suspected to be caused by ranaviruses; however, their success may be ranavirus specific.

**Introduction:** Ranaviruses negatively impact amphibian populations throughout the world and have been associated with population fluctuations and mass mortality events, although host susceptibility differs among species and developmental stages (Miller et al., 2011). This variation in host susceptibility is perplexing, and thus researchers are trying to unravel the pathogenesis of ranaviral disease. A key factor in pathogenesis is identifying the cell types targeted by the virus. Visualizing the virus within cells also provides evidence of infection that can be used in diagnostic investigation of mass die-off events. Immunohistochemical staining is one way to visualize ranaviruses within the cells (e.g., Balseiro et al., 2009, 2010; Cunningham et al., 2008). Further, the OIE has listed immunohistochemical staining as an approved test to use when diagnosing ranaviral disease, especially in areas of necrosis ([http://www.oie.int/fileadmin/Home/eng/Health\\_standards/aahm/2010/2.1.02/RANAVIRUS.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.1.02/RANAVIRUS.pdf)). We have begun a study to investigate the feasibility of using immunohistochemistry to identify cell populations targeted by ranaviruses and the cell damage caused by the viruses. Rabbit antisera was prepared against Common midwife toad virus (CMTV) and Frog Virus 3 (FV3), respectively.

**Methods:** Two New Zealand white rabbits were used to prepare the antisera against CMTV and FV3 (Fig.1). Pre and post-immunization sera were used for immunohistochemical studies. Initial staining was performed on tissues from 3 species of North American anurans (*Hyla chrysocelis*, *Lithobates sphenoccephala*, and *Pseudacris regilla*) and one species of North American turtle (*Trachemys scripta elegans*) that were challenged with 4 different FV3-like ranavirus isolates (FV3, isolate from a ranaculture facility, isolate from an amphibian die-off event, and isolate from a fish die-off event).

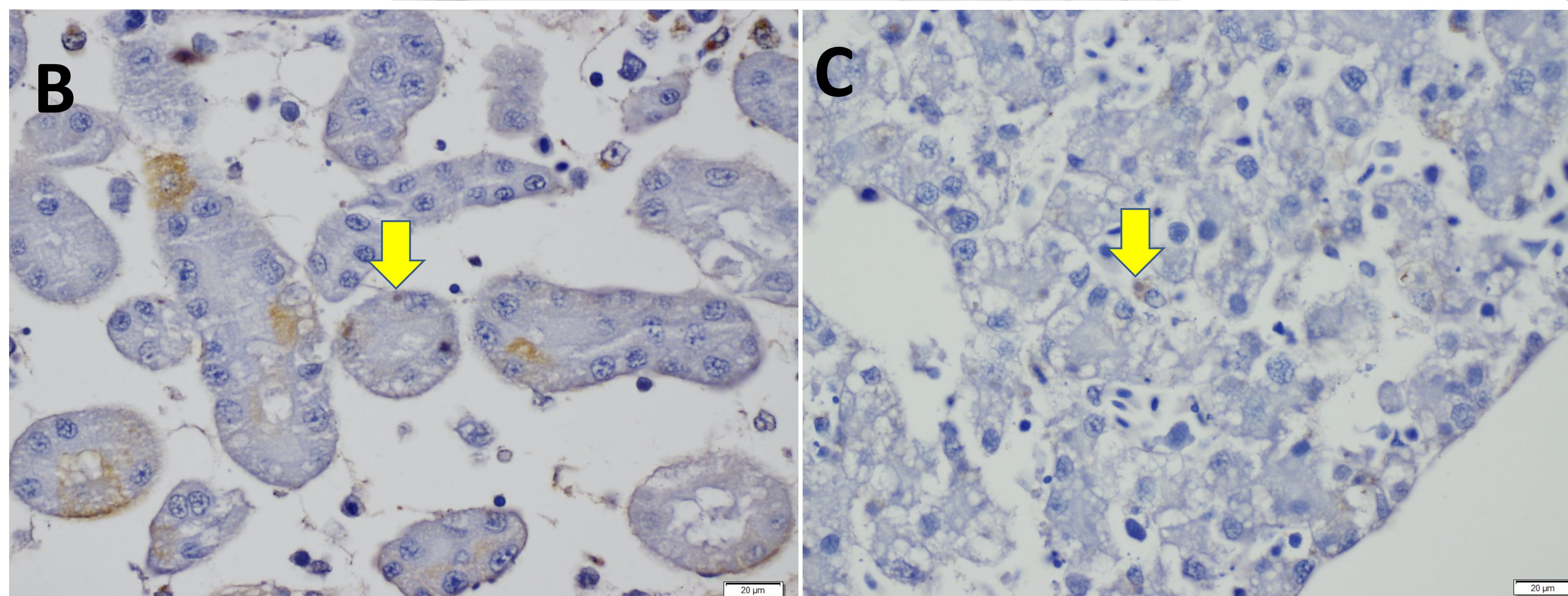
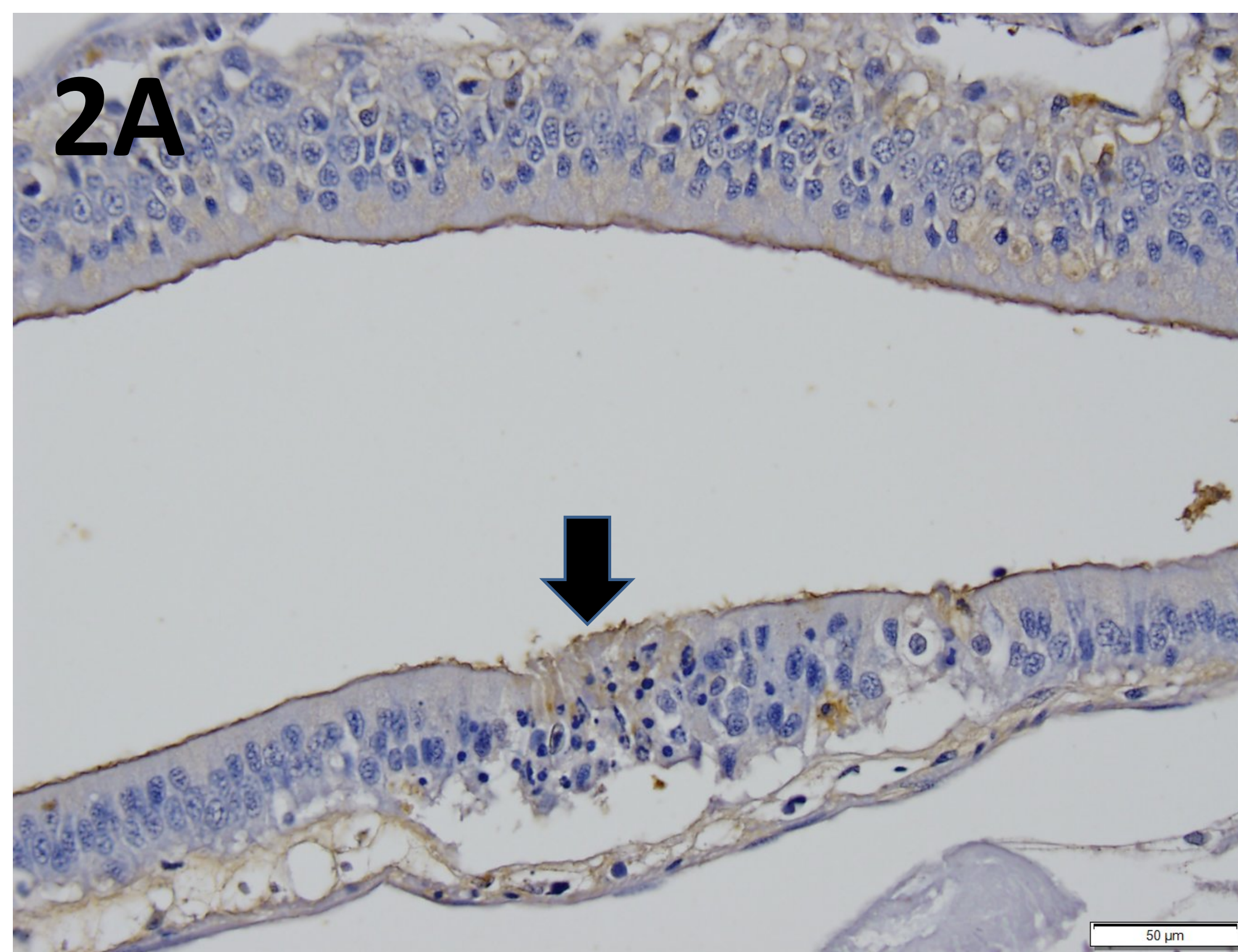
## Methods:

Two New Zealand white rabbits were used to prepare the antisera against CMTV and FV3 (Fig.1). Pre and post-immunization sera were used for immunohistochemical studies. Initial staining was performed on tissues from 3 species of North American anurans (*Hyla chrysocelis*, *Lithobates sphenoccephala*, and *Pseudacris regilla*) and one species of North American turtle (*Trachemys scripta elegans*) that were challenged with 4 different FV3-like ranavirus isolates (FV3, isolate from a ranaculture facility, isolate from an amphibian die-off event, and isolate from a fish die-off event).



**Figure 1:** Preparation of the antisera against CMTV and FV3. Two New Zealand white rabbits were immunized with purified virions emulsified with complete Freund's adjuvant at days 1, 14, 21 and 28. A final booster was administered intravenously at day 35 and serum collected on day 38.

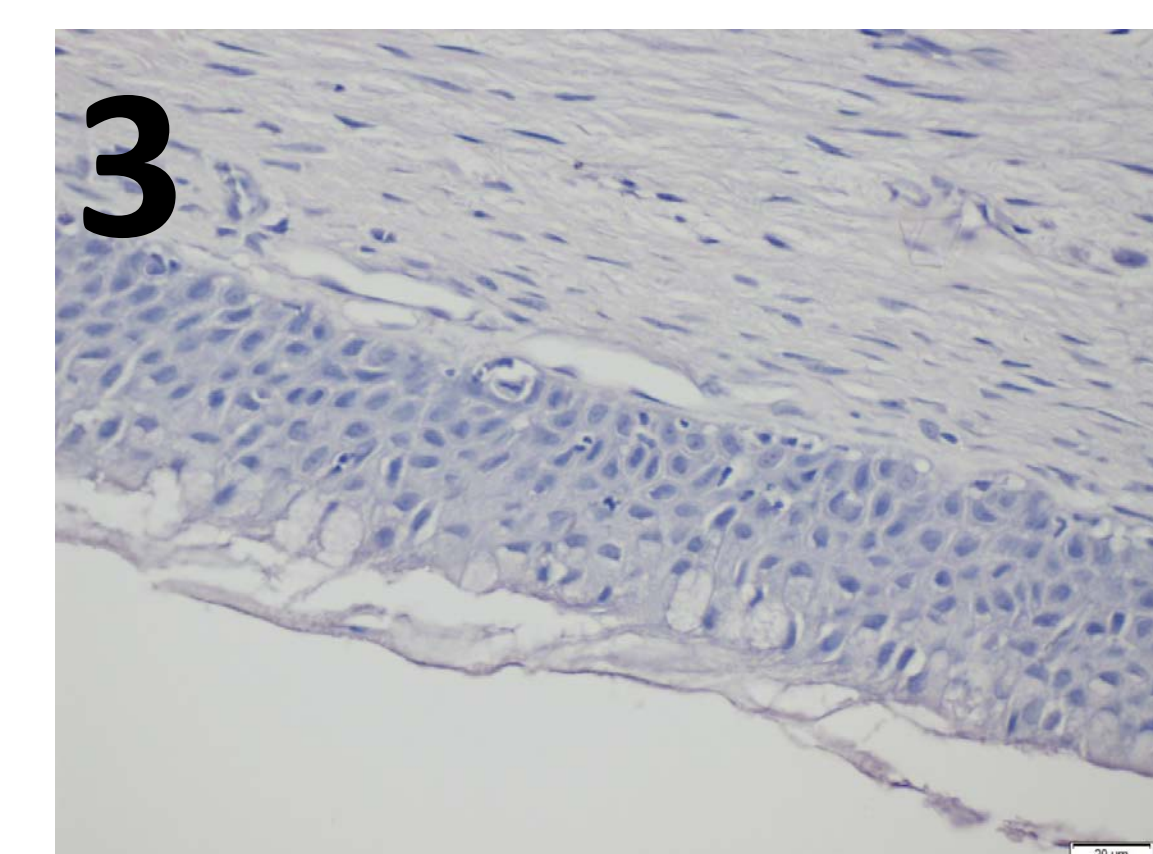
**Results:** Positive staining was achieved using the FV3 antibody but not the CMTV antibody. Positive staining was observed in various cell types including hepatocytes, renal tubular epithelium and intestinal epithelium, and cytoplasmic inclusions also displayed positive staining (Fig. 2). Staining success did not vary by ranavirus isolate. Although positive staining was observed in all amphibian species, no staining was observed in the tissues of the red eared slider (*Trachemys scripta elegans*; Fig. 3). Nonspecific staining was observed in the tree frog (*Hyla chrysocelis*).



**Figure 2.** Photomicrographs of tissues collected from a Southern leopard frog (*Lithobates sphenoccephala*) tadpole that was experimentally challenged with a ranavirus that was obtained from a mortality event in a ranaculture facility. The ranavirus antigen is visualized by the brown staining. A. Intestinal epithelium demonstrating the brown staining that is especially prevalent within areas of necrosis (black arrow). B. Tubular epithelial cells of the mesonephros contain varying amounts of virus and scattered viral inclusion bodies (yellow arrows). C. Hepatocytes within the liver generally displayed faint staining, and scattered viral inclusion bodies were seen (yellow arrows).

**Discussion:** Immunohistochemical staining can be successfully used to demonstrate the presence of Ranavirus antigens within tissues of animals that are infected with ranavirus. The intensity of staining varied among hosts, which may reflect quantity of viral antigen present, variation in host compatibility with the test, or variation in host-antigen interaction. This technique may be especially useful to identify inclusion bodies within areas of necrosis, as this can be challenging when abundant cellular debris is present.

**Future Direction:** Currently, sample sizes are being increased to explore the staining variability observed in host tissues and to confirm the effectiveness of detecting multiple ranavirus isolates within various species of hosts.



**Figure 3.** Negative immunohistochemical staining of the oral mucosa of a *Trachemys scripta elegans* that was experimentally challenged with ranavirus and developed ranaviral disease, including necrosis and sloughing of the oral mucosa. Staining attempts were negative for all virus isolates within this host.

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## References:

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