Ranaviral Disease Pathology

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Basic Outline

- Key Points-Dr. Miller
- Amphibians-Dr. Miller
- Chelonians-Dr. Allender
- Squamates-Dr. Marschang
- Fish-Dr. Miller

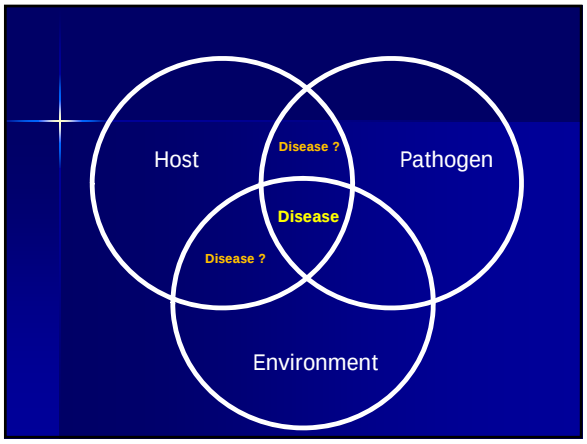
Key Points

Infection vs. Disease

- Infection = pathogen is present. This says nothing about disease
- Sub-clinical=infected but appear normal
- Clinical = infected and there is disease
- So what is Disease???

What is Disease?

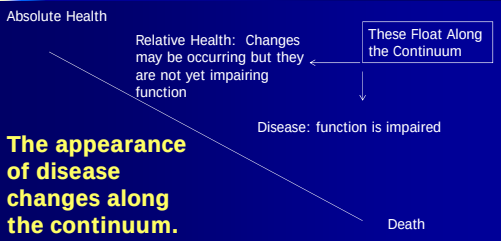
- Any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects; or combinations of these factors (Wobeser 1981)
- Short definition: a condition that impairs normal function.



Concepts of Disease

- Disease is measured in terms of impairment of function rather than by the death of individuals (it occurs along a continuum from absolute health to death)
  - The appearance of disease changes along the continuum.
  - In reality, it is the way the body responds to the damage to a cell or organ (lesion) that results in disease (e.g., common cold, flu, cancer)
- Factors that cause disease may be either intrinsic (e.g., inherited defect) or extrinsic (e.g., virus, bacterium, or contaminant).

What does disease look like?



The appearance of disease changes along the continuum.

Disease: is a continuum and often multifactorial

- **Host Factors**
  - **Age or development stage**
  - **Population dynamics/density**
  - **Possibly, prior exposure**
- **Pathogen Factors**
  - **Primary vs Secondary**
  - **Virulence**
  - **Concurrent pathogens**
- **Environmental Factors**
  - **Water quality and availability**
  - **Contaminants**
  - **Competition for resources (may relate to density)**
  - **Human traffic/movement**

Other Definitions and Points

- **Morbidity = sickness**
- **Mortality = death (a moribund individual is near death = death is imminent)**
- **Pathology = absence from normal.**
- **Pathogen = organism that is capable of causing disease (viruses, bacteria, fungi, parasites)**
- **Not all pathogens cause disease all of the time**
- **Not all diseases are caused by pathogens (ex: diabetes, most cancers)**

What information do we get when we identify lesions (pathology)?

- Presence of disease (response of the individual to a pathogen/agent)
- NOT exact etiology (generally not) but often a 'list of differentials (possible causes)'
- Insight into what might be happening to function (organ, system) within the individual (physiology)

Ranavirus

3 Classes

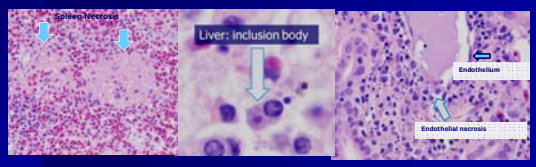
- Amphibians: Anurans and Caudates
- Reptiles: Turtles and Squamates (Lizards, Snakes)
- Fish: Boney fish

General Comment on Pathology

- Tissue are can be similar across classes [e.g., hemorrhage, swelling and necrosis (tissue death) are common gross changes] but the appearance of the changes or locations can vary.

Histology

- Probably the most typical lesions:
  - Cellular necrosis of the hematopoietic tissue, vascular endothelium and epithelial cells and intracytoplasmic inclusion bodies are common microscopic lesions



Amphibians

- Development Stage
  - Egg, Embryo
  - Metamorph, Juvenile
  - Larvae
  - Adult

Amphibians: eggs and embryos

- The vitelline membrane (protein membrane) and/or the mucopolysaccharide/mucoprotein capsule (jelly-like substance surrounding the membrane) coating the surface of the egg seem to protect the embryo from infection
- If exposed, embryos tend to die quickly with hemorrhage, epithelial sloughing and melanosis (see: Tweedle and Granoff 1968 and Haislip et al. 2011)

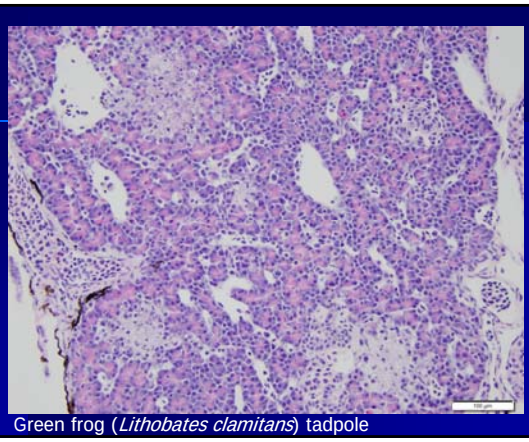
Amphibians: larvae

- Swelling due to edema
- Hemorrhage
- Necrosis (e.g., liver, spleen, epithelial and endothelial cells, hematopoietic tissue)
- Polyps [seen in Hellbender (*Cryptobranchus alleganiensis*) larvae challenged with ranavirus (unpublished); also reported in Tiger salamanders (*Ambystoma tigrinum*) with ATV and Chinese giant salamanders (*Andrias davidianus*) with Chinese giant salamander virus]

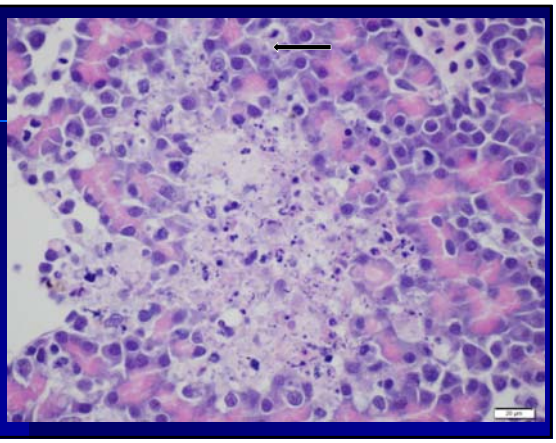
Amphibians: larvae

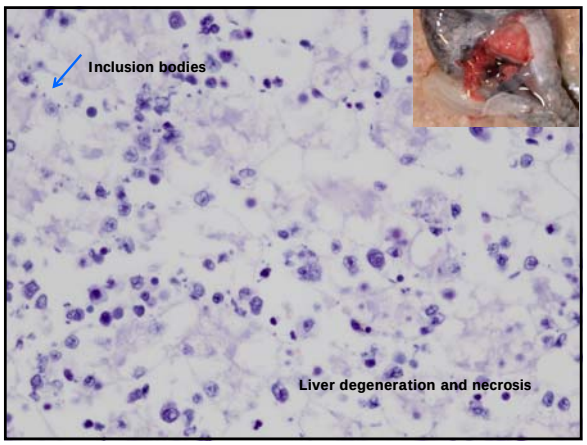
Histology

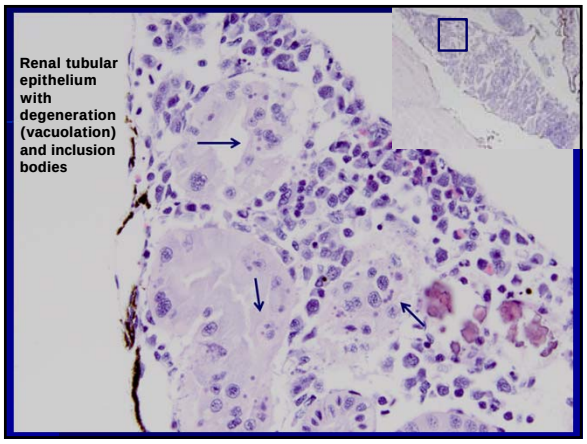
- Typical lesions:
  - Cellular necrosis
    - Organs, such as spleen and liver
    - Hematopoietic tissue
    - Vascular endothelium (with subsequent hemorrhage)
    - Epithelial cells (e.g., renal tubular epithelium, gastrointestinal epithelium)
  - Intracytoplasmic inclusion bodies

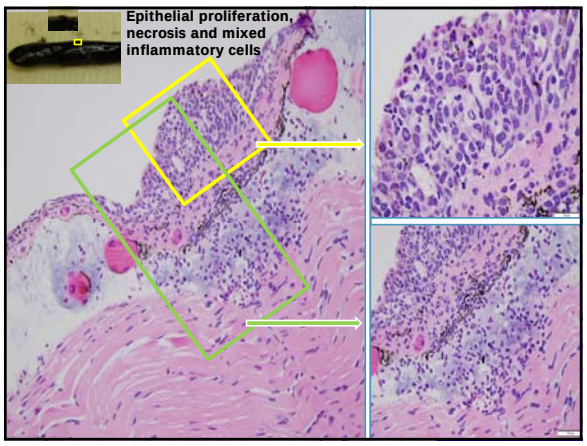


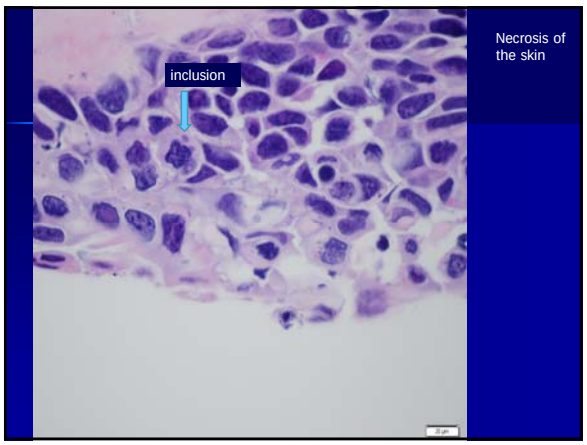
Green frog (*Lithobates clamitans*) tadpole

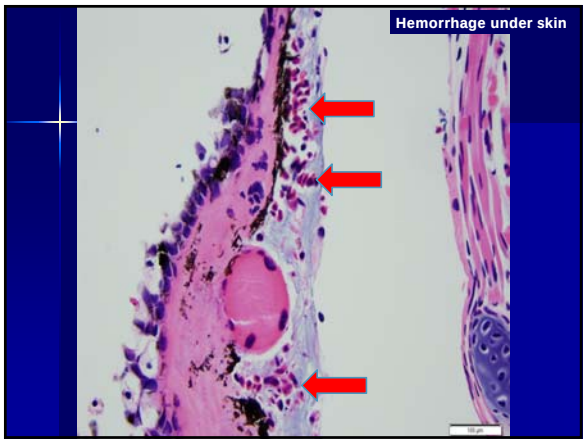



Inclusion bodies
Liver degeneration and necrosis

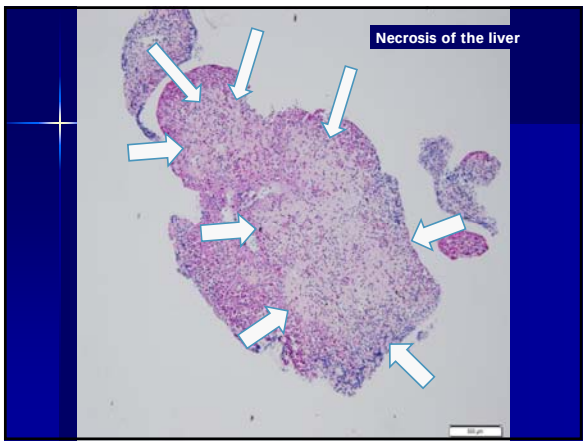

Renal tubular epithelium with degeneration (vacuolation) and inclusion bodies

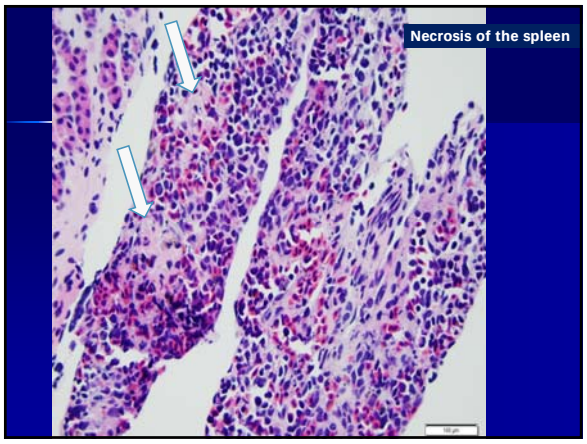

Epithelial proliferation, necrosis and mixed inflammatory cells

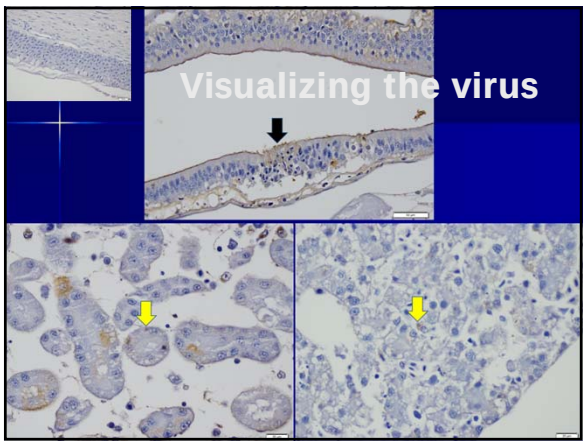




Intracoelomic changes (i.e., changes within the body cavity)


Necrosis of the liver


Necrosis of the spleen

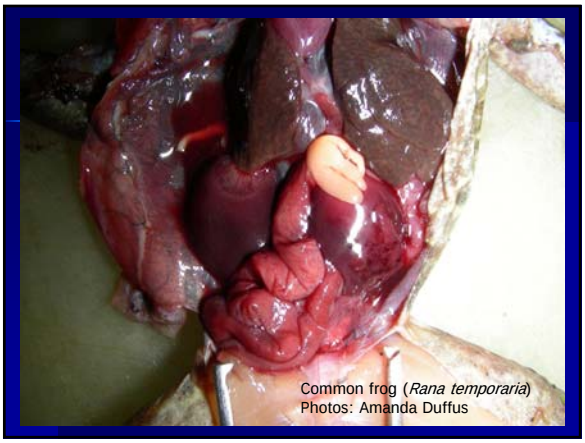

Visualizing the virus

Amphibians: metamorphs

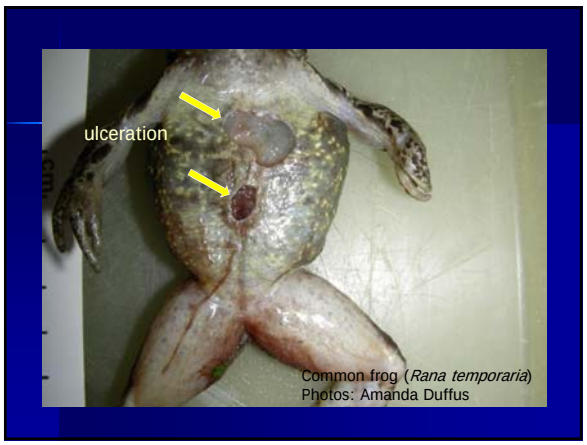
Photo: Jordan Chaney
Boreal Toad (Bufo boreas boreas)

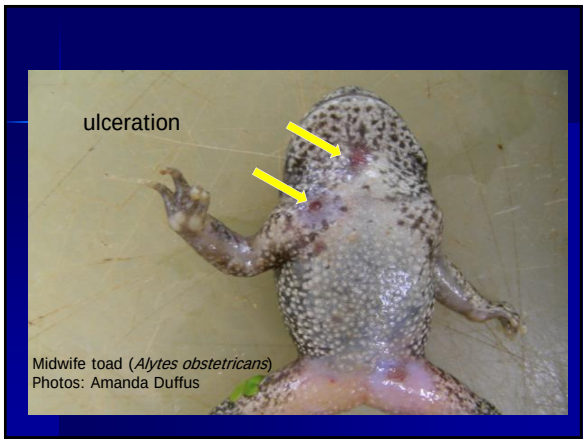
Amphibian: adults

- Europe: 2 presentations reported in adult common frogs (*Rana temporaria*; see Cunningham et al. 2007)
  - Systemic hemorrhages
  - Extensive cutaneous ulceration
  - Both can present in the same animal



Common frog (*Rana temporaria*)
Photos: Amanda Duffus





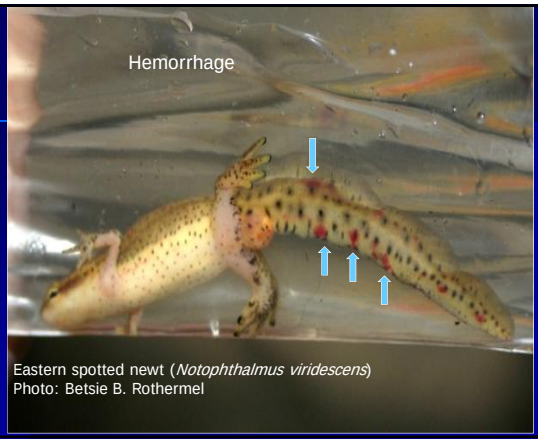
Gross lesions in other species

- **Hemorrhage**
- **Necrosis**
- **Edema**


Hemorrhage
Gopher frog (*Lithobates sevosus*)
Photos: B Sutton and R Hardman


Friable spleen


Rear foot with edema



Eastern spotted newt (*Notophthalmus viridescens*)
Photo: Betsie B. Rothermel

Is this ranaviral disease?

Maybe, but be careful! Many things can cause severe weight loss.

Cryptobranchus alleganiensis alleganiensis
Photo: Dale McGinnity and Sherri Reinsch

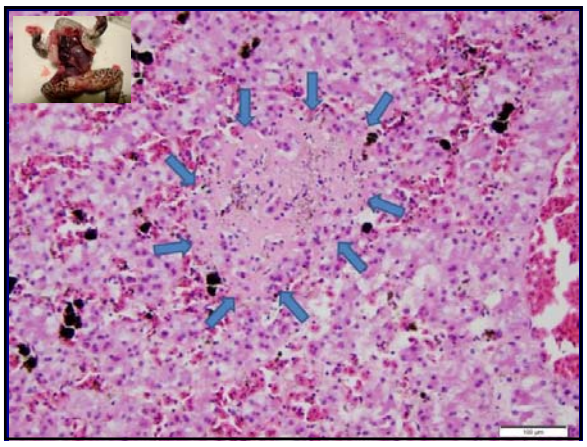
Another presentation:

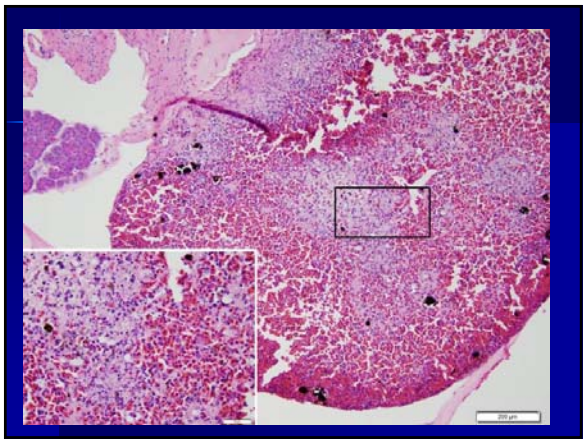
Vestibular syndrome

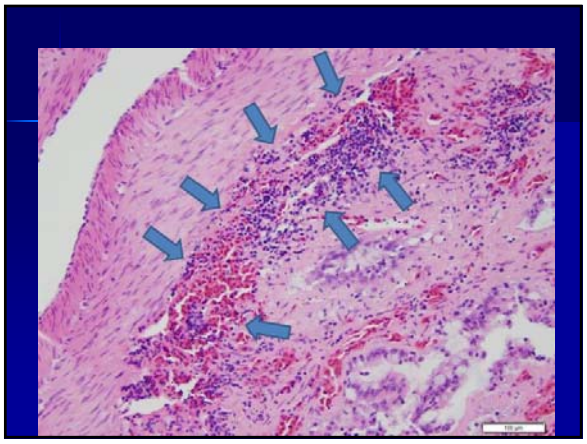
Vestibular syndrome in farmed bullfrogs

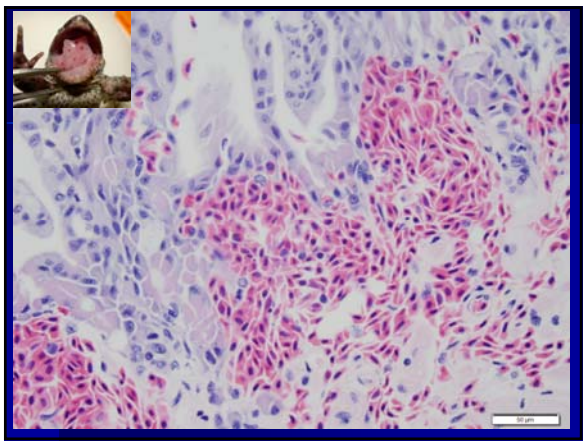
Photo: Rolando Mazzoni

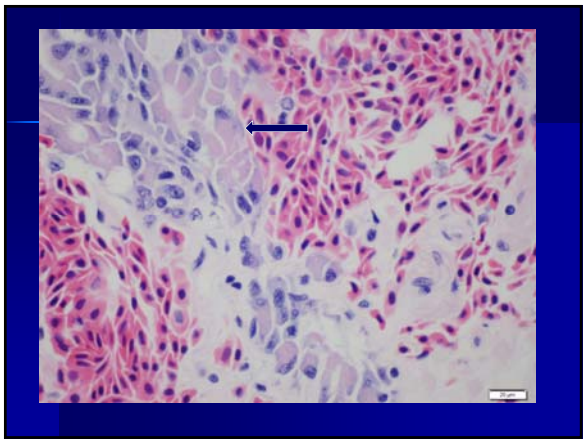
Histology



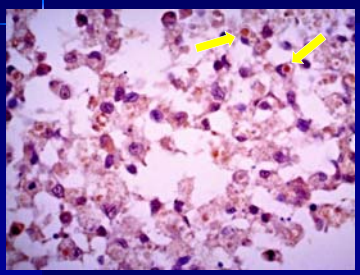








Vestibular syndrome: observed by Rolando Mazzoni

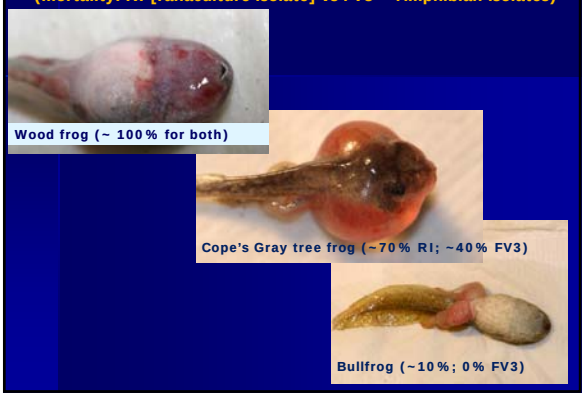


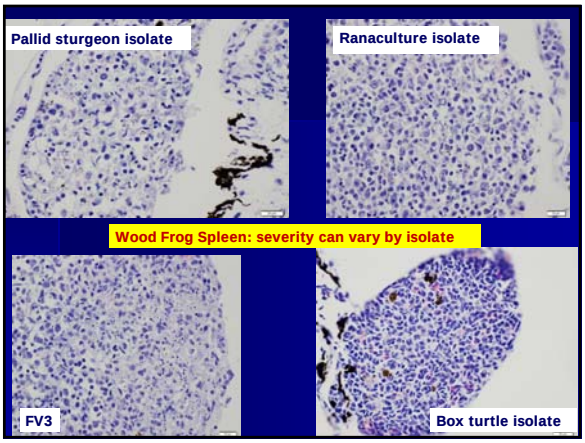
See also: Robert et al. 2016; disseminated ranavirus in the brain of *Xenopus* tadpoles (but not adults)

Things to Consider

Severity varies by host (species)-susceptibility & isolate

(mortality: RI [ranaculture isolate] vs FV3 = Amphibian isolates)





And what role do ectoparasites (leeches) play?

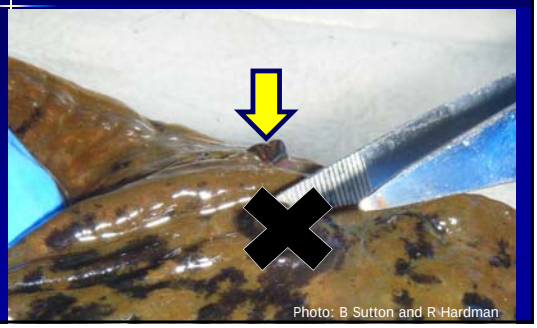
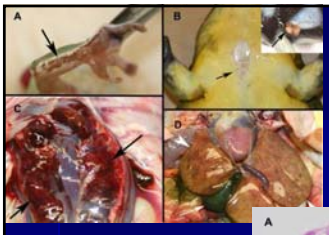


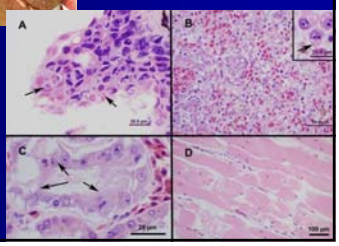
Photo: B Sutton and R Hardman

Regarding Inclusion Bodies

- Inclusion body is a generic term and may be caused by various things (viruses, parasites, clumping of cellular material)
- Ranaviral inclusions are intracytoplasmic (but intranuclear have been reported but are rare and we are uncertain what they represent)
- Inclusion bodies in red blood cells may be a virus (within Iridoviridae) other than ranavirus (Wellehan et al. 2008 ; Grosset et al. 2014).

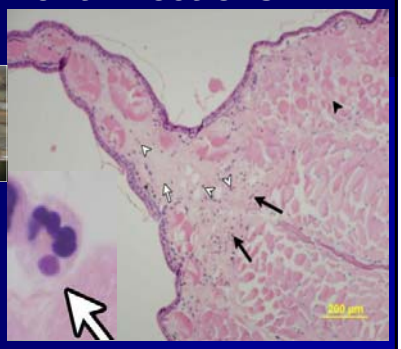


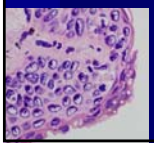
Concurrent Infections



Miller et al. 2008. J Zoo Wildl Med 39:445–449

Concurrent infections

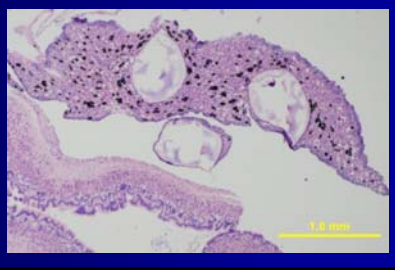




Concurrent infections

***Ranavirus*-positive animals plus: Parasites increased**

In Tennessee: Die-off in *L. clamitans, L. catesbeianus, N. viridescens*



Questions?