

Emergence of Ranaviruses in Japan.

Yumi Une, DVM, Ph.D.

Labratory of Veterinary Pathology,
School of Veterinary Medicine,
Azabu University une@azabu-u.ac.jp



In December 2006, we discovered the first case of chytridiomycosis of captive frogs in Asia.

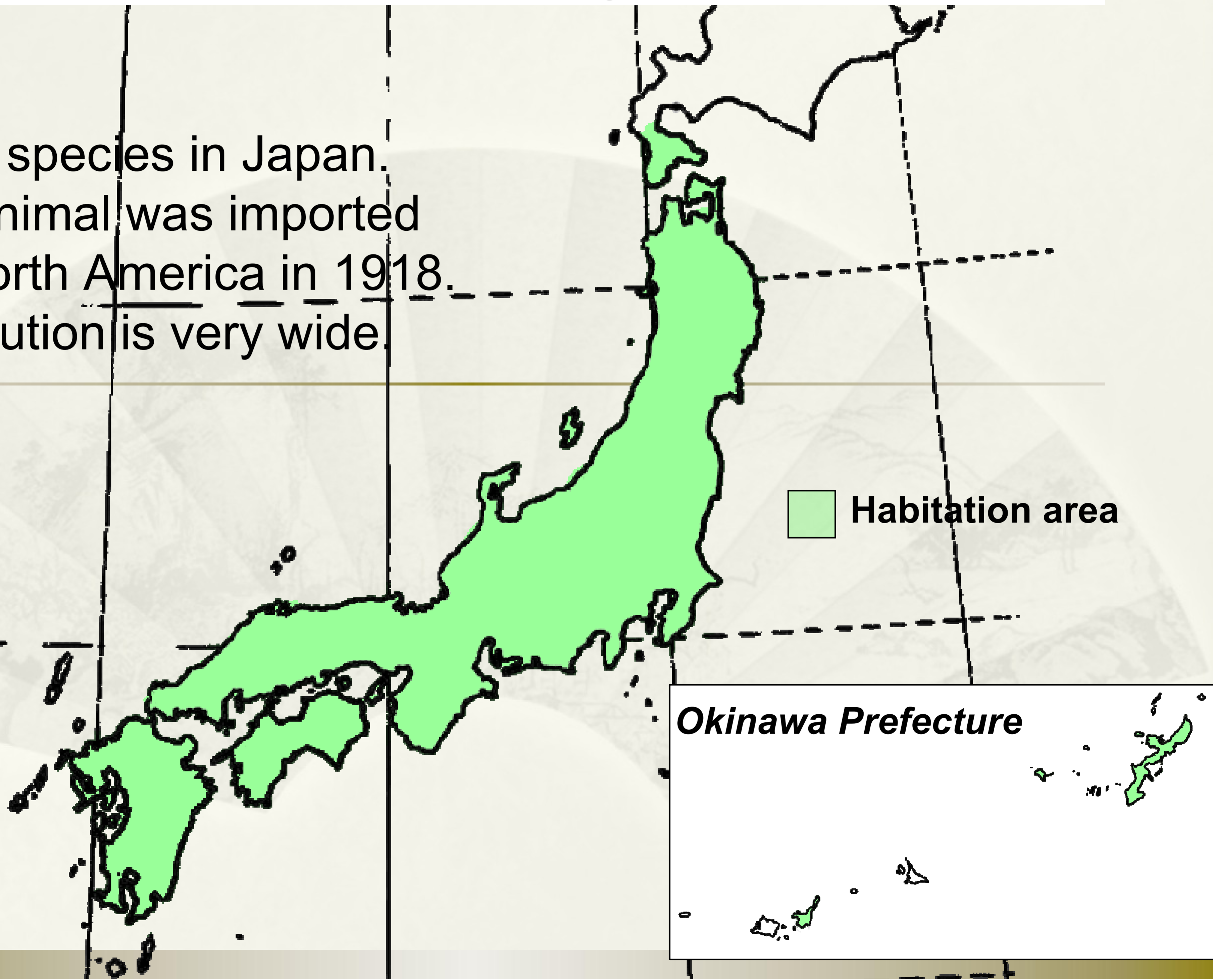
Our aim was risk analysis of *Batrachochytrium dendrobatidis* for native amphibians in Japan.

Action plan

1. Survey of distribution of Bd in nature (in free-living amphibians)
2. Analysis of pathogenicity of Bd for native amphibians using experimental infection
3. Analysis of mass die-off of amphibians

Distribution of the bullfrog in Japan

- Exotic species in Japan.
- This animal was imported from North America in 1918.
- Distribution is very wide.



Episode No.1

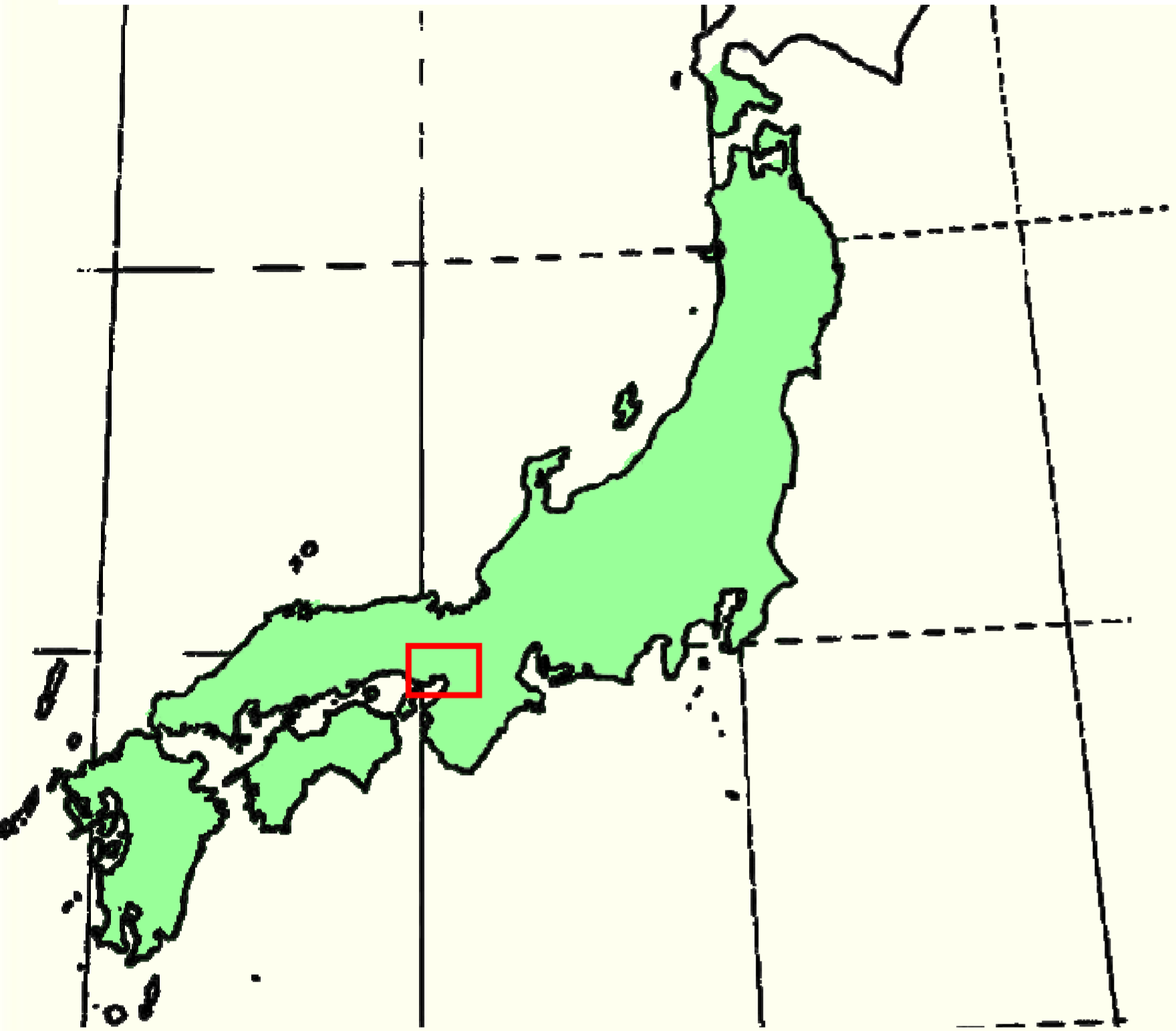
1. in Western Japan
2. September-October 2008.
3. Bullfrog (*R. catesbeiana*)
4. Larvae
5. Mass die-off
6. No abnormality of fish or adult bullfrogs.

This episode was the first time the owner of the farm experienced a mass die-off of frogs .

Affected animals



■ : range of American Bullfrogs in Japan



Episode No.2 & 6

Carcasses of bullfrog larvae and fish

Episode No. 5



Episode No. 3



Episode No.4





Abdominal edema, petechiae, erythema



Tail necrosis

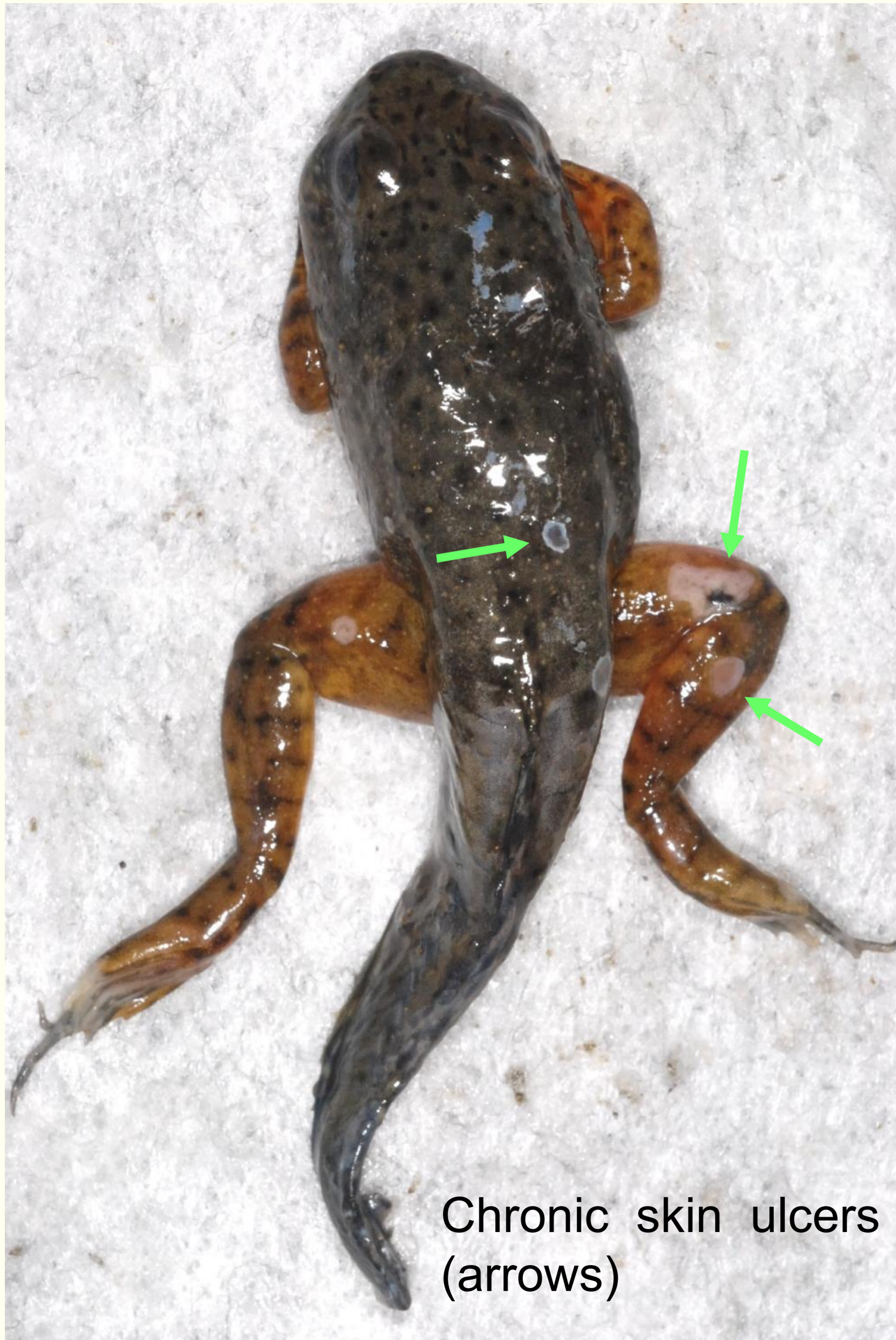


Skin ulceration

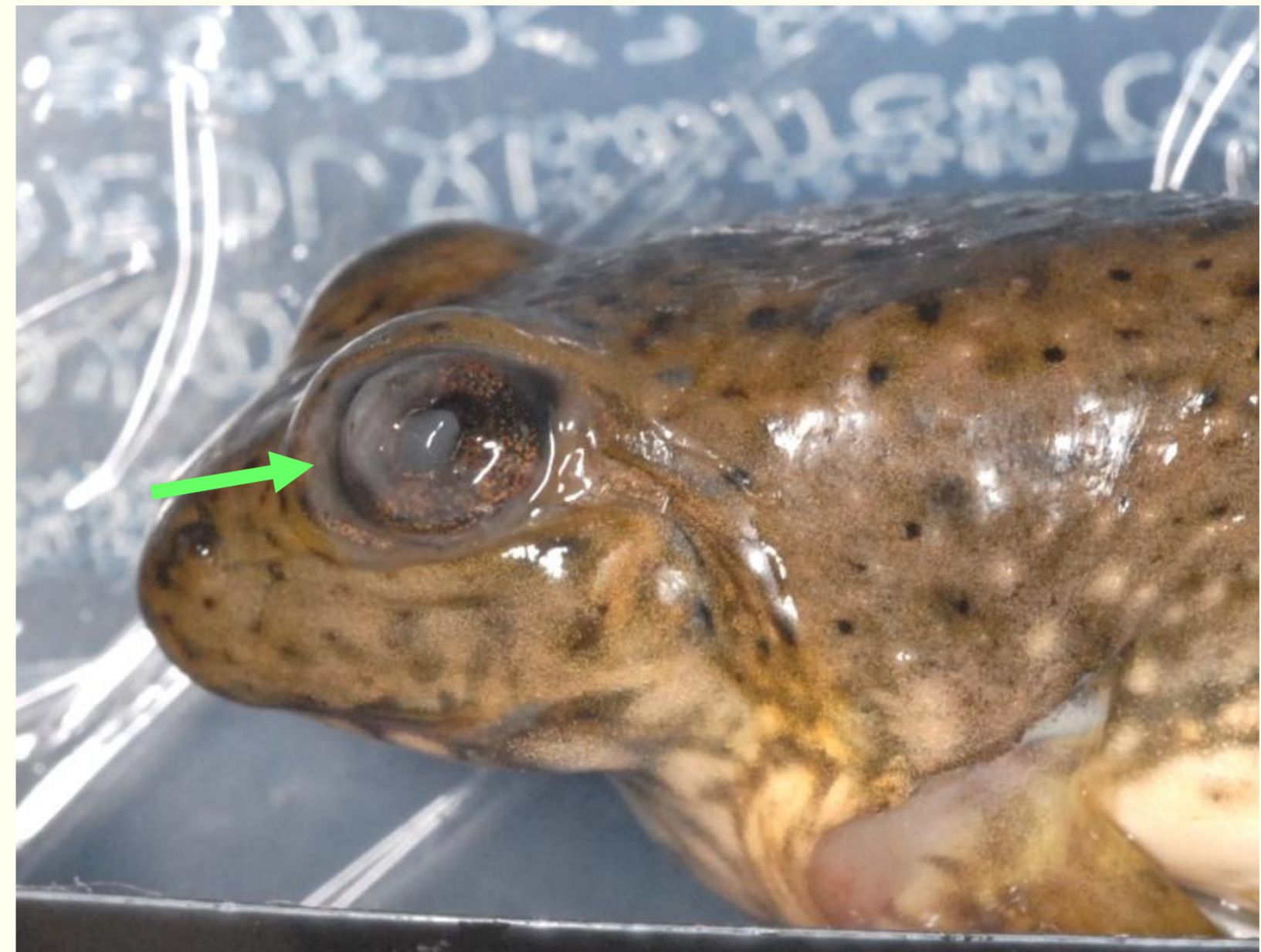


Limb necrosis, emaciation

Episode No.1



Chronic skin ulcers and scars (arrows)

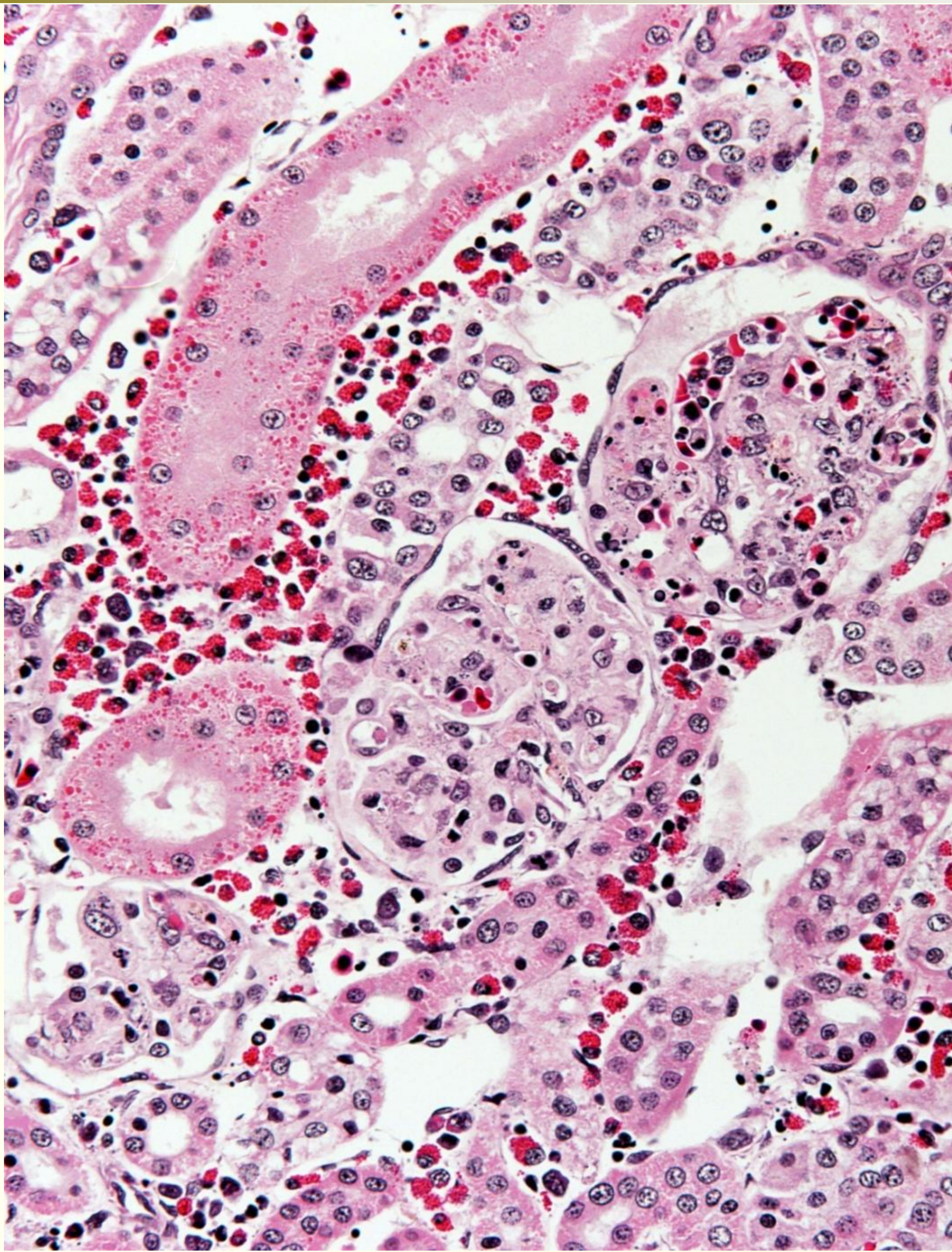


opacity of the cornea

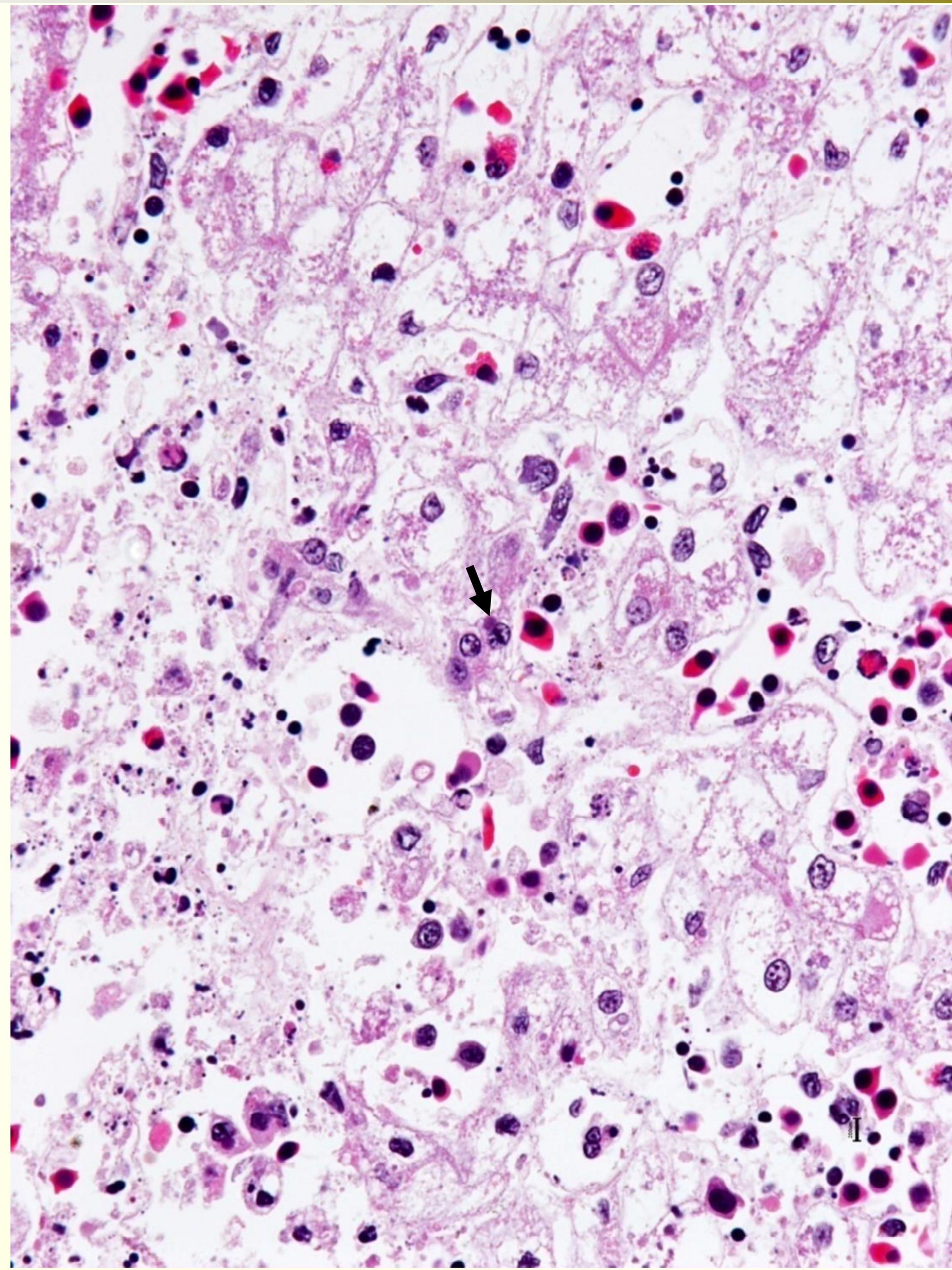


pseudomembranous keratoconjunctivitis

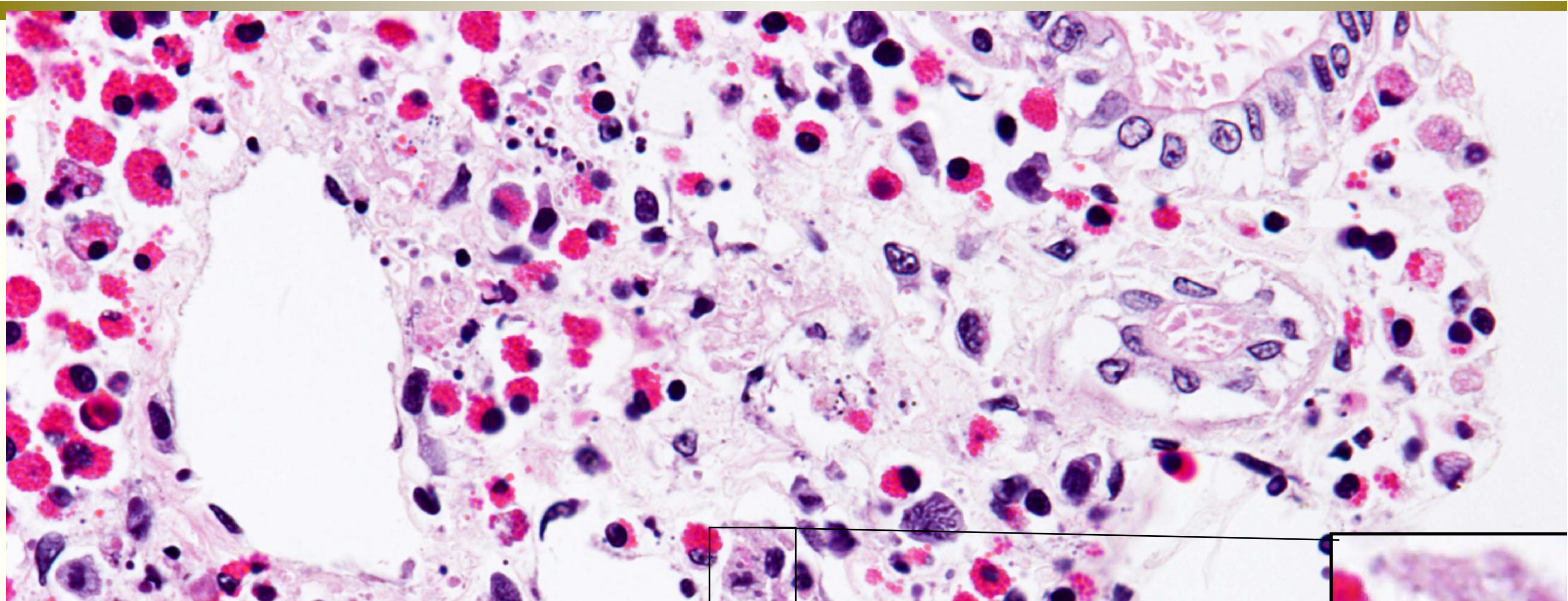
Episode No.2



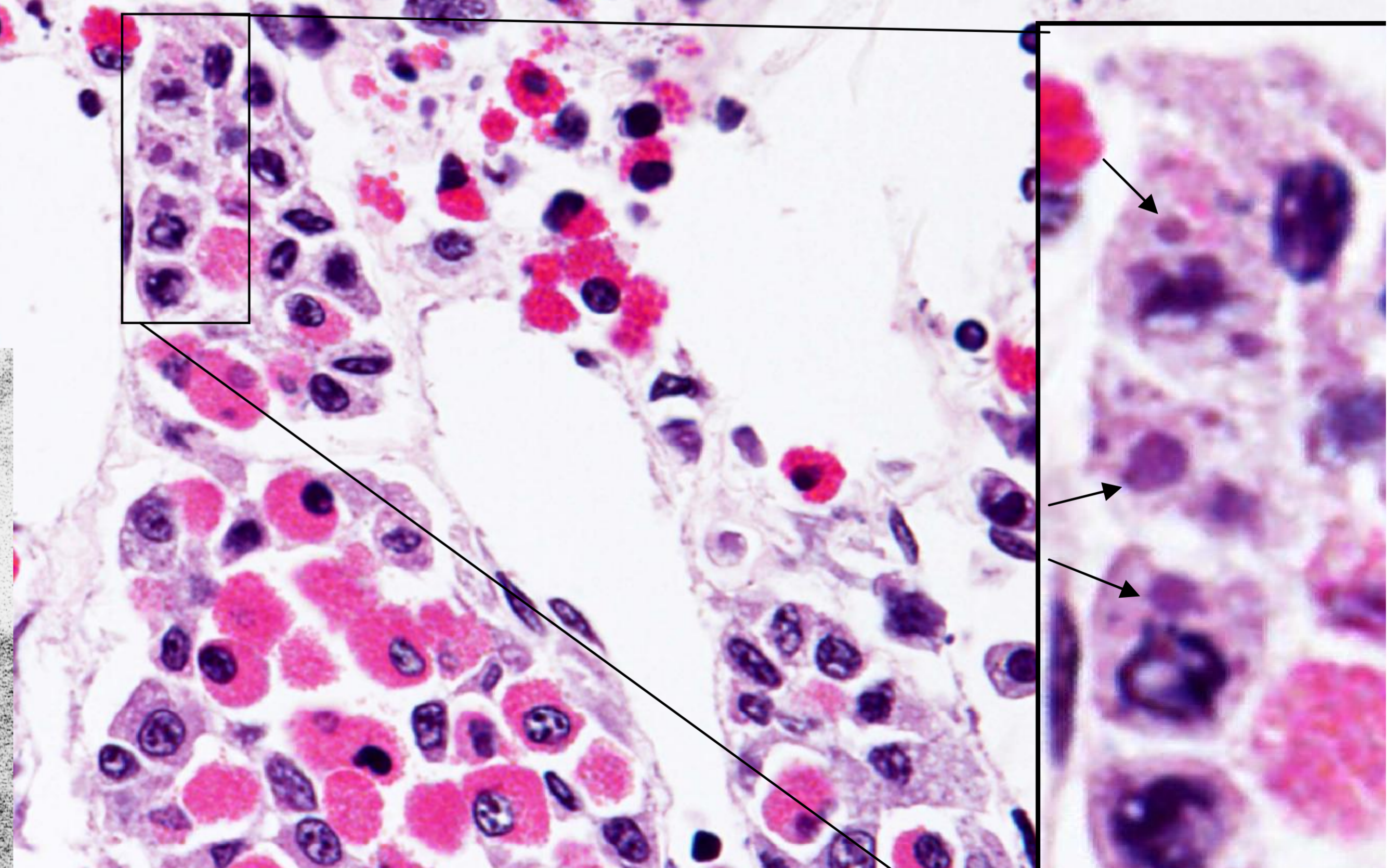
Kidney: glomerular necrosis
Renal tubular hyaline droplets



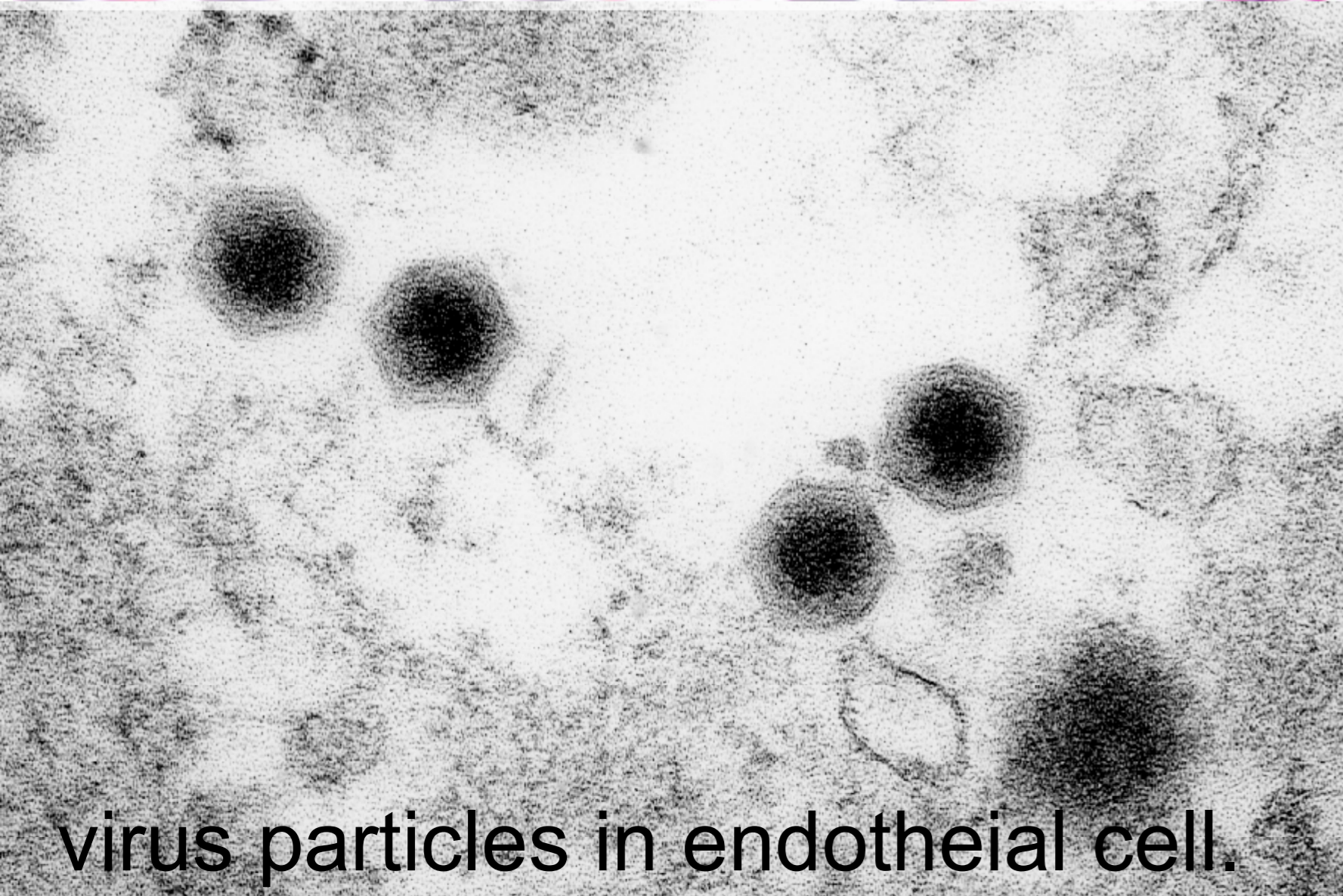
Liver: severe necrosis of hepatocytes
Arrow shows inclusion body



Kidney: Necrosis of tubules and inclusion body formation (arrows)



Basophilic intracytoplasmic inclusion bodies



virus particles in endothelial cell.

Episode No.8

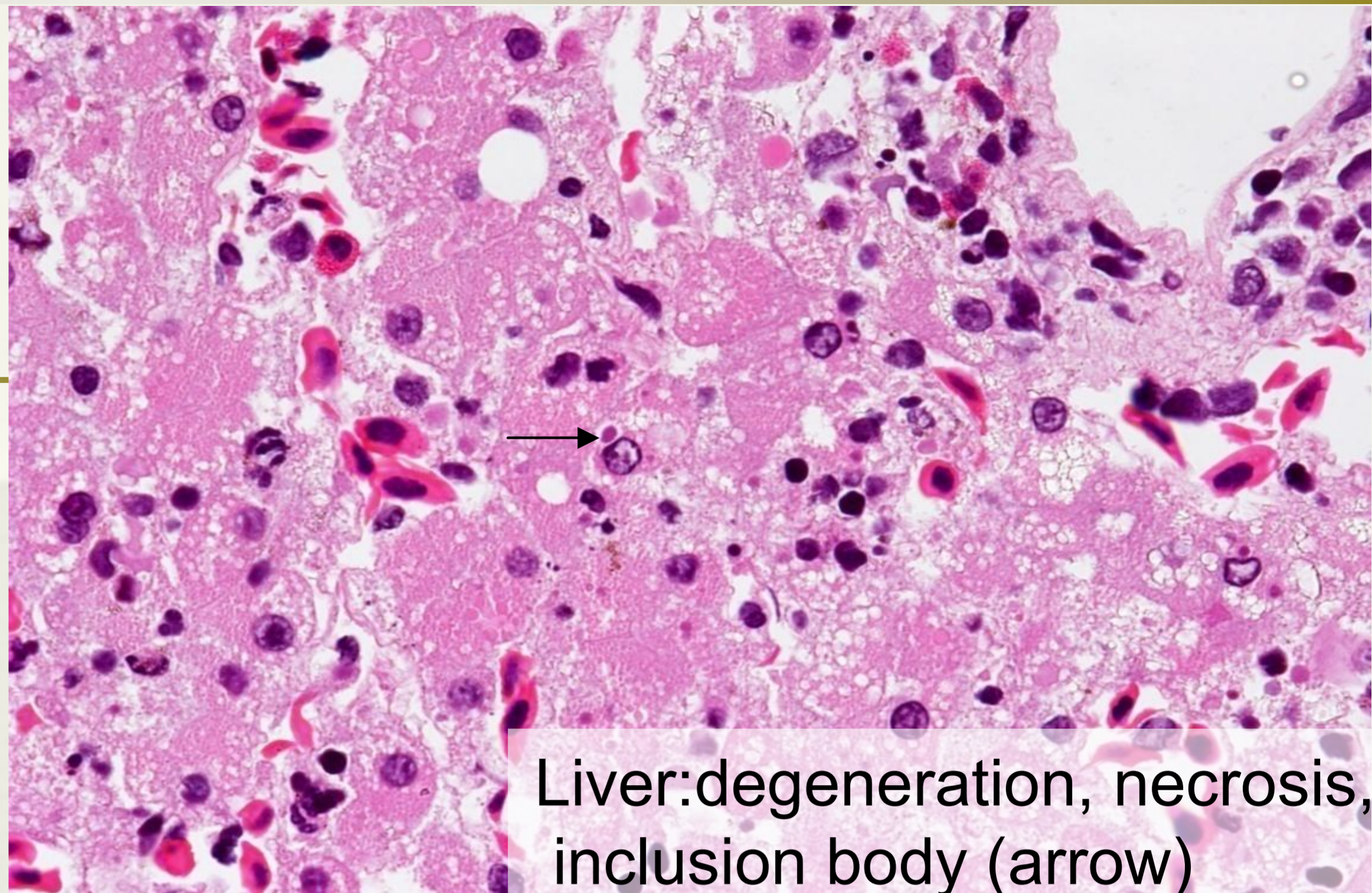
Ranaviral disease in salamanders

Western Japan.

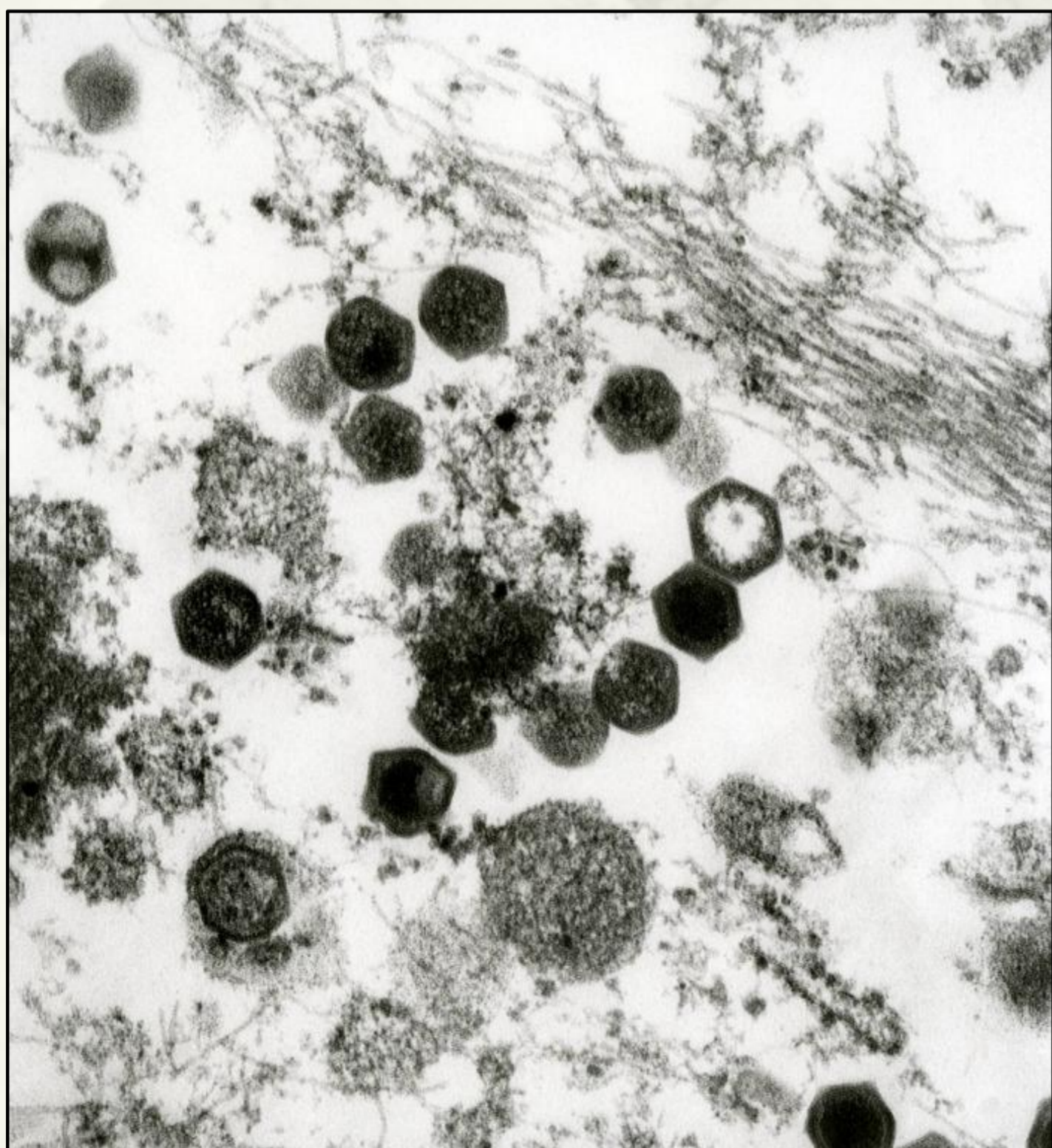


Clouded Salamander
Hynobius nebulosus
70-150mm

Skin lesion (ulcer)



Liver: degeneration, necrosis, inclusion body (arrow)

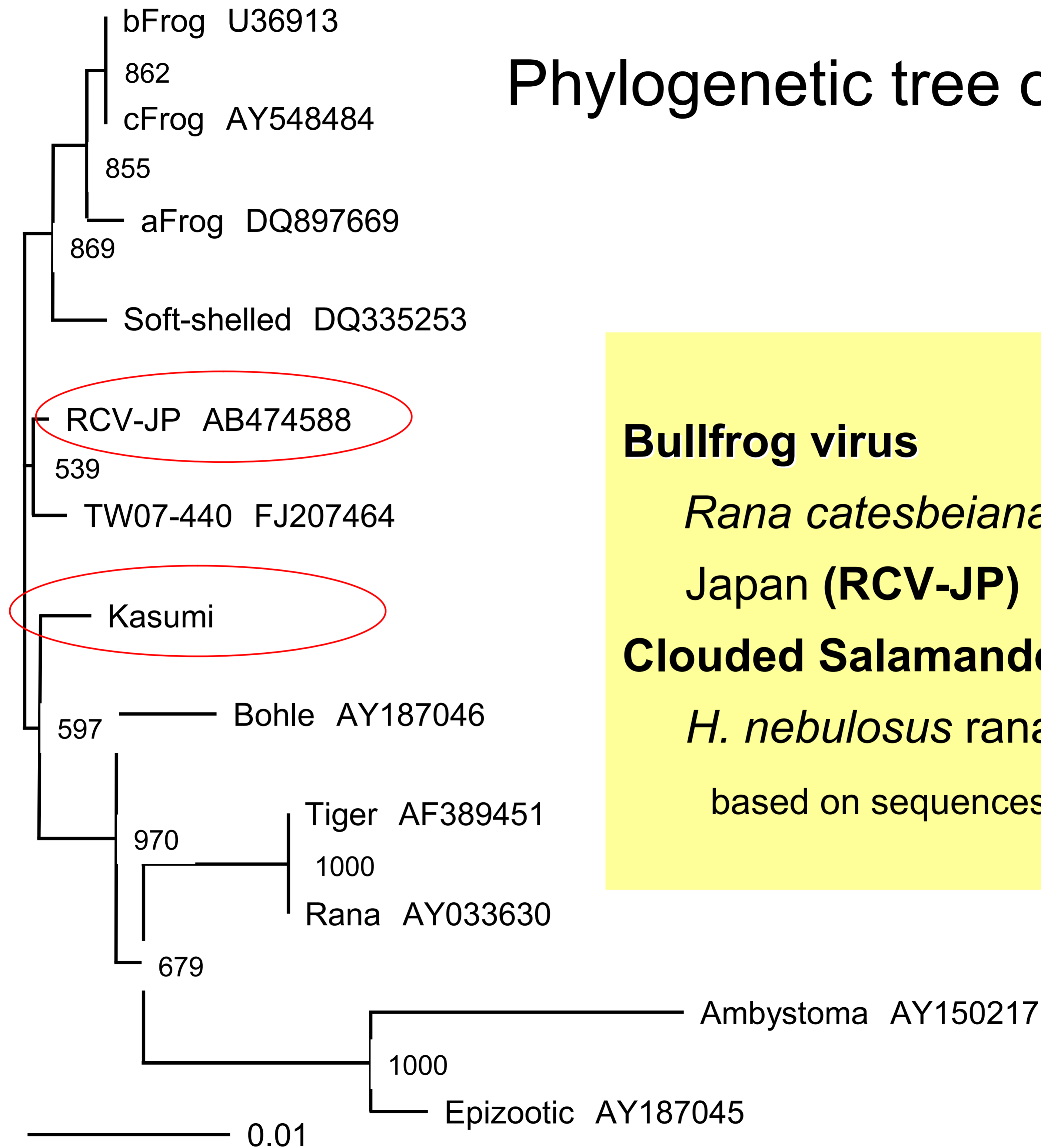


Virus particles in interstitial cells.



Kidney: glomerular necrosis
Renal tubular hyaline droplets

Phylogenetic tree of ranavirus



Bullfrog virus

Rana catesbeiana ranavirus

Japan (**RCV-JP**)

Clouded Salamander virus

H. nebulosus ranavirus (**HNV**)

based on sequences of the MCP gene.

Survey of ranavirus in wild amphibians in Japan

- about 1,200 wild amphibians
- PCR method

HNV is not found.

RCV-JP(+)

- Bullfrog (larvae)
- *Cynops ensicauda*
(adults)
- *Hyla japonica* (adults)
- *Rhacophorus arboreus*
(larvae)



carcass



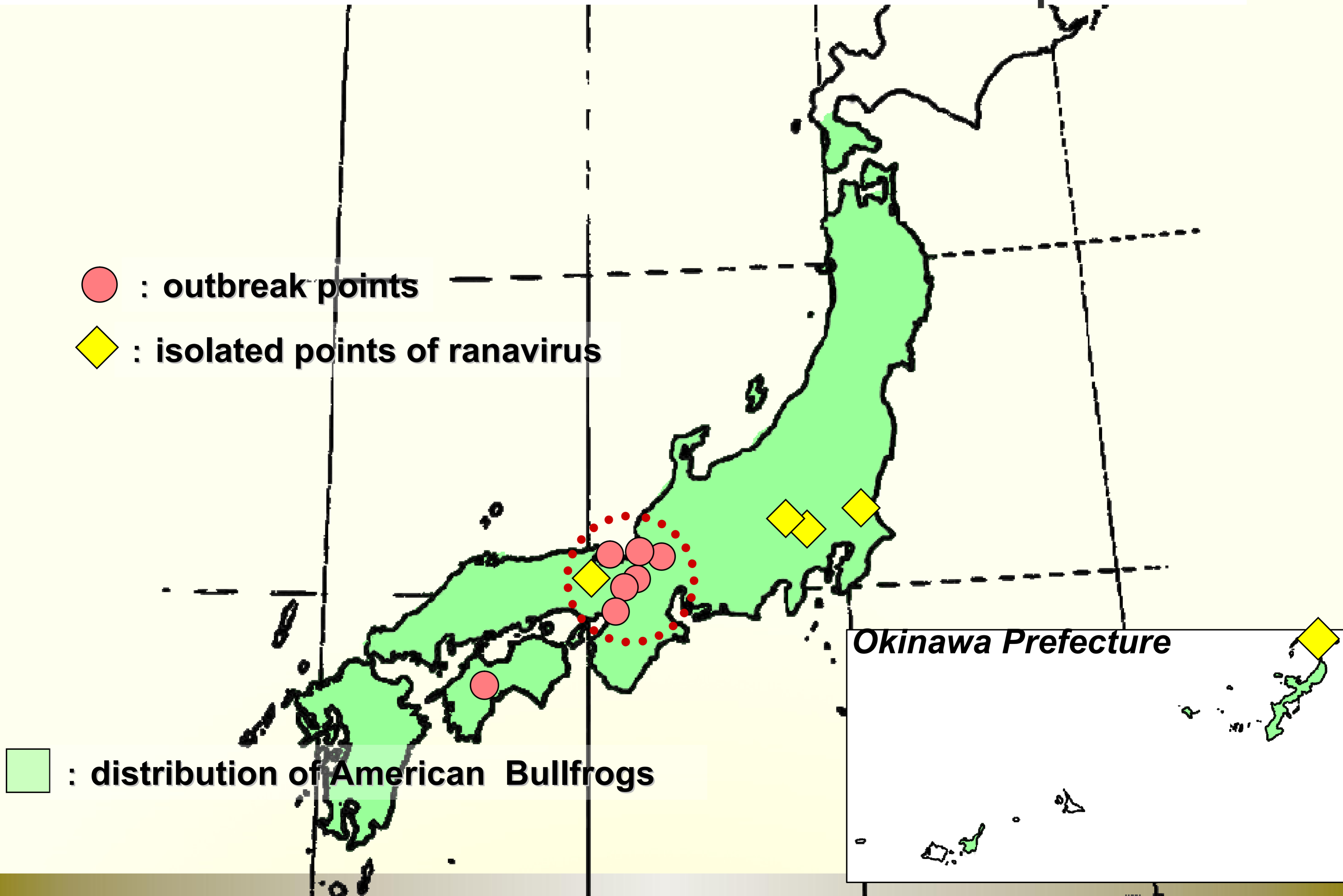
Tiger frog virus (+)

- *Fejervarya limnocharis*
(Cricket Frog, adults).



All infected animals appeared healthy except for *H. japonica*

Distribution of ranaviruses in Japan



Pathogenicity of ranavirus (RCV-JP).

	Species	Infected No. (control No)	Age	Temperature	Mortality rate
Salamander	<i>Cynops pyrrhogaster</i>	1(0)	adult	22°C	100.0%
	<i>Cynops ensicauda</i>	7(1)	adult	22°C	100.0%
	<i>Hynobius nebulosus</i>	53(20)	adult	22°C	100.0%
			larvae	22°C	100.0%
	<i>Hynobius nigrescens</i>	3(1)	adult	22°C	66.7%
			larvae		100.0%
	<i>Hynobius lichenatus</i>	33(13)	larvae	22°C	100.0%
				15°C	100.0%
<i>Hynobius tokyoensis</i>	40(20)	larvae	22°C	100.0%	
			15°C	80.0%	
Frog	<i>Microhyla ornata</i>	4(1)	adult	22°C	66.7%
	<i>Rana brevipoda porosa</i>	4(1)			100.0%
	<i>Rana limnocharis limnocharis</i>	5(1)			66.7%
	<i>Bufo gargarizans miyakonis</i>	4(1)			33.3%
	<i>Bufo japonicus formosus</i>	88(38)	larvae		100.0%

Total 11 species, 242 individuals

Inoculation : bath or intraperitoneally

Pathogenicity of ranavirus (HNV)

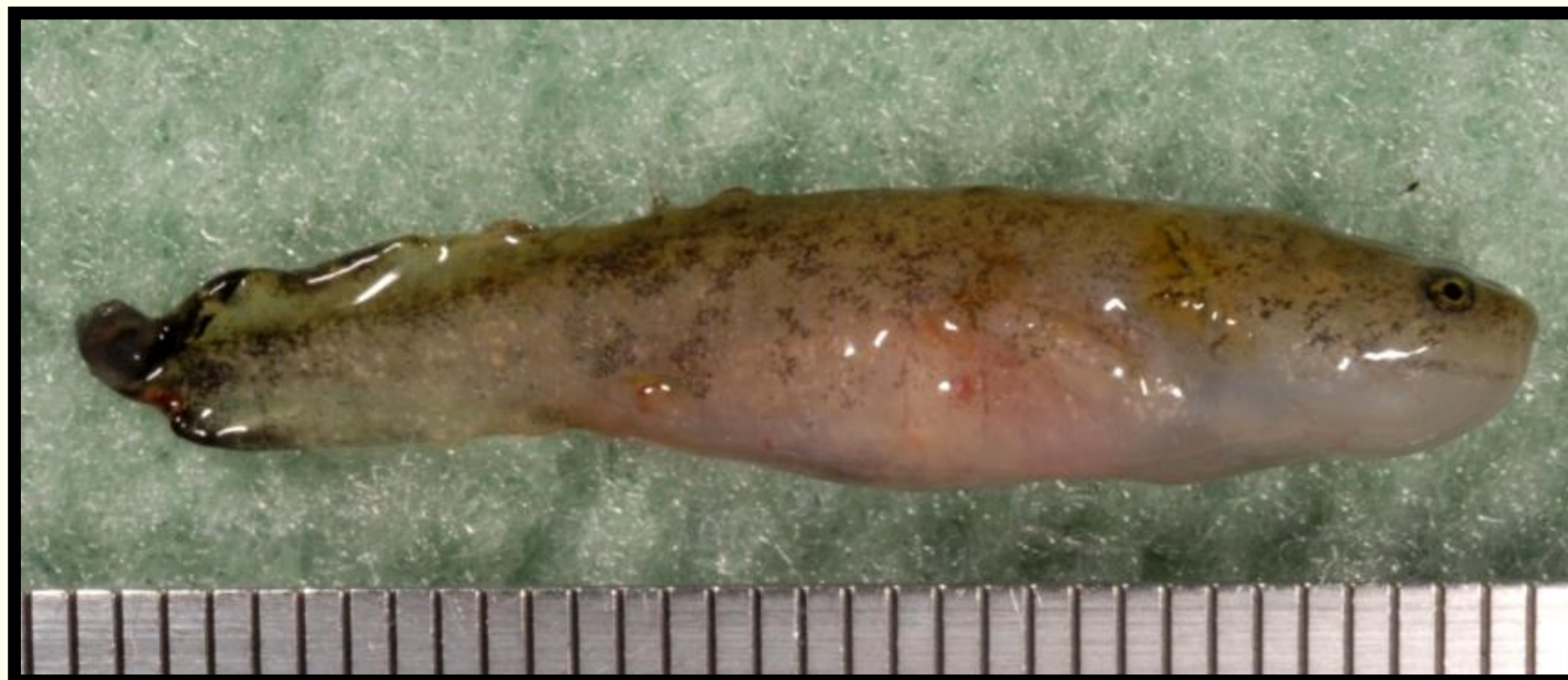
	Species	Infected No. (control No)	Age	Temperature	Mortality rate
Salamander	Cynops ensicauda	8	adult	22°C	100.0%
	Hynobius nebulosus	20	larvae	22°C	80.0%
				15°C	100.0%
	Hynobius nigrescens	5	larvae	15°C	0.0%
					40.0%
	Hynobius lichenatus	20	larvae	22°C	100.0%
				15°C	90.0%
	Hynobius tokyoensis	11	larvae	22°C	81.8%
	Hynobius dunni	20	larvae	22°C	100.0%
				15°C	100.0%
	Hynobius retardatus	20	larvae	22°C	100.0%
15°C				10.0%	

Inoculation : bath or intraperitoneally

Macrofindings in affected salamanders (Larvae)



hemorrhage



Loss of pigmentation, edema, necrosis of tail

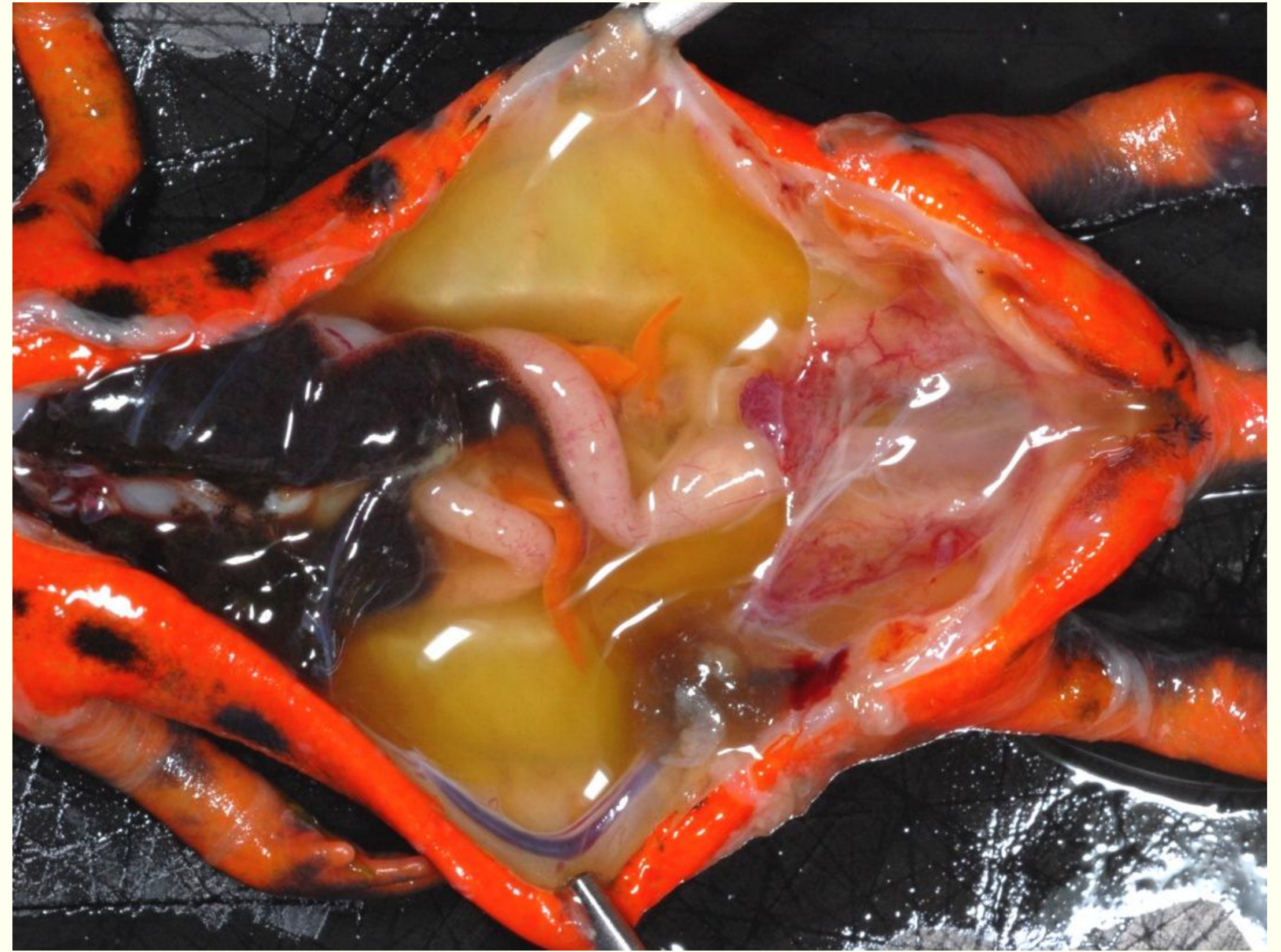


Abdominal edema

Macrofindings in affected salamanders (Adults)



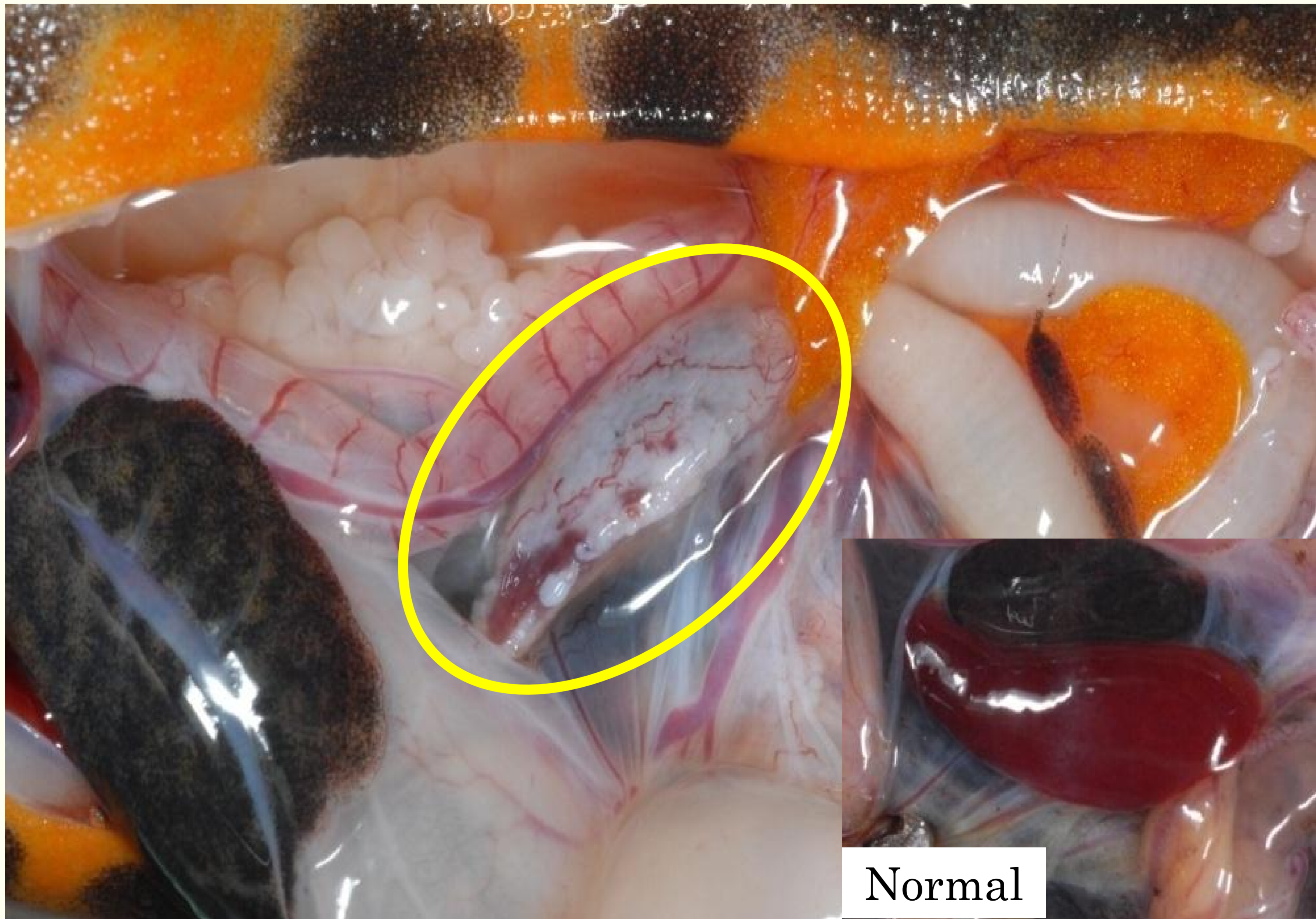
Abdominal edema



Abdominal edema



Skin ulcer



Atrophy and fibrosis of spleen.

Future agenda for ranavirus in Japan

1. Clarify:

- * Distribution of ranavirus in nature
- * Source of ranavirus (native or exotic ?)
- * Risk for native species.

Evaluate pathogenicity of each ranavirus (RCV-JP, HNV, TFV) for native species

- * Disease carriers (reservoirs)
- * Pathogenesis of mass die-offs

2. Draw up:

- * Measures to fight ranavirus in the field.



Thank you for your attention.

Contact: une@azabu-u.ac.jp