





SEVERE PERKINSEA INFECTION

Wood frog (Rana sylvatica) larva with petechial hemorrhages (arrows) and edema from Severe Perkinsea Infection.

Photograph by M. Isidoro-Ayza

CAUSE

Severe Perkinsea Infection (SPI) is linked to an infectious alveolate protozoa in the clade Novel Alveolate Group 01 (NAG01 or Pathogenic Perkinsea Clade) which belongs to the phylum Perkinsozoa. Protists of this group possess several distinct life-stages associated with infections. The transmission route is not well characterized, but infection may occur by direct animal-to-animal transmission, food, or via the environment. Infections occur most commonly in anuran larva and are rarely reported from adults. The pathogenesis of SPI is also not well characterized, and the primary site of infection seems to be the liver. After infection, the microbe proliferates resulting in degradation of the infected tissue, swelling of the abdominal region, and behavioral changes which eventually leads to host death.

There are many different freshwater Perkinsozoa species worldwide and the species linked to severe disease presentation in amphibians appears to be limited to North America and, more recently, in captive reared frogs. However, organisms belonging



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Distribution of molecular detection and mortality events caused by the infectious alveolate protozoa that causes Severe Perkinsea Infection in North America, 1999-2018. Adapted with permission from Isidoro-Ayza et al. 2017.

to the Pathogenic Perkinsea Clade and other groups of Perkinsozoa have been detected at multiple sites globally not associated with disease. The taxonomic classification and phylogenic relationship of amphibian Protozoa parasite to other alveolate taxa is still being resolved, but it is also distantly related to a commercially important marine parasite of oysters.

SIGNIFICANCE

Amphibians have faced large population declines across the world with infectious disease one of the key drivers of declines. Severe Perkinsea Infection is an emerging disease that has a high mortality rate (as high as 95% in recorded events). Emergence of this new lethal amphibian pathogen is worrisome, making research in surveillance and effective control increasingly important. Severe Perkinsea Infection has already created a large conservation impact in the recovery of the critically endangered dusky gopher frog (*Rana sevosa*) by eliminating entire cohorts of larvae in the few remaining breeding ponds.

SPECIES AFFECTED

Severe Perkinsea Infection affects a wide range of frog species, although disease is predominantly observed in larvae and metamorphs. To date, there have been 11 different Ranidae and Hylidae species reported with infections and disease susceptibility may extend to other anuran families.

DISTRIBUTION

As of 2021, Severe Perkinsea Infection mortality events have been diagnosed in 10 different U.S. states, from Florida to Alaska. One instance of SPI associated disease has been reported from captive reared animals in the U.K.

TRANSMISSION

It is suspected that transmission occurs through the aquatic environment. The spores are able to survive a wide range of temperature, pH, and salinity. The spores are also able to survive desiccation. When returned to an aquatic environment, the spores are likely to be able to develop into the infective zoospore stage.

CLINICAL SIGNS

Affected larva that have not yet died might swim erratically with buoyancy problems and seem lethargic. Occasionally, hemorrhages can be seen on the ventral body surface. The external signs of Severe Perkinsea Infection are similar to ranavirus in larval anurans.

Internally, the liver and other internal organs are severely enlarged with paleyellow discoloration and total effacement of the organ. Histologically, these organs are marked by replacement of 50-90% of the host tissue with necrotic debris, hemorrhage, and the Perkinsea organism's spore-like stage and trophozoite stage.

DIAGNOSIS

Severe Perkinsea Infection (SPI) is characterized by the case definition (Isidoro-Ayza el al. 2018):

- 1. Confirmed SPI: histopathologic evidence of SPI and a positive PCR test for NAG01 DNA from one of the internal organs.
- **2.** Suspected SPI: only histopathologic evidence, but no detection from a PCR test.
- 3. SPI organism Present: a positive PCR test for NAG01 DNA from one or more internal organs with no histopathologic evidence of SPI (not observed or unsuitable specimen)

Histopathologic evidence includes multiorgan necrosis with the organ tissue being replaced by extracellular and intracellular Perkinsea-like protozoa.



This photomicrograph shows the liver of a frog with a severe Perkinsea infection. Arrows indicate Perkinsea protozoa. (USGS, public domain)

TREATMENT

While there has been some research done on controlling and treating the related marine Perkinsus protist that infects oysters, there are no treatments for Severe Perkinsea Infection in amphibians.

MANAGEMENT

Current management consists of continued surveillance and monitoring for cases and the event of Severe Perkinsea Infection. Surveillance can be assisted by the development of multiple quantitative PCR methods, some of which are amenable to in field molecular diagnostics and allow detection of both SPI and other Perkinsea groups. Any sightings of large tadpole die-offs or other suspected Severe Perkinsea Infection events should be reported to appropriate wildlife personnel.

SUGGESTED READING

Isidoro-Ayza M, Lorch JM, Grear DA, Winzeler M, Calhoun DL, Barichivich WJ (2017) Pathogenic lineage of Perkinsea associated with mass mortality of frogs across the United States. Sci Rep 7:10288. https://doi.org/10.1038/s41598-017-10456-1

Isidoro-Ayza M, Grear DA, Chambouvet A (2019) Pathology and case definition of severe Perkinsea infection of frogs. *Vet Pathol* 56:133–142. <u>https://doi.org/10.1177/0300985818798132</u>

Karwacki EE, Atkinson MS, Ossiboff RJ, Savage AE (2018) Novel quantitative PCR assay specific for the emerging Perkinsea amphibian pathogen reveals seasonal infection dynamics. *Dis Aquat Organ* 129:85–98. https://doi.org/10.3354/dao03239

Atkinson, M.S. 2019. The Protistan Pathogen Perkinsea (a.k.a. Dermomycoides, Perkinsus-like organism, and alveolate pathogen) and its Impact on Southeastern Amphibians. Southeastern Partners in Amphibian and Reptile Conservation, Disease, Pathogens and Parasites Task Team, Information Sheet #20

Chambouvet A, Smilansky V, Jirků M, Isidoro-Ayza M, Itoïz S, Derelle E, Monier A, Gower DJ, Wilkinson M, Yabsley MJ, Lukeš J, Richards TA (2020) Diverse alveolate infections of tadpoles, a new threat to frogs? *PLoS Pathog* 16:e1008107. <u>https://doi.org/10.1371/journal.ppat.1008107</u>

Smilansky, V., Jirků, M., Milner, D.S., Ibáñez, R., Gratwicke, B., Nicholls, A., Lukeš, J., Chambouvet, A. and Richards, T.A. (2021) Expanded host and geographic range of tadpole associations with the Severe Perkinsea Infection group. *Biology Letters*, 17(6), p.20210166. <u>https://doi.org/10.1098/rsbl.2021.0166</u>