RANAVIRUS

Animal Group(s) Affected	Transmission	Clinical Signs	Severity	Treatment	Prevention and Control	Zoonotic
Amphibians, especially larvae and metamorphs; fish, and reptiles.	Transmission can occur through direct contact with infected animals; contact with contaminated water or substrates; ingestion of infected carcasses.	Infection can be sub-clinical. Enlarged, reddened abdomen; skin ulceration; epithelial proliferation; and emaciation. Stomatitis in chelonians.	Infection with *Ranavirus* is an important cause of mortality in wild amphibians, and possibly chelonia; only occasional reports of this infection in captive animals.	None.	Screen incoming amphibians for history of clinical signs consistent with disease.	No.

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Fact Sheet Reviewed by: Allan P. Pessier; Kathryn C. Gamble

Susceptible animal groups: All types of amphibians including caudates (salamanders and newts), and anurans (frogs and toads). Larvae and metamorphs are most often associated with morbidity and mortality. Adult morbidity and mortality occurs less often, but has been observed in zoo collections. Some species may have covert infections and be able to shed and transmit virus to other susceptible animals without ever exhibiting clinical signs. It may be associated with mortality events in wild chelonia. Amphibians may serve as a reservoir host for susceptible chelonians. Ranaviruses are also found in other poikilothermic vertebrates including reptiles and fish.

Causative organism: *Ranaviruses* are members of the Iridoviridae, a group of double stranded DNA viruses. Numerous strains are identified; however, viruses related to the *Ambystoma tigrinum* virus (ATV) and Frog virus 3 (FV3) appear to be the most important in North America. The Bohle iridovirus (BIV) from Australia also is of concern. Some ranaviruses may be able to infect animals from more than one class (e.g. amphibians and reptiles or amphibians and fish)

Zoonotic potential: None

Distribution: Worldwide although hotspots have been identified in recurrent mortality events. In some areas, it may be an emerging disease.

Incubation period: Variable - 5 days to several weeks. It possibly is affected by ambient temperatures, dose of virus exposure, immunosuppression, developmental stage, and species differences in susceptibility to different *Ranavirus* strains.

Clinical signs: In amphibians, the abdomen may become enlarged and reddened (red leg-like signs) and skin ulceration and/or epithelial proliferation may be seen. Infection does not always cause clinical disease. Long-term nonclinical carriers have been identified. In chelonian, nasal discharge, conjunctivitis, caseous plaques in the oral cavity and subcutaneous edema of the palpebra and neck have been seen.

Post mortem, gross, or histologic findings: In amphibians, necrosis and/or hemorrhage is present in multiple tissues, especially skin, liver, kidney, spleen/ hematopoietic tissue and gastrointestinal tract. In chelonians, observations are found of necrotizing and fibrinous stomatitis/esophagitis, splenitis and vasculitis. Histologically intracytoplasmic inclusion bodies may be seen. However, this finding is unreliable as they are

difficult to identify, are not always due to virus, and may be absent or inconspicuous in many cases.

Diagnosis: PCR is the most useful test and is becoming more widely available. Real-time PCR techniques allow detection of smaller amounts of virus, but to identify the group type (ATV or FV3 virus-like) of *Ranavirus* present conventional PCR with DNA sequencing is required. Determining the specific species of *Ranavirus* usually requires cell culture, virus isolation, and molecular characterization. These techniques are not widely available outside of research laboratories. Conventional PCR can provide false-positive results if confirmatory DNA sequencing or Southern blot analysis is not performed. Histopathology is helpful to screen for lesions in sick animals, but lesions tend to be nonspecific unless intracytoplasmic inclusion bodies are seen. Virus isolation, immunohistochemistry, transmission electron microscopy, cell culture, and serology (not widely available or validated for most species) have also been used to identify infected animals.

Material required for laboratory analysis: The best choice is tissue samples collected at necropsy, especially liver, kidney and, if lesions are present, skin. Frozen tissues are required for virus isolation and are generally best for molecular analysis; however, freezing is not acceptable for histology. For histology, tissues should be submitted fresh or fixed in 70% ethanol or 10% neutral buffered formalin. Ethanol-preserved tissues may be used for some molecular testing. Formalin-fixed tissues may also be used for some molecular testing if the length of time in formalin is minimal at days to weeks but it is possible to perform PCR on paraffin embedded tissues. Samples can also be attempted from clinically ill living animals such as cloacal or pharyngeal swabs, tissue biopsy (tail clips) or blood. Plastic handled, rayon tipped swabs are preferable for collection of PCR samples. If living animals are tested, results should be interpreted with caution recognizing test limitations (e.g., a positive test result is more meaningful than a negative test result). Test sensitivity for antemortem PCR increases with time post-exposure and development of clinical signs of illness. Contact individual laboratories for more information regarding screening.

Relevant diagnostic laboratories: http://fwf.ag.utk.edu/mgray/ranavirus/RanavirusTestingLabs2013.pdf

Diagnostic or research submissions on a fee-for-service and collaborative basis: qPCR, conventional PCR and sequencing (MCP, Pol and NF) for characterization of positive samples (e.g. FV3-like vs. ATV-like); PCR from ethanol or formalin-fixed paraffin embedded tissues; and histopathology:
Amphibian Disease Laboratory
c/o Allan Pessier
San Diego Zoo
15600 San Pasqual Valley Road
Escondido, CA 92027
(760) 291-5471
http://www.sandiegozooglobal.org/News/Amphibian_Disease_Laboratory/

Diagnostic or research: PCR, qPCR, virus culture, MCP sequencing, histopathology:
University of Tennessee Center for Wildlife Health
274 Ellington Plant Sciences Building
2431 Joe Johnson Drive
Knoxville, Tennessee 37996-4563
(865) 974-7948
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qPCR, cell culture, genomic sequencing and speciation:
Zoo Medicine Infectious Disease Lab
c/o April Childress

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2015 SW 16th Ave
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Gainesville, FL 32608
Phone 352-294-4420
Fax 352-392-5464
http://labs.vetmed.ufl.edu/sample-requirements/microbiology-parasitology-serology/zoo-med-infections/

qPCR:
Zoologix
9811 Owensmouth Avenue, Suite 4
Chatsworth, CA 91311-3800
Phone: 818-717-8880
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Email: info@zoologix.com

Treatment: None in amphibians is available. Antiviral therapy and supportive care has been attempted in reptiles.

Prevention and control: The major concerns in captive programs are that mortality will occur in a valuable species or population or that subclinically infected animals will expose naïve wild populations. The prevalence of infection in captive animals is not yet known. Disease has likely gone unrecognized due to clinical and pathological similarities to other diseases in amphibians. Captive amphibian populations can be surveyed continuously for disease by PCR and histopathology testing of samples collected at necropsy. Once a population or individual has been found positive by PCR the disposition of these animals will depend on careful risk assessment. A positive test does not distinguish between a lethal infection and a subclinical carrier. Factors to be considered include their importance to the survival of the species, the presence or absence of pre-existing infection in captive and wild populations and results of follow-up histologic and PCR testing. In some cases, the animals or a population may be managed in permanent isolation from the general amphibian population. Further prevention measures include quarantining all incoming animals. The health history of animals being brought into a population needs to be reviewed- if there have been deaths or illness due to confirmed or suspected *Ranavirus* in the prior 6 months the risk of disease transmission with introduction is considered higher. Animals dying during quarantine can be screened using PCR and histopathology. Strict biosecurity measures must be followed to avoid transmission of infection to other amphibians or susceptible classes of animals (fish, turtles, tortoises).

Suggested disinfectant for housing facilities: 1% Potassium peroxymonosulfate (Virkon®), 3% sodium hypochlorite and 1% chlorhexidine have been reported to be effective at inactivating *Ranavirus* after 1 min. contact duration. Some *Ranaviruses* were found to remain viable for 113 days on dry surfaces and 2 weeks in water. Amphibians are sensitive to disinfectant residues- thorough rinsing is required after use. Biosecurity measures must include treatment of waste and effluent from *Ranavirus* infected animals.

Notification: Infection by a *Ranavirus* is classified as a reportable disease by the OIE requiring proof of *Ranavirus*-negative results before commercial shipment of amphibians (OIE 2008). http://www.oie.int/eng/normes/fcode/fcode2008/en_chapitre_2.4.2.htm. A reporting mechanism (e.g. via USDA-APHIS) has not been announced for the US at this time.

Measures required under the Animal Disease Surveillance Plan: Currently none. See http://www.oie.int/eng/normes/fcode/fcode2008/en_chapitre_2.4.2.htm as Article 2.4.2.10. states that importation of live aquatic animals intended for use in zoos from a country not declared free from *Ranavirus* should be followed by lifelong holding of the animals in biosecure facilities for continuous isolation from the

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local environment and treatment of all effluent and waste materials in a manner that inactivates *Ranavirus.*

Measures required for introducing animals to infected animals: Animals should not be introduced to those showing clinical signs of disease or with exposure to known infected animals.

Conditions for restoring disease-free status after an outbreak: None established.
See: http://www.oie.int/eng/normes/fcode/fcode2008/en_chapitre_2.4.2.htm

Experts who may be consulted:

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