SAN DIEGO ZOO INSTITUTE FOR CONSERVATION RESEARCH

AMPHIBIAN DISEASE LABORATORY NEWSLETTER

Vol. II - February 2011

The Amphibian Disease Laboratory (ADL) has been hopping!

Written by: Megan Jones, DVM, DACVP, Amphibian Pathology Fellow


Photo by K. Benson

Since beginning operation in February, 2009, we have tested over 7000 samples for the amphibian chytrid fungus *(Batrachochytrium dendrobatis or Bd).* Using information in our diagnostic testing database, we are beginning to shed light on the patterns of *Bd* infection in captive amphibian populations.

How common is *Bd* in zoo collections?

We partnered with seven zoos or aquariums that performed complete surveillance of their entire amphibian collections. The animals sampled in this survey were healthy and exhibited no symptoms of disease. *Bd* was detected in four of the seven zoos, with a range of 2 to 3% positive. These findings confirm that *Bd* is present at low levels in some zoos, and underscore the critical importance of disease surveillance, quarantine, and other biosecurity measures to identify subclinical carriers and prevent transmission of disease to susceptible species, especially those in survival assurance populations or reintroduction programs.


Photo by K. Benson

Which animals may be more likely to test *Bd*-positive?

On our submission forms, we always ask for your reason for testing. Testing categories include routine surveillance, quarantine screening, sick animal, or pre-shipment/pre-release. Preliminary analysis suggests that samples from amphibians in quarantine and from sick animals test positive for *Bd* more often than those taken for the purpose of routine collection surveillance. Not surprisingly, animals tested because of suspected exposure to other known *Bd*-positive animals are the most likely to test *Bd*-positive.

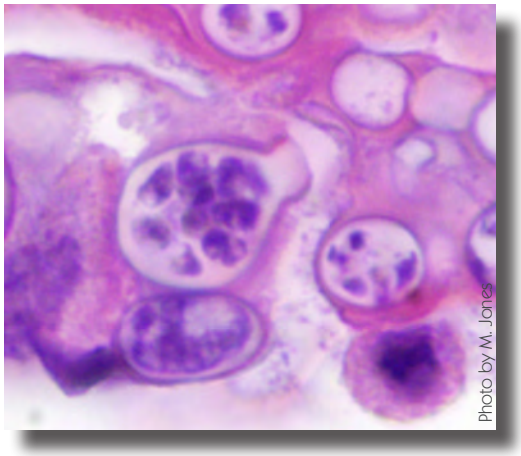


Photo by M. Jones

Continued on page 2....

....Continued from page 1

Treatment of *Bd* Infection

Based on follow-up information kindly provided by our clients, the antifungal drug itraconazole has been the most commonly used treatment for *Bd* infection in U.S. zoos. Most facilities report using the drug at the "standard" dose of 0.01% (100 mg/L) administered as five-minute daily baths for ten days (see Pessier and Mendelson, 2010). However, effective treatment has also been achieved using the lower dose of 0.005% (50 mg/L). Low dose treatment success has been confirmed through serial post-treatment PCR testing in two different captive amphibian populations. Please note that itraconazole is known to be potentially toxic to tadpoles and new metamorphs at standard dosages, and occasional adverse effects are reported in adult amphibians. There is still an urgent need for controlled clinical trials for itraconazole and other *Bd* treatments, but it appears that lower doses could be both effective and safe.

Testing Strategies: Serial Samples

Previous experimental data have shown that, in cases of low-level infections, individual animal PCR test results can vary over time (Hyatt et al, 2007). Data we have collected from real-world samples support this finding: a single subclinically infected animal can vary between PCR-positive and PCR-negative over the course of several weeks of infection. This is because in low level infections, it is possible to swab an animal during a phase of the fungal life cycle where most or all of the fungal zoospores have been recently discharged, leaving behind very little or no fungal DNA to detect by PCR. In this case, a skin swab will test negative, though the animal is actually infected (false negative). Thus, we recommend that you consider serial PCR testing in certain cases. While we recognize that this may not be economically feasible for all situations, we suggest a cost/benefit analysis, and consider sampling animals multiple times in higher-risk situations. These situations may include wild caught animals, animals with an uncertain health history, animals with known exposure to *Bd*-positive populations, and in cases of exceptionally valuable animals where a risk of missed infection is unacceptable (such as with survival assurance populations of endangered species).

Conclusions

Preliminary findings from our database suggest that *Bd* may be present and undetected in some zoo collections. This highlights the importance of collection screening and biosecurity. The information you provide on our submission form, and the time you take to provide follow-up information to us, are greatly appreciated, and are helping contribute to our ongoing efforts to improve our understanding of the amphibian chytrid fungus in captive amphibian populations.

Hyatt, A.D., D. G. Boyle, V. Olsen, D. B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F. Gleason and A. Colling. 2007. Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. Diseases of Aquatic Organisms 73: 175–192.

Pessier, AP, and JR Mendelson (eds.). 2010. A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs. IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, MN.

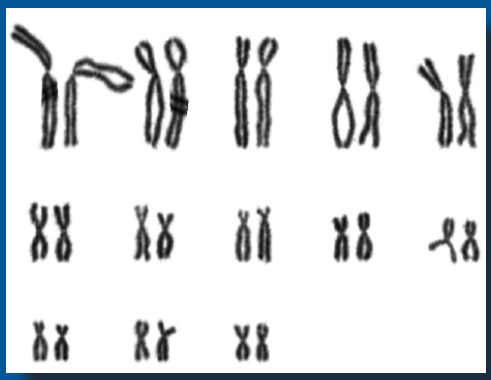
The **AMPHIBIAN DISEASE CONTROL MANUAL**

is a new, valuable resource for people who work with captive amphibians.

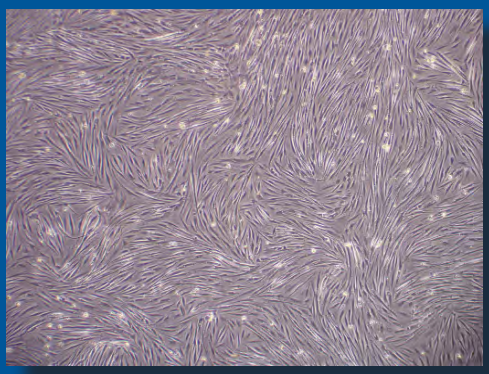
Click on the image for a complete, free PDF copy of the manual!

Genetics

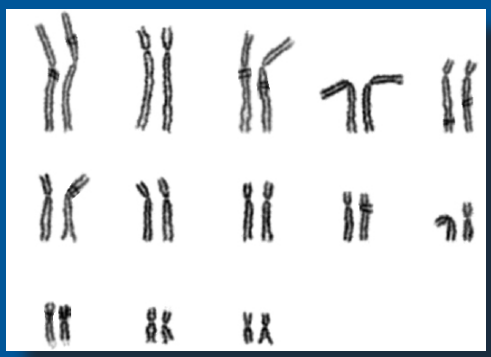
Written by: Andrea Johnson, Research Technician



American Bullfrog Karyotype
Photo by A. Johnson



White's Tree Frog Tongue Cells
Photo by S. Charter



White's Tree Frog Karyotype
Photo by S. Charter

It was a productive year for the Genetics team in our pursuit of frog samples and cell lines. We processed over 250 samples from about 75 individual animals. We made an important discovery when we were able to grow cells from a piece of African bullfrog tongue that had been frozen in liquid nitrogen for four months! This validates our strategy of collecting pieces of frozen tissue from as many species as possible. Important species that we can't grow cells from now can be "tissue pieced" to be thawed and cultured when we have come closer to perfecting amphibian cell culture methods.

Four new amphibian species were added to our tissue piece collection during this last year: axolotl, vermiculated tree frog, Japanese giant salamander, and Chiricahua leopard frog.

We also successfully froze an axolotl cell line, for a total of nine species represented by viable frozen cell lines. That may not sound like many, but considering that tissue culture has never even been attempted on the vast majority of amphibian species, it's something we are proud of.

Fresh samples are still hard to come by so.....

we still need your help!!

We are always grateful to receive pieces of tissue when you have a frog die. Each attempt teaches us a little about what to do (and what not to do!). Thank you for all the help and support you have given so far.

To learn how to collect samples, please contact the

San Diego Zoo's Cytogenetics Lab
at
(760) 747-8702 x5716
or email
Andrea Johnson ajohnson@sandiegozoo.org
or Marlys Houck mhouck@sandiegozoo.org

You can also request a tissue culture sample kit to be included with your chytrid and/or ranavirus testing kit.

We offer free kits containing stardarized tubes and swabs for our clients. Just send us an email at

AmphibianLab@sandiegozoo.org

and let us know how many samples you are planning to submit and we will send you a package.

WE TAKE CREDIT CARDS!

Call our finance guru

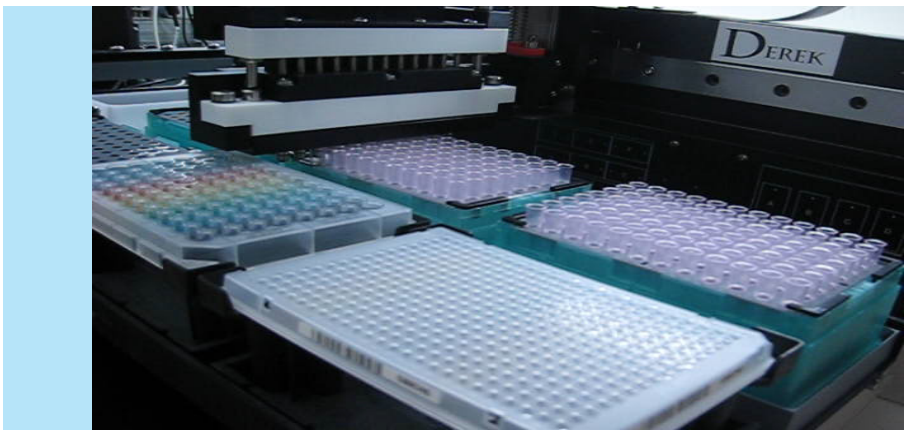
Karen Neely

if you would like to use this form of payment.

(619) 231.1515 x4190

THANK YOU

TO THE EDYTH BUSH CHARITABLE FOUNDATION

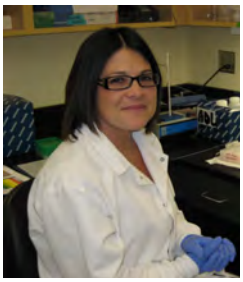


We are sincerely grateful for for the generous donation of the BioTek Precision Liquid Handler and many other contributions that make every day in the lab more efficient and productive, and look forward to their continued support in our efforts to help you maintain a healthy and happy amphibian community!

Amphibian Disease Lab

Allan Pessier.....Project Director
Megan Jones.....Amphibian Pathology Fellow
Mark Schrenzel...Head of Molecular Diagnostics
Bruce Rideout.....Director Wildlife Disease Labs
Tammy Tucker.....Wildlife Disease Lab
Jennifer Burchell.....Wildlife Disease Lab
Isamara Navarrete.....Wildlife Disease Lab
Kristin Benson.....Data Coordinator
Karen Neely.....Finance
Oliver Ryder.....Director of Genetics
Marlys Houck.....Genetics Lab
Andrea Johnson.....Genetics Lab
Suellen Charter.....Genetics Lab

Meet Our Newest Member



Please welcome our new laboratory technician

Jennifer Burchell!!!

The Amphibian Disease Laboratory is supported by grant LG-25-08-0066 from the Institute of Museum and Library Services. Any views, findings, conclusions or recommendations expressed in this publication do not necessarily represent those of the Institute of Museum and Library Services.

AMPHIBIAN DISEASE LAB

Mailing Address:
Amphibian Disease Lab
15600 San Pasqual Valley Road
Escondido, CA 92027

Billing Address:
San Diego Zoo
Attn: Karen Neely/Finance Department
PO Box 120551
San Diego, CA 92112

Think Of The Environment; Please Don't Print This Document Unless It's Absolutely Necessary