SECOND INTERNATIONAL SYMPOSIUM ON RANAVIRUSES

DIAGNOSIS, TREATMENT AND MANAGEMENT OVERVIEW DISCUSSION SESSION

LOCATION: Parlor 2, Holiday Inn World's Fair Park, Knoxville, TN

TIME AND DATE: 14:45 – 15:45, July 28, 2013

LEAD BY: Dr. Allan Pessier, San Diego Zoo

MINUTES TAKEN BY: Dr. Ann Duncan, Detroit Zoo

POTENTIAL ACTION ITEMS:

1. Research Needs:
    - Development of an accurate and reliable test for ranaviral infection in live animals.
    - Development of tests that permit for strain identification.
    - Effective use of antiviral drugs in all taxa affected by ranaviruses.
2. Outreach:
    - Explain the different goals/outcomes/meanings of different tests for ranaviruses.
    - Explain the difference between certification of disease free and disease diagnosis.
    - Explain the difference between diagnostic sensitivity and specificity.
3. Tasks:
    - Proficiency of labs at tests for ranavirus and standardization of procedures between labs.
    - Guidelines for surveys to permit for comparison between studies and regions.
    - Exploration of the potential removal of ranaviruses from the OIE listing as many countries do not follow recommendations anyhow.
    - Establishment of common diagnostic methods.

MINUTES:

1. **What should the gold standard be in diagnostics?**

    The test to use depends on the question that needs to be answered. There is a big difference between detection and diagnosis.

    This question may arise mostly from people wanting to know which test to run in different situations, rather than expecting one a gold standard. There are different tests recommended for determining prevalence, or whether ranaviris is the cause of an outbreak. Diagnosing disease versus certifiying freedom disease also requires different testing. There is currently no good diagnostic test for live animals that are free of clinical disease. The advantages and limitations of each test have to be described.

    Suggested a proficiency test process. A quality control method to compare labs, or a ring trial. Run blinded samples at many different laboratories internationally. May be able to avoid permitting if use DNA instead of animal samples. This process may help flesh out which tests work best for certain strains.
    To undertake this would need a lab to get the isolates up and running, make it a blind study, keep track of data, etc. There is a certification program in Canada for all kinds of diagnostic tests. They might have a system that can be utilized to coordinate this kind of study. Have a web-based program that is international. Everyone enters their own data, so that helps with labor and security.
    Koi herpes virus is a cautionary tale against this all of the different labs using different methods.

Diagnostic sensitivity and sensitivity. The sensitivity and specificity question would be answered by standardization and comparison of testing methods.
PCR based methods depend on viral shedding, and the persistently infected are not going to be found. Persistence might be determined through ELISA- don't have a good test for this. Methods for identifying subclinical animals need to be developed, and then used to follow animals long term to determine the length of shedding/carrying status.

Should we have recommendations that allow us to tease out the strains.
How can we use all of the genome we have already to come up with a list of the regions that work best to answer different questions?

Guidelines for how to do surveys of areas so that comparisons can be made. So that regions can be compared.

It is possible that ranavirus could be taken off of the OIE list in order to not have to prove freedom for movement? Could be similar to AI where it's not the disease but the specific strain that matters. OIE is not being followed by many countries.

2. **OIE methods versus other commonly employed methods?**

The consensus in the room is that hardly any of the labs are using the OIE methods- they are similar but not identical. Why are we not using the method, and should we suggest a method to the organization?
We are sequencing on our own now, so not as much of an issue. OIE methods are not straightforward, and are not instructive. The OIE lists available methods. Have specific recommendations regarding the PCR primers that can be used. These are not the primers that are being used, MAO. MCP, restriction enzyme recommendations, RFLP.
VI is the gold standard, and have to have histo as well.
VI, are no reptilian satellites.

There is a designated OIE reference lab- Australian Animal Health Laboratory. Alex Hyatt, is now retired. Don't know who is in charge of the lab now.

3. **What about PCR specificity and sensitivity?**
See above

4. **Prospects for vaccines and antiviral drugs?**

What is the application of a vaccine?
Could use in animals being reintroduced. Especially chelonians, maybe box turtles will need a vaccine in the near future.
For the amphibian programs, they are presently not in need of a vaccine. Their programs are not being threatened by RV, so they would not want to use a vaccine any time soon.
Vaccine use is probably not applicable for ranaculture and aquaculture.
Decided that there is no immediate need for a vaccine, but may need to start work now in order to develop something in the future. More is needed to know about immune systems and responses.
Vaccine development would require getting the right attenuation of the virus to produce protection without disease. Also don't want exposure of animals in the environment. Could vary species by species.

Treatment

Are you trying to treat sick animals or asymptomatic animals to make sure they are clear?

An antiviral could be useful in the captive situation. It is good to start this work. Need cell culture trials and exposure trials. There is still a lot to figure out in terms of temperature. Temp might be the easiest tool. Hard to do pharmacokinetics in amphibians d.t. sample size limitations.

Chain terminators have been screened in cell culture- acyclovir
Sedofavir- different method of action than the chain terminators. UF is doing work with this drug, in vivo first to see if it seems to work.
Could use interferon treatments. Most of the viruses tested seem to be susceptible.

5. **Develop IHC methods for RV?**
   OIE standards have recommendations.