

Rounds

Animal Health
Department

Medical Rounds

"medicine for all"



Caring for Stranded Marine Animals

NATIONAL
MARINE
L I F E
CENTER

Notes

Veterinary Research
Department

Under the microscope

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Rounds Notes is a report on the health of animals at the National Marine Life Center from Sea Rogers Williams VMD for the staff, volunteers, and community of the center including professionals involved the captive care of similar species, the views expressed are not necessarily that of NMLC. Information in Rounds Notes should be considered confidential and used solely to benefit the health of aquatic animals everywhere.

July 21, 2011

Rounds Notes

6: 16-20 (2011)

Clinical Update: Cooters still hypocalcemic, but stronger mom said 'drink your milk', I'd rather have you chew on a cuttlebone

I've seen the little cooters eating cuttlebone, I think it's great and they seem to like it.

Bruce is doing very well, normal ionized calcium, the wavy appearance of the carapace is improving, the cooter is strong and growing, the plastron is not impinging on the limbs and appears to be less soft then when admitted. The smallest, Vecuvius, was tested at Rounds this week and still does not register an ionized calcium level, so we will continue with oral calcium glubinate, weekly oral calcium carbonate and D3 gavages, and improved husbandry. Both little cooters are making clinical progress but we still have a way to go.



Terrapins, Cooters, and Turtles, oh my . . .

Catch's final Cornell Results

Chrysosporium anamorph of a fungi closely related to *Aphanoascus* species (91% homology) which is insufficient to confirm the genus identification, with Heavy contamination with a mixed bacterial population, and *Fusarium solani*.

What is important is that they isolated the fungus, it has compatible morphology to our findings, and it appears stable in sub-culture. This is HUGE. We will have Cornell hold on to one sub-culture, and send the other to the Fungal Testing Laboratory, University of Texas Health Science Center, a world renowned center on clinical fungal identification. So hang on to your seats, more to come in 2-3 weeks, while we let the folks in Texas work on this problem.



To recap: For now let's call the carapace culture results *Chrysosporium*, and the DNA probe from the biopsy results 99% homology with *Trichosporon dermatis* and *Trichosporon mucoides* [GenBank & CBS fungal database]. All the results are not in, but for those of you (including myself) who can't wait, this is time to ponder *Chrysosporium* in general, *Chrysosporium* anamorph of *Nannizziopsis vriesii*) and just how we diagnose and treat infectious diseases in general.

Headlines News: Who won the revolution ?

how medical science and DNA have changed the way infectious disease is identified

The advent of molecular technology to concentrate minute traces of DNA, an organism's defining and signature molecule(s), by polymerase chain reaction (PCR), and compare the nucleotide sequence (sequencing) to other related and not so related (out group) organisms has revolutionized how infectious diseases are identified, which in turn is revolutionizing how infectious diseases are diagnosed and treated.

The old school microorganism identification was based on growth of the organism in culture be it viral, bacteria, fungal or protozoal. Once the organism was present in pure culture it could be examined for morphologic characteristics (cocci vs rods), induced physical characteristics (such as bacterial cell wall composition as identified by a staining procedure, i.e. Gram positive, Gram negative), and biochemistry (hydrogen sulfide production, gelatin hydrolysis, catalase reaction, glucose fermentation and many others). But morphology is a slippery handle on organisms' ID. Many of these organisms, such as bacteria, do not have a lot of visible morphological markers, even with the best optics in modern microscopes. Further many parasites, fungi, and protozoa have life stages that have completely different anatomy, in the same way a butterfly is related to the caterpillar stage of it's life cycle. As a result some parasites and fungi and other microorganisms have been given separate scientific names at different life stages as if the organisms were not even related. And few organisms have a completely unique chemistry capabilities. What is constant then ? well, it's DNA.

The real problem with the current system is often in step one, growing the little buggers. Failure to grow an organism is not proof of the absence of the organism. Some estimates have the total number of microorganisms that can be cultured as less than 10% of the microorganisms present in nature or in the host. Culture methods have been developed specifically for looking to human pathogens, which works pretty well in most mammal systems at 37°C, but aquatic reptile pathogens with fluctuating thermal and salinity environments can be hindered by standard laboratory procedures set up to deal with terrestrial mammals. For example, the unfamiliarity with many growth requirements of reptile pathogens, and overwhelming bacteria levels normally found on the shell of aquatic turtles hinders shell fungal isolation.

The old school approach to determining infection was to make a test (often an ELISA) to detect the host specific antibodies to a specific part of the pathogen, this was measurable and objective, and could determine if the host had been exposed to the organism. There are of course several problems here too. If the host was immuno-compromised, the host may not make a strong antibody response, and even under the best of circumstances there is a lag time from exposure to antibody production, particularly if it is the first time the host is exposed to the antigen. Thus, paired serum antibody titers were the gold standard, with a typical separation of 2-4 weeks

between tests. Further, this method measures exposure and response, it did not detect if the organisms was still present in the host, something a wildlife rehabilitation organization might like to know. Has PCR come to the rescue? We can now detect traces and remnants of an invading organisms DNA in blood, tissues, biopsies, etc, but it's still not a perfect world. Failure to detect the DNA of an organism by PCR is similarly not proof of the lack the organism in the host. The the presence of an organism does not mean that it is have a specific biological effect (like disease), sometimes other organisms live on and inside of us without doing any harm at all. Think about all the millions of bacteria in our digestive systems, mouth, skin, etc. And the tests are still only as good as the laboratory that designs these DNA probes. A host is awash in it's own DNA so the ability to amplify only the target DNA is critical as well as the distinction from other forms. And while closely related organisms can have vastly different morphology (phenotype) closely related organisms have very similar DNA sequences (genotype), where the difference of one or a few nucleotide changes can make the difference between friend and foe. Laboratory contamination is also possible as these test become sensitive to the most minute level stray DNA. There are advantages as well. There may a decreased need to actually grow dangerous bacteria or viruses in the lab for diagnostic purposes. *Brucella* is a genus of bacteria that colonize marine mammals and some are capable of causing serious human disease. If suspected in a clinical case it is recommended not to culture the bacteria as it is very dangerous in laboratory, but detection of the DNA make give us greater specificity and the ability to diagnose the bacteria without having to culture the organism.

So how does this all relate to Catch-22 ?

It still requires a trained medical professional to determine which samples need to be collected and how they should be tested, and most importantly how to interpret the results.

Chrysosporium vs *Trichosporon* the jury is still out and we have more to learn. The practice of medicine will always involve the art of high quality medical care, but should be enforced by latest science in our understanding the individual, pathogens, and the environment that we all share.

Where in the World:

Buzzards Bay ? Dr. Williams teaching teachers about Medical Technology used when rehabilitation seals, sea turtles, and cetaceans

Dr. Williams presented a talk "Marine Animal Rehabilitation & Medical Technology" to a group of 25 high school teachers as part of the National Marine Life Center Education Summer Series. Use of advanced medical imaging, such as how CT helped us diagnosis the location of the gas in Caveman and manage Fletcher when she ate the rubber stoppers and use of modern molecular methods of infectious disease identification.



Under the Microscope: Charismatic Mico-fauna: parasite or not, the epibiota of sea turtles is fascinating world of life

(from WikiPedia) The most widely known version appears in Stephen Hawking's 1988 book *A Brief History of Time*, which starts:

A well-known scientist (some say it was Bertrand Russell) once gave a public lecture on astronomy. He described how the earth orbits around the sun and how the sun, in turn, orbits around the center of a vast collection of stars called our galaxy. At the end of the lecture, a little old lady at the back of the room got up and said: "What you have told us is rubbish. The world is really a flat plate supported on the back of a giant tortoise." The scientist gave a superior smile before replying, "What is the tortoise standing on?" "You're very clever, young man, very clever," said the old lady. "But it's turtles all the way down!"[1]

The National Marine Life Center has been asked to help assess the health of a sample population of wild Loggerhead sea turtles briefly collected by NOAA Fisheries in the northeast region. A part of this project is the identification of epibiota sampled from the backs of sea turtles. Of course everyone knows the cosmological significance of studying the life on the back of turtles is really a study of life, the universe, and everything [see sidebar].

We found something different. I saw sea turtle specialist amphipods like *Podocerus chelonophilus* found only on the backs of sea turtles, and the more common and somewhat more general generalized *Caprella andreae*. Crabs like *Planes minutus* are commonly found as part of the epibiotic infanua, like the barnacles *Chelonibia testuinarum*, and some surprises as well, but you'll just have to wait for the full report.



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Sea turtle epibionts

