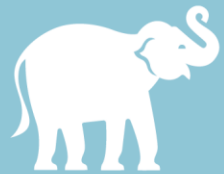




Erin Latimer, Smithsonian's National Zoo

The EEHV Consortium at the National Elephant Herpesvirus Laboratory Update



IMLS UPDATE: LAUREN L. HOWARD, DVM, DIPL. ACZM

The Houston Zoo, Baylor College of Medicine, and Johns Hopkins University are making significant progress in the fight against EEHV. We received a three-year federal grant for \$459,147.00 from the Institute of Museum and Library Services (IMLS) in August 2013.

The mission of the IMLS is to inspire libraries and museums to advance innovation, lifelong learning, and cultural and civic engagement. It is a federal agency that provides leadership through research, policy development, and grant making. One of the many key issues identified as a national concern is care and preservation of collections. The Houston Zoo, like many zoological institutions throughout the U.S., is registered as a museum, and our living "collection" of elephants in North America is what we are striving to care for and preserve. The Houston Zoo partnered with Baylor College of Medicine in 2008 and has enjoyed many successes under the leadership of Dr. Paul Ling, a human virologist with a focus on Epstein Barr research. Dr. Ling works closely with Dr. Gary Hayward at Johns Hopkins University, who has been heavily involved with EEHV research since the virus was first identified at the National Zoo in the 1980's. Together, our three institutions applied for IMLS funding to tackle three important research aims: 1. To determine if any of the currently available anti-herpesvirus drugs have efficacy against EEHV; 2. To develop sensitive tests to evaluate specific immune responses to EEHV; 3. To continue attempts to grow the virus in the laboratory.

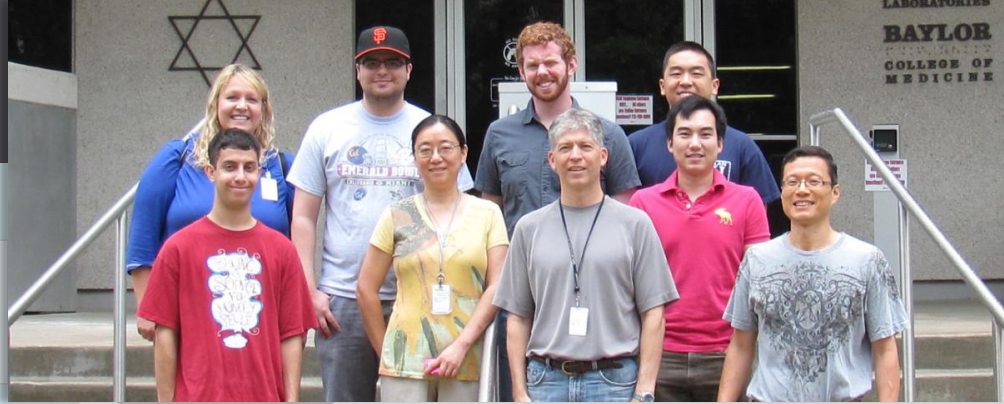
We were thrilled to receive an award notification from IMLS in August 2013, and Dr. Ling and I traveled to Washington DC, where we met up with Dr. Hayward and attended the IMLS award ceremony. Funding began in December 2013 and lasts until November 2016.

The IMLS funds come to the Houston Zoo, which is listed as the primary investigator on the grant. The majority of the funding goes right back out of the Houston Zoo to Baylor College of Medicine and Johns Hopkins University. The funds provide salary for a full time laboratory technician at Baylor College of Medicine and partial salaries for several research positions at Johns Hopkins University. The Houston Zoo provides the matching funds for the grant through the purchase of supplies for sample collection, partial funding of the 2015 and 2017 EEHV Workshops, and with Houston Zoo staff time spent on sample collection and organizing the project. The Houston Zoo is happy to provide samples from our herd of 8 Asian elephants, which are used by the researchers at Baylor College of Medicine to develop immunity assays and refine diagnostic techniques.

Continued on page 4



Meghan Murphy, Smithsonian's National Zoo



Back row left-to-right: Sally Nofs, David Garcia, Joseph Hicks, Daniel Wang
Front row left-to-right: Benjamin Hornstein, Jie Tan, Paul Ling, Jatrong Sewatanon, RongSheng Peng

Photo courtesy of P. Ling

For more information about Dr. Ling's work, please visit www.bcm.edu/departments/molecular-virology-and-microbiology/index.cfm?pmid=15204

COLLABORATION: PAUL LING, BAYLOR COLLEGE OF MEDICINE

How did you first become involved with EEHV research? What interests you about EEHV?

In the fall of 2008, the Houston Zoo lost a young 2-year-old calf named Mac to EEHV-associated disease. Mac was a very charismatic elephant and the loss was devastating to the local community and especially for the elephant keepers and the zoo. The zoo had lost several calves to EEHV over the last few years, so they felt the time had come to be more proactive in doing something to address this issue. My laboratory at Baylor College of Medicine has been investigating herpesviruses for over a decade and we happen to be located adjacent to the Houston Zoo in the Texas Medical Center. I had done my postdoctoral research at Johns Hopkins with Diane Hayward, Gary Hayward's wife, so I was actually familiar with the EEHV story as I kept in touch with the Haywards after I left Baltimore. When I was contacted by the Houston Zoo, I was definitely intrigued and of course, all it took was a visit to the zoo and the elephants convinced me that I should help them out!

There are several aspects of EEHV research that intrigue me. First, most herpesviruses are relatively benign to their natural hosts, so it's unusual to see a herpesvirus that has such a lethal effect, particularly on young animals. Second, EEHV is a unique and novel virus. Mammalian herpesviruses are categorized into three major sub families. When we recently sequenced the entire genome of EEHV1, the most common strain found in EEHV-associated deaths, it is significantly diverged from each of the subfamilies. Third, Asian elephants are an endangered species. EEHV undoubtedly is contributing to captive Asian elephant population declines and recent evidence suggests that it can cause death in range countries too. I believe that our EEHV research efforts might someday help be a part of protecting and saving this amazing species.

What projects / grants are you currently working on?

Our current research efforts are focused on understanding both humoral (antibody) and cellular immune responses to EEHV. We would like to determine the sequence of EEHV strains other than EEHV1, which have been associated with causing disease or death in Asian elephants. We also are interested in trying to grow this virus in the laboratory.

We are grateful for funding from the Houston Zoo and the Institute of Museum and Library Services, which support our research efforts. Past funding from the Elephant Managers Association and the International Elephant Foundation has been extremely important too.

What is the biggest challenge facing EEHV research today?

To make significant progress on the EEHV problem will take significant financial resources. There really is no other way around it. Experienced researchers with proven track records of accomplishment and access to high quality scientific environments and infrastructure are usually located at institutions where the bulk of support for research activities must come from external grants. I think the elephant community has recognized this, but fundraising for research is hard work, especially when the pool of money available is small and there are many interesting and worthwhile wildlife problems that need to be solved. I'm biased of course, but I think elephants are worth it!

Where do you see EEHV prevention /diagnosis / treatment in five years from now? What progress do you think will be made by then?

Several groups, including ours, have pretty good diagnostic tests for EEHV. In addition, many talented clinical veterinarians in the zoo and wildlife field have developed pretty good protocols for managing an animal that has or potentially has EEHV-associated illness. However, we still have several challenges. First, early and aggressive supportive care is probably important, but this can only occur if institutions housing elephant herds regularly monitor their animals for EEHV or make sure to have access to a facility that can do this for them in a timely manner. We do routine monitoring for Houston and occasionally for other institutions. I am encouraged to see that several institutions caring for elephants have recently signed up to receive EEHV detection services from the NEHL. I hope that in the near term we can more fully assess which, if any, of the currently available anti-herpesvirus drugs might work for treating EEHV. I also think advances in our understanding of elephant immunity to EEHV will help us develop tests that might determine which elephants might be most vulnerable to EEHV infection. It might be a stretch, but hopefully in 5 years the conversation will be about potential vaccines or novel treatments like adoptive cellular therapy (i.e., transfusion of T cells specific to EEHV).

What would you investigate with unlimited money?

For the moment, we are doing our best to work on the most pressing issues facing us with EEHV. I think the difference with having "unlimited" money would be that we could hire more people to do the necessary work and this would accelerate the pace of progress.

THANK YOU: MEMBERSHIP HELPS TO PREVENT ELEPHANT DEATHS

THE BUFFALO ZOO	MARYLAND ZOO
CARSON & BARNES CIRCUS	OKLAHOMA CITY ZOO AND BOTANICAL GARDEN
THE CLEVELAND METROPARKS ZOO	THE OREGON ZOO
COLUMBUS ZOO AND AQUARIUM	RINGLING BROS. AND BARNUM & BAILEY
THE DALLAS ZOO	THE ROSAMOND GIFFORD ZOO AT BURNET PARK
HAVE TRUNK WILL TRAVEL, INC.	THE SAINT LOUIS ZOOLOGICAL PARK
THE HOUSTON ZOO	SMITHSONIAN'S NATIONAL ZOO AND CONSERVATION BIOLOGY INSTITUTE
THE INTERNATIONAL ELEPHANT FOUNDATION	THE TULSA ZOO
JACKSONVILLE ZOO & GARDENS	WOODLAND PARK ZOO



Amanda Perez, Smithsonian's National Zoo

TRUNK WASH SCREENING: HOW & WHY

What is trunk wash (TW) screening?

TW screening refers to the testing of multiple sequential TW samples from an elephant, looking for the shedding of EEHV in the trunk secretions. Usually 1-2 TWs are collected per week for 1-2 months.

How is it done?

The TW sample is collected in almost the same way it is collected for TB testing (Stanton JJ et al. 2010. *Am J Vet Res.* 71(8): 925-33.). Briefly, sterile saline solution is poured into the nares of the elephant; the proboscis is elevated for 20-30 seconds, and the elephant is instructed to blow the saline solution into a sterile bag. This effluent is transferred to a sterile tube, and then centrifuged. The supernatant is discarded, and the remaining cell pellet is stored at -80C for further processing. DNA is prepared from this pellet; the DNA is tested by PCR for all of the EEHV types. If EEHV is detected, sequencing of the viral DNA is performed to allow for comparison with other EEHV isolates, from within the herd and from other herds and EEHV cases.

What is its value?

It is thought that most adult and subadult elephants are latently (quiescently) infected with one or more of the EEHVs, much like many humans are latently infected with several human herpesviruses. By testing a herd through TW screening, we can determine which EEHVs are present in the herd (with the caveat that if an elephant doesn't shed during the screening period, we cannot detect any EEHV from it). Detecting shedding of a particular EEHV in a juvenile elephant may bring a measure of comfort to the elephant staff, as it may be a sign that the elephant was previously infected by that type of EEHV and recovered and has likely acquired some level of immunity. Often, a primary infection by an EEHV causes an initial transient viremia (viral DNA detectable for a few days or weeks by PCR in the blood), followed several weeks later by shedding in the trunk secretions. Again this is transient and completely resolves within one to two months. Thereafter, the animal is likely latently infected although occasional intermittent reactivated shedding of the virus or viral DNA may occur periodically throughout the life of the elephant. It is this intermittent shedding that we can detect through TW screening. Several Asian elephants have been observed to undergo sequential non-overlapping episodes of this type involving a second and even third type of EEHV without having serious disease.

Is TW screening a substitute for screening of the blood?

No, it is still important and useful to screen calves regularly for EEHV. Whereas sudden high levels in blood during primary infection could presage acute disease, high spikes in the TW do not seem to have any connection to acute disease. Therefore, TW screening does not substitute for either pre-emptive or reactive monitoring of the blood for acute disease. We encourage all holders of calves to arrange for regular (weekly) screening of the blood if possible.

What more needs to be known?

At present, little is known about how frequently shedding occurs in either adult or juvenile elephants, nor whether reactivated shedding includes viremia or not. Preliminary evidence indicates that periodic shedding can sometimes also be detected in saliva and that African elephants also periodically shed other types of EEHVs that are not found in Asian elephants.

EEHV ADVISORY GROUP FORMATION

Several of us in the EEHV community have formed an EEHV Advisory Group, which will be a subcommittee under the umbrella of the Elephant TAG/SSP Veterinary Advisory committee that is led by Michele Miller. In order to fulfill the group's goals and to better serve the worldwide EEHV community, we have a panel of advisors who are willing to provide their expertise and advice to our colleagues. We have 4-5 advisors in each of the following areas: Veterinary, Research, Elephant Management, Pathology, and Public Relations/Education. We are in the process of updating the eehvinfo.com website to include more content and contact information for the Advisory Group. In the meantime, if you have any questions, please email EEHVinfo@si.edu.

Our Mission

To decrease elephant morbidity and mortality due to EEHV while supporting elephant-holding institution programming by:

- Disseminating knowledge of current best practices for prevention, diagnosis, and treatment of EEHV
- Providing private and public elephant-holding facilities with technical assistance
- Facilitating research by building international collaborations

Goals

1. Recommend husbandry and veterinary management protocols as they pertain to EEHV
2. Develop priorities for diagnostic and research goals between the NEHL Consortium and other research labs
3. Coordinate research sample requests
4. Provide media assistance
5. Assist in the identification of necropsy teams as needed
6. Coordinate EEHV fund-raising
7. Assist with proposal submissions for EEHV research projects
8. Proactively provide elephant holding institutions and the general public with current EEHV information:

Continuously update information on the EEHV Advisory Group website

Manage EEHV listservs—one for advisors and one for advisors plus elephant community (elephant managers/keepers, vets, researchers, etc.)

Steering Committee

Erin Latimer
Lauren Howard
Ellen Weidner
Debbie Olson
Harry Peachey
Paul Ling
Gary Hayward



IMLS UPDATE: LAUREN L. HOWARD, DVM, DIPL. ACZM

Continued from page 1

Dr. Paul Ling and his lab have already made progress towards developing reagents that will be needed for generating tests to detect elephant antibodies to EEHV. There are different types of antibodies that most likely provide protection against lethal EEHV infections. These antibodies can be found in blood and milk. In elephants, only one of three major antibody types has been confirmed to be present. Moreover, our ability to detect these antibody types is limited. To address these issues, Dr. Ling's lab confirmed that elephants do indeed make three important antibody types, known as IgG, IgM, and IgA, and they generated reagents that can detect these antibody types in elephants. This work has been compiled into a research paper that is currently under review with the Journal of Veterinary Immunology and Immunopathology and is titled "Characterization of antibodies against Asian elephant (*Elephas maximus*) IgG, IgM, and IgA". Dr. Ling's group is now poised to use these novel reagents to generate specific tests to evaluate the EEHV antibody response in Asian elephants. These tests will be useful for determining whether or not certain juvenile elephants may be susceptible to EEHV infections and to evaluate the effectiveness of future EEHV vaccines.

We are very grateful to have this grant supporting our work, and are already seeking other sources of support to continue our momentum. There is so much we don't know about EEHV. Most of what we don't know can be learned, if we can develop the right resources and keep asking the right questions.



Photo courtesy of L. Howard

Pictured left to right: Gary Hayward, Susan Hildreth (IMLS Director), Lauren Howard, Paul Ling.



Smithsonian's National Zoo

NEW ELEPHANTS AT THE SMITHSONIAN'S NATIONAL ZOO!

The Smithsonian's National Zoo is excited to announce the addition of three new Asian elephants to our herd. Kamala (39), Swarna (39) and Maharani (23) joined our current herd of Ambika (66), Shanthi (38), Bozie (39) and Kandula (12) and will live in the state-of-the-art exhibit Elephant Trails, which opened last year. The three girls hail (and are on loan) from the Calgary Zoo, which decided in 2012 to find a new home for the elephants, based on their long-term welfare needs.

Planning for the trip took nine months; keepers and vets from the National Zoo visited the elephants in Calgary to meet them and learn about their care, Calgary Zoo staff trained the elephants to prepare them for the journey, and registrars from both zoos handled the paperwork for the international transfer of the endangered animals and the many research samples that had been collected.

Bozie, Shanthi, Kamala, and Swarna all spent time at the Pinnawala Elephant Orphanage in Sri Lanka, although it is not known if they will recognize each other when they are reintroduced. After a standard minimum 30-day quarantine, the elephants will be slowly introduced to one another, with the hopes of forming one large matriarchal herd. Kandula, the young male, will be housed separately.

