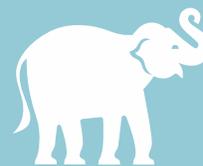


THE EEHV CONSORTIUM
PO BOX 37012, MRC 5508,
WASHINGTON, DC 20013-7012
[NEHL at the National Zoo](#)
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DR. JENNIFER D'AGOSTINO, OKC ZOO

The EEHV Consortium at National Elephant Herpesvirus Laboratory Update



THE OKLAHOMA CITY ZOO: Research partners in effective EEHV monitoring

DR. JENNIFER D'AGOSTINO, OKC ZOO

The Oklahoma City Zoo has a long history with elephants but up until 2011 the zoo had never had a calf born. On April 15, 2011 Malee was born, the first elephant calf in the zoo's history. By this time elephant endotheliotropic herpesvirus (EEHV) was a well-known disease with significant research efforts occurring across the globe. The OKC Zoo is committed to providing the best possible care for our herd of elephants and dedicated in helping to learn more about EEHV. Prior to the birth of the calf, the zoo developed a plan to monitor for EEHV and provide as much data for research as possible.

Our screening protocol for EEHV started prior to the birth of the calf. The zoo's two cows, sisters Asha and Chandra, have a known history with EEHV1A as Chandra is the first documented survivor of the acute disease. Several months prior to Asha giving birth, keeper staff collected weekly trunk wash samples from both cows to document shedding of EEHV. Results of PCR testing indicated that both cows intermittently shed very small amounts of EEHV 1 and Asha shed a minute amount of EEHV 5 at one time point. This was very useful information as EEHV 5 had not been identified from these individuals in the past. Once Malee was born, our screening efforts continued. Staff collects trunk wash samples on the entire herd including the calf

once per week and samples are banked. Blood is also collected on a weekly basis from the adults and at one year of age, keepers were reliably getting weekly samples on Malee as well. In addition to biological samples, we decided to collect a variety of physiologic data to monitor trends and note any abnormalities that might indicate early disease. Once a week, keepers collect indirect blood pressure readings, pulse rate and fecal bolus temperature on each elephant. A complete blood count (CBC) is run on Malee once per month to monitor for abnormalities. Finally, the vet staff and keeper staff participate in quarterly live EEHV drills in order to be fully prepared in the event of a clinical case.

In 2013 the zoo joined the National Elephant Herpesvirus Laboratory (NEHL) as a Research Partner in the EEHV Consortium. As a Research Partner we are able to monitor our herd effectively for EEHV while gathering important data to further our knowledge of this disease. Whole blood and serum samples are sent to the lab weekly from the calf for EEHV PCR screening while samples from the adults are banked. Once a year, we send in two month's worth of trunk wash samples from each elephant to monitor for shedding. Thankfully, we have not had a clinical case to date but through our preparation and screening efforts we hope that we will be able to identify the disease early and respond effectively.

Ed. Note—Congratulations to the OKC Zoo on the birth of their elephant calf, Achara, on Dec 22, 2014.

USEFUL LINKS FOR EEHV PREPARATION

PASSWORD:
E3HVGROUP

[EEHV
PLANNING
PROTOCOLS](#)

[EEHV TESTING
LABORATORIES](#)

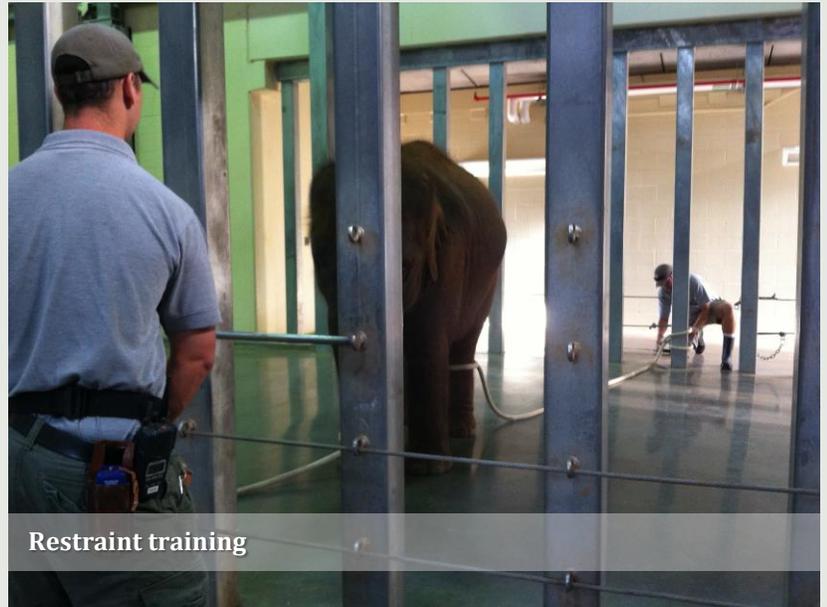
[EEHV
WORKSHOP-FEB
2015](#)



OKLAHOMA CITY ZOO: SCREENING METHODS IN ACTION



Supply boxes for EEHV case, with contents listed on the outside



Restraint training



Restraint training



Rectal fluids training



Cardiac ultrasound



Restraint training



It's been an exciting year at the NEHL. We finished out our first fiscal year (6/1/13 – 9/30/14, 16 months instead of 12 months because we gave the charter members the extra four months between our launch in June 2013 and the start of the federal fiscal year on Oct 1, 2013) as the EEHV Consortium, with 17 zoos and circuses as inaugural members. We're starting our second year with 22 members and are working on signing up more. We are especially hopeful that breeding facilities with calves on the ground or on the way will take advantage of our unlimited diagnostic testing and calf-screening program.

The NEHL was the grateful recipient of a StepOnePlus real-time machine, donated by the International Elephant Foundation. We are now providing qPCR testing for EEHV1, 2, 3-4, 5, and 6, in addition to our usual cPCR testing for all of the known EEHVs (and any as-yet-unknown EEHV types) and gammaherpesviruses. We worked closely with Dr. Paul Ling's lab at the Baylor College at Medicine to validate the EEHV qPCR assays at the NEHL. Thank you to Jie Tan, Paul's fantastic lab tech for her help with protocols, advice and start-up reagents for QC testing.

In the last 12 months, we received 140 packages, consisting of almost 600 samples; they were tested with cPCR and/or qPCR. Most of the samples were tested for the full panel of EEHV types (EEHV1, 2, 3-4, 5, and 6), so approximately 3000 PCR reactions were run and analyzed. These samples were sent to the NEHL for routine monitoring, diagnosis of acute EEHV, necropsy testing, and determination of EEHV shedding in trunk secretions.

It has been shown (Stanton et al, J Zoo Wildl Med. 2013 March; 44(1): 42–54) that EEHV can be detected in blood before the onset of clinical signs, thus allowing increased monitoring and early treatment. Four breeding herds with young calves are being screened regularly for EEHV by the NEHL to detect the early presence of EEHV in the blood. Fortunately, we did not have an acute case of EEHV1 in the US herds this year, although we did have two Asian elephant herds with EEHV5 in more than one elephant. We detected EEHV5 in these herds because their calves were enrolled in our monitoring program. These herds were subsequently followed closely to ensure that if viral loads increased and clinical signs were seen, treatment could be started. Both herds are doing well, although several members of one herd were treated when their viral loads increased past the treatment threshold.

The NEHL was instrumental in the formation of the EEHV Advisory Group over the last year and in the planning of our first meeting of the EEHV Advisory Group Steering Committee and Advisors at the Fort Worth Zoo on August 20, 2014. We had a productive day and have continued the hard work by finalizing the "Minimum Standards of Care for Elephant Calves as Related to EEHV-Preparedness" and updating and adding to the information found on the eehvinfo.org website. We are continuing to improve the website and hope to roll out the redesigned site by the end of Jan 2015.

The NEHL collaborated on two papers published concurrently in the Journal of Virology which provide details about the genetics of EEHV1 – 6 and provide evidence that they may belong to a new herpesvirus subfamily, the proposed *Deltaherpesvirinae*.

****New benefit from EEHV Consortium membership****

In response to requests from our members, the EEHV Consortium is now pleased to provide pre-printed FedEx shipping labels for our frequent shippers. The labels, which cost \$20 each, provide for Priority Overnight shipping of samples to the NEHL and the return shipment of the cooler, so that it can be re-used.

Do you have a benefit you would like to see offered? Please let us know! latimere@si.edu

THANK YOU: MEMBERSHIP HELPS TO PREVENT ELEPHANT DEATHS

| | |
|------------------------------|---|
| THE BRONX ZOO | OKLAHOMA CITY ZOO AND BOTANICAL GARDENS |
| THE BUFFALO ZOO | THE OREGON ZOO |
| BUSCH GARDENS TAMPA | POINT DEFIANCE ZOO & AQUARIUM |
| CARSON & BARNES CIRCUS | RINGLING BROS. AND BARNUM & BAILEY |
| THE CLEVELAND METROPARKS ZOO | THE ROSAMOND GIFFORD ZOO AT BURNET PARK |
| COLUMBUS ZOO AND AQUARIUM | THE SAINT LOUIS ZOOLOGICAL PARK |
| THE DALLAS ZOO | SMITHSONIAN'S NATIONAL ZOO |
| FORT WORTH ZOO | AND CONSERVATION BIOLOGY INSTITUTE |
| HAVE TRUNK WILL TRAVEL | THE TULSA ZOO |
| THE HOUSTON ZOO | UTAH'S HOGLE ZOO |
| JACKSONVILLE ZOO & GARDENS | WOODLAND PARK ZOO |
| MARYLAND ZOO | |



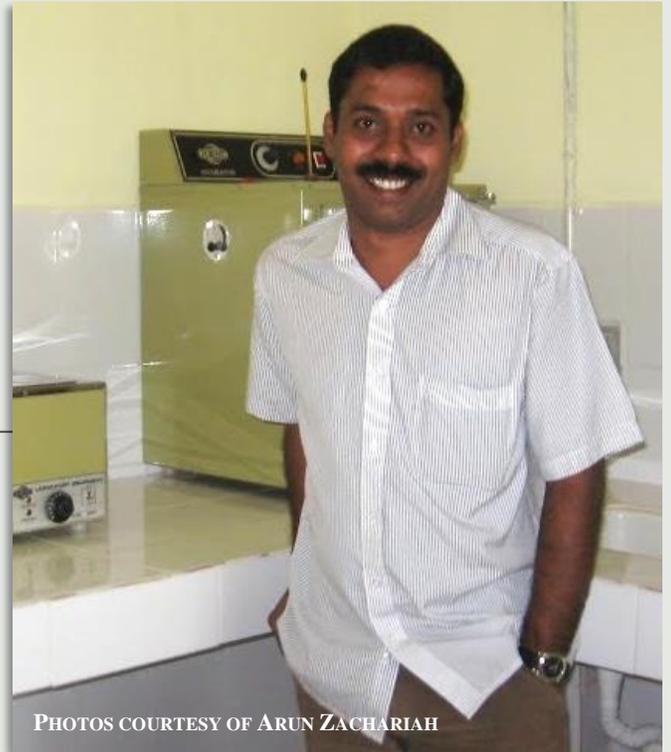


**INTERVIEW WITH
DR. ARUN ZACHARIAH,
BVSc & AH, MS
CENTRE FOR WILDLIFE STUDIES,
KERALA VETERINARY AND
ANIMAL SCIENCE UNIVERSITY
KERALA, INDIA**

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

In 2007 we had encountered the death of an orphan elephant calf (caught from the wild due to the death of its mother which choked on a metal wire). The calf showed typical clinical signs of EEHV and on autopsy multi-organ hemorrhages were evident. Histopathology of the liver revealed intranuclear inclusion bodies. After the 4th day of the calf's death another calf which was housed adjacent to it started showing signs of EEHV infection and succumbed to death within two days. Again typical gross pathological lesions were observed.

In that situation we contacted Dr. Laura Richman and Ms. Erin Latimer [both of the NEHL] for advice. Meanwhile we had encountered a dead elephant calf in the wild with typical gross lesions. Since the chance of contact with African elephants was quite impossible in the cases of captivity and the death of a calf in free ranging situation also complicated the situation.



PHOTOS COURTESY OF ARUN ZACHARIAH

With the help of NEHL we had set up a small PCR laboratory in the Wayanad Wildlife Sanctuary (pictured above and below left). In June 2008, Dr. Laura Richman, Ms. Erin Latimer and Prof. Gary Hayward of Johns Hopkins visited the facility and reviewed the cases. DNA extraction, PCR and sequencing were performed and confirmed that the causative organism was EEHV1A virus.

Then onwards we have started the surveillance program on EEHV both in captive and free-ranging elephants and now we have reported 14 more cases.

What projects / grants are you currently working on?

We are continuing the EEHV surveillance program.

What is the biggest challenge facing EEHV research today?

Inability to culture the viruses.

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

I sincerely hope a vaccine and a handy antigen ELISA can be developed by then.

What would you investigate with unlimited money?

I would like to investigate the evolution of the virus, the mechanism of its pathogenicity and host immune interactions and role/impact of this virus on the future conservation of Asian elephants.

Do you think that it will be possible to treat EEHV in the wild or vaccinate wild elephants in the future?

I don't think that it would be possible to vaccinate wild elephants both due to strategic and ethical reasons.

Do you work with colleagues in other countries, to help them with diagnosing their possible EEHV cases?

Yes, currently we are associating with Vessvic, Sumatra, Indonesia and Myanmar Timber Enterprise. We helped them to set up PCR labs and diagnosed two cases each as EEHV1A.