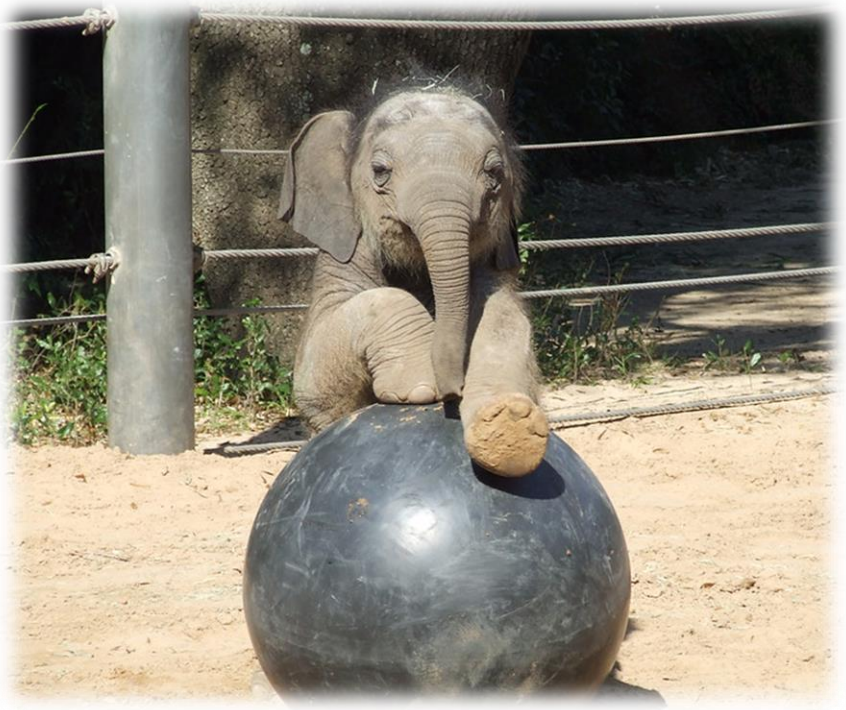
North American EEHV Workshop

March 26-28, 2019

Houston Zoo

Houston, TX



Workshop Proceedings

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North American EEHV Workshop
and
Advisory Group Meeting March 26-28, 2019

Tuesday March 26: Crowne Plaza Hotel – Windsor I and II		
EEHV Advisory Group Meeting (Advisory Group Members Only)		
07:15	Breakfast	
8:30-10:00	First EEHV AG Session	Committee Reports
10:00	Coffee Break	
10:30-12:00	Second EEHV AG Session	AG Activities Continued
12:00	Lunch	
13:00 – 15:00	Third EEHV AG Session	AG Activities Continued
15:00	Snack Break	
15:00 – 17:00	Fourth EEHV AG Session	AG Activities Continued
Tuesday March 26: Crowne Plaza Hotel – Churchill		
North American EEHV Workshop (All Workshop Attendees)		
18:00 – 20:00	Icebreaker	19:00 Welcome from Lisa Marie Avendano (Houston Zoo) followed by opening remarks from Lee Ehmke (Houston Zoo) and Paul Ling (Baylor College of Medicine)

Wednesday March 27, 2019: Crowne Plaza Hotel - Windsor I and II		
North American EEHV Workshop		
7:00 – 8:00	Breakfast	7:15 -7: 45 am EEHV: The Basics *Optional EEHV primer during breakfast* (Howard, San Diego Zoo Safari Park) (30 m)
8:00 – 10:15	Morning session	Opening remarks from Daryl Hoffman (Houston Zoo) (5 m)
		EEHV Advisory Group Steering Committee Update (Howard, San Diego Zoo Safari Park) (15 m)
		EEHV Advisory Group Scientific Program Subcommittee (McClure, Maryland Zoo) (10 m)
		EEHV Advisory Group Research Subcommittee (Bapodra, Columbus Zoo and Aquarium) (10 m)
		EEHV Advisory Group Veterinary Management Subcommittee (Abou-Madi, Cornell University) (10 m)

		EEHV Advisory Group Outreach Subcommittee (Allread, Public Communications Inc.) (10 m)
		Asian Elephant SAFE Program (Felts, Columbus Zoo and Aquarium) (15 m)
		North American Regional Update (Howard, San Diego Zoo Safari Park) (15 m)
		An Update on Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease Cases in Europe 2018-19 (Schaftenaar, Rotterdam Zoo) (15 m)
		EEHV HD in Thailand from 2006 to 2018: What Do We Know So Far? (Sripiboon, Kasetsart University) (15 m)
		African Regional Update EEHV, African Elephants (*Loxodonta Africana*), and the Regional African EEHV Working Group (Howard, San Diego Zoo Safari Park) (15 m)
10:15 – 10:30	Coffee break	*Coffee will be set out at 10 am with a formal break at 10:15 am*
10:30 – 12:00	Mid-morning session	Development of a Disease Risk Analyses Tool to Gain a Better Understanding of EEHV Associated Morbidity and Mortality in the North American Asian Elephant (*Elephas maxiums*) population (Deem, St. Louis Zoo) (15 m)
		Investigating Disease Susceptibility to Elephant Endotheliotropic Herpesvirus in North American Elephants Using a Genome-wide Approach (Prado, Smithsonian Conservation Biology Institute) (15 m)
		Optimization of Non-invasive Techniques for Detection of Elephant Endotheliotropic Herpesvirus in Asian (*Elephas maxiums*) and African (*Loxodonta Africana*) Elephants (Jeffrey, University of California, Davis) (15 m)
		Identification of African Elephant Polyomavirus in Wild Elephants and the Ability of its Early Region Genes Encoding Large and Small Tumor Antigens to Transform Elephant Primary Cells (Pearson, Fox Chase Cancer Center) (15 m)

		Annotated Complete DNA Sequences of Six EEHV 1A Genomes from Lethal HD Cases in Young Asian Elephants from India (Hayward, Johns Hopkins) (15 m)
		Identification of a Novel Highly Diverged Variant of EEHV 1A in Three Lethal Cases of Hemorrhagic Disease From Borneo (Hayward, Johns Hopkins) (15 m)
12:00 – 13:00	Lunch	
13:00 – 15:00	Afternoon session	Fatal Hemorrhagic Disease Caused by EEHV-1A in a 6 Year Old Asian Elephant (*Elephas Maximus*) (Sanchez, Oregon Zoo) (15 m)
		Recent Case Updates (Latimer, Smithsonian's National Zoo) (15 m)
		Five Cases of Hemorrhagic Disease Due to Elephant Endotheliotropic Herpesvirus (EEHV) in a Group of Young Asian Elephants Treated with Aggressive Crystalloid and Colloid Therapy (Gray and Wiedner, African Lion Safari) (30 m)
		Implementation of In-House Testing for Elephant Endotheliotropic Herpesvirus (EEHV) and Potential Benefits for Increased Herd Surveillance (D'Agostino, Oklahoma City Zoo) (15 m)
		Elephant Endotheliotropic Herpesvirus Fatalities in Europe (Perrin, Copenhagen Zoo) (15 m)
		Clinical EEHV1B in Two Asian Elephant Calves (*Elephas maxiums*) – Clinical Presentation and Decision-Making Leading to Survival (Molenaar, Zoological Society of London) (15 m)
		Tough Talk: Bridging Science and Emotion to Communicate about EEHV (Allread, Public Communications Inc.) (15 m)
15:00 – 15:30	Afternoon break	
15:30	Afternoon session	EEHV Funding and Development Support (Espinosa and Jesudason, Houston Zoo) (15 m)
15:45 – 17:00	Panel Discussion	EEHV Preparedness and Best Practices Panel -Daryl Hoffman, Houston Zoo

		-Nick Newby, White Oak Conservation Center -Jennifer D'Agostino, Oklahoma City Zoo -Dennis Schmitt, Feld Inc. -Louis Padilla, St. Louis Zoo -Charlie Gray, African Lion Safari
17:00	Closing remarks	Christine Molter (Houston Zoo)

Thursday March 28, 2019 Crowne Plaza Hotel – Windsor I and II		
North American EEHV Workshop		
7:00 – 8:00	Breakfast	
8:00 – 10:15	Morning session	Opening remarks from Joe Flanagan (Houston Zoo) (5 m)
		Use of Mesenchymal Stem Cell Therapy in Infectious Disease and Potential Utility in EEHV (Johnson, Colorado State University) (15 m)
		The use of Mesenchymal Stem Cells as Part of a Therapeutic Plan for Elephant Endotheliotropic Herpesvirus (EEHV) (D'Agostino, Oklahoma City Zoo) (15 m)
		Using Serum Inflammatory Markers to Investigate the Immune Response to Elephant Endotheliotropic Herpesvirus (Edwards, Smithsonian's National Zoo) (15 m)
		Thromboelastography Use Over Time in Juvenile Asian Elephants (*Elephas Maximus)* (Kiso, Feld Inc.) (15 m)
		Transfusion Medicine and EEHV-HD: Advancing Life-Saving Therapies (Kishbaugh, Bode Vet) (15 m)
		EEHV Infection Challenges in Myanma Elephants and Health Care Management in Myanmar (Zaw, Myanma Timber Enterprise) (15 m)
		Prevalence of EEHV in Captive and Wild Asian Elephants (*Elephas Maximus*) in Assam, India (Mahato, College of Veterinary Science, Khanapara) (15 m)
		What Can We Learn from the Now Many Examples of Identical EEHV 1 Strains Found in Pairs of Unrelated Asian Elephant Calves Afflicted with EEHV-HD at About the Same Time at the Same Housing Facility

		(Hayward, Johns Hopkins) (15 m)
10:15 – 10:30	Coffee break	*Coffee will be set out at 10 am with a formal break at 10:15 am*
10:30 – 12:00	Mid-morning session	Efforts to Increase EEHV Diagnostic Testing Capacity in Elephant Range Countries and Elsewhere (Latimer, Smithsonian's National Zoo) (15 m)
		Moving Elephants Two by Two – Pre-Export Testing and Transfer Training (Dobbs, Twycross Zoo) (15 m)
		Training Benchmarks in Young Elephants (Hoffman, Houston Zoo) (15m)
		Serological Detection of EEHV Infection (Fuery, Baylor College of Medicine) (15 m)
		Towards an EEHV Vaccine (Ling, Baylor College of Medicine) (15 m)
		Towards an EEHV Vaccine_Q&A (Ling, Baylor College of Medicine; Richardson, Pharmoros) (15 m)
12:00	Closing remarks and summary	Christine Molter
12:00 – 13:00	Lunch	
13:30	Travel to Houston Zoo	*The **bus will leave by 13:30** from the hotel for the Zoo and arrive at Gate 9.* *Zoo staff will meet you at the bus and direct you from there to the various afternoon activities.*
14:30 – 17:00	Concurrent Labs, Barn Demo, Tours, and Zoo Free Time Luggage may be stored at the clinic for those leaving directly for the airport (Lindsey Olufsen to manage luggage) Refreshments and snacks are available in the Veterinary Hospital and	**CBC/Crossmatching** -Veterinary Services Building -14:30 – 16:30 -Maryanne Tocidlowski + vet techs (Katie Plaeger, Jason Bartel, Jennifer Atkinson) **Elephant barn training and plasma collection demonstration, barn tour, and EEHV drill walk-through** -Elephant Barn – at barn windows -14:30 – 16:30 -Daryl Hoffman, Joe Flanagan + tech (Andrea Lee) + elephant keepers

	Administration Building and the Elephant Barn	-*Optional barn tour for lab participants and others at 16:30*; meet at elephant exhibit barn door and Lisa Marie Avendano will lead tour **Clinic tours** – Lindsey Olufsen organizer + Clinic Keepers tour guides -Meet in VHAB Conference Room (refreshment room); Keepers to lead tours in VHAB and VSB -14:30 p -15:15 p -16:00 p -16:45 p **BCM Lab and Museum Tours** – Kathryn Lippman + Ling lab members; Kathryn will drive attendees back and forth in zoo's passenger van (already reserved) -Meet in VSB Conference Room -14:00 and be at BCM at 14:15 -14:15-14:50 – tour Ling Lab -15:00 – Genome Center Tour -16:00 – DeBakey Museum Tour -16:30 – return to Zoo **Zoo Day Free Time**
17:30	Travel to Hotel	*The **bus will leave by 18:00** from the Zoo (Gate 9) to travel back to the hotel.* *Zoo staff will help you find the bus following the various afternoon activities.*

26Mar19

On behalf of our colleagues of the EEHV Advisory Group and the Houston Zoo, we welcome you to Texas.

Many of you have traveled far to be with us this week, and your presence underlines the importance of the work being done to protect baby elephants from this deadly virus.

This past Father's Day, our zoo welcomed Tilly, a 345-pound newborn Asian Elephant who brought our herd to 10. Her birth brought joy to a care team devastated by calf losses in prior decades because of EEHV.

But thanks to a partnership created between the Houston Zoo and Baylor College of Medicine after the 2008 death of Mac—a beloved animal known for his playfulness and intelligence—our zoo hasn't lost any elephants to EEHV in the last decade.

Our incredible partnership includes working toward vaccine development. That work is helping save elephant calves around the world, including those in human care and in the wild. The diagnostic and treatment protocols we have developed for zoo elephant calves have been directly applied to wild elephant calves throughout Asia.

These diagnostics and treatment protocols are working; however, we need a vaccine. And we're confident we'll find one. A future vaccine would help prevent EEHV deaths in elephant calves at zoos like ours and could also be administered to elephant calves in the wild. This is both vitally important to the protection of all elephants, and central to our wildlife saving mission at the Houston Zoo.

The lectures and activities at this workshop won't find a cure, but we WILL build stronger relationships for working together toward that eventuality. We hope you end this week knowing that together we will fight this virus for the good of elephants everywhere. And we will win.

Lee Ehmke, President and CEO, Houston Zoo
Lisa Marie Avendano, Vice President of Animal Operations, Houston Zoo

EEHV: THE BASICS

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Abstract

For institutions with elephants, it is not a question of *if* EEHV will cause illness in a herd, but *when*. Elephant endotheliotropic herpesvirus (EEHV) causes acute, often fatal hemorrhagic disease in young Asian elephants (*Elephas maximus*). For zoo veterinarians, this disease can be one of the most challenging and daunting aspects of caring for a breeding elephant herd at their institution. The zoo clinicians' best tools for managing EEHV are preparedness, vigilance, and an early, aggressive approach to treatment. Each elephant-owning institution should develop an EEHV Plan which includes methods of monitoring, treatment and necropsy, and should specifically include feasible means of famciclovir acquisition. Institutions with at-risk elephants (Asian elephants (*Elephas maximus*) aged 1-8 years) should evaluate their elephants regularly, preferably weekly, by testing for EEHV viremia via quantitative polymerase chain reaction (qPCR) and through evaluation of complete blood cell counts (CBC). Routine PCR testing will detect EEHV viremia before clinical signs develop, and allow for early and aggressive treatment. Treatment should include supportive care as well as antiviral therapy, and should be accomplished with standing sedation if it is not possible through training alone. Establishing open lines of communication between the veterinary and elephant teams, and institutional support from administrators and public relations personnel is critical to the successful management of EEHV at any institution.

Key words: Elephant, EEHV, Herpesvirus, Famciclovir, Viremia

INTRODUCTION

Elephant endotheliotropic herpesvirus (EEHV) causes acute, often fatal hemorrhagic disease in young Asian elephants (*Elephas maximus*).[13] For zoo veterinarians, this disease can be one of the most challenging and daunting aspects of caring for a breeding elephant herd at their institution. Based on February 2015 calculations, EEHV was the cause of 40% of deaths in all Asian elephants between 1 and 37 years of age that were born in North America, making it the single greatest cause of death in this cohort.[8] EEHV has caused disease in African elephants (*Loxodonta africana*) as well, and more research is needed to better understand the epidemiology in this species.

The zoo clinicians' best tools for managing EEHV are preparedness, vigilance, and an early, aggressive approach to treatment. This presentation will hit the highlights of these areas below, while many more details and sample protocols can be found at the website: www.eehvinfo.org .

Healthy Asian and African elephants have been shown to shed several of their own species of EEHV virus as part of a natural infection cycle that has evolved over millions of years.[14, 16, 18, 22] While exposure to EEHV appears to be a natural process, Asian elephants between 1 and 8 years of age at are a high risk of developing an aberrant hemorrhagic disease (EEHV HD) associated with EEHV infection.[20] Older Asian elephants and African elephants are also susceptible to EEHV HD, but with less documented frequency thus far. Asian elephants under human care in Europe and in several Asian range countries have succumbed to EEHV HD, [2, 15, 21] and deaths have been documented in wild Asian elephants as well.[10] EEHV is a global issue that we are also fighting at a very local level.

As of February 2016, there have been 32 cases of EEHV HD confirmed in Asian elephants born in North America since 1980. Nine of these elephants survived infection and 23 succumbed (one as recently as January 2016), leading to an overall mortality rate of 72%. It is hoped that, with more research into elephant immunity and EEHV, we may learn why some elephants survive primary exposure to EEHV, often without any clinical illness at all, and some elephants develop widespread and often fatal hemorrhagic disease. Until more is known about this aspect of the virus, the recommendations listed below represent the EEHV community's best attempts at increasing young elephant survival in face of the constant threat of EEHV.

EEHV PREPAREDNESS

All institutions housing elephants should establish an EEHV Plan that outlines monitoring for viremia, treatment of ill animals, and necropsy guidelines, and highlights the supplies needed for each. Drug acquisition should be thought out ahead of time; famciclovir is the antiviral drug most often used to treat EEHV, and the amount of required to treat an elephant (15 mg/kg PO or rectally TID) [3] is not readily available on short notice. All protocols should be developed jointly with veterinary and elephant care teams, with support of key zoo administrators. Decision making strategies and communication tactics should be discussed ahead of time so that critical time is not wasted on long meetings when an elephant is ill. Preparation for EEHV can be a costly and time consuming process and requires the full cooperation of all stakeholders. The Houston Zoo runs yearly EEHV drills, as it does drills for other catastrophic events such as opioid exposures, animal escapes, and venomous snake bites.

A potentially overlooked hallmark of EEHV preparedness is to cultivate and maintain open lines of communication between the veterinarians and the elephant care team. This is critical to allow for bilateral flow of important information, as well to streamline the process of discussions and decisions that will be part of any EEHV case.

EEHV VIGILANCE

Historically we have seen that elephants die of EEHV HD rapidly, often within 24 hours of showing clinical signs of illness.[12, 20] By the time the virus has caused enough internal damage for

illness to be perceptible in these stoic, frequently inaccessible patients, the damage is often irreversible, even with treatment. Recent research has shown that elephants ill from EEHV HD are viremic up to two weeks *prior* to the onset of clinical signs.[17] Recent clinical experience has shown that elephants with EEHV HD often demonstrate changes in their hemograms early in viremia, also prior to the onset of clinical signs.[1, 5, 6] The changes include mild to moderate leukopenia, particularly monocytopenia, and thrombocytopenia. These subtle changes are most notable when compared to the individual elephant's own complete blood cell counts (CBC) ranges, and can be overlooked when compared to more general elephant reference values.

Regular measurement of fecal bolus temperature, body weight, non-invasive blood pressure and heart rate, respiratory rate, and oral mucosa coloration are important steps in establishing normal value ranges for each elephant, which will allow for easy identification of subtle changes that can be a first clue to a more serious illness.[7]

The recommendation of the EEHV Advisory Group is to monitor at-risk elephants (1-8 year old Asian elephants) routinely (weekly is ideal) for EEHV viremia via whole blood quantitative polymerase chain reaction (qPCR). This is the best way to detect EEHV HD early and allow for early, aggressive treatment. Additionally, the Houston Zoo measures CBC's of at-risk elephants weekly, which helps to establish individual reference ranges for key parameters (white blood cell count, monocytes, and platelets) and helps us identify subtle decreases that may be associated with early viremia. If at-risk elephants show any signs of abnormal behavior, including decreased appetite, changes in sleep patterns, lameness, or changes in mentation or training, blood is collected immediately for EEHV qPCR and CBC, even if this requires standing sedation to accomplish.

EARLY, AGGRESSIVE TREATMENT FOR EEHV

All ill young elephants should be considered as possible EEHV HD cases until proven otherwise by the results of whole blood qPCR testing. Treatment should be initiated rapidly and often before confirmation of EEHV qPCR results is possible. Famciclovir (FCV) is the antiviral most often used in North America and in Europe to treat EEHV HD, and acyclovir and ganciclovir have also been used. The efficacy of FCV against EEHV has not been proven. To date, however, there is no peer reviewed data available to establish that famciclovir **does not** have effect against EEHV.[9] Until proven otherwise, it remains best practice to treat EEHV HD cases with FCV. Pharmacokinetic data of FCV in Asian elephants is available.[3] Antivirals are only one aspect of treatment, and it is becoming apparent that supportive care is just as important, if not more so, in the management of an EEHV HD case.[5, 6, 20] Rectal fluids can be initiated immediately and have a striking ability to improve an elephant's hydration and demeanor. At the Houston Zoo, we have administered fresh and frozen-thawed elephant plasma, along with crystalloids, via IV boluses under standing sedation, every two to three days as indicated by clinical condition and CBC status. Antibiotics for secondary infections, anti-inflammatories (at low doses in well hydrated animals), and opioids have also been given to elephants with EEHV HD. Full details on treatment protocols can be found at www.eehvinfo.org, or by contacting the author.

When to treat an elephant with EEHV HD is as important as how to treat one. With weekly monitoring of CBC's and EEHV qPCR in at-risk elephants, the zoo clinician can identify a case of EEHV HD early, often prior to the onset of any visible clinical signs of illness. Treatment with FCV and fluid therapy should be instituted in cases with viral loads above 5,000 vge/ml and/or with rapidly increasing EEHV viral loads, and in any animal that has EEHV viremia combined with an abnormal CBC, even if the elephant appears clinically normal. Low level EEHV viremia should be monitored closely with serial whole blood qPCR and CBCs. It is important to note that most of the data collected thus far is based on experience with EEHV1A and EEHV1B, while interpretation of viral loads for EEHV4, EEHV3 and EEHV5 are not as well established.

Even conscientious monitoring and timely treatment cannot guarantee a successful outcome. Necropsy of an EEHV HD case is an important opportunity to gain more information on this devastating disease. Full necropsy guidelines are available on www.eehvinfo.org.

FUTURE DIRECTIONS

Though our understanding of EEHV has grown astronomically in the past 5 years, there is still very much we do not know about this virus. Current research efforts of the Houston Zoo and Baylor College of Medicine are focused on T cell assays to better characterize the immune response and develop ways to measure vaccine response, as well as fine tuning EEHV HD treatment recommendations. Some headway is being made into understanding the elephant immune response, though there is much more to learn.[4, 11, 19] An ultimate goal of the EEHV community is to develop an EEHV vaccine to decrease the severity of clinical illness, if not eliminate illness altogether, but there is much work to be done before that is accomplished. Additionally, a better evaluation of the epidemiology and distribution of EEHV in North America, Europe, and Asian range countries is important to better understand the impact of this disease on wild and captive elephant populations. And finally, education of zoo professionals as well as the lay-public, by sharing our success and advances with EEHV, is critical to establishing public support for elephant institutions and EEHV-related research.

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UPDATE ON THE NORTH AMERICAN EEHV ADVISORY GROUP

The North American EEHV Advisory Group was formed in 2013 and re-organized in the Fall of 2018, in order to better respond to the needs of the elephant community. Our mission is to decrease elephant

deaths due to EEHV while supporting elephant holding institution programming by 1) Disseminating knowledge of current best practices for prevention, diagnosis, and treatment of EEHV; 2) Providing private and public elephant-holding facilities with technical assistance; and 3) Facilitating research by building international collaborations. Our bi-annual meetings also include invited guest experts to advance our mission.

Our new Advisory Group and Committees include:

Steering Committee
Gary Hayward
Daryl Hoffman
Lauren Howard
Erin Latimer
Paul Ling
Deborah Olson

The **Steering Committee** is tasked with overall planning, fundraising for meeting and other needs, organization of the AG meetings, and membership.

Outreach
Jill Allread - Chair
Avery Elander
Kali Holder
Luis Padilla
Rhonda Saiers
Jackie Wallace

Outreach provides clear/consistent speaking points, will develop a deployment plan for messaging, revises/maintains the eehvinfo website, provides assistance to institutions with EEHV-related questions or needs, and does outreach to the elephant and lay communities.

Research
Priya Bapodra - Chair
Sharon Deem
Ramiro Isaza
Michele Miller
Chuck Richardson

Research receives/manages support letter requests, maintains an EEHV-related research database, maintains/updates the EEHV-related Research Priority list

Scientific Program
Mike McClure - Chair
Lauren Howard
Erin Latimer
Christine Molter
Nick Newby
Debbie Olson

Sci Program identifies hosts for the biannual N.A. EEHV workshop and develops the scientific program with the host institution and works with the SC to ensure representation at other meetings at which EEHV should be a topic.

Vet/Management Resource
Noha Abou-Madi – Chair
Charlie Gray
Jaime Landolfi
Carlos Sanchez
Dennis Schmitt

Veterinary and Management Resources maintains and updates treatment and necropsy recommendations and research sample requests, maintains and updates the elephant plasma bank information and the famciclovir share program.

International Liaisons
Sonja Luz
Michele Miller
Willem Schaftenaar
Arun Zachariah

Our **International Liaisons** update us on EEHV-related happenings in their part of the world and facilitate collaborations with our colleagues.

EEHV ADVISORY RESEARCH SUBCOMMITTEE UPDATE

***Priya Bapodra-Villaverde, BVetMed (Hons), MSc, Dipl. ACZM, MRCVS*[*1]**

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Abstract

The EEHV advisory group research subcommittee is responsible for receiving and managing support letter requests, creating and maintaining an EEHV-related research database, and maintaining and updating the EEHV-related research priority list. The current global EEHV project database consists of 50 projects, being conducted in the US, Europe, South East Asia, India and Africa. Top research priorities include identifying risk factors associated with EEHV HD (hemorrhagic disease), evaluating the pathogenesis and pathophysiology of HD, evaluation of early morphological changes to allow timely detection of EEHV-HD and vaccine development. The research subcommittee also accepts and evaluates project proposals requesting EEHV Advisory Group support – submission information can be found at eehvinfo.org.

EEHV ADVISORY VETERINARY AND MANAGEMENT RESOURCE SUBCOMMITTEE UPDATE

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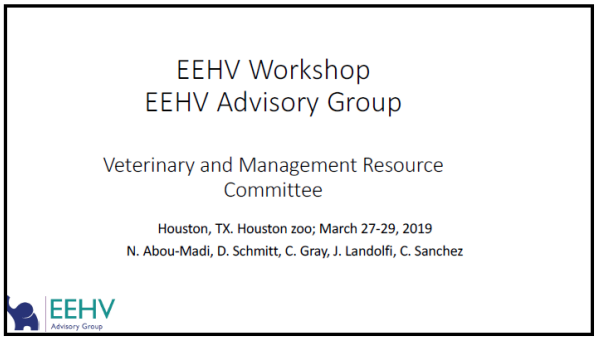

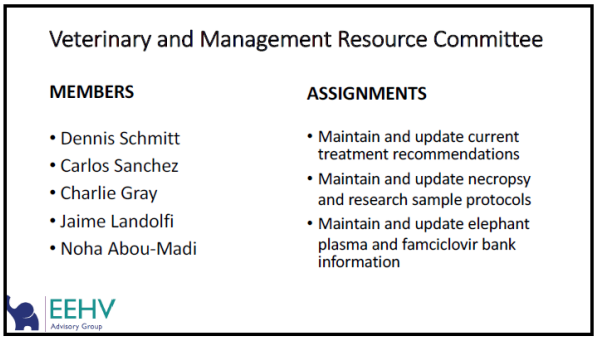

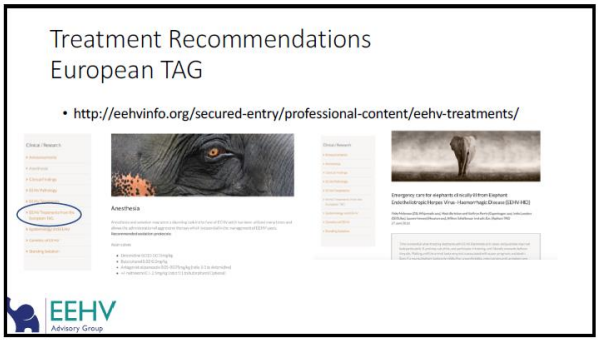

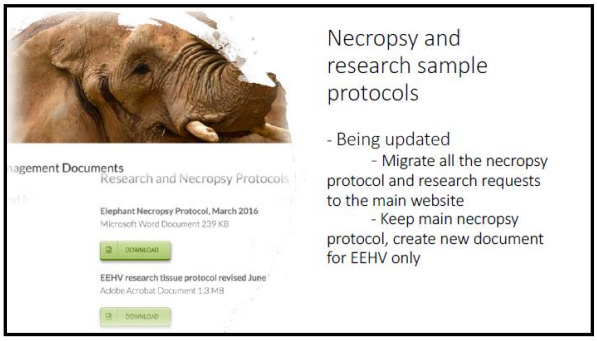

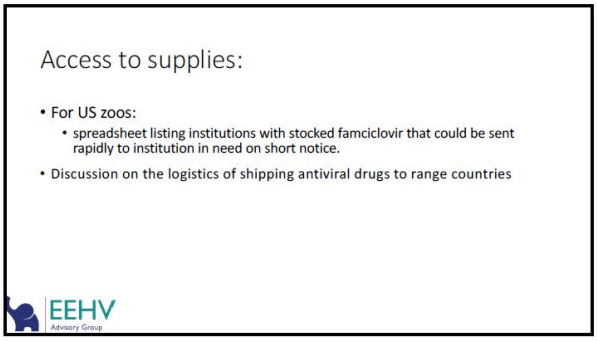

Asian Elephant SAFE Program

Action Plan 2019-2021
Submitted:
Program Leader: Adam Felts
Program Co-Leaders: Nick Newby, and Martha Fischer
with the Elephant TAG, International Elephant Foundation, National Elephant Herpesvirus Laboratory

Program Goals

The goal of the AZA SAFE: Saving Animals From Extinction Asian Elephant Program is to enhance and assist conservation efforts of the Asian elephant within the 13 range countries where the Asian elephant is found, as well as report and celebrate those successes. The IUCN Red List of Threatened Species indicates that the Asian elephant is considered endangered from becoming extinct, with an unknown, but estimated population of less than 40,000 animals (Choudhury et al, 2008). Utilizing the strengths and expertise of the dedicated staff at AZA institutions, the Asian Elephant SAFE Program aims to play a strategic role in contributing and supporting conservation in all 13 range states and within the North American population. Specifically, the Asian Elephant SAFE Program will focus in three key areas:

1. Creation of a registry for human-managed elephant populations in Asia, a priority stated in the Jakarta Declaration for Asian Elephant Conservation,
2. An educational campaign in North America to bring the plight of the Asian elephant to the forefront, and
3. Mitigation and support of emerging diseases in Asian elephant populations.

Human-Managed Elephants

It is estimated that 30% of all elephants in Asia are in human care. By assisting these captive populations, this program will have a direct impact on the global elephant population and conservation of wild elephants. Currently, the registration programs of elephants in human care in Asia are inconsistent across range states leading to difficulties in captive elephant management and therefore their conservation, as the number of animals, distributions, demographics and genetic profiles is incomplete to support best management practices. This topic was a priority at the 2006 Range States Meeting, and again in 2017 with the signing of the Jakarta Declaration for Asian Elephant Conservation, and with Decision 17.217 of CITES (Table A). The Asian Elephant SAFE Program will assist in building a government-supported registration database. Asian Elephant SAFE proposes Indonesia as the site for the pilot program in order to compliment and build upon the current registry program in this country. This registry will be a multi-layered approach using DNA, microchipping, and physical identification cards compiled into a database accessible through user-friendly technology that will ensure each animal is identifiable and verifiable. This effort will lead to a well-documented registration system for elephants in human care in Indonesia, which will then be introduced to other Asian elephant range countries. This will enable governments and stakeholders the enhanced ability to

monitor the numbers and demographics of each elephant population, develop breeding programs, and ensure legal sourcing of elephants transferred in and out of the region.

Table A:

Jakarta Declaration for Asian Elephant Conservation Cooperatively develop captive Asian elephant registration programs including where appropriate microchipping and/or DNA based systems and ensure cross border movements of captive Asian elephants are in compliance with all national and international laws and regulations; Ensure the welfare of captive elephants is maintained at all times.
Decision 17.217 of CITES All parties involved in the trade of live Asian elephants are encouraged to: a) Undertake, as necessary, investigations into the illegal trade in live Asian elephants, and endeavor to enforce and where necessary improve, national laws concerning international trade in specimens of Asian elephants with the explicit intention of preventing the illegal trade in live Asian elephants; b) Develop strategies to manage captive Asian elephant populations; c) Ensure trade in, and cross border movements of live Asian elephants are conducted in compliance with CITES, including the provisions in Article III, paragraph 3, for Asian elephants of wild origin; **d) Collaborate in the development and application of a registry system for registering, marking and tracing live Asian Elephants, requesting as necessary assistance from experts, specialized agencies of the Secretariat and;** e) At the request of the Secretariat, provide information on the implementation of this Decision for reporting by the Secretariat to the Standing Committee.

The Asian Elephant SAFE program identified this priority as an opportunity for collaboration, particularly because AZA institutions have many years of expertise in managing the Asian elephant population in their care, as well as developing and using databases (i.e., studbooks) to help manage a wide range of populations both regionally and internationally.

Understanding that this is an enormous undertaking, strategic partners gathered in February of 2019 to begin to formulate a path forward. The team agreed that Indonesia would be the ideal Asian elephant range country to serve as a registry model for other range nations for several reasons. Specifically, the Indonesian government has already started a registry program, the Indonesian government has jurisdiction over all elephants in Indonesia, and Indonesia's natural heritage includes the critically endangered Sumatran elephant (IUCN Red List). Asian Elephant SAFE will work alongside Indonesian experts to enhance this existing registration program. Working together, we will identify all captive elephants by using DNA analysis of each individual elephant, microchipping, and photo identification. A database with all pertinent information will be developed, as well as, technology that will allow for easy processing and confirmation of each individual elephant identity. With the help and guidance of the Indonesian Government's Ministry of Environment and Forestry (MoEF), the Asian Elephant SAFE will

formalize this process for future use in other Asian elephant range states with captive populations.

The vision of the project is to build upon the existing registry process in Indonesia in order to help facilitate the development of a global registry and identification process for captive Asian elephants in the 13 range states. The goal is to work with the Indonesian government to assist in enhancing the existing registry and process as a model for other range states. Through range-wide participation, the program would:

- Ensure the trade and cross border movement of live Asian elephants are conducted in accordance with national and international guidelines (i.e. CITES)
- Support the population management of Asian elephants throughout their range
- Support law enforcement efforts to ensure the legal trade of Asian elephants
- Support local capacity building through transfer of technology and skills
- Facilitate governance

Asian Elephant SAFE will assist the government of Indonesia in this endeavor by creating a program that can be used throughout the range states. This pilot program could be achieved in Indonesia because of the government's commitment to managing captive elephants in their country.

North American Education Campaign about Asian Elephants

Another goal of the Asian Elephant SAFE program is to raise attention and awareness of guests visiting AZA institutions to the issues facing Asian elephants. Asian elephant conservation is lesser understood by the American zoo-going public than the issues facing African elephant populations, especially the illegal ivory trade. Although the Asian Elephant SAFE program understands this issue cannot be ignored, the program has come to the conclusion that the public are not as aware of the issues such as habitat loss and human/elephant conflict impacting this more threatened and vulnerable species. By creating an educational campaign with consistent messaging focused on Asian elephant conservation, the Asian Elephant SAFE program will identify and separate the issues facing the different species of elephants in order to better educate and engage guests in Asian elephant conservation.

Emerging Diseases

Lastly, the SAFE Asian Elephant Program is committed to advancing the science of emerging diseases that affect both *in-situ* and *ex-situ* populations, including the treatment, management and prevention of these emerging diseases such as elephant endotheliotropic herpesvirus (EEHV). EEHV is a disease that is having negative impacts on Asian elephants in the wild and in human care. The population in human care in AZA and in Asian range states should be managed to be self-sustainable as a reservoir and to prevent this endangered species from extinction. Understanding and finding solutions to diseases such as EEHV is critical for the all populations.

NORTH AMERICAN REGIONAL UPDATE ON ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV)

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Abstract

Elephant endotheliotropic herpesvirus (EEHV) can cause acute, often fatal hemorrhagic disease (EEHV-HD) in young elephants, with Asian elephants (*Elephas maximus*) between 1 and 8 years of age most at risk. Our collaborative, resourceful EEHV community has learned much about EEHV in the last 15 years, and we have a lot more to learn before we have all the answers we need to keep our elephants safe and thriving.

There is immense value in establishing and updating a curated list of EEHV cases so that all members of the EEHV community are communicating the same information. Roadblocks to overcome include concerns about confidentiality, personnel availability for creating and maintaining the list, the need for real time updates to elephant studbook data, and lack of an agreed upon case definition for EEHV HD survivors. Below is an estimate of the EEHV cases and mortality in the North American Asian elephant population as of 3/1/19. This is an estimate based on currently available data, internet search, and personal communication, as the most updated studbook information was not available at the time of this writing.

Of the Asian elephants born in North America between 1/1/80 and 3/1/19, who remain in North America and/or have died in North America (e.g. have not been exported internationally), 67% are still alive and 33% have died. Of the living elephants, 14 elephants (16%) have publicly survived EEHV-HD infection and are still alive ("survivors"). Of the elephants that have died, 27 (64%) have died due to EEHV-HD, thus EEHV remains the single greatest cause of death in this elephant cohort (born in N America since 1980). With 41 total publicly known EEHV-HD cases in North America in this elephant cohort, our current fatality rate (27/41) is 66%. With 41 total EEHV-HD cases, 32% of all elephants in this cohort have been impacted by EEHV HD infection and/or fatality.

Sixteen elephants were born in North America between 2010 and 2017 and are still alive. Eight of these elephants (50%) are EEHV-HD survivors. Six Asian elephants were born in North America in 2018 (between May and November), and they will soon old enough to be at risk for EEH-HD as well. Thus by mid-2019, the at-risk population in North America will include 22 young elephants between 1 and 9 years of age, with 8 of these elephants already having survived at least one episode of EEHV-HD.

Key words: Asian elephant, *Elephas maximus*, EEHV, EEHV-HD, survivor, population

AN UPDATE ON ELEPHANT ENDOTHELIOTROPIC HERPES VIRUS HEMORRHAGIC DISEASE CASES IN EUROPE IN 2018-2019

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Abstract

In 2018 and 2019 (until March) 6 fatal cases of Elephant Endotheliotropic Herpes Virus-Hemorrhagic Disease (EEHV-HD) have been reported in Asian elephants (*Elephas maximus*) in 4 European zoos. Five of the calves were 2-3 yr-old and 1 elephant was 5 years and 9 months old. In 5 cases the course of the disease was acute and death occurred within 1-2 days after the first signs were observed. In 1 case the elephant died 7 days after initial viremia was diagnosed. The interval between the 2 cases in zoo 2 was only 7 days, while the 2 calves in zoo 4 died within a time span of 24 hours. All cases of EEHV-HD were caused by EEHV1a. Two non-fatal cases in Asian elephants were reported from 2 different zoos. One 2 yr-old female showed swelling of the throat area and some hyperemia of the oral mucosa as well as a slightly swollen perineum. The qPCR was positive on EEHV1. Oral treatment with antivirals was initiated but discontinued after a few days. A second non-fatal case was reported in a 2 yr-old female elephant calf, showing hemorrhagic lesions on tongue and vulva. In blood and conjunctival swabs EEHV1b was identified by qPCR. Viral load and white blood cell counts were monitored for 8 days. The viral load in the blood dropped quickly after initiation of treatment (famciclovir, rectal fluids, plasma transfusions and antibiotic treatment).

EEHV HD IN THAILAND FROM 2006 TO 2018: WHAT DO WE KNOW SO FAR?

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Abstract

Thailand is the fourth largest Asian elephant population in the world, which approximately 3,000 individuals remain in the wild and around 3,500 remain in captivity [1]. Elephant endotheliotropic herpesvirus hemorrhagic disease or EEHV HD is one of the most concern threats for Asian elephant conservation in Thailand. The first confirmed EEHV HD case in Thailand was on 2006, and the number had reached 21 cases in 2014 [2].

Thailand EEHV taskforce was established on 2015 which aims to strengthening the collaboration on diagnostic, treatment protocols, data collection and research capacities in Thailand. From 2006 to 2018, we have collected the data from young elephants that showed clinical signs ranging from depression, diarrhea, face swelling, tongue cyanosis to sudden death. Among that, a total of 66 clinical cases were confirmed as positive for EEHV infection by using molecular techniques, of which 47 were dead and 19 were survived (Figure 1). The survived elephants were all treated with either acyclovir (IV), acyclovir (PO), or famciclovir (PO).

Fig1: The number of EEHV HD cases in Thailand from 2006 to 2018.

The most common viral subtypes that were detected in those clinical EEHV cases in Thailand were EEH1A (60%), EEHV4 (29%), and EEHV1B (11%) respectively. Interestingly, EEHV5 haven't been reported in Thailand, either in clinical or subclinical cases; whereas in India, EEHV5 was the most common subtype that found in subclinical cases [3].

The clinical presentations of EEHV1 and EEHV4 positive elephants were quite different. The common clinical signs found in EEHV1-positive cases were depress, inappetite, face swelling and tongue cyanosis; whereas EEHV4-positive cases generally showed GI abnormality signs i.e. bloody diarrhea, bloat and constipation at the beginning of the clinical stage.

The clinical EEHV cases ranged in age from 3 months to 18 years old. The mean affected age was 42±38 weeks and the median affected age was 29.5 weeks. In addition, when elephants were divided by age class structure [4], the EEHV HD was more likely to be occurred in the juvenile, age range 1 – 5 years old (75%), especially between one to two years old (43%). However, no sex predilection was observed. The disease also occurred across geographical locations throughout the country and throughout the year, except clinical EEHV4 cases which found only during April to July.

The data analysis illustrated that approximately 10% of young Asian elephant population (< 10 years old) in Thailand had been affected by this disease and the fatality rate was as high as 70%. As this consequence, without proper disease monitoring and management, this disease could be a potential threat for Asian elephant conservation in Thailand.

Acknowledgement

We would like to thanks all the members of Thailand EEHV taskforce who dedicate their time and skill to help in sample collection, data collection and researching in various topic.

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EEHV, AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*), AND THE REGIONAL AFRICAN EEHV WORKING GROUP

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Abstract

The impact of elephant endotheliotropic herpesvirus (EEHV) on African elephants (*Loxodonta africana*) in free ranging populations and under human care is largely unknown. There have been five cases of clinical illness associated with EEHV hemorrhagic disease (EEHV-HD) in African elephants reported in the literature, with three fatal outcomes (EEHV2 in two cases, EEHV6) and two surviving with treatment (EEHV 3B, EEHV6). Multiple EEHVs have been identified from pulmonary and skin nodules of asymptomatic, free ranging African elephants, as well as in African elephants under human care. Clinically healthy African elephants screened over time have been shown to shed EEHV2, EEHV3, and EEHV6 in either trunk washes, saliva swabs, or both. While the North American African elephant population has been augmented by recent imports, long term sustainability remains threatened due to low cyclicity among breeding aged females, among other factors, and each calf produced has significant impact. To date, the impact of EEHV on our North American population appears low; however, no formal epidemiologic study has been undertaken. In African elephant range countries, even less is understood of the epidemiology and significance of EEHV2, EEHV3 and EEHV6 infections or of EEHV-HD. Frequent anesthetic interventions on adult elephants for translocation/management reasons, and daily care of orphaned elephants are two potential avenues for sample collection and monitoring for EEHV shedding and EEHV-HD. The first regional African EEHV Working Group meeting will take place as part of the International Elephant Foundation's Conservation and Research Symposium in South Africa in October 2019, and will focus on building a communication network, identifying opportunities for collaboration, and identifying gaps in knowledge, laboratory and diagnostic skills, and needed equipment.

DEVELOPMENT OF A DISEASE RISK ANALYSES TOOL TO GAIN A BETTER UNDERSTANDING OF EEHV ASSOCIATED MORBIDITY AND MORTALITY IN THE NORTH AMERICAN ASIAN ELEPHANT (*ELEPHAS MAXIUMS*) POPULATION

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Abstract

As the single greatest cause of death in North American Asian elephants, elephant endotheliotropic herpesvirus (EEHV) threatens the sustainability of Asian elephants under human care. The increased calf monitoring and trunk wash testing that is being performed has provided valuable data on exposures, infections, and viral levels throughout calf-hood. More work is needed on the classification of clinical EEHV and what risk factors determine whether a calf that has been exposed will recover without apparent illness or will go on to EEHV Hemorrhagic Disease.

We now face a need to gain further insight into determining the risk factors for progression from EEHV viremia to clinical hemorrhagic disease. To do this requires a multifactorial approach. Firstly, we must refine the case definition used for EEHV, provided by the initial working group discussions. Secondly, we propose a prospective tracking system of Asian calves born in North America, to collect the required information on risk factors that may be associated with EEHV disease morbidity and mortality later in life, from the time of birth. This survey-based disease risk analyses, in conjunction with biomaterial sampling of EEHV status, will help us unravel the mystery of why some calves with EEHV viremia have no clinical signs while others with viremia quickly die. These data used along with ongoing genetic and immunological studies will allow us to tease out specific at-risk individuals or populations.

In this talk, we will present the template for data collection that may move this forward within the community, with the talk objective to garner discussion from the audience as we fine tune this instrument. With a further understanding of those risk factors that lead to EEHV morbidity and mortality, we may be better able to adjust management strategies and laboratory testing to aid in the sustainability of Asian elephant populations.

Key words: Case definition, Disease risk analysis, EEHV, Elephant endotheliotropic herpesvirus, *Elephas maximus*

INVESTIGATING DISEASE SUSCEPTIBILITY TO ELEPHANT ENDOTHELIOTROPIC HERPESVIRUSES IN NORTH AMERICAN ELEPHANTS USING A GENOME-WIDE APPROACH

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Abstract

Ex situ elephant populations serve as important reservoirs to support declining *in situ* populations, allow the generation of new knowledge, and act as conservation ambassadors to raise awareness for the decline of their wild counterparts. However, North American populations of African (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) are not self-sustaining; high infant mortality and reduced adult survival survivorship are curtailing conservation efforts for both genus. Due to high incidences of mortality and morbidity in both captive and wild populations, recent elephant health research has focused in part on elephant endotheliotropic herpes virus (EEHV). Susceptibility to viral (e.g., EEHV) agents arises from the complex interaction of the pathogen and individual genetic factors. There is huge variation in the individual outcomes that follow exposure to potentially life-threatening pathogens, and this difference in susceptibility illustrates the functional genetic diversity of the immune response. Our aim is to better understand the function and diversity of the elephant immune system, how it is regulated, and the underlying mechanisms of host defense against infections like EEHV. We hypothesize that there will be genetic differences in immune system and regulatory genes, between elephants that survive EEHV infection, and those that succumb to the disease. We will construct a high-density in-solution SNP capture assay to screen ~300 elephants in the North American zoo population for genetic differences that may be related to disease susceptibility. This includes elephants have tested positive for EEHV (n=35), seventeen of which died as a consequence. Whole genome sequencing, assembly, and alignment of female genomes, and SNP discovery has been completed. Our highly-contiguous, phased, whole-genome *de novo* assemblies represent the highest-quality elephant genomes sequenced to date. The results of this study will enable us to understand the genetic underpinnings of immune system function and disease susceptibility in elephants.

Key words: Conservation, Genomics, Zoo

OPTIMIZATION OF NON-INVASIVE TECHNIQUES FOR DETECTION OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS IN ASIAN (*ELEPHAS MAXIMUS*) AND AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

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Abstract

Elephant endotheliotropic herpesvirus (EEHV) poses a significant risk for the health of captive Asian elephants (*Elephas maximus*) in North America due to its virulence and high fatality rates, and an unknown risk to working and wild elephants in range countries. Current EEHV detection requires invasive sampling methods, which are not feasible for surveying untrained captive or wild populations. To investigate whether chewed plant and fecal samples could provide alternatives to blood, trunk wash, and oral swab samples for EEHV detection, and also to determine which non-invasive sample type is best for detection, Asian elephants (n = 9) at the Houston Zoo and African elephants (*Loxodonta Africana*, n = 12) at the San Diego Zoo Safari Park were sampled once weekly for a minimum of five weeks. Conventional and quantitative PCR detected EEHV positives in 18 invasive samples, and none in non-invasive samples. Elephant gammaherpesvirus 1 DNA was detected in six chewed plant samples, indicating the potential for non-invasive herpesviral detection in elephants. In order to evaluate which sample type may be most useful for detecting EEHV DNA as well as elephant DNA for other population genetics studies, we tested for the presence of mammalian ferritin as an indicator of DNA quality. Ferritin was five times more likely to be detected in feces ($P < 0.001$) compared to other non-invasive samples, suggesting feces may be preferred to chewed plants as a non-invasive sample for collection. When chewed plants are more accessible than fecal samples, results suggest that hay, oak, hackberry, mulberry, or elm are better than bamboo. Further studies are planned to evaluate EEHV detection at different time points and assess other methods for DNA extraction to optimize fecal and chewed plant sampling for EEHV detection.

IDENTIFICATION OF AFRICAN ELEPHANT POLYOMAVIRUS IN WILD AFRICAN ELEPHANTS AND THE ABILITY OF ITS EARLY REGION GENES ENCODING LARGE AND SMALL TUMOR ANTIGENS TO TRANSFORM ELEPHANT PRIMARY CELLS

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Abstract

In addition to intense poaching for their ivory, habitat fragmentation and increasing human-elephant conflict, microbial infections such as elephant endotheliotropic herpesviruses (EEHVs) and elephant gammaherpesviruses (EGHVs) have emerged as serious threats to wild and captive elephant populations. However, study of these pathogens has been severely limited by lack of established and validated cell lines that support their *in vitro* propagation and study. In 2013, a novel African Elephant Polyomavirus, (AelPyV-1), was identified in a captive African elephant, *Loxodonta africana*, in Europe. This virus was associated with protruding hyperplastic fibrous trunk lesions (nodules). Subsequently, we used primers specific for five AelPyV-1 genes, polymerase chain reaction (PCR) and Sanger sequencing analysis to screen biopsies of similar nodules we had collected from nine wild elephants in Botswana and South Africa in 2013 and Kenya in 2011. Nodules from six of nine elephants tested positive for one or more AelPyV-1 genes. Previously, we had analyzed these nodules for elephant herpesviruses, finding one or more species of EEHVs and/or EGHVs in every nodule. The AelPyV-1 genome contains open-reading frames coding for classic large (LTag) and small (STag) tumor antigens. Some polyomavirus LTag proteins - notably the SV40 large tumor antigen from the SV40 polyomavirus - are oncoproteins that can induce neoplastic transformation in the host cell. We

cloned the entire early region spanning the LTag region and the overlapping STag region of a wild isolate of AelPyV-1 found in one of the wild elephants in Botswana. We used this plasmid construct pAelPyV-1-LTag to transform primary endothelial cells derived from an Asian elephant, *Elephas maximus*, umbilical cord. These transfected cells tested positive for the AelPyV-1 LTag transgene by PCR and Sanger sequencing analysis following seven passages *in vitro* over 150 days under antibiotic G418 selection, whereas the untransfected cells *in vitro* reached senescence and died after seventeen passages over 70 days. Reverse transcriptase PCR (RT-PCR), gel electrophoresis and Sanger sequencing analysis validated expression of the expected AelPyV-1 LTag as well as the STag in these transformed cells and in two new pAelPyV-1-LTag transfections of primary endothelial cells derived from umbilical cords of two additional Asian elephants. Current efforts are aimed at establishing whether AelPyV-1 LTag or STag alone or in combination is responsible for cellular transformation and immortalization of elephant primary cells. We expect that our novel plasmid construct pAelPyV-1-LTag will aid in the study of elephant-specific infectious agents, particularly elephant herpesviruses, as well as contribute to our understanding of the potential mechanisms for the surprising resistance of elephants to cancer.

Key Words: African Elephant Polyomavirus, Elephant herpesviruses, Oncoprotein

ANNOTATED COMPLETE DNA SEQUENCES OF SIX EEHV1A GENOMES FROM LETHAL HD CASES IN YOUNG ASIAN ELEPHANTS FROM INDIA

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Abstract

Whilst two complete genomes of EEHV1A and one each of EEHV1B, EEHV5A and EEHV4B from North American and European zoo cases have all been complied and annotated by random next-generation Illumina approaches, standard Sanger PCR sequencing of multiple selected small segments from up to 30 other strains examined of EEHV1A have revealed an extraordinary level of hypervariability and subtyping in certain loci scattered across that genome. To evaluate and compare more of that divergence and to include strains from both wild and camp-reared cases in Asia, we attempted to obtain and assemble the complete genomic sequences from necropsy tissue DNA obtained from seven previously described cases from the state of Kerala in Southern India (three free-ranging, three rescued orphans and one captive born). To do so, we first generated long-range PCR amplicons (10-kb in size) based on both EEHV1A and EEHV1B then pooled the resulting overlapping segments of each genome for high-throughput Illumina sequence runs. Together with some additional Sanger PCR to join across gaps and to reevaluate ambiguous regions (especially within inverted repeat ori-Lyt and the tandemly repetitious terminally redundant regions), the genomes of five independent strains (IP43, IP91, IP143, IP164 and IP165) each totalling 176,300 +/- 850-bp were successfully and directly assembled and annotated. Completion of the sixth similarly sized genome required pooling of data from two nearly identical cases occurring nearly simultaneously at the same camp (Aswathi IP06 and Nirangen IP07).

The new data revealed that only IP06/IP07 and IP91 were closely related within the 10.5-kb multigenic R2 or vIgFam region, with the other three displaying the same extremely high levels of divergence in both gene content and homology there as seen in all previously examined strains. Interestingly, although there were no new never-before-seen subtypes in any of the variable genes, several examples of the rarest subtypes were found amongst the six India genomes, including two more examples of vOX2/3-subtype C, three of the ORF-O, ORF-P, ORF-Q subtype F-H2-E complex and two of E56-F (= vGPCR12), each seen just once or twice

previously e,g, in Singgah only, Preya/Singgah and Kala/Mac. The analysis also revealed one last new additional hypervariable locus encompassing E30/E31 (1.8-kb), in which again the same three India genomes (IP43, IP91 and IP165, subtype D) matched the rarest previous pattern (Preya only). Overall, it is evident that the extant population of EEHV1A strains worldwide display extraordinary levels of divergence and scrambled subtyping, indicative of ancient possibly composite origins and extensive recombination.

Key words: EEHV1 HD in India, Gene content, Genetic variability, Rare gene variants, Whole genome comparisons

IDENTIFICATION OF A NOVEL HIGHLY DIVERGED VARIANT OF EEHV1A IN THREE LETHAL CASES OF HEMORRHAGIC DISEASE FROM SABAH, MALAYSIA

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Abstract

Three cases of lethal sudden onset acute hemorrhagic disease (HD) with typical gross clinical symptoms occurred in May 2016 in two-year-old rescued calves being reared at an elephant orphanage in Sabah, Borneo. All proved to have high levels of EEHV1-like virus DNA in necropsy tissue and a fourth herdmate without symptoms apparently survived the infection. Because these were the Borneo-specific sub-species *Elephas maximus borneensis*, questions arose about whether the viruses involved might, like their hosts, be genetically distinct from mainland Asian elephant EEHVs. PCR DNA sequencing was carried out across eight amplified conserved and variable gene loci from pathological heart tissue from one of the lethal cases, as well as at selected loci in samples from the other cases. The results revealed that exactly the same strain was present in all four afflicted calves. As anticipated, the viral nucleotide sequence proved to be novel and differed significantly by between 2.0% to 5.4% from the prototype USA genome EEHV1A(Kimba) at each locus examined. Remarkably, the DNA differences, although predominantly syngeneic, were most dramatic within two of the "oldest" most conserved segments of the genome (POL and TER) where the Borneo version falls intermediate between the previously well-defined EEHV1A and EEHV1B chimeric subtypes. At the more hypervariable loci examined (U48/gH, E51/vGPCR1, E54/vOX2-1), the Borneo version shows both DNA and protein level differences that more closely resemble specific modern subtypes of EEHV1A rather than of EEHV1B. These results provide evidence that this novel strain of EEHV1 initially co-evolved together with the Borneo subspecies elephant hosts (which evidently diverged from their closest mainland relatives about 300,000 years ago) but may now be chimeric. Furthermore, despite the high levels of nucleotide divergence within the core segment especially (estimated to represent between 500,000 to 1.7 million years since their last common ancestor) the Borneo variant of EEHV1A still retains a similar highly pathogenic phenotype to that of mainland EEHV1A.

Key words: Asian EEHV1A DNA sequences, Bornean elephants, Conserved versus novel genes, EEHV HD, Evaluating divergence

FATAL HEMORRHAGIC DISEASE CAUSED BY EEHV-1A IN A 6 YEAR OLD ASIAN ELEPHANT (*ELEPHAS MAXIMUS*)

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Abstract

A six-year old female Asian elephant died acutely after initial clinical signs of EEHV-HD; an initial routine blood sample yielded an EEHV-1 (+) of 700vge/ml in 1 of 2 wells; test was repeated by the National Elephant Herpesvirus Laboratory (NEHL) in the same sample resulting in 250vge/ml; the calf was clinically normal. The follow up sample submitted the following week was delayed in transit during shipment. A courier was sent to the NEHL with blood from the calf the following morning when she presented clinical signs for the first time: decreased appetite, swelling of temporal glands and mild lethargy. Coincidentally, this calf and mother had been isolated in the indoor area overnight due to a recent positive TB trunk wash in the cow. (This positive trunk wash was proven to be false on the same day as the onsite of clinical signs of EEHV illness in the calf.) Supportive and antiviral treatment started within the next few hours. Rectal fluids, intravenous whole blood Hetastarch® and LRS; rectal and oral famciclovir and fortified plasma from male elephant (bull administered 30mg/kg famciclovir PO, plasma collected 90 mins later for transfusion. Dennis Schmitt pers. Comm.) were administered throughout the day. The condition of the calf deteriorated rapidly over the next few hours passing blood clots in feces and red-urine. EEHV-1 levels from the morning sample yielded a 3,400,000vge/ml. Bloodwork at this time showed a significant WBC shift with a marked decrease on monocytes and platelets when compared with previous values for this calf. The severe thrombocytopenia observed (71,000 k/uL) indicated a poor prognosis. The original blood sample that had been delayed during transportation finally reached the NEHL and results showed 4,600,000vge/ml. The elephant calf died at 11:00 pm on the same day it presented clinical signs for the first time. Typical EEHV-HD lesions were found in necropsy and histologic examination of tissues, intra-nuclear inclusion bodies were observed in capillary endothelial cells (Zoo Pathology Program report). Preliminary sequencing shows the virus is a novel EEHV-1A.

Key words: Asian elephant, *Elephas maximus*, Elephant endotheliotropic herpesvirus

FIVE CASES OF HEMORRHAGIC DISEASE DUE TO ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) IN A GROUP OF YOUNG ASIAN ELEPHANTS TREATED WITH AGGRESSIVE CRYSTALLOID AND COLLOID THERAPY

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Abstract

Five female juvenile Asian elephants presented acutely with signs of EEHV at a single zoological facility in Canada. The cases occurred sequentially over the late summer and fall. Four out of five calves survived. Treatment was aggressive, and involved multiple transfusions, often after sedation, using rectal fluids, intravenous plasma, blood, and hetastarch, along with other supportive and antiviral therapies. Care staff provided almost continuous 24 hour around-the-clock care for over four months. Freshly made blood smears were used routinely to assess the elephants' prognosis.

Key words: Blood, EEHV, Fluid therapy, ICU care in megavertebrates

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IMPLEMENTATION OF IN HOUSE TESTING FOR ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) AND POTENTIAL BENEFITS OF INCREASED HERD SURVEILLANCE

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Abstract

In July 2017, the Oklahoma City Zoo implemented in house EEHV qPCR surveillance for the Asian elephant (*Elephas maximus*) herd. Development of the in house lab was in response to the death of a 4.5-year-old calf due to EEHV1A hemorrhagic disease on October 1, 2015. Retrospective trunk wash analysis revealed that one of the adult females in the herd had been shedding the same strain of EEHV1A for 2 months prior to the death. This information initiated the discussion regarding the need for increased herd surveillance and ability to receive results more quickly. A detailed proposal, costs analysis and justification was presented to the Zoo's administrators which resulted in an agreement to proceed with purchase of equipment and implementation of the lab.

With the initiation of the lab, the Zoo developed a surveillance plan which included testing weekly blood and trunk wash samples for EEHV1, EEHV3/4 and EEHV5 on each elephant in the herd (2.5 animals). Blood samples on calves less than 1 year of age are tested opportunistically. Since starting the increase surveillance program, several bouts of trunk wash shedding have been noted within the herd which prompted heightened surveillance in the susceptible calf. Additionally, an episode of EEHV5 viremia was noted in a 15-year-old bull which would have gone undetected if the increased surveillance program was not in place. Even though in house testing is accomplished weekly, the Zoo understands the value in continuing to partner with the EEHV consortium and has maintained it membership at the research partner level.

Key words: EEHV, Elephant endotheliotropic herpesvirus, *Elephas maximus*, PCR, Trunk wash

ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS FATALITIES IN EUROPE

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Abstract

Elephant Endotheliotropic herpesvirus (EEHV) infection is the leading cause of death among captive Asian elephants (*Elephas maximus*). The aim of this study was to summarize data relating to known EEHV fatalities in Europe in order to identify possible risk factors for development of EEHV-Hemorrhagic Disease associated death. Stud book data were collated from all Asian elephants born in Europe during 1985 – 2017. The risk period for EEHV was defined as from nine months of age (the youngest known case), to eight years. Calves were classified as survivors if they exceeded eight years of age by the end of the study period. Fatalities were classified as any calf succumbing to EEHV during the study period. Forty-one institutions recorded a total of 263 birth events, including still births. Fifty-five calves were either born dead or died in the first 24 hours (21 %). Twenty-five fatalities were identified from 14 institutions, at an average age of 2.7 years old. Twenty dams (one to three calves each) and 14 sires (one to five calves each) produced calves that succumbed to EEHV. There was no predilection for either sex. Of the 31 calves that died in the risk period, only six died from non-EEHV confirmed disease, however several of these case had limited information available and therefore EEHV could not be definitely ruled out. Due to the retrospective nature of these data, many questions are left unanswered, however the results of this study will help generate hypotheses for risk factors leading to EEHV-associated death.

Key words: Age, Europe, Fatal, Risk factors, Sex

CLINICAL EEHV1B IN TWO ASIAN ELEPHANT CALVES (*ELEPHAS MAXIMUS*) – CLINICAL PRESENTATION AND DECISION-MAKING LEADING TO SURVIVAL

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Abstract

Two clinical cases of EEHV1B presented at Woburn Safari Park in December 2017 and at ARTIS Amsterdam Royal Zoo in November 2018 respectively. In the first case, significant EEHV-1B DNAemia ($5.30*10^5$ vge/ml) was detected on a routine blood sample of a three-year-old Asian elephant (*Elephas maximus)* in the absence of clinical signs, which rapidly increased to $17.2*10^5$ vge/ml within 72h. Marked leukopenia and thrombocytopenia were present. Twice daily intravenous treatment with ganciclovir and plasma under standing sedation was initiated and viral loads decreased in the subsequent 48 hours. Cephalosporines were added to the treatment. The calf developed lymphocytosis and subsequently clinical signs 6 days after the onset of viremia, prompting treatment with short acting glucocorticosteroids for two days. Within 3 days all clinical signs resolved. Intravenous (ganciclovir) and subsequently rectal (acyclovir) antiviral treatment continued for another 3 and then 2 days respectively. After three weeks, EEHV PCR was negative but three subsequent subclinical episodes were detected five, four and then three weeks later.

In the second case, a 2-year-old female Asian elephant presented with petechiae on its tongue. Sedation of the dam facilitated separation and sedation of the calf, enabling diagnostic sampling and immediate treatment per rectum with famciclovir and fluids, and intramuscular potentiated sulfonamides. The total white blood cell count was raised and qPCR was positive for EEHV-1B ($20.8*10^5$ vge/ml). The calf was subsequently treated for three days with intravenous plasma, intramuscular potentiated sulphonamides, and famciclovir and fluids per rectum. As the viral load decreased and monocytes and platelet counts increased consecutively, the administration of glucocorticosteroids was not considered. Clinical signs were absent after one week.

These cases shed further light on the pathophysiology and pathogenicity of EEHV-1B, and highlight the value of easily accessible, low cost, in-house blood smear analysis in the decision making of both clinical and subclinical EEHV cases.

Key words: EEHV-1B, EEHV-HD, *Elephas maximus*, Hematology

ACKNOWLEDGEMENTS

Special thanks to our supportive veterinary colleagues Frank Verstappen and Marno Wolters, to the elephant keepers at Woburn Safari Park and ARTIS Amsterdam for their input and training to enable animal access, to Tim Bouts and the elephant keepers at Pairi Daiza for their immediate action in obtaining fresh plasma for the treatment of the ARTIS calf, and to all other staff and technicians involved in these two cases.

TOUGH TALK: BRIDGING SCIENCE AND EMOTION TO COMMUNICATE ABOUT EEHV

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Abstract

From detection to treatment, EEHV presents many challenges, not least of which is how to talk about the deadly virus with the public, which is more receptive to soundbites that science. Educating audiences with in the zoological and international elephant communities as well as the public about the facts of EEHV is particularly difficult when anti-zoo groups use EEHV deaths as reason demand the end of elephant breeding programs. Communication strategies and tactics can assist institutions and researchers in more effectively gaining understanding and support for the battle against EEHV.

USE OF MESENCHYMAL STEM CELL THERAPY IN INFECTIOUS DISEASE AND POTENTIAL UTILITY IN EEHV

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Abstract

Over the past several decades, an increase in multi-drug resistant infections and biofilm infections due to orthopedic implants have accentuated the necessity for novel therapeutics to treat infection. The use of therapeutic medications is invariably associated with evolution of the pathogen and resistance and therefore we sought to develop an immunotherapy to enhance the immune system's capability to fight infection. Mesenchymal stem cells (MSC) have been demonstrated to have antimicrobial activity and their properties can be modulated to increase this effect. Several studies have demonstrated the utility of MSC therapy in treating sepsis by decreasing bacterial load and dampening the cytokine storm. Our laboratory has demonstrated the ability of MSC pre-activated with viral analog poly-IC to resolve multi-drug resistant infections and biofilm infections both in an animal model, and a spontaneously occurring natural model in canines. Our laboratory has successfully developed several blood derived mesenchymal stem cell lines from both African elephants (*Loxodonta africana*) and Asian elephants (*Elephus maximus*). These cells have been demonstrated to exhibit the phenotypic and functional properties of mesenchymal stem cells and have been safely administered to 3 African and 1 Asian elephant. Therefore, we hypothesize that the use of pre-activated MSC in Asian elephants suffering from acute hemorrhagic disease caused by elephant endotheliotropic herpesvirus (EEHV) would result in improved clinical outcomes. Many studies have documented the increased anti-inflammatory effect of pre-activation of these cells and our studies in other species have documented increase immune response to infection. We propose to initiate in vitro studies to examine the effect of pre-activation against herpes viruses as a potential novel and effective therapy for acute EEHV infection.

THE USE OF MESENCHYMAL STEM CELLS AS PART OF A THERAPEUTIC PLAN FOR ELEPHANT ENDOTHLIOTROPIC HERPESVIRUS (EEHV)

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Abstract

Mesenchymal stem cells (MSCs) are found in all mammalian tissues.[4] MSCs, considered multipotent stem cells, can be derived from a variety of different tissues such as bone marrow, adipose, umbilical cord tissue (Wharton's jelly), and umbilical cord blood; however the number of stem cells in different tissues varies.[2-4] They are self-renewable and have regenerative, anti-inflammatory and immune-modulatory properties.[2,3] In veterinary medicine, MSCs have been used to treat a variety of degenerative and inflammatory conditions in domestic animals such as arthritis, tendon injury, spinal cord injury and heart disease.[1,4] MSCs have been shown to localize in damaged tissues resulting in regeneration and reduced inflammation suggesting this may be an ideal adjunct therapy for EEHV.[4]

EEHV-1A and EEHV-1B viremias were detected in a juvenile Asian elephant at the Oklahoma City Zoo during routine weekly blood PCR screening. Complete blood count parameters remained within normal range and no clinical signs of illness were observed during either episode. Blood samples were collected 2-3 times weekly to monitor viremia level until resolution. During each episode, a 10-fold increase in viremia was noted therefore empirical therapy was initiated. Under mild sedation, autologous mesenchymal stem cells (originally collected from umbilical cord) were administered IV in conjunction with fresh frozen plasma, rectal and/or intravenous antiviral therapy, and rectal fluid therapy. No adverse reactions were noted and both viremia episodes resolved without incident. Further research is warranted to determine efficacy and effective therapeutic dose of MSCs as an adjunct treatment of EEHV.

Key words: EEHV, Elephant endotheliotropic herpesvirus, *Elephas maximus*, Stem cells

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USING SERUM INFLAMMATORY MARKERS TO INVESTIGATE THE IMMUNE RESPONSE TO ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS

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Abstract

Over the last two decades, numerous elephant deaths have been attributed to the hemorrhagic form of elephant endotheliotropic herpesvirus (EEHV). Herpesviruses in other species are known to suppress the immune system, preventing it from successfully fighting infection; however, the etiopathogenesis of EEHV is poorly understood, and it is still unclear why some animals develop often fatal hemorrhagic disease, while others have only latent or asymptomatic infections. Changes in immune function may be predictive of disease onset or outcomes, and the goal of this study was to measure concentrations of serum immune biomarkers (cytokines [tumor necrosis factor alpha, interferon gamma, and interleukins 1-beta, 2, 4, 6, and 10] and acute phase proteins [serum amyloid A and haptoglobin]) to better understand the response to EEHV viremia in Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. Using serum samples collected from viremic individuals in collaboration with the National Elephant Herpesvirus Lab, cases of controlled infection are being compared to those of fatal and non-fatal hemorrhagic disease, to determine whether immune function differs between asymptomatic, fatal, and non-fatal cases, perhaps indicating that immune suppression may be involved in disease progression. Preliminary data show that acute phase protein and some cytokine responses differ between controlled and un-controlled hemorrhagic disease, and may be useful in furthering our understanding of disease progression and individual susceptibility. However, concentrations are much lower than those measured in adult elephants with other clinical pathology, so longitudinal monitoring is important to understand changes in immune function while we continue to work on more sensitive analysis techniques.

Key words: Acute phase proteins; African elephant; Asian elephant; cytokines; EEHV; immune response

THROMBOELASTOGRAPHY USE OVER TIME IN JUVENILE ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)

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Abstract

Abnormal Thromboelastography (TEG) results has been suggested as a possible predictor of the onset of EEHV-HD. Current literature has documented normal values for Asian elephants. However, current published results were from one time samples primarily from adult Asian elephants. This presentation is from data generated in juvenile Asian elephants over time and during which two cases of EEHV-HD occurred. The analyses of several juvenile Asian elephants confirmed that similar TEG values were detected in both juvenile and adult Asian elephants. The variation between elephants necessitated establishing a normal range for each individual. TEG was not predictive of EEHV-HD, and abnormal values were not observed until well into the haemorrhagic phase of disease. EEHV affected animals were noted with increased clotting time (reaction time) and decreased clot strength (maximum amplitude) compared to normal values established for the individuals prior to clinical disease. In a separate note, ACT tubes were used to assess whether they would detect changes in clotting time. Even during prolonged TEG clotting times (15->30 minutes), there were no differences in clotting time with the ACT tubes between normal elephants and those with clotting deficiencies. This emphasizes the differences in some clotting factors between elephants and most other animals. In these EEHV cases, TEG values did not change during the initial rise in EEHV viral load, and it did not predict the onset of clinical EEHV.

TRANSFUSION MEDICINE AND EEHV-HD: ADVANCING LIFE-SAVING THERAPIES

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Abstract

Transfusion medicine in the veterinary world has undergone rapid advancement in recent years, coming in to sharp focus with emphasis on advanced techniques, novel therapies and expanding treatment options as well as guidelines for blood collection and processing.[3,5] However, in zoological medicine a lack of resources often underscores similar advancements and can limit the care provided to critical cases including hemostatic stabilization of Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease (EEHV-HD). With emphasis on training for blood collection events in EEHV monitoring programs, as well as advanced techniques for the stabilization of blood components for transfusion, the ability of zoos and animal care facilities to pursue transfusion medicine may become more feasible. Transfusions have been given as a component of treatment for EEHV cases and are recommended in some EEHV treatment protocols.[1,2,4] Unfortunately, protocols for Asian elephant whole blood and plasma collection, storage and administration have not yet been standardized. A review of blood collection and banking methods utilized in domestic animal medicine, including the basic materials and techniques required, will provide necessary resources for the EEHV community to explore safe transfusion options in critical case management. In addition, ongoing research endeavors are striving to provide the community with a comprehensive "best practices" guideline for Asian elephant transfusion medicine, a vital resource for the EEHV community. These research projects, including a multi-institutional Asian elephant blood compatibility project, the continuing development of lyophilized blood products, and a survey of current transfusion practices in EEHV case management, require input and collaboration from the EEHV community for success and to encourage the continued application of transfusion medicine practices in these critical cases.

Key words: Blood collection, *Elephas maximus*, Elephant endotheliotropic herpesvirus, Transfusion

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EEHV INFECTION CHALLENGING IN MYANMA ELEPHANTS AND HEALTH CARE MANAGEMENT IN MYANMAR

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Abstract

At the present, there are over 3000 captive elephants in Myanma Timber Enterprise (MTE), a state owned organization, over 2000 elephants privately owned, and nearly 1000 in the Forest department. Hence, the total captive elephant populations in Myanmar are around 6000. Out of them, only 40% are workable ages (between 18-55 yrs) and the rest 60% are old and retired (over 55 yrs), pregnant, disabled, and baby elephants. Most captive elephants being used in timber extraction are due to Myanma selective felling system. It can decreased deforestation compared with machinery extraction. Nowadays, the government wants to sustain the forest cover, therefore the timber extraction was reduced country wide and totally stopped in Bago Yoma area for ten years since 2017 where 600 captive elephant are taking care in the natural forest. In addition, most of private elephants are becoming jobless and facing with many difficulties for their long existence. On the other hand, the wild elephants are also facing with problems such as habitat lost, human and elephant conflict, poaching, and improper protection. The common problems in Myanma timber elephants are work related injuries, wounds, abscess, and parasitic problem (internal and external). The biggest challenges in young elephants (<13 yrs) is Elephant Endotheliotropic Herpes Virus (EEHV). This fatal type of EEHV was confirmed by PCR and stated as EEHV1a at 2014. From 2012 to 2018, out of 3000 captive elephants, 6 to 14 young elephants died in every year. Hence, we are trying to translate the EEHV guideline from English to Burmese. For elephant health care, there are 17 elephant veterinarians and 44 assistant veterinarians in Myanma Timber Enterprise who are taking care the elephants. There are three mobile elephant clinics for providing Myanma Timber Enterprises owned elephants as well as private ones.

PREVALENCE OF ENDOTHELIOTROPIC HERPESVIRUS IN CAPTIVE AND WILD ASIAN ELEPHANTS (*ELEPHAS MAXIUMS*) OF ASSAM, INDIA

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Abstract

Elephant endotheliotropic Herpesvirus (EEHV) has become a major obstacle for the continuation and success of captive breeding programs for the highly endangered Asian elephants because of high morbidity and mortality in elephant calves. Out of the seven known members of the Proboscivirus, EEHV1 A and EEHV1 B are mostly associated with the disease. In the present study, a total of three orphan wild elephant calves were found positive to EEHV with the prevalence of 13.64%. On the other hand, only one adult female was found positive for EEHV while screening 289 captive Asian elephants in Assam, India. The prevalence of EEHV in asymptomatic adult Asian elephant was 0.346% during the two year study. The overall prevalence of EEHV was 1.286% in Assam in wild and captive elephants. The diagnoses of EEHV was carried out based on clinical findings, gross necropsy, and histopathology, and were confirmed by PCR amplification for three selected EEHVA1 gene loci. The amplified PCR product showed the band size of 520, 600 and 930 bp. The PCR amplified product with size 600 bp had shown the gene sequence for EEHV1U77/HEL. Our results also correlate with the gross morphology and histopathological changes of EEHV infection in elephants.

Key Words: Asian Elephant, EEHV, Elephant endotheliotropic herpesvirus, Phylogenetic

INTRODUCTION:

Elephant endotheliotropic herpesvirus – Haemorrhagic disease (EEHV- HD) is a fatal disease of elephants caused by double stranded DNA virus belonging to the subfamily *Betaherpesvirinae* under the genus *Proboscivirus*[11,13]. Till now eight different genotypes of EEHV have been reported [2,3,4,7] out of which EEHV1A and EEHV1B are considered to be the most common cause of high morbidity and mortality in captive Asian elephants.[3, 11] The virus

damages the inner lining of the small blood vessels; primarily the capillaries and is responsible for the rapid onset of acute hemorrhagic disease in both Asian and African elephants generically in juveniles with high mortality rate. The disease is characterized by generalized edema of the head and limbs, oral ulceration and cyanosis of tongue, trachea and death within 7 days.[6] Deaths due to EEHV-associated disease cover approximately 65% of the overall mortality rate of captive-born Asian elephants in North America. [5]

EEHV associated haemorrhagic disease is most common in juvenile captive born Asian elephants in North America.[9] The virus primarily affects elephant calves between one to eight years of age with a fatality rate of 80% from the desktop folder 1st paper. [4,8,10,11] In the year 2008 and 2014, only one lethal case had been reported from North America while the incidence rate of the disease with high mortality had been observed in European zoos over the same time period.[5]

The incidence of EEHV – HD has been reported from India also, the first being in the year, 1997 [14] and later on 9 out of 15 potential cases have been confirmed from Southern India in wild free-ranging calves in Kerala, Karnataka, Tamil Nadu forest reserves and Madras Zoo[14]. A positive case of EEHV-1A infection has also been reported from captive Asiatic elephants of Assam.[1]

As there is a paucity of information about the prevalence, pathogenesis and impact of EEHV in Assam the North Eastern part of India an attempt has been made to investigate the incidence of the disease in both captive and wild elephants.

MATERIALS AND METHODS

Sample collection, storage and processing:

Blood and serum samples were collected from the suspected elephants following the appropriate protocols and were processed in the laboratory of department of Veterinary Epidemiology & Preventive Medicine, College of Veterinary Science, Khanapara, Guwahati for detection of disease and hematological studies. Blood is collected in EDTA vials and SST clot activator serum vials. A comprehensive post mortem procedure following elephant necropsy protocol was carried out within 24 hours. Gross changes in the external body as well as in various internal organs were recorded. Tissue samples were collected and stored at -80°C until analysis. The samples were processed in 4-5 µ thick sections and stained with haematoxylin and eosin stains.

PCR amplification:

Standard operating procedure for detection of EEHV was done as per the procedure described by [1,7,11,12]. Three selected EEHV1A gene loci representing U38/POL, U51/vG and U77/HEL were targeted for PCR amplification. Briefly, the genomic DNA from suspected cases was extracted from blood, tissue and serum samples (Nucleo-pore, Genetix brand). The PCR reactions were performed with a 25 µl total reaction volume resulting from 12.5 µl of master mix (Thermo scientific), 1µl of forward primer and reverse primer, 8.5 µl nuclease free water and 2µl of DNA templates. The details of primer sets used are mentioned in Table 1. Thermal cycling conditions followed were: initial denaturation at 94° C for 5 mins, denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 1 min, final extension at 72°C for 7 mins with 36 cycles. The PCR products were electrophorosed in 1.7% agarose gel ethidium bromide in 1 X tris acetate EDTA (TAE) and visualized on UV tranilluminator as per standard procedures. For size comparison, a 100 bp, DNA ladder marker (Thermoscientific) was run parallel to the PCR amplicons. The amplified PCR product was purified and sequenced by 1st BASE DNA Sequencing, Malaysia. The phylogenetic analysis of the isolated EEHV genome sequence was

done using the MEGAX software (Fig. 5). The presence of EEHV-1 was confirmed from the blood and tissues of the calves and adult elephant by polymerase chain reaction (PCR).

Table 1: Details of primer sets used.

Sl. No.	Gene	Primer Sequence	Product Size	Reference
1.	EEHV Pan Pol./U38 PCR	5’ – GTATTTGATTTYGCNAGYYTGTAYCC-3’	520 bp	Latimer *et al*., 2011; Richman *et al*., 1999; Stanton *et al*., 2010
		5’ – ACAAACACGCTGTCRGTRTCYCCRTA-3’		
2.	EEHV1 U51/vG PCR	5’- GATTGTGAACGCTGTAGTC-3’ 5’-GACTTTCTTCGTCGTAGCCCTCGTCTT-3’	930 bp	Latimer *et al*., 2011; Richman *et al*., 1999; Stanton *et al*., 2010
3.	EEHV1 U77/HEL	5’- GCAAGGTRGAACGTATCGTCG-3’ 5’- CACAG[A/C]GCGTTGTAGAACC-3’	600 bp	Latimer *et al*., 2011; Richman *et al*., 1999; Stanton *et al*., 2010

RESULTS

A total of 289 captive (Departmental/ privately owned) semi adult and adult wild elephants were screened for EEHV during the period of December 2016 to December 2018 in Assam. Besides these 22 numbers of wild orphan elephant calves were also screened and three calves were found positive for EEHV1 with percentage of 13.64. The elephant calves showed severe dehydration, in coordination of movement, sleepy (lethargic) unable to stand, arched back

condition, slight bending of foreleg with dropping of head (Fig. 1), edema of tongue and buccal cavity (Fig.2), protrusion of tongue, stringy saliva with neurological signs. There was edematous swelling in the eyelids and mandibles with drooling saliva accompanied with lethargy. The animal rejected solid food with frequent lying down and getting up (Fig.4). Employing polymerase chain reaction (PCR) in whole blood, tissue and serum sample EEHV-1 was detected in the affected calves. During the second year one of the treated calves (Sonit) suffered again from the disease and died after treatment and another dead carcass of 1(one) year age of CWRC, Bokakhat was also found positive for EEHV. An adult female (Purnima) of 18 yrs of age of Orang National Park was also found positive for EEHV while screening but the elephant did not exhibit any clinical signs. Upon necropsy, EEHV lesions were noted with congested blood vessels of liver (Fig.5), foci of petechial hemorrhage in spleen (Fig.6), congested serosal surface of stomach (Fig.7) and congested mucosa of caecum (Fig.8).

Histopathological examination of EEHV infected cases showed severe intertubular edema in kidneys (Fig. 9) due to increase capillary permeability owing to damage of the capillary endothelium. There were focal areas of degeneration and necrosis of the tubular epithelium (coagulation). Severe hydropic degeneration and fatty change of the hepatocytes were observed along with massive proliferation of fibroblast cells replacing the necrotic hepatocytes in liver (Fig. 10). In the fibrous tissue some biliary epithelial cells were seen which tend to form new acini. The empty capillaries were devoid of endothelium (Fig. 11). Few congested capillaries with endothelium and partially hemolysed erythrocytes with thickened vascular wall were observed. There was depletion of lymphoid cells in the white pulp of the spleen (Fig. 12).

The presence of EEHV-1 was confirmed in the blood and serum sample of the orphan calves by polymerase chain reaction (PCR) for three selected EEHV1 gene loci representing U38/POL,

U51/vG and U77/HEL. The amplified PCR product shown band size of 520, 600 and 930 bp. Nucleotide sequence analysis of amplicons showed identity with the available EEHV sequences from the GenBank. Amplicons of 600 bp were sequenced and checked for similarities with known sequences with the BLAST algorithm of GenBank. The partial nucleotide sequences showed its identity with EEHV genome sequences upon BLAST analysis. The PCR amplified product with size 600 bp had shown the gene sequence for EEHV1U77/HEL. Phylogenetic analysis of polymerase, helicase, GPCR genes showed clustering of the sequences with strains of EEHV1A (Fig.13).

Figure 1. Edema of head region

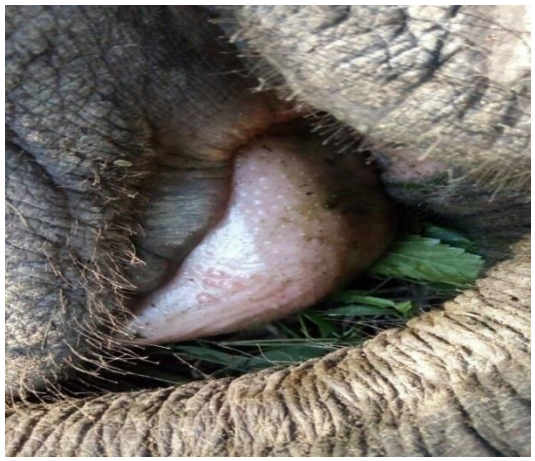

Figure 2. Edema of buccal cavity

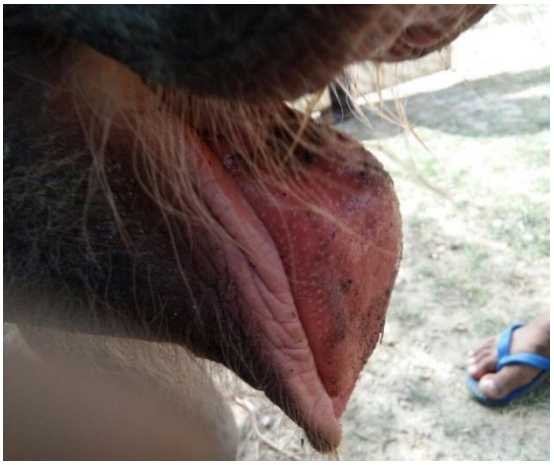

Figure 3. Pinkish discoloration of mandibles

Figure 4. Lateral recumbence

Figure 5. Congested blood vessels of liver spleen

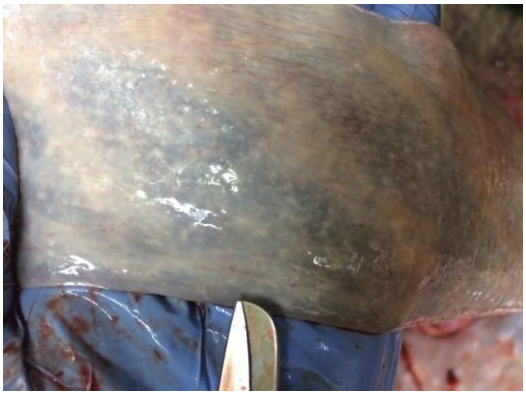

Figure 6. Petechial haemorrgahe in

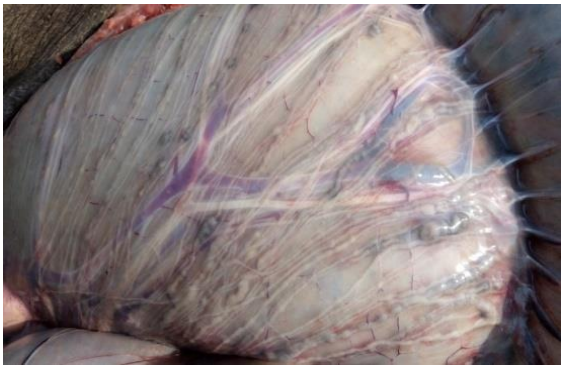

Figure 7. Congested serosal surface of stomach caecum

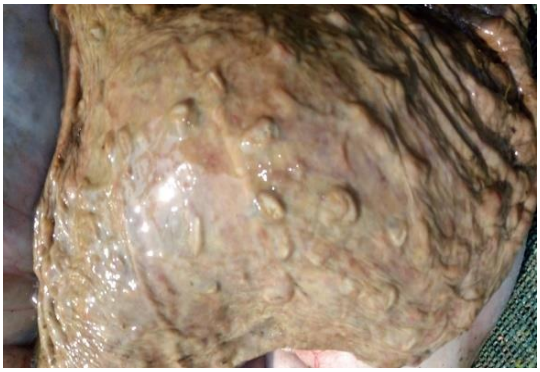

Figure 8. Congested mucosa of

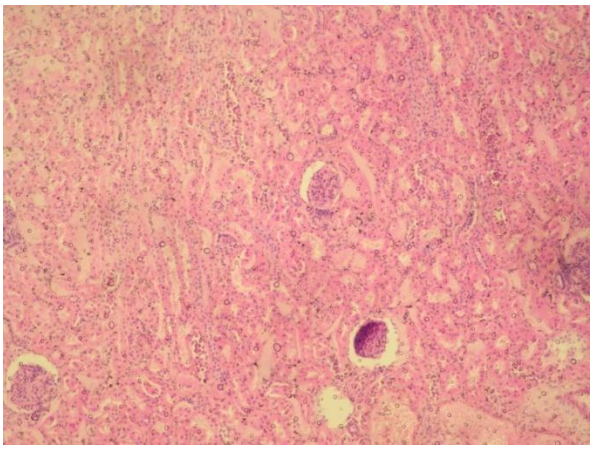

Figure 9: Severe inter tubular edema in kidneys

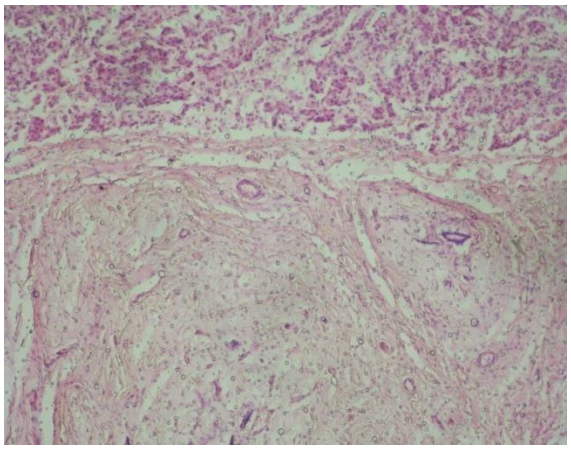

Figure 10 Proliferation of fibroblast cells replacing the necrotic hepatocytes in liver

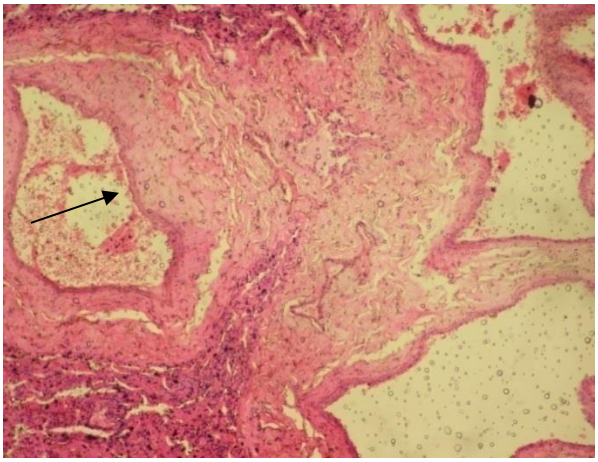

Figure 11. Blood vessels showing loss of endothelium

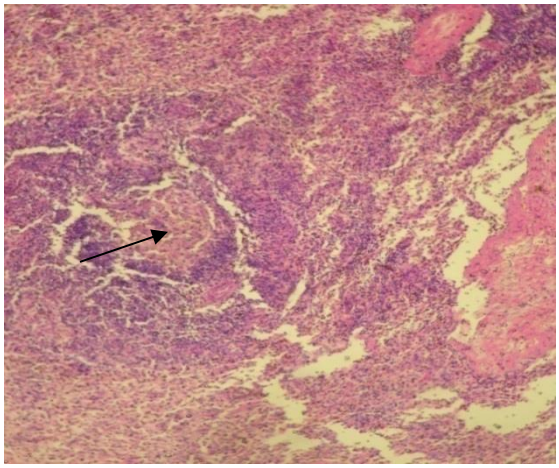

Figure 12. Depletion of lymphoid cells in the germinal centre of the lymphoid follicles of the spleen due to necrosis

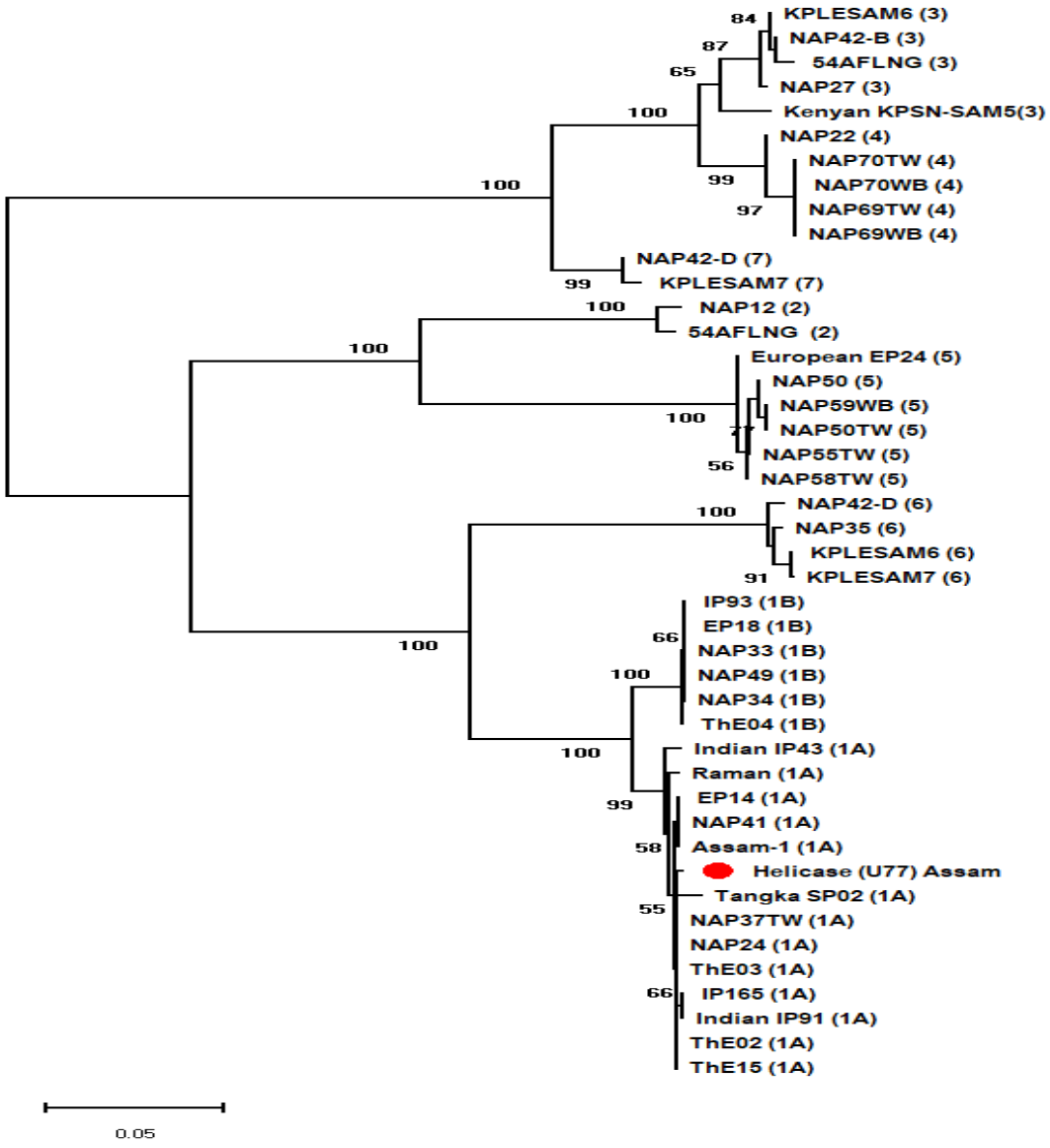

Figure 13. Legend: Phylogenetic tree of elephant endotheliotropicherpesvirus (EEHV) isolated from elephant of Assam based on Helicase (U77) gene. The tree was constructed in MEGAX software by Neighbor-Joining method and substitution model used was Tamura 3-parameter as estimated to be the best fit model in MEGA X software on the basis of Bayesian information criterion (BIC). Different EEHV strains are represented in the figure. Numbers along the branches refer to the bootstrapping value (percentage of confidence). The partial helicase gene sequence (520 bp) of EEHV used in this study is highlighted with red solid circle and found to be clustered with EEHV1A group.

DISCUSSION

The prevalence of EEHV during the study was higher in elephant calves (13.64%) in comparison to adult elephant (0.364%) which was quite low as per the reports of [12,14] has reported that the wild born elephants in North American population carrying EEHV1 strains which is in agreement with our findings because all the positive EEHV cases were wild orphan calves. Although there was evidence of EEHV in captive born elephant calves, not a single positive case was recorded in the present study in captivity. Presence of EEHV strains in adult asymptomatic healthy female was recorded from a captive departmental elephant of Assam and was found negative in subsequent tests. The same female elephant after one year became pregnant. However, symptomatic disease was not recorded in adult captive elephant during the study.

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WHAT CAN WE LEARN FROM THE NOW MANY EXAMPLES OF IDENTICAL EEHV1 STRAINS FOUND IN PAIRS OF UNRELATED ASIAN ELEPHANT CALVES AFFLICTED WITH EEHV HD AT ABOUT THE SAME TIME AT THE SAME HOUSING FACILITY

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Abstract

Over the 23 years since the discovery of EEHV1 in Kumari the first observed case of lethal HD disease in a USA zoo calf in 1995, we have endeavored to analyze at least some of the DNA sequence by Sanger PCR amplification at multiple selected loci across the genomes of all known cases of lethal (23x) and survived (12x) HD occurring across North America. Part of the rationale for this work was to then compare the results with those from strains encountered elsewhere around the world (in both Europe and Asia), as well as from strains associated only with asymptomatic shedding or from routinely encountered mild viremia episodes, as well as with those from strains causing lethal disease in free-ranging wild calves versus captive reared orphans in range countries. One of the most important early findings from these studies of course was that essentially all the viruses found at different involved elephant housing facilities proved to be distinct and that no two facilities anywhere in the world proved to harbor exactly the same strains of either the EEHV1A virus or its chimeric variant EEHV1B. Even although that same result of different strains often also applies to cases occurring at different times at the same facility, on the other hand, it has proven perhaps universally true that when two or more cases occur nearly simultaneously at the same facility they have proven to involve exactly the same virus strain, irrespective of whether the calves involved are closely related genetically or not. Here, we will review all 13 instances that we have studied or been involved with of multiple identical strains being identified at the same facility (three in Asia, two in Europe and six in USA) and attempt to explain why these results and others lead us to conclude that severe viremic cases of HD may all represent primary uncontrolled infections, not reactivated latent infections. Several other strange examples of partial identity between virus strains, especially within the E54(vOX2-1) gene will also be discussed.

Key words: DNA genome identity, Lethal versus surviving cases, Multiple PCR loci, Lethal versus surviving cases, Range versus captive cases

EFFORTS TO INCREASE EEHV DIAGNOSTIC TESTING CAPACITY IN ELEPHANT RANGE COUNTRIES AND ELSEWHERE

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Abstract

Elephant Endotheliotropic Herpesvirus (EEHV) is a major cause of death for young Asian elephants, with deaths and illness also occurring in young African calves and older Asian elephants, although to a much smaller extent. Early advancements in EEHV knowledge came from elephants in human care; for the last several years, there has been a push to ascertain the extent of EEHV in elephants in the range countries.

EEHV testing capacity is vital for both monitoring/early diagnosis for elephants in human care and for prevalence studies/diagnostics in range countries. Since 2008, the National Elephant Herpes lab at the Smithsonian's National Zoo, and colleagues at Johns Hopkins University and Baylor College of Medicine have worked to increase EEHV molecular testing capacity in labs in Asian and African range countries and in Europe and the US. Factors to be looked at while planning for testing capacity include what resources are present in a given area (lab space, personnel, equipment, reagents, funding), what resources are needed, likelihood of sustainability of the testing capacity, and follow-up training/consultation needed.

At first, labs were provided with protocols, reagents, and training on a piecemeal basis, as the desire for training was expressed. Requests from the EEHV Asia Working Group led to a recent train-the-trainers workshop in Thailand that has allowed for a substantial increase in colleagues trained and labs capable of testing for EEHV. This talk will provide some details on a few of the past and recent collaborations to increase EEHV testing capacity in India, Thailand, and Zambia.

Funding has come from the Smithsonian Institution, EEHV Consortium, Baylor College of Medicine, the US State Department, Asian Elephant Support, Wildlife Reserves Singapore, Kasetsart University, Zoological Parks of Thailand, Game Rangers International, IFAW, and International Elephant Foundation.

Key words: Elephant endotheliotropic herpesvirus, EEHV, PCR, *Elephas maximus*, Range countries, Testing capacity

MOVING THE ELEPHANTS TWO BY TWO – PRE EXPORT TESTING AND TRANSFER TRAINING

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Abstract

Twycross Zoo made the decision in March 2017 to move their four Asian elephants (*Elephas maximus*) out of the collection. The 18 months that followed involved months of planning, preparation and positive reinforcement training before this goal was achievable.

The four female elephants at Twycross Zoo were held in a protected contact environment, although prior to 2012 they were free contact. Two of the elephants had always lived at Twycross Zoo, one of which was a 4-year-old calf. One of the other adult elephants was particularly difficult for all training.

Pre export testing involved positive reinforcement training for trunk washes and blood sampling. The four-year old calf was already having weekly preventative trunk and blood swabs taken for elephant endotheliotropic herpesvirus (EEHV) monitoring. These swabs showed variable results from negative to weak positive but throughout the training and moves, she showed no clinical signs of disease. Oral famciclovir was given around the move dates as a precaution.

Crate training started in October 2017 and all training was via positive reinforcement. The first elephant was successfully moved in January 2018, with the matriarch of the herd moved six weeks later. The dam and calf required extra training, as they needed to travel in separate crates. They were successfully moved on 29th August 2018 and mixed back with the rest of the herd within a few days. No sedation was needed on any of the moves and all elephants remained calm throughout the process.

Since arriving at their new collection blood swabs have been taken regularly from the calf and these have remained EEHV negative.

Key words: Asian elephant, Crate training, EEHV monitoring, *Elephas maximus*, Positive reinforcement

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The authors would like to thank the elephant keeper team at Twycross Zoo and Blackpool Zoo, Albert Pamies (Barcelona zoo) and Alan Roocroft for their hard work to achieve this goal.

TRAINING BENCHMARKS IN YOUNG ELEPHANTS

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Abstract

A solid training foundation is the backbone to a successful elephant program. Solid training basics set the stage for success in all aspects of advanced care for animals especially when preparing for and treating an active EEHV case. Training is vital to maximizing preparedness and minimizing stress for both the animals and the caregivers during these life-threatening events. Using AZA's required behavioral components as a starting point, the Houston Zoo team came up with a list of behaviors that they focus on to train all elephants by the time they reach one year of age. Training to achieve these behaviors reliably, regardless of the circumstances, directly relates to our ability to care for animals when they are sick, tired, not hungry and least likely to want to participate in their care. This is a critical component in providing for the welfare of the elephants entrusted to us.

SEROLOGICAL DETECTION OF EEHV INFECTION

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Abstract

An understanding of the serological response to EEHV infection has remained largely elusive. Such an understanding is likely to help us better understand the susceptibility of some elephants to EEHV hemorrhagic disease (HD). Hurdles in detecting antibody responses to EEHV include a lack of tools such as species-specific reagents to measure them. Here we utilize a novel assay that has been used to study serological responses to several human infections, including herpesviruses, to investigate EEHV antibody responses in elephants. Through careful selection of EEHV proteins both broadly conserved amongst EEHV types and specific for EEHV1, we have been able to establish the EEHV infection history of several elephants from four different captive herds. Most strikingly, we have found that most elephants who died from EEHV HD are completely sero-negative to all EEHV proteins studied, indicating that they likely died from primary infection. We also observed that antibody responses to some EEHV proteins decline during the first two years of life, suggesting that maternally transferred immunity may play a role in protection against EEHV primary infection associated with HD. The assay presented is likely to pave a way towards a greater understanding of EEHV serology, where it may be employed as a diagnostic tool for zoos and other institutions who house elephants. In addition, this assay will be particularly useful when we begin evaluating the effectiveness of an EEHV vaccine.

TOWARDS AN EEHV VACCINE

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Abstract

Despite the availability of sensitive tests and improved protocols for treating EEHV-associated illness, these measures are not always effective. The best line of defense would be a preventive vaccine. Vaccines target specific pathogen proteins to "educate" the vaccine recipient's immune system to react to the same protein in an invading virus or bacteria. However, development of an EEHV vaccine presents many challenges, including: 1) What does a protective immune response look like?, 2) How do we identify and select target viral antigens for the vaccine?, 3) What is the best vaccine platform to induce a protective response?, 4) How transferable is small animal immunogenicity to use in Elephants?, and 5) What are the regulatory requirements for worldwide use? We have developed a rational strategy towards development and implementation of an EEHV vaccine based on available information and evidence, that addresses each of these challenges. We will present our overall plan and details will be provided for parts of our approach in which we have made tangible progress.

COMPLETE BLOOD COUNTS (CBC) AND CROSS MATCHING REVIEW LABORATORY

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Abstract

Complete blood counts (CBC) are an integral part of health monitoring and management of Asian elephants. This laboratory test becomes more important in monitoring cases of Endotheliotropic Elephant Herpes Virus (EEHV) infections. Watching for consistency and trends within the CBC helps to monitor the clinical course and prognosis of the infection. Often, when elephants are infected with the virus, the CBC's will reflect the infection through decreased and/or increased total white blood cell counts, absolute monocyte counts, platelet counts, and hematocrits.

Plasma transfusions may be used as part of the treatment of Asian elephants (*Elephas maximus*) during elephant endotheliotropic herpes virus (EEHV) viremia. This may be elected when the elephant shows clinical signs, has an abnormal complete blood cell count (CBC), or when the viral load is elevated. Fresh plasma is a colloid that contains antibodies, clotting factors, is isotonic for the elephant, and may be advantageous to the recipient. At the Houston Zoo, plasma transfusions have been administered to viremic elephants without complications and contributed to positive outcomes. Intravenous administration of plasma, at the earliest stages possible, appears to have contributed to sustaining the calves through the worst stages of the disease. To be able to give blood products safely, the plasma of the donor should be compatible to that of the recipient as determined by a minor cross match. To give a whole blood transfusion safely, both a major and a minor cross match, with the donor and recipient blood and plasma, should be carried out. Cross matching can be time consuming (1-2 hrs.). It is therefore prudent to carry it out at the time of plasma collection for banking, so that banked plasma is labelled with recipient's suitability. This will save precious time during treatment of a clinical EEHV case. This cross-matching technique can be used for any animal that needs a transfusion.

Key words: Complete blood count, cross match, elephant, *Elephas maximus*, white blood cells

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