Development of Freeze-Dried Platelets to Combat Elephant Endotheliotropic Herpes Virus

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Project Start Date: January 2016
Project End Date: January 2017

List of overall and specific conservation needs this project addressed

- Provide evaluation of a treatment option to be on hand at critical hemorrhagic phase of EEHV infection in young Asian elephants to stabilize hemostatic imbalance and assist in supportive care
    - Proof of concept studies in manufacturing and developing an infusible freeze-dried platelet product from elephant blood
    - Successful production of freeze-dried Asian elephant platelet product:
        - stable at room temperature
        - long shelf life, goal of 24 months
        - lightweight
        - portable
    - Taking steps to demonstrate utility in clinical treatment setting

Updated timeline

Due to paperwork delays, this project was postponed from the originally proposed dates, but the initial stages have been successfully completed.

Objectives and updates

<u>OBJECTIVE 1</u>: Utilize proprietary lyophilization technology to develop a freeze-dried platelet-derived hemostatic agent (FDPDHA) for use in the treatment of critically ill elephants infected with EEHV.

- Progress and successes:
    - Blood collected from donor elephants
    - Blood separated into components and standards for Asian elephant blood processing was successfully established based on adaptation of existing techniques for human and equine blood processing
    - First freeze dried elephant platelets have been produced following species specific Asian elephant platelet cell characterization and the initial evaluation of procedures for obtaining platelet extraction from elephant whole blood
    - Preliminary information includes identification of a suitable centrifugation procedure to recover platelet rich plasma from Asian elephant whole blood; evaluation of the agonist-induced aggregation potential of elephant platelets in plasma and physiological buffer; isolation of human derived antibodies reacting with elephant platelet cell signaling proteins; and thromboelastography measurement standards for the raw platelet product and lyophilized platelet product. The markers for platelet identification and functionality include Anti-CD61, Annexin V, and anti-CD63 clone CC25.

- Next steps:
    - The initial product processing is currently being duplicated to increase the sample size prior to publication, providing accurate quality control measures that will lead to large scale production pursuing clinical trial studies

<u>OBJECTIVE 2:</u> Evaluate the produced blood product for shelf stability, contamination, viability, and utility for future use in ill elephants.

- Progress and successes:
    - Initial evaluation of platelet product has indicated that it passes FDA sterility standards
    - Product meets FDA endotoxin requirements for infusible products.
    - Assessment of size and number of particles is within goal parameters.
- Challenge: Validation for identity and activity using antibody testing is problematic due to the exotic species. Antibodies commonly used for human medicine not always effective. Evaluation of broadly-active antibodies for utility in confirming identity and activity of the platelet product has been successfully completed, and is pending publication of results.
- Next steps:
    - Assess in vivo activity.
    - Develop protocol for clinical trial safety and efficacy.

<u>OBJECTIVE 3</u>: Develop a rapidly-deployable communication and distribution plan through a global collaborative network of elephant-housing facilities for the sharing of information, blood products, and experiences in the use of FDPDHA product in the treatment of EEHV HD.

- Progress and successes:
    - Project includes stakeholders from the National Elephant Herpes Laboratory as well as several organizations that have Asian elephant collections – National Zoological Park, Houston Zoo, Ringling Brothers, and Fort Worth Zoo.
    - The project will be been featured in the EEHV newsletter which includes all participating zoos and facilities which take part in the EEHV consortium
- Next steps:
    - As progress is made on the product, investigators will continue to reach out to additional zoos and elephant holding facilities with at-risk herds to determine their interest in participating in clinical trials of the product.

<u>OBJECTIVE 4</u>: Prepare a manuscript and oral presentation for publication and presentation of data and results to the elephant conservation community.

- Progress and successes:
    - Project was presented at the American Association of Zoological Veterinarians Annual Meeting. Support from the IEF is prominently noted.
    - The project was presented at the International Elephant and Rhino Conservation and Research Symposium in Singapore last fall.
    - The project has been highlighted in an accepted publication for JAVMA, “Do lyophilized platelets hold promise for treatment of hemorrhagic diseases in wild

animals?" with recognition of support from the IEF in pursuing this treatment option in Asian elephants

    - A poster has been presented to the Elephant Managers Association October 2017 discussing the research and emphasizing the initial successes, highlighting support from the IEF.

- Next steps:

    - Manuscript and additional data collection is currently underway to publish the results of elephant platelet characterization and coagulation characteristics discovered during this project

Human interest story

It's 4 am, and you are heading to the airport with an empty biological cooler. You will fly over a thousand miles today on a whirlwind round trip to a Florida elephant sanctuary. You are a veterinarian researching a cutting-edge treatment to save baby elephants from a devastating viral disease.

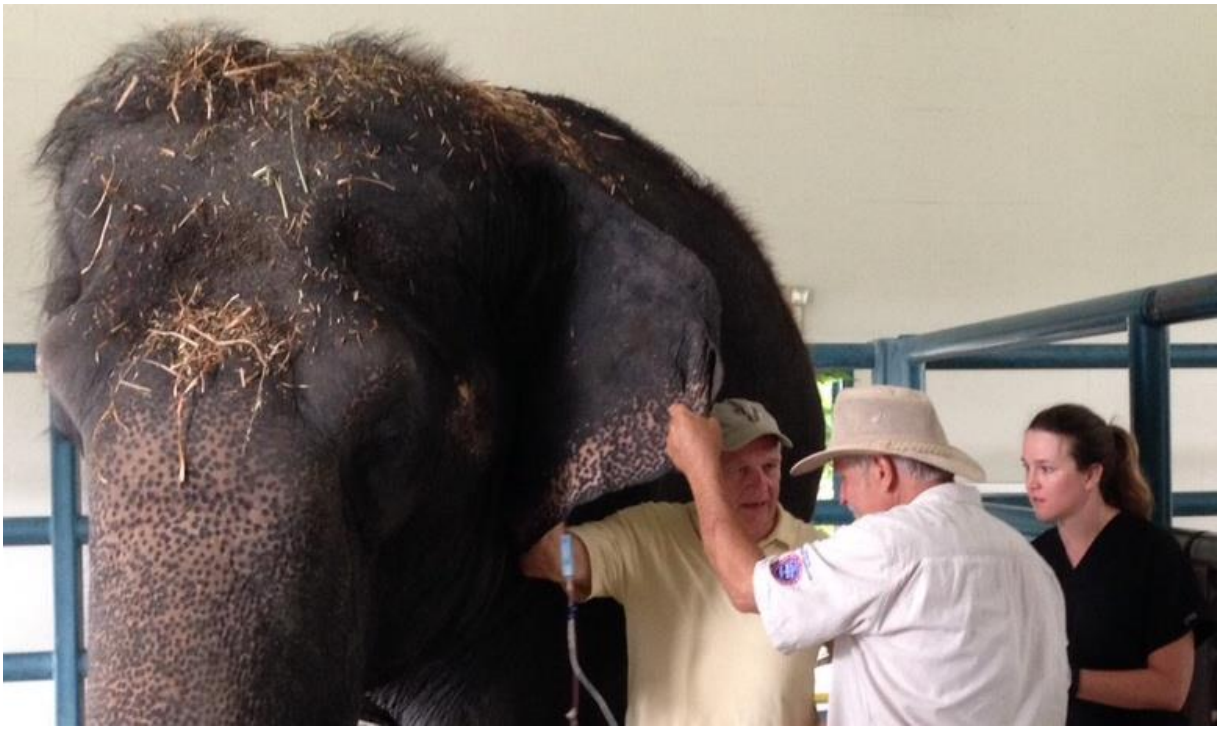
The airport is crowded, and thunderstorms at your destination could mean your flight will be one of the many with "CANCELLED" blinking next to it on the departure announcement screen. The cooler gets some curious looks from security agents and sleepy travelers, and a few hear your abbreviated itinerary for the next 24 hours: fly to the sanctuary, work with the vets there to collect blood from donor elephants, fly home, and hand off the blood to a research team who will rush the blood to the laboratory to stabilize the precious material in the dark hours before dawn. From elephant to stabilization, the process must be complete in 12 hours, or the entire operation was a waste. The flight south is over two hours, but you catch a little rest before the turbulence makes sure the whole plane is awake.

Despite the bumpy landing, the liaison with sanctuary vets goes smoothly, and the rainy drive to your enormous patients is lightened by shop talk. Everyone is excited about what this project could mean: a way to combat the deadly Elephant Endotheliotropic Herpesvirus, a way to pull platelets – the clotting particles – out of elephant blood and store them until they are needed, a way to keep baby elephants alive when the virus that targets them causes uncontrolled bleeding.

In the cavernous, hay-scented elephant barn, the massive creatures are unbothered by the weather. A trunk slyly checks you for apple slices during the donation procedure. Eventually, you have what you came for: 4 precious liters of elephant blood. It's time to hurry back to the airport, but not before thanking your donors with apple bits and kind words.

The storms are still raging and the flight home is running late. The clock is ticking on your cargo, and the weather delay is eating into the narrow buffer the schedule allows. There is enough time for phone calls; the team awaiting your return needs an update on the new complications. Eventually you are airborne, tired but wide awake, tensely watching rain slide down the plane's round window. The blood in the cooler at your feet is silently losing vitality, while you check your watch. The weather has added in three hours, but if everything goes right from here, there's a chance you can deliver in time for the lab to work its magic.

It's after midnight. You are met personally by the CEO of the biotech company that is donating its expertise to the mission. He relieves you of the cooler and shakes your hand before charging off. The blood will arrive at the lab in under an hour, and the scientists there will have just enough time to process it for stabilization. The platelets in the blood will be turned into a treatment that stops bleeding, even in the face of a viral onslaught from EEHV. There's a growing hope for young elephants afflicted with this terrible disease.



Progress and results achieved (<500 words)

Thanks to a grant from the International Elephant Foundation, biotechnology company Cellphire, Smithsonian Global Health Program, Ringling Bros. Center for Elephant Conservation, Fort Worth Zoo, and Houston Zoo have made great strides in developing a novel treatment for a lethal virus afflicting baby Asian elephants around the globe. Elephant Endotheliotropic Herpesvirus (EEHV) is the leading cause of death among captive Asian elephants under 12 years of age. This virus attacks the blood vessels and frequently causes uncontrolled hemorrhage, causing the victim to die of blood loss. The innovative treatment under development builds off of cutting-edge research in human platelet-products, a specialty of Cellphire. This ground-breaking treatment is shelf-stable and can stop bleeding from trauma and internal damage. The human product is targeted at situations as diverse as battlegrounds and Ebola outbreaks. Until now, there has not been a version for elephants. The biotech firm is aided by the extensive elephant health expertise from partner zoological veterinarians at diverse institutions. To date, blood from two Asian Elephants has been collected, stabilized, and prepared for separation into component parts. Separation was successful, and scientists isolated platelet-rich plasma, which was then freeze-dried using a proprietary process. The

resulting lyophilized platelets are being subjected to a battery of quality control tests to evaluate shape, size, identity, activity, and purity. So far, the platelet product reconstitutes appropriately and contains adequate size and number of particles. Samples have been tested for sterility, and the product meets or exceeds FDA regulations for an injectable blood product's sterility. The product also passes tests for bacterial endotoxin. Partners are currently focused on optimizing protocols to appropriately utilize antibodies for confirmation of identity and purity, and completing assessment of vitro potency for activation and clot formation. These complicated laboratory procedures are designed to ensure that the final result is a treatment that is safe, effective, and reliable for contributing to stabilization of the hemostatic imbalance caused by EEHV.

Progress and results achieved (<50 words)

This project has made enormous advances in developing a potential treatment for a fatal viral hemorrhagic disease targeting young elephants. Platelets were isolated and freeze-dried from elephant blood using proprietary techniques in order to transform them into a shelf-stable product to save baby elephants from an uncontrolled bleeding crisis.

Organizations and roles

Organization: Cellphire, Inc.
Role(s):

- Expertise in platelet product isolation for human medicine
- Laboratory capacity
- Experience and equipment for quality control of platelet products

Organization: Smithsonian Institution
Role(s):

- Expertise in elephant health
- Expertise in veterinary pathology
- The National Elephant Endotheliotropic Herpesvirus (EEHV) Laboratory

Organization: Fort Worth Zoo
Role(s):

- Expertise in elephant health
- Blood donor elephants

Organization: Houston Zoo
Role(s):

- Expertise in elephant health
- Blood donor elephants

Organization: Ringling Bros. Center for Elephant Conservation
Role(s):

- Expertise in elephant health
- Blood donor elephants

Financial Report

Budget Item	Amount Requested	Total Costs
Travel	$2,000	$2,000
Blood collection supplies	$2,000	$2,170
Sample collection and management	$4,000	$3,830
Lab supplies	$1,500	$1,000
Publication, outreach, and conference costs	$1,500	$2,000
Shipping fees	$750	$750
Total	$11,750	$11,750

Presentations resulting from study (see attached files)

- Freeze-dried platelets: A Novel Treatment for Elephant Endotheliotrophic Herpes Virus Hemorrhagic Disease – International Elephant and Rhino Conservation and Research Symposium, Singapore, 2016
- Hemostatic Agents as a Novel Treatment for Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease – AAZV Conference presentation, Atlanta GA 2016
- Kishbaugh, J., Valitutto, M., Ober, J., Zimmerman, D., Howard, L., Schmitt, D., Sanchez, C., and Murray, S. (2017). Do lyophilized platelets hold promise for treatment of hemorrhagic diseases in wild animals? *JAVMA.* In press.
- Investigation of a Novel Treatment for Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease – EMA Conference poster presentation, Columbus OH 2017