EVALUATION OF PLATELET TRANSPORT IN ASIAN ELEPHANTS (*Elephas maximus*) AS PART OF AN ONGOING STUDY TARGETING NOVEL EEHV-HD TREATMENT

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Platelet Characterization and Coagulation

- Increased utilization of coagulation parameters in veterinary medicine
- Drops in platelet count are a feature of EEHV-HD
- Viral damage may disrupt coagulation, leading to DIC in EEHV cases
- Need for studies of coagulation parameters and platelet function, and potential application to EEHV (Thromboelastography application)

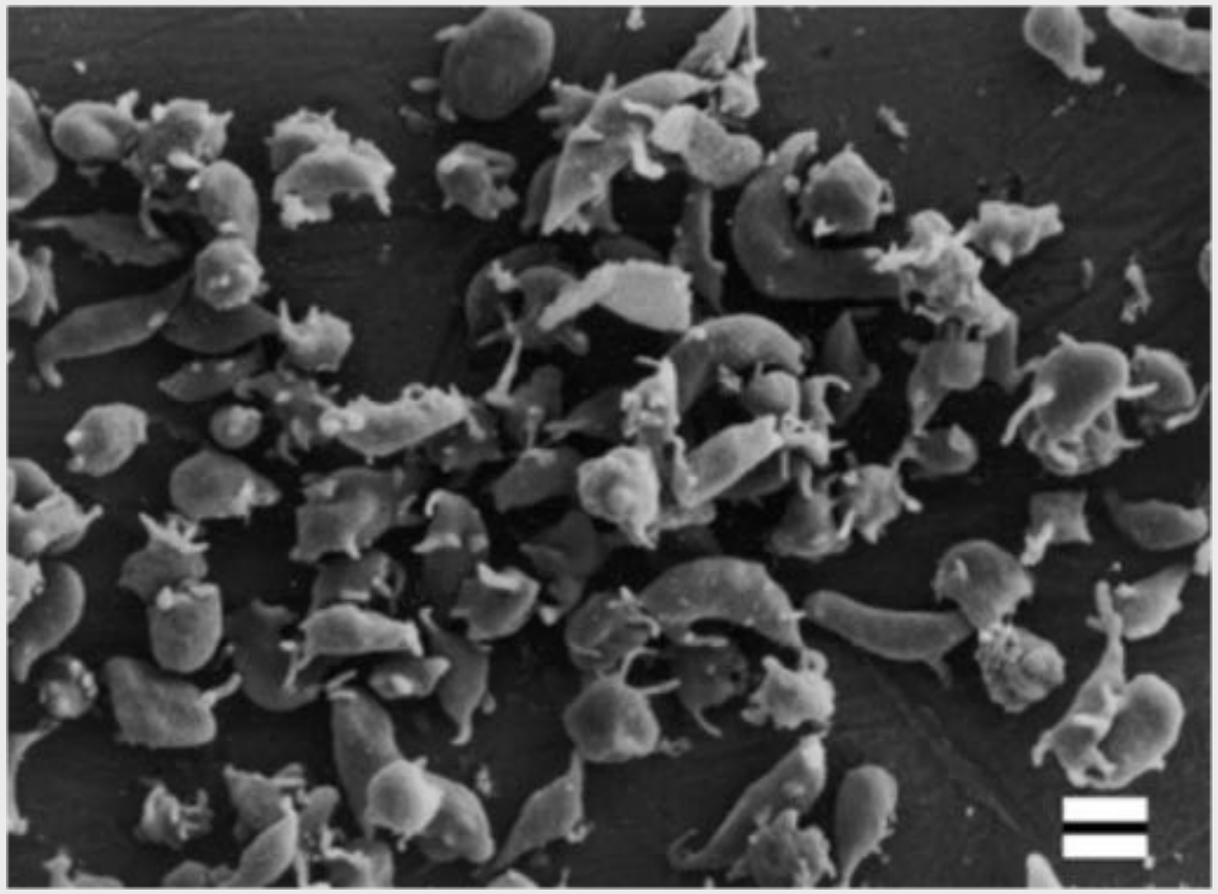

Image: Elephant platelets displaying various forms and numerous pseudopodia. SEM. Bar, 1mm.
Du Plessis and Stevens, 2002

Stability of Blood During Transport

- Maintaining platelet stability during sample storage and shipping is notoriously difficult
- A 2018 study by Perrin *et al* evaluating thromboelastography (TEG) demonstrated poor results following delayed sample analysis
- Application of whole blood transport methods used in transfusion medicine may give improved results

Image: https://www.health.harvard.edu/blog/blood-test-results-not-quite-normal-201606029718

Study Outline: Whole Blood Transport

Image: Meg Murphy, SCBI, National Zoo

- Study Objective: Evaluate an alternative whole blood storage and transport method from a comparative medicine approach with the goal of improved platelet stability

- 8 Asian elephant participants in pursuit of developing a freeze-dried platelet product for the treatment of EEHV-HD

Methods: Blood handling

- Whole blood collected in ACD-A anticoagulant, stored within Saint Gobain blood bags and transported within CREDO cube shipper - not commonly utilized in clinical diagnostic medicine
- Observed differences:
  - Blood collection method
  - Time of transport to laboratory (24-48 hours)
- Data logger temperature monitoring during transport

Image: iMini Temperature Logger

Methods: Blood evaluation

- Blood samples in laboratory evaluated for:
  - Optical Density Aggregometry - measure of platelet aggregation in response to different agonists
  - Thromboelastography - measure of clot formation and stability based on cellular and plasma components
  - Thrombin Generation Assay - ability of platelets to produce thrombin clot
  - Flow Cytometry - measure of platelet identity and activation

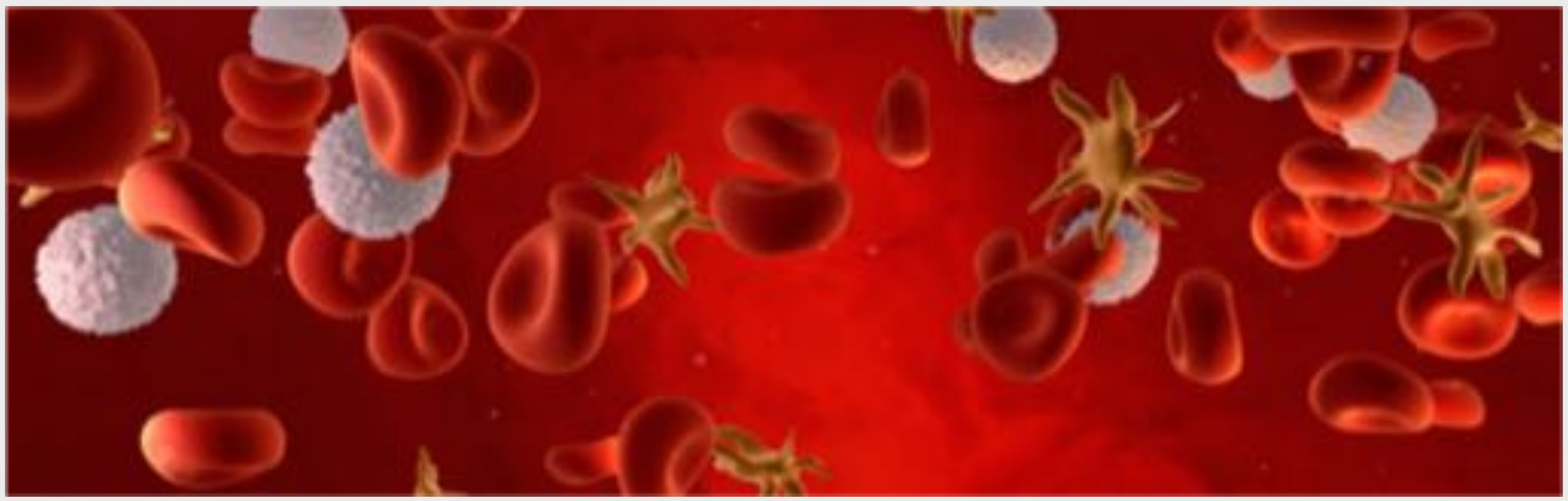

Image: Cellphire, Inc.

ACD-A: Acid-Citrate-Dextrose-A

- Anticoagulant used in many blood banking operations for the generation of platelet rich plasma (PRP)

- Contains an acid, citrate anticoagulant, and dextrose for glycolysis and ATP production

- ACD-A demonstrates whole blood viability for 21 days in human studies

Image: ACD-A anticoagulant tubes with Asian elephant whole blood
© Stephanie Adams, Houston Zoo

Saint-Gobain blood bags

- Blood transferred from tubes to blood bags
- Porous bags compared to blood tubes which restrict cellular respiration
- Decrease anaerobic respiration and lactic acid production, minimizing cellular storage lesions and excessive platelet activation

Image: ACD-A and Asian elephant whole blood within Saint Gobain blood bags
© Stephanie Adams, Houston Zoo

Credo Series 22-248 Platelet Shipper

Image: CREDO cube shipper
http://pelibiothermal.com/products/credo-cube-series-22-248

- Contains thermal isolation chamber panels with integrated 22°C phase-change material
- Blood maintained at steady state temperature for 96 hours according to manufacturer instructions
- Systems used for transport of temperature sensitive materials including biologics, pharmaceuticals and food

Results

- Platelet Count: 305 x $10^3/\mu$L
  - (prior reports range 200-447 X $10^3/\mu$L)
- Platelet Size: 0.5 - 2.5 μm
- Platelet Surface Markers: CD61, CD41a, CD9
- Obtained Asian elephant PRP which generated diagnostic TEG

Data courtesy A. S. Hale; full data set currently in manuscript submission

Viable Whole Blood Shipment Method

- Successful extraction of PRP
- Responsive, viable platelets 48 hours after collection as determined by platelet functionality testing
- Potential to improve diagnostic evaluation of platelets utilizing alternative whole blood storage and shipping technologies

Image: Asian elephant whole blood draw
© Stephanie Adams, Houston Zoo

Future Directions

Image: Developmental Lyophilized Elephant Platelet Product, courtesy BodeVet™

- Indications for large volume whole blood shipment
- Need for controls and studies on coagulation measures utilizing comparative technologies
- Explore similar studies to optimize diagnostic samples
- Results of study guiding elephant lyophilized platelet treatment production

Thank You!

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