IEF Annual/Final report—Ling 2020

a. Project title: Realization of an Effective Vaccine Against Elephant Endotheliotropic Herpesvirus

b. Annual/Final report

c. Investigators

Name & Title: Paul D. Ling, PhD, Professor (Tenured)
Institution: Baylor College of Medicine
Institution Address: Department of Molecular Virology and Microbiology, One Baylor Plaza, Houston Texas 77030
Phone, Fax, email: (PH) 713-798-8474, (Fax) 713-798-3170, pling@bcm.edu

Name and Title: Jennifer Lynn Spencer Clinton, PhD, Postdoctoral Fellow
Institution: Baylor College of Medicine
Institution Address: Department of Molecular Virology and Microbiology, One Baylor Plaza, Houston Texas 77030
Phone, Fax, email: (PH) 713-798-5090, (Fax) 713-798-3170, Jennifer.CLINTON@bcm.edu

d. Project Start Date: January 1, 2020

e. Project completion date: December 31, 2022

2. Conservation needs this project addresses

EEHV is the single largest cause of death for captive juvenile Asian elephants in North America and Europe. Furthermore, EEHV-associated deaths have now been documented in wild Asian elephants in their natural range countries, adding yet another threat to this endangered species. Recent EEHV-associated deaths documented in captive African elephants are now raising concerns about EEHV in this species as well. In contrast to Asian elephants, much less is known about the EEHV viruses that are naturally found in African elephants.

Our lab has spent the last several years developing the tools needed to discover what parts of the EEHV virus might useful for developing a vaccine that can induce protective immunity. These tools will be used for evaluating anti-EEHV vaccines and will be adapted for use in African elephants.

The EEHV vaccine represents a truly innovative endeavor because it is considered an experimental vaccine developed specifically for use in an endangered species. If promising results are realized with vaccine trials in the Houston zoo elephants, we envision distribution of the vaccine to other institutions. Sequencing of the African elephant-specific EEHV types will provide the fundamental basis for multiple research activities, including adaptation of the tests and assays to the African elephant system that were developed for understanding Asian elephant anti-EEHV immune responses. Long-term, strategies developed for an anti-EEHV vaccine in Asian elephants will be leveraged for a similar vaccine in African elephants.

The first 12 months of this project have been significantly impacted by Covid 19. On March 20, 2020, Baylor College of Medicine shut down all nonessential research activities (known also as phase 0), which included our laboratory. On May 21, the school began phase 1 of a return to research plan, that restored activities/occupancy to 25% of normal operations. As of August 1, the school will move to phase 2, restoring activities/occupancy to 50% of normal operations.

Nonetheless, we managed to complete sequencing of African elephant EEHV genomes 3A, 3B, and 2. We have also made significant progress sequencing the genome of EEHV6. This information was used to develop a first generation serology assay for EEHV3 (see below items 5 and 15), which was responsible for the deaths of 3 African elephants in 2019. We initiated a preclinical vaccine study in mice with one of our prototype vaccines just before the March 20th shutdown, but were allowed to keep the vaccinated mice going, with various groups of mice being euthanized at different time points. Unfortunately, complete evaluation of this trial was not feasible because access to some of the core instrumentation required to accomplish this was prohibited during the Covid shutdown. However, we now have limited access to some of the required instrumentation. Initial results indicate that mice generate good antibody responses to the vaccine and have good T cell responses, which are known to be critical for immunity to herpesviruses (and as an aside, similar to the types of immunity required for SARS CoV 2/Covid 19). We have now initiated a second preclinical vaccine trial in mice with a different vaccine platform with the intention of comparing its efficacy to the first vaccine and to determine whether both vaccines used in combination might be better than either one alone.

3. Goals and objectives of the study

Goals:

1. Develop an anti-EEHV vaccine for Asian elephants
2. Sequence EEHV viruses endemic within African elephants and develop tests to understand African elephant immunity to EEHV (as done previously for EEHV in Asian elephants)

Objectives:

Asian elephant EEHV vaccine-related activities

1. Preclinical studies in mice: Test two vaccine types (a recombinant vector and a subunit vaccine) expressing an EEHV protein known as glycoprotein B (gB) for immunogenicity and toxicity
2. Produce clinical-grade vaccine(s) sufficient to pass regulatory requirements
3. Test the vaccine(s) in Asian elephants housed at the Houston Zoo and monitor their ability to induce immune responses

African elephant EEHV activities:

1. Sequence the genomes of EEHV2, 3 and 6, which are endemic within African elephant populations
2. Utilize the information from genome sequencing to develop tests for understanding anti-EEHV immunity in African elephants, similar to those done already in Asian elephants.

Specific actions taken to achieve objectives

Elephant necropsy samples were identified and characterized by a variety of measures to determine suitability for sequencing. From this analysis, we submitted samples to the genome center at Baylor College of Medicine to determine the first genome sequences of EEHV2 and 3A and 3B. We have now completed a first attempt at sequencing EEHV6. Because the samples available for this virus were from an elephant that survived EEHV-associated illness, we only have blood samples available for sequencing. Compared to necropsy samples, blood generally has lower levels of virus specific DNA, which reduces the probability that enough virus sequence will be generated during sequencing to assemble a complete genome sequence. To make matters more challenging, the level of viremia experienced by the EEHV6 positive elephant was rather modest. Nonetheless, our first sequencing attempt yielded a genome sequence that is about 80-90% complete. Rather than trying to complete this sequence by "manually" conducting specific PCR sequencing, we identified a second sample from the EEHV6 positive elephant that had about 5-fold more EEHV6 DNA and decided to conduct a second sequencing run with the Baylor human genome sequencing center. We just received very promising results from this attempt and are hopeful that it might speed up completion of the EEHV6 genome sequence significantly. Preclinical studies, which involve testing of a prototype EEHV vaccine in mice, were initiated. As mentioned previously, these studies have been delayed due to the Covid 19 pandemic. However, partial results from this study have now been completed and our ability to fully evaluate these first mouse experiments has been completed. A second vaccine trial in mice has now been initiated to compare and contrast a second EEHV vaccine type.

4. Activities that differ from original proposal

One small modification was made from our original proposal. We initially wanted to sequence the genome of EEHV3 from one of the cases at the Indianapolis zoo. However, it became apparent that the EEHV3 genomes from cases at the Indianapolis zoo were quite different from those of an earlier case at the Maryland zoo (a survivor) and a case at the Fresno zoo, even though both fell under the category of "EEHV3". We decided to sequence this EEHV3 variant due to its divergence from the strain found in the Indianapolis zoo and some difficulties that our colleague, Gary Hayward at Johns Hopkins, was having with sequencing this variant. Tentatively, these EEHV3 strains are being referred to as EEHV3A and EEHV3B. At the moment, we think it may be possible that infections from 3A might not completely protect against disease caused by 3B and vice vera. This is similar to the situation observed for 1A and 1B in Asian elephants. Knowledge of the genome sequences from these two African elephant EEHV strains will help us generate tools to discriminate which EEHV strain, 3A or 3B, an elephant has been infected with and possibly identify whether an elephant is vulnerable to infection with one or the other or both.

5. Conservation outcomes for elephants, other wildlife, habitat and human communities, and other major findings and accomplishments to date

Below are summarized findings and accomplishments to date:

African elephant genome sequencing and serology tests

1. The full genome sequences of EEHV3A (Nyah- from the Indianapolis zoo), and EEHV3B (Ms Bets—Fresno zoo) have now been completed. Sequencing was accomplished by the Human genome sequencing center here at Baylor College of Medicine and Gary Hayward has done a considerable detailed analysis of the genomes. Briefly, the divergence of these genomes is somewhat greater than we see for EEHV1A versus 1B seen in Asians. One speculation is whether this represents two distinct EEHV3 species; one that coevolved with Loxodonta Africana and the other with Loxodonta Cyclotis. The level of divergence of these viruses hints at that, but more evidence is required to make a stronger case.

A draft of the complete annotated sequence of EEHV3A has already been submitted and is publicly available in GenBank, accession number MN373268.1.

2. The entire genome sequence of EEHV2 (from Kijana) has also been completed, also here at Baylor. Analysis and annotation of it is in progress.

3. A first sequencing attempt for EEHV6 was made from a blood sample from Ms Bets when she endured viremia from EEHV6. Unlike samples for EEHV2, 3A, and 3B, which are from necropsy samples, the only available samples for EEHV6 are from blood. Blood samples, even from lethal cases, are generally not preferable to tissue samples as they contain lower EEHV levels. Over the last year the human genome sequencing center at Baylor College of Medicine has upgraded its sequencing instrumentation. These new instruments can produce 10-40 fold more data than before, so despite the lower abundance of EEHV6 in blood samples from Ms Bets, we were hopeful that that the new technologies could overcome this issue. Sequencing and assembly of EEHV6 has proceeded well, but there remain some small parts totaling perhaps 10% of the genome that have to be resolved. We have now identified a second blood sample from Ms Bets that has more abundant EEHV6 DNA and subjected that sample to a second sequencing run, which generated significantly more data. We are in the process of evaluating the quality of this data, and are hopeful that it might be enough to fully resolve the entire genome sequence of EEHV6.

In summary, and in close collaboration with Gary Hayward at Johns Hopkins, we have completed the sequencing and annotation of the EEHV3A genome and are on target to complete the annotation of the genome sequences for EEHV3B and 2 shortly (Annotation is the process of identifying and delineating genes from the raw DNA sequence). We are hopeful that the sequence for EEHV6 will be completed in the coming weeks. In short, complete annotated genomes of all major EEHVs endemic within African elephants should be finished in the coming months. This effort will provide an essential source of information for investigation of EEHV biology within African elephants for years to come.

4. We have generated an EEHV3 serology test based on the immunoreactivity to the EEHV3 E34 protein, which we derived from sequencing the genomes of EEHVs endemic within African elephant populations. Initial results, which we have presented at the EAZWV conference in July 2020 and in the Journal of the Elephant Managers Association (JEMA) indicate that we can detect antibodies from elephants who have been previously infected with EEHV3. This test was used to determine that all three elephants that died from EEHV HD caused by EEHV3, were sero-negative for prior EEHV3 infection. These results suggest that lethal infection in these elephants resulted from primary infection. Notably, we have obtained similar findings in Asian elephants (Fuery et al , J. Virol 2020).

Clinical illness and death have now been attributed to both EEHV3A (Indianapolis) and EEHV3B (Fresno, Maryland). To better understand the prevalence of these subtypes and to quickly diagnose viremia caused by them, we generated **new qPCR tests** that can distinguish infection (or shedding)

from these viruses. Knowledge of the complete genome sequences of these virus subtypes was critical for designing these new tests. We have shared these new tests with Erin Latimer at the NEHL for use in screening samples from African elephants.

One issue that has emerged from our serology studies from the recent EEHV outbreaks is that Sampson (Maryland), Amahle (Fresno via Dallas), and three others at Indy (Kedar, Zahara, and Ivory) all got clinically ill but survived. Why? By serology, and in contrast to the three that died, they all had specific antibodies to EEHV3 prior to clinical illness. We need to go back to the 3A and 3B story now. The tests right now can't detect the difference between a 3A infection versus a 3B infection. We are now working on that—and the sequencing of the complete genomes has given us the information and tools to at least make new reagents to try and develop such a test. Amahle and Sampson got sick from 3B, while the Indy elephants (clinical survivors that is) from 3A. If we can develop a **specific serology test** for each, our current hypothesis is that the Indy elephants had 3B prior and got sick from 3A and Sampson and Amahle had 3A prior and got sick from 3B. It is the simplest explanation for now, but the data will tell us if we are right or not. I liken it possibly to what we have seen in Asians—elephants that got infected with 1A first were less sick when infected with 1B and vice versa (St Louis zoo Jade and Maliha and Barack at Feld are examples)—so in this case there may be some cross-protection offered by prior infection with a more closely related EEHV.

Asian elephant EEHV vaccine:

1. In January and February, we initiated experiments with a prototype EEHV1A vaccine in mice. The goal was to see how robust of an immune response was generated in an animal (mouse) when given a vaccine that contained one of the EEHV proteins known as "gB". (In fact you may have heard of Covid 19 vaccines made up of the SARS CoV 2 "spike" protein---consider EEHV gB somewhat analogous to the Covid 19 Spike protein). Before the shut down in March we had vaccinated a few dozen mice, and had planned on boosting them as well. Baylor allowed us to do this, even though they were encouraging everyone to wind down animal experiments. So vaccinations went as planned, and Taylor Pursell (the other postdoc in the lab) was able to harvest mice at our planned time points. The good news is that we were able to assess antibody responses to the vaccine throughout the entire planned experiment—mainly because serum is easily obtained and stored. I can say with confidence that the vaccine worked as hoped for this part in the experiment.

However, an important part of the vaccine design was to also induce another type of immunity mediated by a cell type called T cells. T cell mediated immunity, also called cell mediated immunity (CMI) is important for defense and clearance of herpesviruses. This part is tricky to evaluate and requires specialized expensive equipment. At Baylor we have a core service with such equipment, that unfortunately during the Covid shutdown, was also closed for business. So we were unable to conduct CMI analysis for the vaccine in real time. Taylor froze down all of the relevant tissues and cells harvested from the mice with the hope that we could maybe get data for this part "later". This fall, we were able to gain limited access to the Baylor Flow Cytometry core and complete analysis of T cell responses to the vaccine. Like the antibody responses, mice generate strong T cell responses to the recombinant MVA gB vaccine.

6. Approximate number of humans/communities impacted by the project and approximate numbers of elephants impacted.

Knowledge of the complete genome sequences of the EEHVs endemic within African elephants will be a significant knowledge base for anyone doing research on African elephants and EEHV. Our first generation serology assays will also be useful for screening elephants in captivity and those in the wild. When the Covid 19 pandemic ends and travel restrictions ease, we anticipate that we will collaborate with our colleagues in South Africa (Dr. Michelle Miller) for example, to conduct surveillance for anti-EEHV antibodies on collections of serum samples from wild elephants. Our

vaccine development for EEHV in Asian elephants is in early stages. Success with this effort will impact all institutions that care for Asian elephants, especially ones that have breeding programs.

7. Problems discovered during grant period

Except for delays caused by the Covid 19 pandemic, we are on target to accomplish our original goals and objectives.

8. Project success evaluation.

We identified samples appropriate for determining the genome sequences of EEHVs 2, 3A, and 3B and are working on EEHV6. Thus, we have almost completed the goal of determining the genome sequences of all of the major EEHVs endemic within African elephants. In addition, we developed a first generation serology assay for detecting anti-EEHV3 antibodies in African elephants. Thus, within the first year we have nearly completed goals outlined for research on African elephant EEHVs. A significant first step in the development of a vaccine is to test its ability to stimulate an immune response in animals and to assess it for potential toxic effects. In this regard, our MVA-gB recombinant vaccine showed no ill effects in mice and induced the mice to make antibodies and T cell responses towards the EEHV gB protein, which we believe will confer at least protection from lethal infection with EEHV (i.e., in an elephant).

9. What is the next step for this project and what are the implications for future conservation actions?

We intend to complete the sequencing of the EEHV6 genome by mid-year 2021 and publish the sequence along with those of EEHV2, 3A, and 3B. We also intend to publish results of our EEHV3 serology assay and alert the African elephant community that we can provide antibody surveillance of their elephants, if needed. Finally, we are adding additional EEHV proteins to our vaccine (glycoproteins gH and gL) with the idea that vaccination with more than one part of the virus may be required to provide protective immunity. We are going to repeat our mouse experiments with these newer “multivalent” vaccines. Furthermore, with the rapid development of new technologies related to the Covid 19 vaccine effort, we intend to add at least one of these approaches to our preliminary vaccine studies in mice. The approach we are considering is to try a vaccine known as an mRNA vaccine, popularized recently by Moderna, and their early success with an mRNA vaccine against SARS CoV 2.

10. Human interest story.

The story of Tupelo:

In 2009, following the death of a young elephant named Mac from EEHV-associated hemorrhagic disease, I was approached by the Houston zoo to see whether I might help them find ways to mitigate disease cause by this newly recognized herpesvirus of elephants. After some consideration I decided “who wouldn’t want to help save baby elephants”? Shortly after I made a commitment to investigate EEHV, two elephants were born at the Houston zoo. Baylor, born in May of 2010 and named after our unique collaboration, and Tupelo, born in October of 2010. Looking back, Tupelo’s story is remarkable for several reasons. First, Tupelo is one of those elephants who responds to training with robust enthusiasm. In this regard, she was able to provide blood samples from an early age resulting on one of the most extensive blood sample collections from birth that I’m aware of. Thus, Tupelo provided a sample set that allowed us to interrogate antibody transfer from her Dam (Tess) and determine that elephants are more like people--they transfer antibodies to their young trans placentally (before birth), with colostrum contributing little or no antibodies (Nofs et al, JII 2013 and Fuery et al JV 2020). Until this time, it was anticipated that maternal transfer of antibodies from Dams to their calves was more in line with that of horses or cows. Tupelo’s sample set also allowed us to visualize the temporal decline of anti-EEHV antibodies over time, which may provide an important insight into why elephants become susceptible to EEHV HD after 1.5-2 years of age. Second, Tupelo experienced two bouts of clinical illness from EEHV. One from EEHV4 and the other from EEHV1B. From these events, we

were able to describe and characterize EEHV infections in Asian elephants with substantial thoroughness (Fuery et al JZWM 2016 p311 and Fuery et al JZWM 2016 p319). For example, we reported onset of viremia in the blood through its decline during recovery and associated changes in several blood values. The onset and duration of shedding in trunk washes was followed in detail and levels of penciclovir in the blood were determined in a clinically ill elephant following rectal administration of the anti-herpesvirus drug famciclovir (most prior studies were done in healthy elephants). Furthermore, the samples collected during these clinical events were used to establish serology assays to detect EEHV infections in Asian elephants (Fuery et al JV 2020). Tupelo's contributions towards our understanding of multiple aspects of elephant biology and EEHV are extensive and the result has been generation of knowledge and tools (e.g., EEHV qPCR) which have been incorporated into life-saving EEHV protocols for elephants around the world. Even more remarkable is that Tupelo's first AI was successful and she has now come full circle with an expectant birth of her first calf this month **(see pictures of Tupelo March 1, 2021 with Paul Ling)**. Together with her mother Tess, who is due in April, the Houston herd has gone from 5 elephants to now an expected 13 elephants since 2010. We would like to think that together with special elephants like Tupelo, we have contributed to this success when previously, at least six elephants born at the Houston zoo succumbed to EEHV HD.

11. Organizations associated with this project and their roles.
Baylor College of Medicine: All vaccine related experiments were conducted, analyzed, and evaluated in Dr. Paul Ling's laboratory. All sequencing was done through the Human Genome Sequencing Center (HGSC) at Baylor College of Medicine with assistance from Dr. Vipin Kumar and Xiang Qin (also at BCM).
Houston zoo, Indianapolis zoo, Fresno Chaffee zoo, Maryland zoo, Oakland zoo: These zoos provided serum samples or necropsy samples used for determination of genome sequences for African elephant EEHVs and for development of an EEHV3 serology assay.

12. Itemized financial report.
See separate page, as requested.

13. Five high resolution photos:
(attached in email).

14. 2 minute video clip
(attached in email)

15. Publications and/or conference presentations:

Pursell, TP, and PD Ling. Early efforts to generate EEHV serology assays for African elephants. Journal of the Elephant Managers Association (EMA), Spring 2020.

Manuscripts in preparation:

1. Pursell, TP., Clinton J , Hayward, GS, and PD Ling. 2021. Lethal hemorrhagic disease caused by EEHV3 in African elephants (*Loxodonta africana*) is caused by primary infection. (In preparation).

2. Pursell, TP, Clinton J., and **Paul D. Ling**. 2021. Modified Vaccinia Ankara vaccine expressing EEHV gB induces humoral and cellular immune responses in mice. (In preparation)

Conference presentations:

Development of EEHV-specific serology assays in Asian and African elephants.
Pursell, T, Fuery, A, Hayward, GS, Heaggans SY, Menon, VK, Qin, X, Worley, KC, Ling, Paul D

EAZWV, virtual conference (in lieu of the physical conference in Emmen, NL from May 2020). EEHV session, day 3, July 22, 2020.

**Generating an immunogenic elephant endotheliotropic herpesvirus (EEHV) vaccine.
Clinton, J.L.S., Pursell, T., Tan, J., Peng, R., Ling, P. Oral (virtual) presentation at the MD Anderson 10th Annual Postdoctoral Science Symposium in Houston, Texas, October 2020.

**Third place, basic sciences category.

16. Media coverage.

Interview with Lorena Villanueva-Almanza of the Indianapolis Star for a piece about EEHV:
https://www.indystar.com/story/news/environment/2020/08/11/indianapolis-zoo-elephants-died-they-may-help-save-others-virus/5527343002/

17. Social media associated with work supported by IEF

Interview series from the International Elephant Foundation (IEF); #AskAConservationist, April 24, 2020:
https://www.youtube.com/watch?v=n2Dha8idn_E&feature=youtu.be&fbclid=IwAR3fA4FVrinyzbRiZnzKwdyc5ep15dtHKFFGLNKJE2tOTxAEorpzf1oRwmM

"Pachy Chat" podcast: episode 16 on EEHV:
https://podcasts.apple.com/us/podcast/episode-16-dr-ling/id1503609586?i=1000495176915

Itemized Financial report

Personnel costs January 1, 2020-December 31, 2020: $51,441.40
Supplies/services January 1, 2020-December 31, 2020: $8820.41
Total: $60,261.81
A general summary is below:

Layout	CJI3	/JDIGGS		TOTAL BY G/L	
Object		WBS 1383012508		INTERNATIONAL ELEPHANT FDN - Y	
Cost Element		40031600	To 73642000	PRIVATE AWARDS...	
Posting Date		01/01/2020	To 12/31/2020		

RefDocNo	Posting Date	Cost Elem.	WBS Element	DocTyp	BusA	Σ	ValCOArCur	Cost element name
		40031600					60,261.81-	PRIVATE AWARDS
		63270000					11,670.14	BENEFITS - STAFF
		67000000					39,771.26	SALARY - STAFF
		73320000					419.85	INSUR-STUDENT MED.
		73530000					126.06	SERVICES - COURIER
		73548000					7,398.30	SERV.-LAB ANALYSIS
		73642000					876.20	CHEMICALS
							0.00	

Itemized costs, not including personnel are below (Some of the charges from the human genome sequencing center (HGSC) have not been posted yet for costs associated with sequencing EEHV6):

Run Date: 02/26/2021
Run Time: 11:12:50

Baylor College of Medicine
Detailed Project Expense (Direct Cost Only)
For the Period 01/01/2020 - 12/31/2020

WBS Element: : 1383012508 INTERNATIONAL ELEPHA
Principal Investigator: 00037755 Paul Dalling Ling
Termination Date : 12/31/2021

Doc Type	Document Number	PO Number/ Req Number	Posting Date	Item Description	Vendor	GL Acct	Order	Actual	Encumbrance
	245229		03/28/2020	Payroll for posting period =09		73320000	100000	419.85	0.00
				Sub Total for GL Acct 0073320000 INSURANCE-STUDENT MEDICAL				419.85	0.00
KR	190539816		08/27/2020	FedEx 395891572832 08/17/2020	FEDEX ERS	73530000	100000	8.86	0.00
ZX	14123158		10/13/2020	00029546/99786/Courier/Shippin		73530000	100000	18.17	0.00
ZX	14123158		10/13/2020	00029546/99786/Courier/Shippin		73530000	100000	32.34	0.00
ZX	14123158		10/13/2020	00029546/99786/Courier/Shippin		73530000	100000	14.49	0.00
ZX	14161456		12/07/2020	00029546/104644/Courier/Shippi		73530000	100000	22.24	0.00
ZX	14161456		12/07/2020	00029546/104644/Courier/Shippi		73530000	100000	29.96	0.00
				Sub Total for GL Acct 0073530000 SERVICES-COURIER				126.06	0.00
ZI	2500037565		04/27/2020	TSF PYMT FOR HUMAN GENOME SEQU		73548000	100000	763.46	0.00
ZI	2500037631		05/19/2020	TSF PYMT FOR HGSC-SSF FEB. 20		73548000	100000	181.47	0.00
ZI	2500037631		05/19/2020	TSF PYMT FOR HGSC-SSF APR. 20		73548000	100000	60.61	0.00
ZJ	14146307		11/11/2020	TSF PYMT FOR HGSC-SSF SEPT 20		73548000	100000	27.08	0.00
ZJ	14158458		12/04/2020	TSF PYMT FOR HGSC-SSF OCT 20 I		73548000	100000	6,365.68	0.00
				Sub Total for GL Acct 0073548000 SERVICES-LABORATORY ANALYSIS				7,398.30	0.00
	523090057	5601590335	10/27/2020	UMNSAH/DF-1	AMERICAN TYPE CULTUR	73642000	100000	876.20	0.00
				Sub Total for GL Acct 0073642000 SUPPLIES-CHEMICALS				876.20	0.00
				Total For Funds Center 1383012508				8,820.41	0.00
				Actual Expense + Encumbrance For Funds Center 1383012508					8,820.41

Legends for photos

Steps for conducting serology for African elephants:
1. Jie Tan washing plate with samples to be measured in Luminometer

2. RongSheng loading plate into automated Luminometer plater reader

3. Jennifer Clinton, PhD. Reads data generated by the Luminometer

Paul Ling with Tupelo, a gravid female due to give birth in March 2021
4. Paul with Tupelo

5. Paul with Tupelo and Joy and Nelson, who wanted in on the action!