THE EEHV CONSORTIUM
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NEHL at the National Zoo
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PHOTO COURTESY OF THE MARYLAND ZOO

The EEHV Consortium at National Elephant Herpesvirus Laboratory Update

PROFILE:

Dr. Byron Martina, The Erasmus University & Artemis One Health Research Institute

DR. BYRON MARTINA IS A RESEARCHER IN THE DEPARTMENT OF VIROLOGY, THE ERASMUS UNIVERSITY AND ARTEMIS ONE HEALTH RESEARCH INSTITUTE, BOTH IN THE NETHERLANDS, WORKING ON EEHV AND OTHER VIRUSES.

(1) WHERE DID YOU GO TO SCHOOL AND HOW DID YOU GET STARTED IN RESEARCH?

I started my study in Biomedical Sciences in The Netherlands in 1991. In 1996 I did my MSc in Infection & Immunity in Leicester, UK. From 1998-2003 I performed my PhD studies at The Erasmus University in Rotterdam, The Netherlands.

(2) HOW DID YOU GET INVOLVED IN EEHV WORK?

I did my PhD study on seal herpesviruses. I studied the epidemiology of two seal herpesviruses and I worked on development of a vaccine against one of the herpesviruses. In addition, I studied the potential of the viruses to induce cancer formation. Several herpesviruses are known to cause cancer in humans or animals. So when a case of elephant herpesvirus was suspected in the Rotterdam zoo in the Netherlands, I was asked if I was interested to help with the diagnosis.

(3) DESCRIBE THE EEHV STUDIES YOU ARE CURRENTLY DOING.

Currently, my group is involved in developing a serology test that would allow us to perform epidemiological studies and evaluate the response of elephants to a vaccine. In addition, we are investigating the possibility to develop a vaccine or an anti-viral against the virus. We realize however, that it is a long way to go before a possible vaccine can be implemented, but we have reasons to believe that a vaccine could work, if we are able to fill some key gaps in our knowledge.

(4) WHAT ARE THE OTHER PROJECTS IN YOUR LAB?

My group focuses on understanding the pathogenesis of zoonoses (viruses that resides in animals and are transmitted to humans) and arboviruses. The viruses that we are studying at the moment can be divided in those that cause a hemorrhagic disease (e.g. Hantavirus, dengue virus, chikungunya virus) and those that cause neurologic syndrome (e.g. West Nile virus, rabies virus, entero-71). The elephant herpesvirus causes a syndrome similar to the hemorrhagic fever viruses that we are studying.

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USEFUL LINKS FOR EEHV PREPARATION

- EEHV PLANNING PROTOCOLS
- EEHV TESTING LABORATORIES
- PROCEEDINGS OF THE FEB 2015 EEHV WORKSHOP.

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(5) WHERE DO YOU SEE EEHV RESEARCH GOING?

I think that the amount of groups working on EEHV is still too small to be able to solve some of the key questions in EEHV research. In order to push the research on EEHV forward there is a need for funding of several lines of research. In addition, a closer collaboration between groups in Europe as well as groups worldwide is imperative to prevent unnecessary duplication of work and enhance focus on the priority questions. But I do not think that we can solve the problem for now. In the human field, several of the same questions that we have with regards to disease mechanisms and risk factors remain incompletely understood and no effective treatment has been developed. I do not expect the situation for EEHV to be different.

(6) WHAT PROJECT WOULD YOU DO IF GIVEN THE FUNDING TOMORROW?

I would invest more time using state-of-the-art technology to understand the immune response of elephants and I would try to isolate the virus from biological samples or rescue the virus from a molecular clone. Analogous to the other hemorrhagic fever viruses such as Ebola, Marburg, Crimean-Congo, or even bacterial sepsis, I believe that the virus is an important trigger of the disease, but that the immune response to the infection has the most important contribution to disease. Having the virus would allow us to test antivirals, which can be applied to save the life of animals even if we do not understand the pathogenesis.

(7) DO YOU THINK A VACCINE WILL BE DEVELOPED FOR EEHV?

I think it is easy to develop a candidate vaccine. It is much more difficult to predict if the vaccine will be effective. We do not know what kind of immune response would be protective (antibodies or T cells) against EEHV. We do not know against which part of the virus a response should be directed. We do not have a system to test the vaccine before it is administered to elephants. Vaccines against some other herpesviruses have shown protective efficacy, but there are also herpesviruses against which no effective vaccine could be developed. If we isolate the virus, a killed virus vaccine could be developed, which can be used and studies should be conducted to evaluate its efficacy in preventing disease of lethal infection. In my opinion we should consider developing a vaccine based on what we know from other herpesviruses and test if it works in the field. This is better than doing nothing because research trying to understand the mechanisms of disease per se will not solve the problem on the short term.

(8) WHY DO YOU THINK EEHV IS SO SERIOUS IN ASIAN ELEPHANTS, WHEN HERPESVIRUSES REALLY SHOULDN'T CAUSE DISEASE?

It is difficult to say. The easiest explanation was that the virus is not of the Asian elephant origin. This explanation seems not to hold true. It is interesting to hypothesize that the host genetic background may be an important determinant of disease outcome. There is some circumstantial evidence to support this.

(9) WHY IS IT IMPORTANT TO STUDY EEHV?

EEHV is an important threat to elephants. If we want to tackle the problem we need to understand the epidemiology of the disease, the immune response to the virus and the mechanism of disease. The latter could also help us understand the other hemorrhagic diseases.

(10) DO YOU THINK FAMCICLOVIR AND GANCICLOVIR ARE EFFECTIVE AGAINST EEHV?

There is little convincing evidence for this. As a matter of fact, the majority of the data suggest these medicines do not work. But, unless we have convincing evidence that they do not work, I think empirical use is still justified because doing nothing is not an option.

PHOTOS COURTESY OF PETRA VAN DEN DOEL
Petra van den Doel, Erasmus University and Dr. Willem Schaftenaar, Rotterdam Zoo

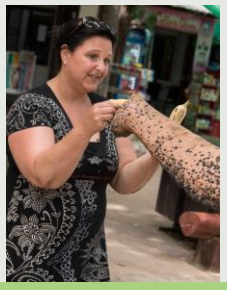

PHOTO BY EURYDICE MARTINA
Stephanie Lim, Byron Martina, Loubiela Joseph; Artemis One Health Research Institute

A recent paper, A novel antigen capture ELISA for the specific detection of IgG antibodies to elephant endotheliotropic herpes virus, from Dr. Martina's laboratory at Erasmus University and collaborators Petra van den Doel and Willem Shaftenaar, and others, details some of their work on an EEHV ELISA

ABSTRACT

BACKGROUND

Elephants are classified as critically endangered animals by the International Union for Conservation of Species (IUCN). Elephant endotheliotropic herpesvirus (EEHV) poses a large threat to breeding programs of captive Asian elephants by causing fatal haemorrhagic disease. EEHV infection is detected by PCR in samples from both clinically ill and asymptomatic elephants with an active infection, whereas latent carriers can be distinguished exclusively via serological assays. To date, identification of latent carriers has been challenging, since there are no serological assays capable of detecting seropositive elephants.

RESULTS

Here we describe a novel ELISA that specifically detects EEHV antibodies circulating in Asian elephant plasma/serum. Approximately 80 % of PCR positive elephants display EEHV-specific antibodies. Monitoring three Asian elephant herds from European zoos revealed that the serostatus of elephants within a herd varied from non-detectable to high titers. The antibody titers showed typical herpes-like rise-and-fall patterns in time which occur in all seropositive animals in the herd more or less simultaneously.

CONCLUSIONS

This study shows that the developed ELISA is suitable to detect antibodies specific to EEHV. It allows study of EEHV seroprevalence in Asian elephants. Results confirm that EEHV prevalence among Asian elephants (whether captive-born or wild-caught) is high.

Annual Report

On Sept. 30, 2015, we finished our second fiscal year and are now well into our third fiscal year. We finished the fiscal year with 23 EEHV Consortium members and have just signed up two more. A major push of the year was to increase the number of elephant calves that we monitor weekly by qPCR. We finished out the year with eleven calves participating, with two more young calves being trained for blood collection; they will soon become part of our weekly group.

Our calf monitoring program consists of weekly qPCR testing of whole blood; Asian calves get screened for EEHV1, 3-4, and 5 and African calves get screened for EEHV2, 3-4, and 6. Sick elephants get tested for all of the known EEHVs. Zoos at the Platinum level of membership get two months of weekly trunk wash testing to detect possible latent EEHVs that are shed in the trunk secretions.

In fiscal year 2015, we received 219 shipments from elephant-holding facilities, with anywhere from one sample to 56 samples per shipment, for a total of about 900 samples. These samples were sent to the NEHL for routine monitoring, diagnosis of acute EEHV, necropsy testing, and determination of EEHV shedding in trunk secretions.

PHOTO BY NICOLE FURST
Nicolas Cruz, NEHL intern

Our FedEx shipping program has become very popular; we have seven zoos that use our pre-printed labels for shipping weekly samples. It's a great savings for the participating zoos: a pre-printed label costs $20 and covers Priority Overnight shipping of a cooler to the NEHL and the return shipment of the cooler to the sender. Normal shipping costs for Priority Overnight can exceed $100.

The NEHL collaborated with colleagues in the Netherlands, Spain and the US, leading to the following two publications during FY2015:

1. van den Doel PB, Prieto VR, van Rossum-Fikkert SE, Schaftenaar W, **Latimer E**, Howard L, Chapman S, Masters N, Osterhaus ADME, Ling PD, Dastjerdi A and Martina B. A novel antigen capture ELISA for the specific detection of IgG antibodies to elephant endotheliotropic herpes virus. *BMC Veterinary Research* 2015, **11**:203 doi:10.1186/s12917-015-0522-6
2. Ortega J, Corpa JM, Orden JA, Blanco J, Carbonell MD, Gerique AC, **Latimer E**, Hayward GS, Roemmelt A, Kraemer T, Romey A, Kassimi LB, and Casares M. Acute death associated with *Citrobacter freundii* infection in an African elephant (*Loxodonta africana*)**.** J Vet Diag Inv. July 25, 2015 doi: 10.1177/1040638715596034

PHOTO COURTESY OF NZP
NEHL intern, Nicole Furst

We have another two publications that have been accepted and will be published during FY2016.

We updated the EEHV Research and Tissue Request document, developed the EEHV Monitoring and Testing guidelines for the North American herd and helped our European colleagues develop their Monitoring and Testing guidelines. The EEHV Research and Tissue Request document and the Monitoring and Testing guidelines for North America were approved by the EEHV Advisory Group and Elephant TAG Steering Committee.

Donations from Ringling Bros. and Barnum & Bailey and the International Elephant Foundation and EEHV Consortium membership dues supported two interns during the summer of 2015; Nicole Furst (now at University of Florida College of Veterinary Medicine) worked on an EEHV prevalence study and Nicolas Cruz (Center for Conservation Medicine, Cummings School of Veterinary Medicine (Tufts)) worked on an EEHV multiplex assay.

The eehvinfo.org website has proven to be very popular; I get multiple requests for the member password every month, including requests from many of our international colleagues. It is still a work in progress and we welcome any suggestions for it (new content, organization, etc). My favorite sections are the planning documents from two zoos, and documents describing recommended EEHV monitoring, calf training, treatments and standing sedation.

The NEHL was featured in a story in The Scientist magazine and the accompanying video was chosen as one of the Best of Multimedia 2015 by the magazine (scroll down to the bottom of the page for the video).

Thank You: Membership helps to prevent elephant deaths

- The Bronx Zoo
- The Buffalo Zoo
- Busch Gardens Tampa
- Carson & Barnes Circus
- The Cleveland Metroparks Zoo
- Columbus Zoo And Aquarium
- The Dallas Zoo
- Denver Zoo
- Dickerson Park Zoo
- Fort Worth Zoo
- Have Trunk Will Travel
- The Houston Zoo
- Jacksonville Zoo & Gardens
- Maryland Zoo
- Oklahoma City Zoo and Botanical Gardens
- The Oregon Zoo
- Point Defiance Zoo & Aquarium
- Ringling Bros. and Barnum & Bailey
- The Rosamond Gifford Zoo at Burnet Park
- Saint Louis Zoo
- San Diego Zoo Global
- Smithsonian's National Zoo and Conservation Biology Institute
- The Tulsa Zoo
- Utah's Hogle Zoo
- Woodland Park Zoo

RECENT MEETING OF NOTE

FROM NOV. 5TH TO 7TH, 2015, WILDLIFE RESERVES SINGAPORE HOSTED THE 1ST ASIAN EEHV STRATEGY MEETING. A WORKING GROUP WAS FORMED THAT IS DEVELOPING PLANNING DOCUMENTS AND PROTOCOLS SPECIFIC FOR THE COUNTRIES IN SOUTHEAST ASIA. A SUMMARY STATEMENT IS INCLUDED BELOW.

ASIAN EEHV WORKING GROUP
GROUP STATEMENT

A recently recognized herpes virus, EEHV (elephant endotheliotropic herpes virus), can cause severe hemorrhagic disease in elephants, and is associated with a high fatality rate in young Asian elephants (1-8 years of age). Death frequently occurs within 1-2 days of the first visible signs, and early diagnosis and treatment is critical to survival.

The prevalence of EEHV in captive Asian elephants in North America and Europe has been well characterized, with an estimated mortality rate of 70% in captive born elephants that become ill from the virus. Little is known about the prevalence and impact of EEHV on captive and wild elephant populations in Asian elephant range countries.

From Nov. 5th to 7th, 2015, Wildlife Reserves Singapore hosted the **1st Asian EEHV Strategy Meeting.** For 3 days, 38 (wildlife) veterinarians, researchers, conservationists, and elephant specialists shared information, identified regional needs, and prioritized future EEHV-related projects. Eight Asian elephant range countries were represented (Thailand, Myanmar, Indonesia, Cambodia, Sri Lanka, India, Vietnam, and Malaysia) along with delegates from Singapore, the United States, Canada, and the Netherlands.

As a result of this 1st Asian EEHV Strategy Meeting, an **Asian EEHV Working Group** was formed which together recognized:

- The epidemiology of EEHV in elephants in Asia and its impact on populations is currently unknown. Within the last 10 years, 59 fatal cases of EEHV disease in Asian elephants have been identified within the eight range countries represented at our meeting. Twelve of these deaths were wild elephants.
- The identification of EEHV-associated deaths in wild elephants in Asia is significant and it is the opinion of the Working Group that EEHV is a conservation concern requiring close monitoring and further study.
- Early diagnosis of EEHV-associated disease in young elephant calves allows early treatment and a better chance of a successful outcome. Therefore an important consideration is that the examination, sample collection, and treatment of young calves depend on the ability to handle and manage the calf from a very young age (less than 1 year old).
- Laboratories are critical to the routine monitoring, detection, and post mortem evaluation of elephants affected by EEHV. Currently, of 13 Asian elephant range countries, only 3 (Thailand, Indonesia, and India), have laboratories capable of confirming EEHV.

Based on the above concerns, the Asian EEHV Working Group seeks the support of regional governments and international stakeholders in the following areas of immediate focus:

- To build capacity and increase awareness and education of EEHV amongst elephant care staff in Asia including keepers (mahouts), veterinarians, and government officials.
- To develop region-specific medical protocols, "standard operating procedures" that outline routine monitoring, rapid and accurate detection, and appropriate treatment of EEHV-associated disease.
- To closely collaborate within the region and internationally to identify and implement research projects to continue to advance the understanding of EEHV.

CHARTER MEMBERS OF THE ASIAN EEHV WORKING GROUP

Dr Sonja Luz	Director C&R	Wildlife Reserves Singapore, WRS
Dr Abraham Mathew	Senior Veterinarian	Wildlife Reserves Singapore, WRS
Dr Chia-Da Hsu	Pathologist	Wildlife Reserves Singapore, WRS
Mr Saravanan Elangkovan	Curator	Wildlife Reserves Singapore, WRS
Mr Kalirathinam Udhaya Kumar	Junior Animal Management Officer	Wildlife Reserves Singapore, WRS
Ms Paige Lee	Officer C&R	Wildlife Reserves Singapore, WRS
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Dr Preecha Phoungkham	Veterinarian	Friends of Asian Elephant Foundation
Dr Taweepoke Angkawanish	Manager	National Elephant Institute, FIO
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Ms Supaphen Sripiboon	PhD candidate/University lecturer	Murdoch University/Kasetsart University
Erica Ward	veterinarian	
Mr Pallop Tunkaew	Research scientist	Faculty of Veterinary Medicine, CMU
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Dr Muhammad Agil	Reseracher	Veterinary Faculty of the Bogor Agriculture Institute
Dr Adin Priadi	Reserche scientist	Satwa Duta Medical Lab for Animal health, Bogor, Indonesia
Dr Zaw Min Oo	Assistant Manager (Veterinary)	Myanma Timber Enterprise
Dr Ye Htut Aung	Professor	University of Veterinary Science
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U Myo Thant	Deputy General Manager	Sagaing Extraction Agency
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Dr Oung Chenda	Head Veterinarian	Wildlife Alliance
Nick Marx	Wildlife Rescue Director	Wildlife Alliance
Mr Sitheng	Head Keeper	Wildlife Alliance
Dr Vatsana Chanthavong	Deputy of Division of Veterinary Services/Vet technician of ElefantAsia	ElefantAsia Project, Division of Veterinary Services, Department of Livestock and Fisheries
Dr Senthivel Nathan	Assistant Director	Sabah Wildlife Department
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Dr Diana A. Ramirez Saldivar	Assistant Manager	Wildlife Rescue Unit
Dr Arun Zachariah	Assistant Professor	Kerala Veterinary and Animal Sciences University
Dr Kalaivanan	Veterinary Assistant Surgeon	Tamil Nadu Government, Department of Animal Husbandry
Dr Kushal Konwar	Professor and Head of Department of Surgery & Radiology	Assam Agricultural University
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Dr Chandana	Veterinary Surgeon	Pinnawala
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Dr Ellen Wiedner	Veterinarian	Point Defiance Zoo and Aquarium/Northwest Trek
Ms Heidi Riddle	Co-founder and Director of Operations	Riddle's Elephant and Wildlife Sanctuary (International Elephant Foundation)
Dr Lauren L Howard	Associate Veterinarian	Houston Zoo
Dr Wendy Kiso	Research and Conservation Scientist	Ringling Bros. Center for Elephant Conservation
Dr Janine Brown	Research Scientist	Smithsonian Conservation Biology Institute
Dr Dennis Schmitt	Chair of Veterinary Services and Director of Research	Ringling Bros. Center for Elephant Conservation
Dr Paul D. Ling	Associate Professor	Baylor College of Medicine
Dr Willem Schaftenaar	Veterinarian/Veterinary Advisor	Rotterdam (Blijdorp) Zoo/European Elephant TAG