ABSTRACTS

Abstracts

2013 International
Elephant & Rhino
CONSERVATION & RESEARCH
Symposium

2013 International Elephant & Rhino CONSERVATION & RESEARCH Symposium

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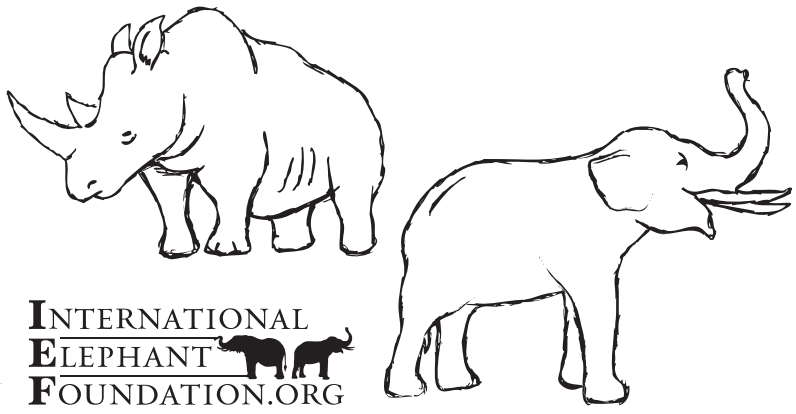

INTERNATIONAL RHINO FOUNDATION

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Abstracts

Session I: Human - Wildlife Co-existence

Securing Human–Elephant Coexistence

Simon Hedges, *IUCN/SSC Asian Elephant Specialist Group*

Elephants are wonderful creatures with complex social lives, play a dominant role in the ecosystems in which they live, and act as flagships for the conservation of biodiversity wherever they occur. Elephants are also of great economic significance: for example, they do great damage to crops, especially large-scale plantations, but they are also sources of income for many people through wildlife-viewing-based tourism, especially in Africa. In addition, elephants are of tremendous cultural and religious significance across Asia. It has been said that, "One can start from Ganesha and work from there in an unbroken line to almost any aspect of Indian culture." Similar observations could be made about the role of elephants in the cultures of many other countries in Asia. Unfortunately, elephants are threatened by habitat loss, fragmentation, and degradation; illegal killing for ivory and other products or in retaliation for human–elephant conflict; and, in some regions at least, a loss of genetic viability resulting from small population size and isolation. Moreover, a lack of good-quality data on population size, distribution, and even basic biology hampers effective elephant conservation in many areas. Furthermore, in too many range States a lack of capacity in wildlife departments also handicaps effective conservation. But it does not have to be like this. There is still time to turn this situation around; there are still enough elephants and enough wil d places for the species to thrive again.

To address the various threats, it is necessary for the international community to work with range State governments to secure large new protected areas for elephants and other wildlife; to quantify and reduce illegal killing through improved law enforcement measures; to assess and field-test a range of both traditional and novel approaches to reducing human–elephant conflict, promoting those methods shown to be effective; and to evaluate, and promote new techniques for elephant monitoring. However, while actions at the site and landscape level are necessary to save elephants they are not sufficient. It is also necessary to act outside of sites and landscapes at the provincial, national, and global scales. Many important threats to elephants including trade in elephants and elephant parts (especially ivory) and the impacts of the large scale extractive industries and of infrastructure developments cannot be addressed at the site or even landscape level. It is necessary, therefore, to identify and disseminate best practices and to work to directly influence policy in those arenas particularly important to elephant conservation.

Fortunately, there are signs that evidence-based approaches to elephant conservation are having an increasing, if overdue, impact in Asia and Africa and that the global community is once again acting in the face of the new African elephant poaching crisis, and so we may yet secure human–elephant co-existence across large parts of their range. These welcome developments as well as the remaining very significant challenges are reviewed.

An Update on the Asian Elephant Conservation Fund Supported Projects

Mini Nagendran, *Asian Elephant Conservation Fund - U.S Fish and Wildlife Service*

The Asian Elephant Conservation Fund of the US Fish and Wildlife Service has provided support for field conservation projects in the 13 Asian range countries since 1999. The primary threat to Asian elephants is continued habitat loss leading to increasing human-elephant conflict. The presentation will discuss success stories, a range-wide estimate of wild Asian elephant populations, threats and challenges.

Rhinos: Are We at the Tipping Point?

Susie Ellis, Ph.D., *International Rhino Foundation*

Rhinos have walked the Earth for more than 50 million years, but today, all but one species faces extinction, possibly within our lifetimes. Organized poaching in Africa and India is taking place at nearly unprecedented rates, and Indonesian rhinos face a host of existing and emerging threats. But the news is not all bad. This presentation provides an update on the current situation facing the five rhino species and outlines some of the most urgent rhino conservation and research needs – requiring all our collaborative efforts to prevent extinction.

The Africa Asia Human Elephant Conflict Education & Resolution Project

Ravi Corea, *Sri Lanka Wildlife Conservation Society*

From time immemorial people have been fascinated and been in awe of elephants as well as terrified of them. In Asia this fascination had led to the development of one of the most unique relationships that had ever evolved between a wild animal and a human. In Africa most unfortunately the fascination for the elephant is mostly to do with its ivory rather than for its' enigmatic and wondrous nature. Today irrespective what their relationships with people had been historically, both species of elephants are in critical trouble. The Africa Asia Human Elephant Conflict Education & Resolution Project will be a pioneering effort—for the first time African and Asian conservationists will partner to develop effective measures to address the decline of both species due to intense human-elephant conflicts (HEC).

Poaching for ivory is the biggest crisis for the African elephant, yet HEC is a covert and equally lethal process that decimates elephants over the long term. In Asia HEC contributes the most to elephant mortality yet poaching still occurs and is a threat with the potential to increase influenced by events happening in Africa and due to the increasing demand for ivory. Basically elephants are being mowed down from both ends and at this rate of killing it is doubtful whether both species will survive to see the dawn of another century. The Africa Asia Human Elephant Conflict Education & Resolution Project is strategically important due to the following reasons:

1. The current crisis the African elephant is facing due to poaching and HEC.

2. The increasing HEC in Asia due to exponential development and population growth.

3. The lack of collaboration between Asian and African counterparts.

4. The project is about protecting the planet's natural resources.

5. Killing of elephants is also a national security issue, a public health issue and an economic security issue since dead elephants will affect the tourism dollars of Africa and Asia in the long term.

The Sri Lanka Wildlife Conservation Society has been addressing HEC mitigation for the past 17 years and the society's Saving Elephants by Helping People Project received a UNDP Equator Prize in 2008. The SLWCS is keen to use its experience as well as learn about HEC resolution in Southern Africa. There must be HEC situations in Africa and Asia that are similar but are looked upon and addressed differently due to widely differing social, cultural, economic, management, and political perspectives. Therefore combining our collective experience and knowledge would provide inspiration to develop several innovative measures to establish the world's first such joint effort to benefit both African and Asian elephants.

The project brings together five conservation groups: the Sri Lanka Wildlife Conservation Society, Integrated Rural Development and Nature Conservation in Namibia, the Elephant Pepper Development Trust in Zimbabwe and the Okavango Elephants & People Research Project in Botswana in a unique partnership where we will work together to develop new models to mitigate human elephant conflicts. The project will create a platform to assess and discuss ideas in the field. One of the important outcomes would be the publication of a human elephant conflict field manual. This would be an illustrated guide drawn in the same way airline safety cards are: A simple easy to understand drawings with minimum text. The Field Manual will provide graphically illustrated information about HEC and how to address it. The Field Manual will be designed so that it can be easily adapted to regional conditions and languages. The guide will be translated into regional languages, and laminated to withstand the rigors of remote wilderness application. These field manuals will be distributed to areas most affected by human elephant conflict, and provide practical advice on how to lessen the frequency of HEC incidents such as safeguarding crops and property and protecting human life with minimum harm to elephants.

The seed money for this seminal project was provided by the International Elephant Foundation.

The Elephants and Bees Project: Using Bees as a Natural Deterrent for Crop-Raiding Elephants

Lucy E. King [1,2]* ,Iain Douglas-Hamilton[1,2] and Fritz Vollrath[1,2]
[1] *Animal Behaviour Research Group, University of Oxford*
[2] *Save the Elephants*

Increasing elephant populations in Kenya since 1989 have been widely praised as a conservation success story. However,where elephants and agricultural land overlap, incidents of human–elephant conflict are on the increase. Wildlife managers and farmers are now trying different farm-based deterrents to keep elephants out of crops. Here, we present data on the effectiveness of a novel beehive fence deployed in a Turkana community of 62 commu nally run farms in Kenya. Specifically, 1700-m of beehive fences semi-surrounded the outer boundaries of seventeen farms,and we compared elephant farm invasion events with these and to seventeen neighbouring farms whose boundaries were 'protected' only by thorn bush barriers. We present data from 45 farm invasions, or attempted invasions, recorded over 2 years. Thirteen groups of elephants approached the beehive fences and turned away. Of the 32 successful farm invasions, only one bull elephant broke through the beehive fences. These results demonstrate that beehive fences are more effective than thorn bush barriers at deterring elephants and may have a role to play in alleviating farmer–elephant conflict. Additionally,the harvesting of 106 kg of honey during the trial period suggests that beehivefences may also improve crop production and enhance rural livelihoods through honey sales.

Session II: Illegal Trade

Distinguishing Between Genuine and Imitation Rhinoceros Horns and Horn Artifacts

V.M. Blount, B.C. Yates, and E.O. Espinoza, *National Fish and Wildlife Forensics Laboratory*

The National Fish and Wildlife Forensics Laboratory assists U. S. Fish and Wildlife Service agents investigate criminal operations involved in illegally obtained rhino horns for sale in the global wildlife trade. This contraband includes raw horns, fragments of horns, carvings, and artifacts. Successful prosecution depends on correct taxonomic identification of each evidence item. Forensic protocols include morphological identification, genetic assessment, and keratin characterization via Fourier Transform Infrared Spectroscopy. Items are examined initially for probable cause using both covert and in situ photographic assessment documenting morphological details, and after seizure, physical and instrumental analyses are performed. Results of these analyses reveal trafficking of both black and white rhino horns, as well as an assortment of deliberate fabrications of rhino horn fakes.

Assigning the Provenance of African Elephants Using Mitochondrial DNA

Yasuko Ishida[1], Nicholas J. Georgiadis[2], Tomoko Hondo[1], Alfred L. Roca[1]
[1]*Department of Animal Sciences, University of Illinois*
[2]*Puget Sound Institute, University of Washington*

African elephant mitochondrial (mt) DNA follows a distinctive evolutionary trajectory. As females do not migrate between elephant herds, mtDNA exhibits low geographic dispersal. We therefore examined the effectiveness of mtDNA for assigning the provenance of African elephants (or their ivory). For 653 savanna and forest elephants from 22 localities in 13 countries, 4258 bp of mtDNA was sequenced. We detected eight mtDNA subclades, of which seven had regionally restricted distributions. Among 108 unique haplotypes identified, 72% were found at only one locality and 84% were country specific, while 44% of individuals carried a haplotype detected only at their sampling locality. We combined 316 bp of our control region sequences with those generated by previous trans-national surveys of African elephants. Among 101 unique control region haplotypes detected in African elephants across 81 locations in 22 countries, 62% were present in only a single country. Applying our mtDNA results to a previous microsatellite-based assignment study would improve estimates of the provenance of elephants in 115 of 122 mis-assigned cases. Nuclear partitioning followed species boundaries and not mtDNA subclade boundaries. For taxa such as elephants in which nuclear and mtDNA markers differ in phylogeography, combining the two markers can triangulate the origins of confiscated wildlife products. Published in Evolutionary Applications 6(2):253-265, 2013. Supported by USFWS African Elephant Conservation Fund Grant Number 0554-96200-0-G051.

Ivory and Rhino Horn: Legal Sale Versus Illegal Poaching

W.R. Allen
The Paul Mellon Laboratory

The unsustainably high levels of poaching of elephant and rhino blighting many African countries at present are driven by deeply rooted traditional pressures in China and other Asian countries for intricately carved ivory ornaments and an imagined pharmacological panacea. The present CITES listing of elephant and rhino in Appendix 1 effectively bans all international transport of, and trade in, ivory and rhino horn. Clearly it is failing badly and it fuels the existence of the ruthless middleman racketeer element which sustains the poaching industries at both the supply and user ends of the chain.

This paper reviews current methods and levels of poaching and illegal sale of ivory and rhino horn and it argues for a radical new approach to address the problem. First, dialogue with authoritative representatives of both the ivory carving and traditional medicine industries in China/Asia to determine realistic figures for present demand for both products and likely future requirements were they to become legally available at reasonable prices. Second, dialogue with high ranking CITES representatives to determine the theoretical and administrative difficulties of instigating an experimental, say 5 year, relaxation of the existing blanket ban on the sale and transport of ivory and rhino horn. Third, dialogue with senior and knowledgeable representatives of National Game Parks, Hunting Conservancies and Governments of the major sub-Saharan African countries affected by the current poaching storm, to list their present stocks of ivory and rhino horn, estimate the numbers of live “useable” elephant and rhinos surviving in their country and calculate how much of both products they might be able to sell sustainably. Fourth, discuss with appropriate authorities and governments the establishment of a pan-African tightly controlled and fully transparent marketing organization to regulate the legal sale of ivory and rhino horn at reasonable prices to reputable dealers in China and elsewhere, with equable return of income to the supplying countries and to their farmers and/or indigenous peoples who have produced or protected each species to enable such humane and sustainable sale of their products.

Can sufficient rhino be farmed commercially for their horn, along similar lines to the production of Red deer velvet in New Zealand? Could enough ivory be gathered from existing stocks, natural deaths and population control culling to meet reasonable demands of the user markets in Asia. Most important, could the proposed marketing organization be established and run well enough to knock the bottom out of and sink the illegal poaching industry?

Is Habitat or Food Plants Responsible for Rhino Poaching in Kaziranga National Park, Assam, India

Abhijit Rabha, *Additional Principal Chief Conservator of Forests, Department of Environment and Forests, KAAC, Karbi Anglong*
Bhrigu Prasad Saikia, *Research Scholar, Centre for Animal Ecology and Wildlife Biology, Department of Zoology, Gauhati University*

The Indian rhino has been listed as Vulnerable in the IUCN Red Data Book (2012), CITES Appendix-I species and Schedule-I species of the Indian Wildlife (Protection) Act, 1972 to ensure the conservation of this mega-herbivore. The Kaziranga National Park, Assam, has the highest population of Indian rhino, which are heavily threatened by poaching pressure.

There have been 115 Indian rhinos killed during the last 10 years in Kaziranga National Park (KNP), Assam, through the year 2012. The records suggest that poaching increases as the number of Indian rhinos increase within Kaziranga National Park. It has also been noted that most Indian rhinos are killed in the habitats along the boundary line of the KNP. Hence, the present study was conducted with the objective to evaluate the habitat utilization and feeding patterns of stray Indian rhinos in KNP during the year 2011-2012.

The representative habitats present along the boundary line of KNP have been identified and GPS data were collected for the classification of the habitat using ERDAS and ArcGIS 10 software. The habitat utilization pattern data were collected by using the 'dawn to dusk' scan animal and *ad libitum* sampling methods for each of the habitats utilized by Indian rhino. The feeding pattern study wjouas also conducted by 'dawn to dusk' scan animal and ad libitum sampling methods; data on the food plant species utilized during the time they strayed outside the KNP were also collected by direct observation.

It has been found that, the Asian elephant utilizes the grassland habitat in Kaziranga National Park but occupies the woodland habitat outside of park boundaries. In contrast, stray rhinos mostly feed in the grassland, utilizing habitats in a mosaic manner outside the KNP boundary. During the present study it was noted that the distribution of the stray rhinoceros depends on habitat and food availability. There was a strong association observed between the Indian rhino poaching sites and the presence of grassland and wetland in the surrounding areas. Habitat and food shortages lead to increased straying behavior of the Indian rhino in Kaziranga National Park, which negatively impacts Indian rhino conservation because they are more likely to get killed while visiting these grasslands outside the protected areas. However, annual floods also force the Indian rhino to come out of the protected boundary of KNP making it vulnerable to poaching.

Hence, for the conservation of the Indian rhino outside the boundary of the Kaziranga National Park, the grassland and wetland habitats need to be protected. The grassland should be managed properly to ensure food availability for the Indian rhino. The Indian rhino does not have any political or legal protection when it strays outside the KNP boundary to fulfill its ecological needs; therefore protection should expand to include areas beyond the KNP boundaries during times of ecological crisis like annual floods, food shortages, etc. when Indian rhinos are likely to stray outside the Kaziranga National Park.

Conserving African Elephants Through Walking and Talking Over 2500-km in Kenya:

Jim Nyamu

Kenya continues losing at least 7-10 elephants per week. In the year 2012, Kenya lost 450 elephants with most parts of the protected areas losing more than 3 elephants per day. At least 145 elephants have been poached this year and if the status remains by the end of the year, more than 300 elephants will be killed. In 1979 Kenya had 167,000 elephants that were drastically reduced to 16,000 by 1989. The population has gradually been increasing to 30,000 currently (2013) with threatening poaching and habitats degradation all over the country.

I have walked over 2,500-km across Kenyan coast, Rift Valley, Northern Kenya, Eastern and Central Kenya using "Ivory belongs to elephant" slogan. During the 64 days walk I had three objectives (1) Raising awareness on elephant poaching (2) Raising awareness on elephant values in regards to economic and ecological values and (3) Engaging local communities on how to mitigate human-elephant conflict. It's apparently clear that 78% (n-2030) people do not want to conserve our precious elephants despite the fact that 80 % of wildlife in Kenya is found outside the protected areas.

The walk brought together over 50 national and international conservation organisation who pledged to support the upcoming walk in East Africa in October 2013 as we establish community based conservation program.

Session III: *In situ* Population Dynamics and Conservation

Conservation Genetics of Greater One-horned Rhinos in India – from Counting Numbers to Determining Conservation Priorities

Udayan Borthakur, *Aaranyak*
Pranjal Kumar Das and Bibhab Kumar Talukdar, *Aaranyak*

Being a major flagship species in its range distribution and emerging from a catastrophic population decline in the past, the Greater One-horned Rhino (Rhinoceros unicornis) population management and conservation requires a multi-disciplinary effort, including the use of molecular tools for genetic monitoring of natural populations. In India, seven protected areas harbour more than 70% of the extant global population of this species. The contemporary populations of rhino are distributed across a landscape of fragmented habitat patches of protected areas, with restrictions to the natural movement and gene flow among them due to various land use patterns. This demanded an investigation on whether this distribution pattern facilitates any demographic and

genetic exchange of rhinos among the protected areas in India and whether any genetic management of these populations are required in order to ensure their future survival.

Keeping this in view, we have initiated genetic population monitoring of rhinos in 5 different protected areas of India, Viz. Kaziranga National Park, Orang National Park, Pobitora Wildlife Sanctuary of Assam and Gorumara National Park and Jaldapara National Park of West Bengal, with 98% of the total rhinos present in the country. Microsatellite markers were optimized to identify individual rhinos and Y-chromosome linked markers to identify gender from noninvasively collected dung samples, along with optimization of sampling techniques to meet the requirements of genetic studies. Using these techniques, we have successfully carried out genetic census of rhinos in Gorumara, while using the same techniques to carry out a wider investigation of genetic population structuring and gene flow among the protected areas.

The result of our genetic census of rhinos in Gorumara was in concordance with the hear-count census carried out by the West Bengal Forest Department, and we have further confirmed that the population has a skewed sex ratio and a comparatively low level of genetic diversity. Genetic monitoring based on dung DNA analysis revealed direct evidence of demographic exchange of rhinos among the protected areas of Assam. In overall, genetic monitoring of rhinos revealed moderate to high levels of genetic diversity spreading across the rhino bearing protected areas, with a high degree of structuring observed. We are of the opinion that the techniques of genetic monitoring adopted by us, with further developments on a robust sampling strategy, can be used for estimating population size of rhinos in all the protected areas in future. These genetic techniques may further be useful in forensic investigation of rhino poaching cases, which is on a peak in Assam for past few months. Further, our analysis of spatial distribution of genetic diversity of rhinos in India shows the need of restocking the West Bengal population, in view of the lack of natural connectivity with other source populations.

Revealing Cryptic Forest Elephant Behavior Through Acoustics and Thermal Imaging

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Andrea K. Turkalo[1,2] and Elizabeth D. Rowland[2]
[1]Wildlife Conservation Society; [2]Cornell University

A sufficient understanding of the ecology and behavior of forest elephants *(Loxodonta cyclotis)*, even for developing basic conservation strategies, has been difficult to achieve. These elephants spend the majority of their time in small, dispersed groups, or solitarily within rainforest habitats where direct observation is severely limited and dangerous. Although forest clearings often attract large numbers of elephants, observation at such sites has been limited by a number of factors, not the least of which is that elephants prefer to visit these clearings at night. Autonomous acoustic monitoring has proven valuable in both forest settings and at forest clearings for continuous tracking of relative abundance and to characterize patterns of both diel and seasonal activity. The addition of thermal imaging provides a means to probe nocturnal behavior and to interpret acoustic patterns for use in distributed monitoring situations.

This paper draws on seven years of acoustic monitoring to quantify patterns of forest elephant behavior that in some cases were suspected and in other cases unknown. In the forest, where these elephants spend close to 95% of their time budget, activity is nearly equally divided between daytime and nighttime periods, with significant but modest seasonal differences. However, activity in forest clearings is overwhelmingly nocturnal—assumed to be a response to the increased risk of being hunted in such sites. The proportion of all activity occurring at night, and changes in this proportion, could be valuable indicators of hunting pressure. Acoustic monitoring provides a metric of activity that is unbiased with respect to time of day, or phase of the moon, and should be implemented with or without observation teams at specific clearings. Change in the mix of acoustic signals produced by elephants in clearings at night suggests that different behaviors may be involved or perhaps a change in the mix of sex and age classes entering the clearing. A recent study that paired acoustic recording with thermal imaging confirmed that the number of elephants simultaneously in the clearing increased dramatically after dark, but that this increase was due disproportionally to female-calf groups. The number of adult males increased only slightly. Sexual behavior was an order of magnitude more frequent at night than during the day and was correlated with the previously detected change in vocalization types at night across multiple sites, providing the first indication that clearings may indeed function as mating hotspots.

Focus on Black Rhino *(Diceros bicornis bicornis)* Population Dynamics and a Formula for Successful Conservation of the Species 2002 - 2012

S.P. Downie; A. Mavrandonis
David Shepherd Wildlife Foundation

In 1989 South Africa had less than 20 *Diceros bicornis bicornis*. This project alone has helped quadruple that number. The Biodiversity Management Plan for the Black Rhinoceros in South Africa 2011 – 2020, (BMP) (Government Gazette, 25th January 2013), calls for 260 by 2020, and a longer term goal of 500.

This project, registered with South African National Parks, supports a number of strategic objectives of rhino monitoring and management by studying separate sub-populations in four smaller parks within their historical range. The accumulated data assesses and measures performance towards the BMP goals.

Although data is available for over 21 years, and the first founder population was introduced to one study area in 1999, regular ground monitoring commenced in 2002, and numbers have increased from 5 to 72 rhinos. All are individually identifiable. The objectives were to monitor developmental stages, study all performance indicators, assess dispersal into new habitats, determine success/failure of translocation strategies, study behaviour and develop genealogy.

The experience with Shibula, returned to southern Africa from Lisbon Zoo in 1991, has been an inspiration, was invaluable, and is comprehensively documented.

Monitoring involved locating, identifying and observing individuals using a combination of methods and techniques: telemetry, tracking spoor, searching from vantage points and once located, a spotting scope used to identify individuals by means of unique ear-notches.

Accomplished 130 field trips, over 1'000 days in the veld and over 1'200 hours observing rhino. Over 60'000 photographs taken and 90 hours of video filmed. On average, identified 2 at least 75% per field trip, located 100% each year, and during 80% of sightings ensured rhinos were undisturbed and therefore observed natural behaviour and social interaction.

Average annual growth rate was 15% over 14 years in one area with the most rhinos. The study highlights the problems of developing small populations, and the impact of management interventions. Population constraints were forecast and relocations recommended. As poaching reached record levels in 2010, emphasis moved to focus on safety and security, which led to the project initiating specific plans, supplying equipment, manpower and anti-poaching training.

This study highlights the success of undisturbed populations, careful unobtrusive monitoring and the importance of security. These four sub-populations will contribute significantly to the overall growth and survival of the sub-species, and exceed by far the growth targets set by the BMP. The project methods have proved to be a valuable formula for the future.

This monitoring and security project was funded by the David Shepherd Wildlife Foundation and ourselves personally.

Reproductive Parameters in Wild Asian Elephants in Southern Sri Lanka Estimated Through Individual-based Longitudinal Monitoring

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*[1]Colorado State University, [2]Elephant, Forest and Environment Conservation Trust, [3]Stirling University, [4]University of Colombo, Colombo, Sri Lanka, [5]Trunks & Leaves Inc., Somerville, MA, USA, *Presenting and corresponding author*

High-resolution demographic data on wild Asian elephants has been difficult to collect due to the habitat characteristics of much of the species' remaining range. Such data, however, is critical for understanding and modeling population processes in this endangered species. I present data from six years of an ongoing study of Asian elephants *(Elephas maximus)* in Udawalawe National Park, Sri Lanka. This relatively undisturbed population is individually monitored, providing cohort-based information on survival and fecundity. Reproduction was seasonal, such that most births occurred during the long inter-monsoon dry season and peaked in May. The average age at first reproduction was 11.4 years with an average inter-birth interval of 3.9 years. Birth sex ratios did not deviate significantly from parity. Fecundity was relatively stable throughout the reproductive life of an individual (ages 11-60), averaging 0.15 female offspring per individual per year. Mortalities and injuries based on carcasses and disappearances showed that males were significantly more likely than females to be killed or injured through anthropogenic activity. Overall, however, most observed injuries did not appear to be fatal. This population exhibits higher fecundity and density relative to other Asian elephant populations including those in captivity, possibly enhanced by present range constriction. Understanding the factors responsible for these demographic dynamics can shed insight on the future needs of this elephant population, with probable parallels to other populations in similar settings.

Determining Potential Environmental and Social Factors Affecting the Success of the Black Rhinoceros in Addo Elephant National Park, South Africa

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[1]Lincoln Park Zoo, [2]Addo Elephant National Park, [3]Western Kentucky University, [4]George Mason University

Factors, including intra- and interspecies conflicts, may make it more difficult to increase population numbers of the critically endangered black rhinoceros (rhino). Using non-invasive methods to study this elusive species can assist with determining factors that limit its success in the wild. Our goal was to determine the health and success of a population of southwestern subspecies of black rhinos (Diceros bicornis bicornis) in Addo Elephant National Park (AENP). Camera traps were placed on rhino middens to identify individuals and facilitate the collection of fecal samples to measure adrenocortical activity (via fecal glucocorticoid metabolites, FGM). Four camera traps were set up in two park sections, Addo's main campus (Addo; n=2 camera sites) and Nyathi (n=2 camera sites), which were separated by a highway and differ with respect to numbers of competitors, predators and tourists, which were higher in Addo than Nyathi. The photos and feces were used to investigate the relationships among season (wet vs. dry), number and sex of the rhinos that came to each site (n=4) and FGM concentrations. Additionally, GIS data from each site were used to determine the influence of landscape cover (xeric vs. mesic) and type of roads (staff vs. concession) on FGM concentrations. Fresh fecal samples (n=167) were collected, processed and sent to the United States for FGM analysis. Results demonstrate that individuals in Addo had higher ($P<0.001$) FGM concentrations compared to Nyathi rhinos. Specifically, one site in Nyathi had the lowest ($P<0.001$) FGM compared to the other three sites; however, these FGM values were not influenced by the number of different individual visitors to the sites, which ranged from 4 to 14 rhinos; nor by sex (ratio of females to males ranged from 0.33 to 3.0). In Addo, GIS data and type of roads did not influence ($P>0.05$) FGM values. However, neither of these factors differed among the camera trap areas in Nyathi (all were mesic and concession roads only); so comparisons could not be made. Irrespective of section, FGMs were higher ($P=0.05$) in wet versus dry season. Yet, the season did not seem to influence the number of rhinos at each site even the two sites with waterholes. In conclusion, our results may be limited by the camera trap placement on middens because the rhinos may be avoiding the highly disturbed areas (near tourists and heavily used roads) and other environmental pressures due to competitors and/or predators. However, overall, rhinos residing in Addo, the more disturbed section of AENP, did have higher adrenocortical activity suggesting interspecies conflicts may be negatively impacting the health of the population. Results can be used to assist AENP managers with improving the success of their rhino population.

White Rhinoceros Reproduction: Insights from the Wild and Semi-wild

Ronald R. Swaisgood, Shannon Chapman and Lisa Nordstrom, *San Diego Zoo Institute for Conservation Research*

Due primarily to poor reproduction among females born in captivtity (F1 generation) the southern white rhinoceros (Ceratotherium simum simum) captive population is not self-sustaining. We devised a detailed in-person questionnaire to evaluate histories of individuals reared in varying conditions in small South African game reserves that lie on a continuum between wild and captive, with the goal of determining how social environment, reserve size, and husbandry factors (during development and as adults) influence reproduction. We obtained interviews from 96 properties. For the individual-female data, 96% of F1's and 92% of F2's reproduced successfully. For population-level data, 92% of the respondents indicated that their F1's were reproducing. We also examined the reproductive rate of females and inter-birthing intervals, but found differences between F1 and F0 females did not approach significance. These data are markedly different from those in captive populations.

We also analyzed the effects of 9 independent variables that we hypothesized may influence rhino reproduction: (1) supplemental feeding intensity, (2) supplemental feeding frequency, (3) male social environment, (4) female social environment, (5) overall social density, (6) male social density, (7) female social density, (8) rainfall, and (9) property size. Analyses show that only two of these variables had significant effects: social density and supplemental feeding. Overall social density and female density had a significant effect, with female reproductive rates increasing with increasing density. This finding runs counter to the hypothesis that suppression by older females is reducing reproduction in F1 females in zoo environments; if dominance-mediated aggression and suppression were negatively impacting these semi-wild populations in South Africa, the opposite effect is predicted. Increased supplemental food was associated with higher reproduction. None of the independent variables had a differential effect on reproductive success between the generations (i.e., statistical interactions not significant), indicating that factors affecting reproduction affect both F1 and F0 females in the same way.

To evaluate possible developmental effects of the rearing environment, we examined the same 9 factors during the first 5 years of life. Our results mirror the adult statistics, with a positive relationship between rate of reproduction and social density measures, in contradiction to what would be expected if high densities during development negatively affected F1 reproductive success.

Some have hypothesized that zoos should keep more than one male to encourage reproduction in F1 females, but several populations in our study contained only a single male and had successful F1 reproduction. Thus, male-male competition and female mate choice are not prerequisites for reproduction, as has been suggested, and we should probably look elsewhere for better explanations.

Session IV: Reproduction I

Gestational Pattern in Elephants - Their Consequences for Pregnancy and Birthing Management

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Among our living terrestrial vertebrates, the three elephant species *(Elephas maximus, Loxodonta africana, Loxodonta cyclotis)* hold a unique position and differ in many ways from the general mammalian model. A fact that is most striking is in regard to their reproductive physiology. Elephants, with an average gestation of 21 months, have the longest pregnancy of all mammals. For this reason, their fetal development has always been of academic interest. Due to poor reproductive performance in captive elephant breeding programs caused by spontaneous embryonic resorption, late gestational abortion as well as fatal dystocia there is also a high demand for practical knowledge how to manage pregnant elephants from day first until birthing. In addition, the calculation of the birthing time by determination of gestational age creates often a significant challenge for the elephant staff and the zoo management. The extraordinary size of the animals and the exceptional female anatomy hampered the observation of pregnancies with conventional methods in the past. In comparison to other terrestrial mammalian species, early embryonic development appears delayed in elephants. Before 8 weeks, ultrasonographic detection of embryonic structures is impossible even with ultrasound systems generating resolutions below 1mm. After transient progesterone drop 8-9 weeks post-conception and subsequent recovery, an embryonic vesicle of about 10 mm in diameter can be detected followed 1 week later by the first differentiation of embryonic tissue. By contrast, embryos/foetuses in other mammalian species have reached sonographically measurable crump-rump-lengths (~40 mm cattle, ~70 mm in sheep, ~40 mm in horse) at this same stage of gestation. There are morphological evidences that elephants evolved from an aquatic mammal due to their specific embryogenesis. Using transrectal ultrasonography, the embryogenesis be can meticulously monitored. The implantation has been documented as central and superficial, with mesometrical orientation of the yolk sac and the embryo being antimesometrial in location. The elephant placenta is characterized as a Placenta zonaria endothelialchorialis forming a ring of villious tissue, which is anti-mesometrically divided into either three or four major placental islands. The use of the new 3D-ultrasound technique can expand our ability to characterize fetal development by creating near life-like images. The organogenesis is estimated to be finished at around 125 days of age when the transition from embryo to fetus takes place. At that stage the fetal length is approx. 70 mm. Hormonal fetal sex determination becomes possible in Asian elephants by measuring the maternal testosterone levels in the peripheral maternal blood serum with a gestational stage of 12 months. Ultrasonographic sex determination in elephants like common practice in horses and cattle has been not successfully applied yet. Gestational luteal activity in elephants can be monitored based on the analysis of progestins and its metabolites in blood, urine and feces. There is a broad range of individual variation in gestation length (623 – 729 days in the Asian and 640 – 673 days in the African elephant), making an individual prediction of parturition necessary. This can be done on the basis of the detection of functional luteolysis resulting in a decrease in progestin secretion usually

occurring 2-5 days prior birth. Besides the analytical approach, the upcoming birth can also be predicted by daily transrectal ultrasound assessments. The frequent perinatal examinations requires, conditioning of the pregnant cow including rectal palpation and to foreign people as well as technical equipment of at least 6 months prior to the calculated birth date. The ultrasound examinations can be performed in direct and protected contact settings. The presentation will demonstrate a variety of data collected during routine ultrasound examinations in pregnant wild and captive elephants combined with pregnancy associated endocrine profiles and the corresponding gestational disorders observed at specific pregnancy stages.

Relationship of Salivary Hormone Concentrations to Urinary Hormone Excretion Profiles in the Indian Rhinoceros *(Rhinoceros unicornis)*

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Improperly timed introductions for mating can lead to severe aggression between male and female Indian rhinos. To minimize this risk, pairings or artificial insemination (AI) must be timed to ovulation as accurately as possible. Currently, urinary concentrations of estrogen conjugates (EC) and progesterone metabolites (PdG) are used to monitor ovarian function and time breeding or AI. Collecting clean urine samples consistently can be a major challenge with some rhinos, and has limited the number of individuals for which hormone monitoring can be used. Reproductive hormones can also be detected in saliva, and have been previously measured in a female Indian rhino. However, before saliva can be used as the sole biological sample for monitoring ovarian function in this species, it must be proven to be reliable, accurate and reflective of urinary hormone profiles.

Matched urine and saliva samples were collected from two multiparous female Indian rhinos (#137, #189) and analyzed using enzyme immunoassay (EIA) techniques. Samples were obtained during morning hours and frozen (-20°C) until analysis. Urinary EC (R522), PdG (R13904) and cortisol (R4866) concentrations were indexed by creatinine. Saliva samples were centrifuged (1500g) and the supernatant analyzed for testosterone (R156/7) and cortisol. A portion of salivary supernatant was ether extracted, concentrated and analyzed for progesterone (P4; Salimetrics, State College, PA). Three estrous cycles were monitored per female. Female #137 was paired for natural breeding during one estrous cycle, and female #189 had a non-anesthetic AI performed on each cycle. An ovulation inducing agent (Sucromate™; 2.1cc IM) was administered during two estrous cycles in female #189. Transrectal ultrasonography was performed (#189) to monitor follicular growth and verify ovulation. Ultrasonography was also employed (#137, #189) 21 days post-breeding/AI to diagnosis pregnancy. Urine and salivary steroid results were aligned with day 0 corresponding to day of behavioral estrus or day of sucromate injection. Pearson product moment correlation coefficients were calculated between urinary and salivary hormone concentrations.

Matched values of urinary EC and salivary testosterone were correlated throughout the estrous cycle ($r=0.815$; $P<0.05$). Profiles of urinary PdG and salivary P4 were also highly correlated ($r=0.804$; $P<0.05$). Despite the fact pooled saliva produced a displacement curve parallel to the standard curve for cortisol ($r=0.99$; neat to1:8), salivary cortisol concentrations did not correlate ($r=0.058$; $P=0.698$) to urinary concentrations of cortisol. Urinary cortisol has been validated in this species using the R4866 antibody, but it appears additional research is needed to produce reliable salivary cortisol results. As a by-product of this study, we determined a cyclic variation in urinary cortisol concentrations occurs during the Indian rhino estrous cycle. Concentrations of urinary cortisol peaked on day 0 (45.14 ± 7.96 ng/mg Crt). Urinary cortisol and EC were positively correlated ($r=0.681$; $P<0.05$) throughout the estrous cycle, while urinary cortisol and PdG were negatively correlated ($r=-0.398$; $P<0.05$). Although successful ovulation was documented in female #189, only female #137 became pregnant. Results from this study demonstrate that Indian rhino saliva samples yield temporal patterns of testosterone and P4 profiles consistent with urinary EC and PdG.

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Attempt to Control Estrus and Ovulation in White Rhinoceros Using a Synthetic Progestagen and Slow-release GnRH Analogue

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Breeding in captivity may play an important role in the survival of the conservation-dependent white rhinoceros *(Ceratotherium simum)*. However, the overall reproductive rate in capt ivity is too low, and the captive population is not self-sustaining. One related factor is the inexplicable observation at many institutions of an aberrant cycling pattern in females. In order to increase breeding success in captivity, it is important to determine the direct causes of estrous cycle irregularity and also to develop an estrus and ovulation synchronization protocol that allows timed natural breeding and assisted reproduction. Several studies have involved an attempt to induce estrus in the white rhinoceros, but ovulation using those protocols was inconsistent from female to female. This study utilized a progestagen treatment followed by a slow-release GnRH analogue to synchronize estrus and ovulation among 2 southern white rhinoceros. Oral synthetic progestagen (altrenogest, 0.022mg/kg/d) was administered for 21 days after a random start, followed by a single injection of a synthetic slow-release GnRH analogue (deslorelin acetate, 2.5 µg/kg) 9.5 days after discontinuing progestagen treatment. Treatment success was determined using enzyme immunoassay of daily fecal samples and behavioral observations. During the protocol, females were successfully introduced to a bull. Although no mating or estrus behavior was detected during or after treatment, fecal hormone analysis did show synchronized luteal activity in the females. Interestingly, this synchronization did not appear to be the result of the progestagen and GnRH protocol, and other causative factors should be considered.

Correlation Between Serum Progesterone Levels and Luteal Blood Flow in Asian Elephants *(Elephas maximus)*

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Asian elephants *(Elephas maximus)* are one of the most endangered species of animals in the world with less than 30,000 remaining in the wild. An aging captive population along with difficulties in captive breeding has created a need for a better understanding of the Asian elephant's unique reproductive system. Elephants are unique in the fact that they are mono-ovulatory animals yet present multiple corpus lutea both during regular cycling and pregnancy. They also present two luteinizing hormone peaks a feature only found in elephants. Their main excreted progestagen is not progesterone as in most other mammals but it's 5-alpha-reduced metabolites (5-alpha- Pregnan-3,20-dion and 5-alpha-Pregane-3-ol-20-one) that are secreted by the CLs into the peripheral circulation They also possess the longest cycle out of any other land mammal approximately 13-16 weeks. The sequence of events that occur during the elephants' reproductive cycle has been clearly identified. However the specific mechanisms that control these events are still mostly unclear. The one important question that still remains unanswered is: what mechanism controls the luteolytic process? From previous studies blood flow/ vasculature has been identified as one possible mechanism for controlling the luteolitic cycle. Previous research in other species has shown that changes in plasma progestagen changes do not coincide with observed changes in luteal structure. The purpose of this study was to perform a descriptive study that characterizes the relationship between luteal blood flow in growing and degrading CLs with the corresponding changes in plasma progestagen levels using color flow Doppler ultrasonography in Asian elephants. The study subjects consisted of six female Asian elephants ranging in age from 14 years to 50. The subjects were ultrasounded on a weekly basis for almost a year and a half. OvCL's had larger amounts of vasculature than acCL's (25+/-7%, 17+/-5%; t=2.928, df.30) but the number of days post ovulation at which the maximum blood flow occurred did not differ (28.81+/-15.22, 34.88+/-22.05; t=-0.905, df.30). The average maximum progestagen concentrations were 3.56 with a very large standard deviation of 3.83 and occurred at 29+/-10.84 days post ovulation. The ovulatory ovary had a higher maximum level of blood flow than the non-ovulatory ovary (21.+/-3.8%, 15.1+/-4.74%; t= 4.480, df.30). The reproductive cycle was divided in to the follicular and luteal phases and correlation between parameters were not detected. The luteal phase was subdivided into its three components of growth, mid, and regression phase. Within these subdivisions no consistent correlations excited. This study has shown that despite the fact that luteal blood flow, luteal area, and circulation progestagen follow similar distinct cycle patterns, no true correlation exist among the three parameters. It has shown that there are vascular differences between ovulatory ovaries and non-ovulatory ovaries that might suggest a role of ovarian vasculature in dominant follicle selection and ovulation.

Incidence of Reproductive Tract Leiomyoma in Indian Rhinoceroses

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Cited in husbandry guidelines and veterinary textbooks reproductive tract leiomyoma appears to be common in Indian rhinoceros. However, data on the incidence of leiomyoma in the captive population is scarce. In this study 15 female Indian rhinoceros 18.3 ± 2.4 years of age were assessed by ultrasound for their reproductive health and the incidence of reproductive tract tumors. Ten proven, four non-proven and one sub adult were examined. Proven breeders had reproduced their first offspring at 12.1 ± 1.1 years of age. Ultrasound assessment in proven breeders was conducted 7.5 ± 2.6 years after giving birth for the last time. The incidence of leiomyoma in proven and non-proven breeders >12 years of age was 100%. Average number and maximum size of tumors found was 15.4 ± 3.9 and 5.1 ± 1.1 cm, respectively. There was no significant difference in number or size of tumors between proven and non-proven breeders. However the incidence of tumors correlated strongly with age (r= 0.8411). Irrespective of the reproductive performance female Indian rhinoceroses seem to develop reproductive tumors during their lifetime. If leiomyoma development is inevitable in female Indian rhinoceros it is concluded that fecundity can be increased only by breeding this species at the earliest age possible. This conclusion is supported by data from the international studbook.

Session V: Veterinary Care

Manual Restraint and Chemical Immobilization With Xylazine/Ketamine of Wild And Captive Sumatran Elephants *(Elephas maximus sumatranus)* Under Field Conditons

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Conducting medical procedures in elephants often requires manual restraint or chemical immobilization. In modern zoos, sophisticated restraint devices have been developed for manual restraint and a high variety of different tranquilizers are available to achieve reliable and safe sedation and immobilization of elephants in these facilities. But more than 90% of captive Asian elephants live in the Asian range countries, where such sophisticated restraint devices do not exist, and many tranquilizers are not available due to legal restrictions, financial, and logistics limitations.

In Sumatra, as in many other range countries, safety during medical procedures relies on basic traditional tools and techniques for manual restraint of elephants. This includes different kinds of fore and rear foot hobbles, neck and body tethers made from different materials such as ropes, chains, and rattan, and the use of basic "homemade" restraint devices or just tree trunks as tether points. To properly apply restraint methods, the experience of mahouts is

very important and veterinarians have to ensure that restraint devices are used and fitted without causing injuries to the elephants. Well trained koonkie elephants are an important "tool" often used to properly restrain and handle wild or untrained elephants. The effectiveness of a koonkie depends very much on the level of training and experience of the individual elephant and its mahout.

For chemical immobilization the only drugs reliably available on the market in Indonesia are Xylazine and Ketamine. These drugs have been proven to be sufficient for reliable standing sedation in captive and wild Sumatran elephants for different needs such as: light standing sedation for transportation, tusk trimming and wound treatment in unreliably trained animals, deep standing sedation for some basic simple surgical procedures like tail amputations and removal of tumors in combination with local anesthetics in captive elephants, and, in wild elephants fitting GPS collars, treatment of injuries, and translocations. Dosages used vary depending on the condition of the elephant and the level of sedation to be achieved.

In captive elephants we use dosages of 0,08 – 0,15mg Xylazine/kg BW combined with 0,03 – 0,06 mg Ketamine/kg BW by i.m. or i.v. injection. If prolonged sedation is needed in cases of time consuming treatments and surgery, a second injection with 1/3 to 1/2 of the initial dose can be administered about 60 to 90 min after the first injection, and in cases of light sedation (i.e. for transporting untrained elephants) about 2-4 hours after the first injection.

In wild elephants, dosages of 0,16 – 0,36 mg Xylazine/kg BW combined with 0,08 – 0,14mg Ketamine are used in cases of capture for translocation, fitting GPS collars, and treatment of injuries. In some cases 30 to 45 min after the initial injection, a second injection with dosages of 0,06 – 0,2 mg Xylazine/kg BW and 0,02 – 0,07 mg Ketamine/kg BW have been administered to achieve adequate tranquilization. Yohimbine is sometimes used as reversal about 45 to 75 minutes after the administration of the tranquilizer with a dosage of 0.05 – 0.11mg /kg BW.

Standing Sedation in Sumatran Elephants *(Elephas maximus sumatranus)* Using Detomidine and Butorphanol

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Standing sedation was provided for comprehensive health and reproductive assessment and for minor clinical procedures in captive Sumatran elephants *(Elephas maximus sumatranus)*. 33 (4, 29) animals were investigated within three days (11 procedures/day). The elephants (body weight ranged from 650 to 2.800 kg) were managed in free contact hence save access to ear veins was possible in all animal, except in one aggressive bull. However, none of the animals were conditioned to receive transrectal ultrasonography. Therefore standing sedation was imperative. An initial hand-injection of detomidine hydrochloride and butorphanol tartrate (mixed syringe) was administered intravenously (i.v., n=22) with a dosage of 10 - 40 mg/animal (10 - 25 µg/kg, mean 15 µg/kg) and 20 - 50 mg/animal (14 - 60 µg/kg, mean 21 µg/kg), respectively. The initial injection resulted in adequate sedation for initiation and completion of all procedures. No supplemental doses were required. The aggressive bull, not accessible for intravenous injection, received initially 60 mg (34 µg/kg) detomidine and 60 mg (34 µ/kg) butorphanol intramuscularly (i.m.) and a supplemental injection i.v. of 10 mg (5.7 µg/kg) detomidine and 20 mg (11.4 µg/kg) butorphanol after 15 min. Maximal effect occurred at 5 and 20 min after i.v. and i.m./i.v. application, respectively. All animals did not move at all and were standing steady with head down, trunk and tail 100% immobilized while they were able to keep balance without signs of ataxia. From the author's experience from other Asian elephants (not included in this study) ataxia was observed with a dosage >25 µg/kg detomidine in combination with butorphanol > 40 µg/kg body weight. No cardiac or respiratory depression was appreciated. Reversals were applied 30 - 40 min after initial administration of sedatives. They were injected two-third and one-third intravenously and intramuscularly, respectively. Application of atipamizole (10 - 30 mg/animal, mean 8 µg/kg, SD 4 µg/kg) and naltrexone (12.5 - 50 mg/animal, mean 18.3 µg/kg, SD 8.1 µg/kg) resulted in rapid and complete recovery within 2 to 15 min. Animals diagnosed with kidney failures sonographically showed slower recovery (29 to 30 min). In contrast to former results were the authors used xylazine and yohimbine intravenously for short term standing sedation in Asian elephants in Thailand no adverse side effects on gastrointestinal tract (e.g. anorexia, abdominal distention or bloat) could be observed this time. The combination of detomidine and butorphanol is highly recommended for standing sedation in Sumatran elephants. In comparison to intramuscular administration of the sedatives total dosage and induction time was reduced when detomidine and butorphanol was administered intravenously.

Urinary Hormone Concentrations and Pharmacokinetics/pharmacodynamics of Haloperidol in a Female Indian Rhinoceros *(Rhinoceros unicornis)*

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Long-acting neuroleptics (LAN) are frequently used during immobilization and transport of rhinoceroses. A single administration of LAN reduces transport stress and aids boma acclimation in wild African black rhinoceroses. Long-term use (>3 weeks) of LAN's have been reported in several non-domestic species, but have not yet been examined in the rhinoceros. The goal of this study was to assess effectiveness of the long-term use of the LAN haloperidol to acclimate a 6.5 year old female Indian rhinoceros to novel stimuli. Haloperidol is unique in that it can be administered orally. Specific objectives included validating a urinary haloperidol assay to determine pharmacokinetics and pharmacodynamics over time and at different dosages. Urinary adrenal and gonadal hormone profiles were compared before and during treatment. Finally, behavioral correlates related to public exhibition and handling for reproductive assessment (transrectal ultrasonography) were evaluated.

Oral dosing of haloperidol was accomplished by inserting tablets (10mg) into a banana and hand feeding to the rhinoceros. During the first 50 days of treatment, the rhinoceros received 50mg PO. Thereafter, dosage was increased to 80mg PO. After 203 days, dosage was tapered for 34 days to

complete discontinuation of treatment. Urine samples were collected daily. Urine haloperidol was measured using a commercially available enzyme-linked immunoassay (Neogen, Lexington, KY). The assay was validated for Indian rhinoceroses by analyzing urine from two untreated and one treated female (80mg PO). Samples were assayed in duplicate against blanks, a standard curve (0.008-5ng/mL) and controls. Urinary estrogen conjugate (EC; R522) and progesterone metabolite (PdG; R13904) concentrations were measured to monitor reproductive activity. In addition, urinary cortisol (R4866) was measured to reflect adrenal function.

No extrapyramidal side effects were noted during the 240 days of treatment. We found no difference (P=0.16) in background concentrations (0.76 + 0.01 ng/mg Crt; 0.13 + 0.01 ng/mL) of haloperidol between Indian rhinoceroses and both were similar to background values reported in equine urine. Serially diluted urine from the treated female (80mg PO) demonstrated parallelism (r=0.99) to the haloperidol standard curve. There was no difference (P=0.32) in urinary haloperidol concentrations between 50mg (1.98 + 0.22 ng/mg Crt) and 80mg (3.08 + 0.19 ng/mg Crt) dosages. Both treatments were higher (P<0.05) than background levels. A dose dependent excretion effect (P<0.05) was observed during dosage decline, with urinary haloperidol concentrations averaging 4.07 + 1.08, 2.08 + 0.25 and 0.93 + 0.35 ng/mg Crt at 60, 40 and 20mg PO. Concentrations returned to background levels within 2 weeks of treatment ending. Of four estrous cycles that occurred during treatment, the female successfully ovulated once, which was comparable to previous reproductive outcome in this individual. Urinary cortisol concentrations during treatment averaged 50.37 + 1.63 ng/mg Crt and were higher (P<0.05) than those excreted 149 days prior (36.68 + 2.20 ng/mg Crt). A positive correlation (r =0.15; P<0.05) between urinary EC and cortisol was observed, underscoring the need to concurrently evaluate adrenal and gonadal hormone results in this species. This is the first data with regard to urinary pharmacokinetics/pharmacodynamics of the long-acting neuroleptic haloperidol in the Indian rhinoceros.

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Current Studies on Molecular Mechanisms of Iron Homeostasis in Rhinoceroses

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Iron storage disease (ISD) is a hazardous and clinically underappreciated condition commonly acquired by exotic wildlife species when displaced from their natural habitats and confined for even short periods under artificial conditions.

An international symposium recently reviewed and validated evidence that African black and Sumatran rhinoceroses invariably develop progressive ISD commensurate with their times in captivity, whereas African white and Indian rhinoceroses do not (1). Since vulnerability to ISD is a species-wide characteristic, it is likely to have a genetic basis possibly reflecting evolutionary adaptions to differences in iron bioavailability between browser and grazer diets.

As a biologically essential element that is also highly toxic in excess, iron is exquisitely regulated by molecular mechanisms primarily focused on interactions between the peptide hepcidin, (the principal iron-regulatory hormone), and its receptor ferroportin, (the sole channel for egress of intracellular iron into plasma) (2). Iron-regulatory gene sequences from both ISD-susceptible and non-susceptible species were compared to search for possible molecular differences. DNA was extracted from peripheral blood samples from all four available rhinoceros species, and genes encoding hepcidin and ferroportin, as well as modulators hemojuvelin, transferrin receptor 2, and HFE protein, were cloned and analyzed by PCR amplification. Over half of the DNA sequences of these five genes have now been determined without identifying any that could account for disparities in iron loading among the species. Evaluation of the remaining sequences continues, as do studies to determine the responsiveness of rhinoceros ferroportin to hepcidin modulation and quantitative levels of hepcidin expression (3).

In addition, liver and spleen mRNA sequences from African black and white rhinoceroses were assembled using Trinity RNA-Seq software (4) and compared with human sequences using the SIFT algorithm (5). Candidate single-nucleotide polymorphisms were independently validated by genomic sequencing. Mutations were found in four genes that may be associated with primary iron disorders or hemolytic anemia in black rhinoceroses: SLC28a2, EPB41, MTF1, and STEAP4 (6). The functional consequences of these mutations are being determined.

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Issues of Elephant Health Care Management in Myanma Timber Enterprise (MTE) Myanmar

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Currently over 5000 captive elephants still belong to Myanmar; most of these elephants are owned by the government, and some are privately owned. In Myanmar the elephant situation is not so bad compared to our neighbor countries such as India and Thailand. Although, Myanma elephants are still utilized for logging in the forest, most Myanma elephants can live and spend their time freely during resting periods. On the other hand, according to MTE recorded data, the annual elephant death rate is higher than the annual birth rate. Working elephants in MTE are occasionally faced with work related injuries, wounds, swelling on the lower shoulder area, and tumors and fibrosis. According to fiscal year records of MTE, most old elephants die of malnutrition and old age, accidental cases, and a few from suspected infectious diseases. From 2011 to 2013, we notified that some baby elephants (less than12 yr old) died with sudden death. The symptoms of these young elephants before death and necropsy findings after death were similar to those from elephant herpes virus. As prevention for bacterial infectious diseases, MTE elephants have to be inoculated with two kinds of vaccines: haemorrhagic septicemia vaccine (killed vaccine used in cattle) and anthrax vaccine (live spore vaccine special for elephants which is produced in Myanma Livestock Veterinarian Department). Haemorrhagic septicemia vaccine is inoculated twice a year, and anthrax vaccine is inoculated once a year. Under MTE, there are 14 Region and State sub-departments. Under these 14 Region and State sub-departments, there are 43 agencies. Therefore elephants are distributed all over the country. But MTE has only 41 vets especially for elephants; they are veterinarians and para-veterinarians. The responsibilities of vets are not only in elephant health care management but also for wild elephants such as driving back to the forest to solve the problem of human-elephant conflict, and wild elephant translocation projects. The main causes of death of elephants in MTE, Zoos, and private owners are old age and malnutrition deficiency during summer season. The other causes are heat stroke, snake bite, accidents, diarrhea, parasitic infestation, malnutrition, bloat, bacterial infectious diseases, and illegal killing.

Update from the Stakeholders Task Force for the Management and Research Priorities of Tuberculosis for Elephants in Human Care

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There is historical and current evidence that elephants are susceptible to infection by MTB complex. However, only since 1996 have the elephant display and veterinary communities worked closely with the U.S. Department of Agriculture (USDA) to develop protocols for testing and treating elephants infected with MTB, and developed research priorities to learn more about potential risks and possible MTB transmission pathways (i.e. animal to animal, human to animal, and animal to human). There has also been an emphasis on putting the issue in context from both an animal and human health perspective.

In April of 2011, the USDA hosted a seminar at the Animal Welfare Information Center (AWIC) in Kansas City entitled: TB in Elephants: Science, Myths & Beyond. The meeting focused on issues of MTB in elephants, risk of transmission between elephants and to humans, and the role of new serological tests (commonly referred to as ElephantTB STAT-PAK® and MAPIA) in the detection of MTB in elephants. Presenters included representatives from USDA, NIOSH, CDC, University of Illinois, University of Georgia, Colorado State University, AZA, the Elephant TAG, Ringling Bros., the Tennessee Department of Health and several elephant researchers and veterinarians. Focus of the general discussion centered on issues related to diagnosis, treatment, and risk analysis.

At the conclusion of the seminar, Dr. Chester Gipson, Deputy Director of USDA-APHIS/Animal Care, encouraged interested stakeholders to further discuss the issues based on science and not politics and suggested a "stakeholders" meeting to further explore many of the issues raised over the two-day meeting, and to identify next steps.

The primary goal of the Management and Research Priorities of Tuberculosis for Elephants in Human Care Workshop is working with all elephant stakeholders to communicate and share what is known about tuberculosis in elephants and to focus on the science, treatment, research needs and regulatory issues associated with this disease.

Testing for Tuberculosis in Elephants: What is the Evidence?

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Infection with *Mycobacterium tuberculosis* in elephants is a source of concern, due to the organism's potential impacts on public health, animal welfare, elephant conservation, and economic consequences. Diagnosis of tuberculosis infection in elephants has been difficult due to the insidious nature of the disease, and diagnostic limitations based on the animal's size and various ante mortem testing methods. Accurate detection of tuberculosis infections

in elephants will be an important factor in understanding the epidemiology and controlling the disease. Diagnosis is dependent upon the accuracy of diagnostic tests for this agent in elephants. There is controversy regarding the utility of currently available tests, particularly serology, and their proper role in the management of elephants with *M. tuberculosis* exposure or infections. Tools for performing systematic reviews have been developed for resolving similar controversies in the medical care of humans. We performed a systematic review of literature pertaining to diagnosis and prevalence of *M. tuberculosis* in captive and wild elephants. Our systematic review employed four different, but not mutually exclusive, review criteria for assessing the strength of study design and validity of results for each publication. This evaluation identified a number of weaknesses and limitations, including: small sample sizes; combined analysis of different host genera; incomplete differentiation of *M. tuberculosis* from *M. bovis* and non-Tb *Mycobacterium* species; the limitations of the current "gold standard", the triple sample trunk wash; and other study design flaws.

The degree to which the study design flaws that we identified affect the performance of diagnostic tests in target populations requires further investigation. Important concerns include the influence of disease prevalence on positive and negative predictive values (PPV and NPV, respectively) of diagnostic test results. For instance, the PPV decreases as the prevalence of disease decreases. Consequently, studies of elephant populations with artificially high prevalence may provide over-estimates of PPV for a given test. For populations where the prevalence of disease is low, even diagnostic tests with high specificity can yield unacceptably high numbers of false positives. Similarly, high disease prevalence populations may have low test NPVs and false negative results that are unacceptable. Failure to recognize the impact of prevalence on test results can result in incomplete identification of infectious animals, over-diagnosis, increasing treatment and management costs, and/or inappropriate euthanasia.

Many of these study design limitations are difficult to remedy, but must be acknowledged as a part of assessing risks of tuberculosis in individuals and populations of elephants. Candid and balanced assessments of diagnostic tests increase the likelihood that management strategies that are based, in part, on diagnostic test results will be effective for managing tuberculosis in elephants.

Point Prevalence and Incidence of *Mycobacterium tuberculosis* Complex in Captive Elephants in the United States of America

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Captive elephants infected with tuberculosis are implicated as an occupational source of zoonotic tuberculosis. However, accurate estimates of prevalence and incidence of elephant tuberculosis from well-defined captive populations are lacking in the literature. Studies published in recent years contain a wide range of prevalence estimates calculated from summary data. Incidence estimates of elephant tuberculosis in captive elephants are not available. This study estimated the annual point prevalence, annual incidence, cumulative incidence, and incidence density of tuberculosis in captive elephants within the USA during the past 52 years. We combined existing elephant census records from captive elephants in the USA with tuberculosis culture results obtained from trunk washes or at necropsy. This data set included 15 years where each elephant was screened annually. Between 1960 and 1996, the annual point prevalence of tuberculosis complex mycobacteria for both species was zero. From 1997 through 2011, the median point prevalence within the Asian elephant population was 5.1%, with a range from 0.3% to 6.7%. The incidence density was 9.7 cases/1000 elephant years (95% CI: 7.0-13.4). In contrast, the annual point prevalence during the same time period within the African elephant population remained zero and the incidence density was 1.5 cases/1000 elephant years (95% CI: 0.7-4.0). Accurate and species specific knowledge of prevalence and incidence will inform our efforts to mitigate occupational risks associated with captive elephants in the USA.

Session VI: Veterinary Care - Viruses

First Evidence Of EEHV Infection In Sumatran Elephants *(Elephas maximus sumatranus)* in Indonesia

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We describe the first time an incidence of EEHV infection and mortality in the Sumatran subspecies of the Asian elephant *(Elephas maximus sumatranus)* in Indonesia.

In 2005, a captive born elephant calf died at an age of about 2 years after a short illness lasting less than 24 hours, with unclear symptoms including anorexia, drowsiness, and some mild ataxia. Major gross pathological lesions observed internally were edema of the head and cyanosis of the tongue externally, and massive hemorrhage and petechiae on the heart, liver, lymph nodes, stomach, intestinal mucosa and sub cutis. Furthermore the liver was enlarged, the mesentery edematous, and large amounts of clear yellowish serous fluids were seen in the peritoneal and pleural and thoracic cavities and pericardial sac. At that time no proper laboratory facilities were available to run appropriate tests for infectious diseases. Discussing these findings later on

with international experts and the frequent appearance of small ulcers and blisters on the mouth mucosa of several adult elephants in several locations in Sumatra raised a suspicion of EEHV.

Seven years later, in April 2012, in a different location in Sumatra, 2 captive born calves died suddenly only 6 days apart, after showing no or very mild signs of discomfort only a few hours before death. Post mortem lesions were almost identical to the case from 2005. Two sets of tissue samples from all organs with pathological changes, and were collected and preserved in 96% ethanol and deep frozen at -20 C. Due to a lack of diagnostics for EEHV in laboratories in Indonesia the samples could not be tested immediately. About 6 months after collection of the samples a specific laboratory facility for molecular diagnosis of EEHV were established in Bogor. DNA was extracted from both frozen and alcohol preserved samples from heart, spleen and liver. Both cases were identified as EEHV 1 by conventional diagnostic PCR for PAN-EEHV POL and EEHV1-specific POL. These were then subjected to detailed gene subtype DNA sequencing at three key PCR loci, U38/POL, U51/vGPCR, U60/TER. These two cases have identical EEHV1A DNA sequences to one another indicating a common epidemiological source.

Clinical Signs, Diagnosis, and Treatment of the First Clinical Case of EEHV3B in an Elephant

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Elephant Endotheliotropic Herpesviruses (EEHVs) are ubiquitous in both Asian *(Elephas maximus)* and African *(Loxodonta africana)* elephants. While it is well recognized that some EEHV types such as EEHV1 can cause lethal hemorrhagic infections in juvenile Asian elephants, there is comparatively little information on the ability of the other EEHVs to cause disease, especially in African elephant calves. Here we report for the first time that EEHV3 can cause significant clinical disease in a juvenile African elephant. A 5 year old male African elephant presented with mild stiffness in the front leg, lethargy, and reduced appetite. A complete blood count (CBC) revealed leukopenia with a left shift, mild anemia, and high numbers of platelets. Treatment was started with oral famciclovir, as well as oral and rectal fluids. Polymerase chain reaction (PCR) revealed Elephant Endotheliotropic Herpesvirus (EEHV) 3 in whole blood taken at the time of clinical presentation, and genotyping identified the virus as EEHV3B. Quantitative PCR showed viremia as high as 100,000 VGC/ml of blood, which slowly decreased to undetectable levels over the following 35 days of treatment. During the course of the disease, the white blood cell count rose from the initially low level on day 2 to an elevated level until the third week, when the value returned to the animal's normal range. Concentrations of acute phase proteins (serum amyloid A and C-reactive protein) and beta globulins rose initially as well before returning to normal levels after several weeks. During the second week, the calf developed a single vesicle on the tongue and edema on the top of the head, which resolved over the following two weeks. Therapeutic intervention at the first sign of disease, as well as frequent monitoring of CBC, urinalysis, protein electrophoresis (EPH), and acute phase proteins are likely to have contributed to the success of this case.

Seven Species of Elephant Endotheliotropic Herpesviruses (EEHVs) Form a Novel Mammalian Subfamily the Deltaherpesvirinae

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Extensive genetic analysis of elephant endotheliotropic herpesvirus (EEHV) genomes from infected Asian and African elephants has both greatly clarified the evolutionary relationship between the Proboscivirus genus and other mammalian herpesviruses and revealed multiple distinct species and subtypes. In particular, the complete 177-kb genome of the most highly pathogenic species EEHV1A(strain Kimba) had been compiled from infected Asian elephant necropsy tissue. This data has revealed that 64 of the total of 115 genes are novel , including 20 members of the vGPCR family, five IgFam genes, two captured cellular glycosyl tranferases and three vOX2 genes. Analysis of another two strains each of EEHV1A, EEHV1B and EEHV2 (totaling 60 to 65-kb each), as well as three strains of EEHV5 and two of EEHV6 (totaling 25 to 30-kb each) have revealed that all five AT-rich branch EEHV types (which diverge from each other by 15 to 20%) have an inversion of a 40-kb core segment of the genome relative to betaherpesviruses. Three other species EEHV3, EEHV4 and EEHV7 (4-kb each) are even more highly diverged (35%) and form a distinct GC-rich branch of the Proboscivirus genus. The AT-rich branch EEHVs also all encode CPK genes, plus alphaherpesvirus-like TK, RRB, OBP genes and an Ori-Lyt domain that are absent from betaherpesviruses. Most dramatically, all EEHV genes and proteins encoded in common with other herpesviruses are at least 50 to 80% diverged from their nearest orthologues. In both DNA and protein based phylogenetic trees the EEHVs fall into a monophyletic clade branching intermediate between the mammalian gammaherpesvirus and betaherpesvirus sub-families. Therefore, we propose that the Probosciviruses (=EEHVs) should be designated as the prototypes of a new Deltaherpesvirinae sub-family, which we estimate has evolved separately from the three other mammalian Herpesviridae sub-families within Afrotidian hosts, including the ancestors of modern elephants, for more than 100 million years.

Many EEHV species also exhibit multiple chimeric features and subtype variants. All 36 well characterized EEHV1 strains fall into just two major chimeric clusters called EEHV1A and EEHV1B that are diverged by 17% and 32% at the DNA level in segments of gB-POL (3.0-kb) and gN-gO-gH-TK (3.8-kb), as well as in UDG-gL and vFUT9 and at several other novel gene loci, but vGPCR1,5,6 and gH also cluster into 3 to 5 additional subtypes. Similar analyses have revealed two major chimeric subtypes of EEHV5 that differ by 10-20% in gB-POL, TK-U49 and UDG-gL, as well as multi-locus A and B variants of both EEHV3 and EEHV7. Current evidence indicates that EEHV1A and EEHV1B, which have been the cause of the majority of over 80 fatal cases of acute systemic hemorrhagic disease in North America, Europe and Asia, together with both EEHV4 and EEHV5, are likely all natural endogenous viruses of Asian elephants, whereas only EEHV2, EEHV3, EEHV6 and EEHV7 have been found in all African elephants tested with benign lung or skin nodules. Together with five species plus three subtypes of elephant gammaherpesviruses (EGHVs), none of which have yet been associated with disease, a total of 12 distinct species and 19 major subtypes of elephant herpesviruses have now been identified.

Elephant Herpesviruses EEHV2, EEHV3A, EEHV3B *(a new subspecies)*, EEHV6, EEHV7A, EEHV7B *(a new subspecies)* and EGHV1A, EGHV1B *(a new species)*, EGHV2, EGHV4 Found in Tissue Biopsies and Saliva from African Elephants In Kenya and America.

Virginia R. Pearson[1],[2]
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Elephant herpesiruses include Probosciviruses EEHV1A, EEHV1B, EEHV2, EEHV3A, EEHV3B, EEHV4, EEHV5A, EEHV5B, EEHV6, EEHV7A, EEHV7B and Elephant Gammaherpesviruses EGHV1A, EGHV1B, EGHV2, EGHV3A, EGHV3B, EGHV4, EGHV5A, EGHV5B. Herpesviruses generally are species-specific, but if inter-species infection occurs, severe pathology in the non-natural host may be the outcome. It was hypothesized that herpesviruses of African elephants were infecting Asian elephants, causing acute disease and hemorrhagic death, so it was crucial to identify which herpesviruses are endogenous viruses of African elephants. In 1986, viral particles morphologically consistent with herpesviruses were found in nodular raised fibrous cutaneous lesions biopsied from African elephants imported from Zimbabwe to America. In 2009, corresponding author Virginia Pearson observed similar nodules on wild African elephants in Kenya, and, in 2011, led an expedition in Kenya to obtain biopsies

from these skin nodules. In collaboration with Save The Elephants and Kenya Wildlife Service Veterinary and Capture Services Department, we immobilized twelve wild elephants in Samburu and Maasai Mara National Reserves. We collected saliva, blood and exudates from all twelve elephants, biopsies from raised cutaneous nodules on five of the elephants, and lung biopsies from a thirteenth recently dead elephant. By extensive polymerase chain reaction (PCR) and viral DNA sequencing analysis at Princeton University and subsequently at Johns Hopkins School of Medicine, we identified DNA from EEHV2, EEHV3A, EEHV3B (a new subspecies), EEHV6, EEHV7A and EEHV7B (a new subspecies) in skin nodule and lung biopsies and saliva from these wild African elephants. Also, we have found EEHV2, EEHV3A, EEHV3B, EEHV6 and EGHV1B (a new species), EGHV2 and EGHV4 DNA in saliva collected weekly for one year from two captive asymptomatic wild-born African elephants in America, and in saliva samples collected occasionally from an additional two dozen captive African elephants. No EEHV1A, EEHV1B, EEHV4 or EEHV5 DNA sequences have been found in these wild or captive African elephant skin or lung biopsies or saliva samples. Our findings of DNA sequences, together with PCR sequencing from necropsy lung nodule tissues from two culled South African adult elephants and an adult African elephant euthanized in the United States have shown that the subset of Probosciviruses EEHV2, EEHV3A, EEHV3B, EEHV6, EEHV7A and EEHV7B, but probably not EEHV1A, EEHV1B, EEHV4 or EEHV5 are likely to be natural endogenous viruses of all African elephants. We conclude that cross-species infection between the two elephant genera is extremely rare and does not account for the unusually severe pathology and hemorrhagic deaths in juvenile captive and wild Asian elephants. EEHV2 and EEHV3 have caused hemorrhagic deaths in two captive African and one captive Asian elephant respectively. However, is yet to be confirmed whether the presumed endogenous herpesviruses of African elephants cause hemorrhagic deaths in wild African elephants, as has been shown for EEHV1A, EEHV1B and EEHV4 in wild Asian elephants and EEHV5 in one captive Asian elephant. Whether co-infections with Elephant Gammaherpesviruses (EGHVs) affect pathogenesis of hemorrhagic disease in African and Asian elephants is also yet unknown.

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Elephant Endotheliotropic Herpesvirus (EEHV): Where We Are, Where We're Going

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Elephant endotheliotropic herpesvirus (EEHV) is the single largest cause of death in Asian elephants *(Elephas maximus)* born in North America since 1978. In January 2013, the Houston Zoo and the International Elephant Foundation hosted the 9th annual International EEHV workshop, which focused on recent EEHV research and prioritizing needs for the future. Highlights from workshop presentations include:

1. That the EEHV1 genome has been fully sequenced along with partial sequencing of other EEHV types and collectively, based on their significant divergence from other known herpesviruses, should be classified as a new subfamily of Deltaherpesviruses.1
2. That there are currently at least 11 subtypes of EEHV, which, with minor exceptions, are endogenous to Asian elephants (EEHV 1, 4 and 5) and African elephants (EEHV 2, 3, 6, and 7).
3. That healthy African elephants in North America have been shown to shed EEHVs from their trunks, and more information is needed on EEHV in African elephants
4. On preliminary investigations into the immunity of elephants related to EEHV, with much more study needed on this important topic.2
5. On clinical management of EEHV-associated illness in affected elephant calves.3, 4

Research priorities included: 1. Procuring funding for continued research, 2.Continued attempts at culturing the EEHV virus; 3. Studying elephant immunity related to EEHV; 4. EEHV vaccine development; 5. Determining antiviral drug efficacy against EEHV; and 6. Continued epidemiologic evaluation of EEHV.

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Veterinary Treatment and Management for Papiloma Viral Infection of a Captive Sri Lankan Elephant *(Elephas maximus maximus)* In Colombo Zoological Garden

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The Sri Lankan elephant *(Elephas maximus maximus)* is an endangered species in IUCN categories. A new census revealed that the country has around 5800 wild elephants and 213 captive elephants. Due to human-elephant conflict-related reasons, on a daily basis elephants deaths are reported. Out of the captive elephants 86 are in the Pinnawala Elephant Orphanage and 7 are in the National Zoological Gardens in Colombo. Among captive elephants most of them are reaching beyond a young age. Some of them have health problems that are age-related. According to their age, immunity deficiency has occurred and some infectious diseases have been observed. Papiloma viral infection, on the foot, is observed mostly in old elephants, but occasionally in young elephants that had some immune deficiency in captivity. It is a serious health issue and it can damage the foot and simultaneously can incur a podo-dermatitis condition. Severe growth with a bacterial infection leads to damage of the foot and creates serious health problems with the foot as well as with the life of the elephant.

Colombo Zoological Gardens has seven elephants, six females and one old male on location. 'Devi' is a female elephant, 24 years old, with an average body weight of 4000 kg. She was used for elephant performances during the last 17 years. Recently, due to an arthritis condition, she was retired from elephant performances. She was infected by a papiloma viral growth on the foot of the right hind limb. It causes limping and shows a clearly visible flower-like growth on the lateral margin of the foot of the right hind limb. Initially a sample from the growth was removed and sent to the Veterinary Research Institute for confirmation. According to the clinical signs and nature of the lesion, treatments were started. The elephant was trained for foot dipping, by putting a leg into a medicated foot bath which contained 10 L of water mixed with 500 ml of Providone iodine and 2 g of KMNO4 (Potassium Permanganate) and by keeping the affected leg in the foot bath for 15 minutes. After cleaning the area of the lesion a mixture was applied, a combination of which contained 5 g CuSo4 (Copper sulphate), 5 Aciclova tablets powder (anti-viral tablet), and Base of Stalkhalm Tar. The mixture was applied directly on the lesion and the Stalkhalm tar was applied to the rest of the margin of foot. For the bacterial podo-dermatites, she was given a Penicillin Streptomycin Intramuscular injection 80 ml at three day intervals for up to two weeks. She was also given vitamin A, D and E injections two consecutive times at 5 days intervals. The laboratory sample confirmed it as a papiloma viral growth. For preventing the spread to others, the elephant was relocated to a corner, but not completely separated because stress can lead to a drop in the immune response. The elephant was teathered in place to a much drier environment and was fed oral calcium. With treatment for 35 days the growth gradually decreased and was completely cured. Throughout this period to maintain the elephant's immune system, on a daily basis 'Devi' was fed 25 tabs of 100 mg Ascorbic acid, 30 Vitamin B complex tabs, and 10 Folic acid tabs. 'Devi's' papiloma growth was completely cured and for future prevention, Providone Iodine was sprayed on the foot twice weekly.

The Lonely Rhino: Analyzing Anthropomorphism Toward Solitary Animals

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Studying anthropomorphism toward solitary animals can help zoos address concerns about animal welfare and determine appropriate educational responses.

A random sample of 200 guests were asked to complete a survey on 1 of 4 black rhinoceros (Diceros bicornis) located at Brookfield Zoo's Pachyderm House. The topics on the survey ranged from the rhino's behavior and welfare to the guest's environmental viewpoints and their emotional connection to the animal. Survey responses were examined for awareness of the black rhinoceros' solitary lifestyle, satisfaction with the animal's enclosure, and anthropomorphic descriptors. Correlations were investigated with SPSS software.

Analysis revealed 64% of survey respondents were unaware of the black rhinoceros' solitary lifestyle. When perceived as social, guests were more inclined to agree that the rhino appeared lonely (Spearman's rho, $r_s(144)= 0.186$, $P = 0.026$), to disagree with the rhino not needing a companion (Spearman's rho, $r_s(145)= 0.345$, $P = 0.000$), and to agree that the rhino seemed stressed with the presence of visitors (Spearman's rho, $r_s(145)= .166$, $P = 0.046$). When describing the rhino's mood, 15% of guests used negative anthropomorphic descriptors i.e. "lonely", "sad", "bored", "depressed". When describing their own emotional response to the animal's behavior, 2% of guests used negative descriptions i.e. "felt sad for the rhino". The rhino's activity level, exhibit space, and enrichment items also influenced the guest's perception of the animal's welfare.

These findings suggest that educational outreach should be increased to improve guest awareness and satisfaction with solitary animal exhibits. Otherwise, zoos will need to determine how to reconcile visitor preference for multianimal exhibits with the black rhinoceros' solitary lifestyle.

Session VII: *Ex situ* Benefits and Support of *In situ* Conservation

The Bigger Picture – How Captive Elephant Facilities Benefit Wild Elephant Populations.

Sean Hensman
Elephants for Africa Forever

We're living in an imperfect commercial world which is constantly evolving and modernising. Humans are continually putting pressure on wildlife. Ultimately everyone has the same conservation objective; to ensure that our grandchildren's grandchildren are able to experience wildlife better than we currently do, we all just have differing opinions on how to achieve this.

Having had a rich wildlife and conservation orientated upbringing Sean Hensman, from Adventures With Elephants in South Africa shares his family's story on how their tamed and trained elephants are wildlife ambassadors contributing to the wider conservation of elephant. This ranges from interactive and educational tourism, promotional aspects, research on a variety of issues, elephant welfare and finding future potential by harnessing elephant's incredible sense of smell to detect explosives and track people. All these different capabilities give elephant's incredible value, thus ensuring their survival in our future imperfect world.

Contributions to Science and Conservation by Elephant Managers and Captive Elephants

Heidi S. Riddle
Riddle's Elephant and Wildlife Sanctuary, Arkansas and Elephant Managers Association

Captive elephant management has greatly evolved since elephants first came to North America in the late 1700s as part of menageries exhibiting unusual animals. In early zoological management, an elephant keeper's main concern was basic animal care and husbandry; in present times, however, captive elephant managers and their elephants play a significant role in the scientific study and conservation of the species.

In 1988, the Elephant Managers Association (EMA) was established in the United States, and in 2006 the first professional association for elephant managers in Asia was developed in Indonesia as the Sumatran Mahout Communication Forum (FOKMAS). Both groups provide an opportunity for elephant keepers/handlers/trainers (mahouts) to come together, share experiences, and improve elephant management. Captive elephant managers have many roles: we provide care to the elephants, act as educators, conduct some public relations, contribute to scientific research, and we raise awareness, funds, and work to support the conservation of wild elephant populations.

Intensive scientific studies of the captive elephant population over the past 20 years have taught us much about the biology and physiology of the animal. Elephant managers are instrumental in biological studies and elephant health care work – managers train and condition elephants to veterinary procedures such as blood collections, trunk wash sampling, ultrasound or radiographic assessments, as well as for medication routes (i.e. oral, rectal). Without the cooperation and input of elephant managers and the ability to obtain samples for diagnosis, scientists would not be able to study diseases of concern to elephants (i.e. EEHV), and wildlife veterinarians would not be able to implement effective elephant health care programs.

Captive elephant management is an integral part of elephant conservation. Almost one third of the Asian elephant species is in captivity, and most of those animals are in Asia. Captive elephants in the western world and in Asian range countries are kept in a variety of environments; much can be learned from these different situations about elephant care, husbandry, and training. Working together with other elephant managers, groups such as EMA and FOKMAS have not only improved elephant husbandry, but also make important contributions to the scientific study and conservation of these animals. Everyone in the elephant community (both wild and captive) needs to work together to learn from each other and help improve all aspects of elephant management; only then will we truly be successful in our work with and for elephants.

Contributions of the Ringling Bros. Center for Elephant Conservation to Wildlife Management in Sri Lanka

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In order to enhance the management of wildlife in general and the elephant in particular in Sri Lanka, the Ringling Bros. Center for Elephant Conservation® (Ringling Bros. CEC) in Polk City, Florida, USA hosted four Sri Lankan graduate students for one year of training and research, utilizing elephants at the Center, as part of their postgraduate degree program with the University of Peradeniya, Sri Lanka. Three of them have submitted their theses for review, while the fourth student's research was upgraded to PhD. Ringling Bros. CEC subsequently built a bullpen for the Temple of the Tooth in Kandy to manage bulls in musth without them being chained. The partnerships with Sri Lankan universities, the Department of Wildlife Conservation (DWC), and Ringling Bros. CEC led to the surveys of human-elephant conflict in 2008, 2009 and 2011. Ringling Bros. CEC has also established the Ringling Bros. Center for the Study of Asian Elephant at Rajarata University in Mihintale, Sri Lanka and provided funds for student projects. In addition, it has provided resources to conduct a course on Wildlife Conservation & Management at Rajarata University. At the Elephant Transit Home (ETH) in Uda Walawe National Park, Ringling Bros. CEC has been assisting the DWC in monitoring the growth of the orphaned elephant calves by weighing them at monthly intervals for over three years. The data from weighing these calves has become a unique resource. In addition, the program is also assessing the changes in the body condition of wild elephants at monthly intervals. An accurate method to estimate the length and/or height of elephants and other wildlife using laser beams has been introduced. In addition, the decibel levels of the firecrackers that villagers use to ward off wild elephants were tested and found to be very high indeed. Frequent bursting of such firecrackers in close proximity to elephants may impair their hearing. An innovative method to monitor wild elephant movement was initiated using standing sedation during which a home-made GPS/GSM collar was affixed to the bull. The elephant's movement was then tracked on line once every minute for a month. This technology was mainly developed to monitor marauding elephants with the aim of mitigating the human-elephant conflict. Ringling Bros. CEC assisted the DWC in the planning and execution of the First National Survey of Elephants in Sri Lanka in August 2011. It also carried out conservation education programs at several schools and colleges in the island. Students from Missouri State University in USA came over to the Ringling Bros. Center for the Study of Asian Elephant at Rajarata University in the summer of 2012 to observe wildlife (including elephants) and agriculture and to gain an understanding of the culture where elephants are a part of the landscape.

Employing Mahouts and Captive Elephants for Elephant Conservation Programs in Sumatra

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In Sumatra, the management of captive elephant populations and professional mahoutship is fairly recent, and only exists since the mid 1980s when the Indonesian government launched a program to capture wild elephants in Human Elephant Conflict (HEC) areas and brought them into captivity for domestication. Due to unsatisfactory concepts about how to utilize the captured elephants, a lack of sufficient resource for the care and management of these elephants, and a lack of sufficient training and education for the newly recruited mahouts, the management and care of these elephants was initially lacking in many ways.

To address these issue and involve captive elephants and mahouts in elephant conservation strategies, over a decade ago some NGOs, along with government conservation agencies started to develop concepts and programs to establish elephant patrol units where mahouts are trained and employed, and captive elephants are utilized for wild elephant conservation activities. These activities include HEC mitigation and management by monitoring wild elephants and their movements, driving them away from cultivated areas and back into protected forests, or in cases where single elephants have ventured deeper into farmland or settlements evacuate these animals and relocate them back into protected forest areas. Further activities for which mahouts and captive elephants are sometimes employed include the translocation of wild elephants from encroached areas to suitable habitats, education awareness activities, and fitting GPS collars on wild elephants for monitoring their movements to evaluate home ranges, habitat utilization, and HEC management prevention.

During the past years an increasing number of mahouts have been trained and more elephants are being utilized in such patrol units in many provinces in Sumatra. These units are mostly managed in collaboration between conservation NGOs and government conservation agencies. These units significantly contribute to conservation needs for wild elephants and their habitat, and, in areas such as the Way Kambas National Park, have become an important part of the government's HEC mitigation strategies.

To successfully implement these described activities the skills and experience of the mahouts, who in the past were involved in wild elephant captures, is absolutely crucial, They have a very good knowledge about wild and captive elephant behavior and handling in such situations. The training given to these mahouts about field navigation, conservation needs, and regulations in combination with their elephant handling skills have made them valuable and effective field conservation workers for elephant conservation in Sumatra.

Reproductive Assessment of Sumatran Elephants *(Elephas maximus sumatrensis)* in Elephant Conservation Centers (ECC) Across Sumatra

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The Sumatran elephant is a subspecies of the Asian elephant and listed as critically endangered by the IUCN Red list. Besides a wild population of currently 2000-2500 elephants, there are about 650 elephants in captivity. These were mostly wild caught between 1985 and 2000 in order to reduce human elephant conflicts (HEC). The majority is maintained in governmental Elephant Conservation Centers (ECC) across Sumatra. A few elephants are also utilized in Conservation Response Units (CRU) in order to fight illegal logging and poaching.

Fragmentation of habitats, human elephant conflicts (HEC), habitat encroachment, and the illegal killing of elephants, diminish the wild Sumatran elephant population. Utilizing the captive elephants for various conservation activities and programs, and ensuring its long-term survival as a self-sustaining genetically valuable population, will back up wild elephant conservation strategies. To address these strategies, the careful management of the captive population is crucial, requiring ongoing professional veterinary care, consideration, and management support. The *Veterinary Society for Sumatran Wildlife Conservation* (Vesswic) started its *Elephant Health Care Program* (EHCP) in 2006 to provide veterinary expertise for Sumatran elephant conservation in Sumatra. Regular visits of 10 different camp locations, ensure basic veterinary care such as diagnostics and treatment, providing drugs (e.g. antibiotics, dewormers, tetanus vaccination), management and nutrition, foot and wound care (Stremme et al., 2007).

However, to ensure that the valuable genetic pool of the captive Sumatran elephants is represented, breeding of these animals is a priority.

- To ensure the availability of sufficient numbers of healthy elephants to be utilized long-term in programs such as CRU or HEC mitigation and without relying on further capture of wild elephants
- To ensure the long-term existence of captive elephants to function for the public as ambassadors of wild elephant conservation
- As back up for wild populations, e.g. by reintroducing captive individuals or small groups into smaller isolated wild populations to increase genetic variation and thus improve their odds for long-term survival
- For reintroduction into rehabilitated habitats having lost their wild populations, e.g. previously logged forest concessions with secondary forest growth

However, in the past 10 years, there have only occurred 30 births, of which 22 offspring survived (73.2 %). In order to enhance breeding and to be able to monitor the reproductive status of the camp females, VESSVIC implemented ultrasound to their veterinary repertoire. Here we summarize the results from a first survey on the reproduction of the Asian elephants in Sumatra in order to assess the breeding potential.

Basic breeding data

During 2002-2012, a total of 30 births (13 males/16 females/ 1 unknown sex) from 27 different dams were recorded. Of these, 22 calves are presently still alive. Of the 8 calves that died, one was stillborn, 3 died to EEHV in 2 different camps (two cases PCR confirmed), one was killed after birth by the dam and 3 deaths occurred within days or weeks after birth for unknown reason.

If natural breeding occurs, it is usually by wild bulls, because captive bulls are always tethered and social interactions are thus very limited. However, in cases were males or females broke loose or the chains were long enough, also captive bulls were seen mating. In one camp (Tangkahan), an electric fence was established which allowed a male and six females to socialize during the day. This resulted in mating and 3 of the six females became pregnant between 2009 and 2012.

	CRU Sampoinet	ETC Saree	CRU Tangkahan	BBS	ECC Way Kambas	Zoo Lampung	*Total*
Location	Aceh Jaya	Aceh Besar	Lagkat/North Sumatra	East Lampung	East Lampung	Lampung	
Total number of Elephants	4	19	7	5	63	2	*100*
Elephants assessed by ultrasound	3	6	7	2	24	2	*49*
Male US	1	3	1	1	4	1	*11*
Female US	3	3	6	1	20	1	*34*
Calves born	1	1	3	0	5	1	*11*
Calves still alive	1	1	1	0	5	0	*8*
Females currently pregnant	0	0	0	0	6	1	*7*
Females non-pregnant	3	3	6	1	14	0	*27*
Females cycling, healthy	1	1	2	1	6	0	*11 (4 had calves previously))*
Females in lactational anestrus	1	0	1	0	3	0	*5*
Prepubertal females*	0	1	0	0	3	0	*4*
Females post reproductive	0	1	1	1	4	0	*7*

*females younger than 5 years not included

Table 1. Overview of elephant examined in the 6 different camps

During birth, elephants were chained in 25 cases by one hind leg chain. In two camps, birthing paddocks were built and used in 4 cases. No group birth occurred and as of yet, there is no dystocia reported. Mahouts usually found the calf the next morning, only in a handful of cases, mahouts were able to witness a birth.

Ultrasound Assessment

A portable, battery driven ultrasound device (Micromaxx, Sonosite, Fa. Frings Medizintechnik, Germany) equipped with a 2-5 MHz convex probe was donated to VESSWIC in 2012 (through funds from U.S. Fish and Wildlife Service and Asian elephant Support). This US machine is very durable and has an average battery lifespan of 5 hours. Thus, being an ideal tool for the filed situations in Sumatra. For the examinations, elephants were tethered to a tree or, when available, within a restrainer (Fig.1). The rectum was cleaned and an enema given with a hose pipe.

In two trips, a total of 49 elephants were assessed via ultrasound in six different locations from Aceh to Lampung province, of these 32 were females (table 1).

Fig. 1 Female tied in a restrainer (ECC Way Kambas), enema before ultrasound (ECC Saree)

Female ultrasound assessment

During assessment, we found a total of 7 pregnancies. These pregnancies were detected by transrectal ultrasound (3 cases) or transabdominal ultrasound (4 cases). Further indicators of pregnancy were large corpora lutea (CLs) on one or both ovaries, a mucus plug within the vagina and bright cervical folds. The mean number of CLs found in pregnant elephants was 2.8 CLs on both ovaries. This is markedly lower than the number of CLs reported for pregnant African (average of 6 CLs; Allen, 2006) or pregnant, mainland Asian elephants (average of CLs: 5.4; Lüders et al., 2010). However, the CLs were usually quite large, measuring 3.5-4.5 cm in diameter. From the non-pregnant elephants,11 females were cycling and showed a healthy reproductive tract, five were in lactational anestrus with a calf (7-16 months) at foot, four were prepubertal and seven showed pathologies or acyclicity, thus considered post-reproductive (table 1). The ovarian medulla of most elephants showed distinct hyperechoic spots (indicative of dense, possibly fibrotic tissue), but this was irrespective of reproductive status.

Four prepubertal females (age 5-11) were scanned. There was a large mucus plug within the vagina, the uterus appeared dark and no functional structures were seen on the ovaries.

Of the cycling females (mean age: 24 years, range: 12-39 years), 8 were in the luteal phase and only 3 in the follicular phase, with one cow in estrus with a dominant follicle measuring 21.0mm. During the follicular phase, follicles of different sizes and small CLs (mean number: 2.5 CLs) were present, while the uterus appeared bright and convoluted. During the luteal phase, larger CLs, but no follicles were observed. The uterus was usually not visible after the bifurcation, due to its relaxed status.

The seven post reproductive females aged 33-45 years (mean: 38 years) and showed no ovarian activity (n=4) or ovarian activity, but reproductive tract pathology (n=3) such as uterine leiomyoma, cysts in the uterus or ovary, cervical leiomyoma or a combination of these pathologies.

Male ultrasound assessment

Bull elephants examined aged between 10 and 28 years. The lower reproductive tract organs, such as bulbourethral glands, prostate, ampullae and seminal vesicle were evaluated. Testicles could be reached with the hand held ultrasound probe or with the help of a plastic extension (older, larger bulls). In all but one bull, the seminal vesicles yielded no fluid filling, the ampullae were generally small and anechoic, the prostate not distinct and the testicles rather small (10-15cm in diameter).

Discussion

The overall reproductive parameter in Sumatran elephants investigated in this survey resembled those described for mainland Asian elephants. Remarkable was the lower number of corpora lutea (usually 2-3 CLs) and the prominent hyperechoic spots found in the ovaries of mature cows. These may be fibrotic foci, however their meaning and origin are unknown.

Of all females scanned, ranging from 5 to 45 years, only 7 females were considered post-reproductive due to uterine or ovarian lesions or acyclicity. However, the majority of females is reproductively healthy and still young enough to breed. Eleven females were showing ovarian cyclicity (four of these previously had calves) with another four females prepubertal and five in lactational anestrus. Therefore, in the potential breeder (n= 11) and proven breeder group (n=16) we found a total of 27 elephants ranging from 5-33 years. Thus, 79.4 % of the females in this survey are considered fertile and may reproduce.

Of all non-pregnant females, only four were acyclic for an unknown reason. This is 11.8% of females investigated and is close to the 14% of acyclic Asian elephant females recorded in North American zoos aged >30 years (Brown et al., 2004). Although malnutrition, parasite load and distress related to a lack of social interactions, constant tethering, and the inbreaking procedure through the mahouts, are obvious, acyclicity has only been observed in four elephants. This occurred exclusively in the older age class (>33 years). The low fecundity of the captive population appears therefore not related to fertility issues. The main problem is likely the lack of undisturbed interaction possibilities for these elephants, thus lack of breeding opportunities. This is a management issue. With the ultrasound at hand now, it will be possible to determine which females should be given opportunity to be breed. As persuaded in Tangkahan, paddocks for temporary mixing of elephants would be ideal.

Another problem may be the suppression of males in a captive situation. Reportedly, males adapt not as good to the captive environment compared to the females. Also too many bulls are kept within close vicinity in some camps (e.g. Way Kambas, were about 30 males are kept). Fighting and injury, sometimes even fatally, occur between bulls that broke loose or were chained to close together. Due to the presence of more than one adult bull as well as the poor nutritional status, most males appeared reproductively suppressed. This was reflected in the overall small testicle size and diminished filling of accessory sex glands. In result, only few males will be capable of breeding in these camps.

In conclusion, breeding needs to be promoted in order to keep the genetic diversity in the ECCs at high level and to establish a self sustaining captive population. Mahouts must be educated as to when allow males to have access to females. Birthing and mating paddocks need to be established in each facility.

Although the number of births did increase during the past four years, there is a higher potential in the captive Sumatran elephant pool.

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What Elephants Can Teach Us About Preventing Cancer

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Peto's Paradox describes the puzzling observation that the increased cellularity of larger animals does not correlate with the expected increase in cancer risk. This is particularly true in elephants where there is only a 4% cancer incidence compared to the 30% seen in most other mammals, including humans. Evolutionary biologists have proposed one explanation for this paradox in which better functioning DNA repair mechanisms may provide larger, long-lived mammals with an improved ability to counteract cellular stresses that may cause mutations. One gene that may contribute to this increased DNA repair robustness is the known tumor suppressor gene, TP53. TP53 has been called the "guardian of the genome" and counteracts DNA damage by initiating DNA repair or alternatively by causing damaged cells to undergo cell death (apoptosis). While most organisms encode only one copy of TP53, African elephants encode this copy as well as 18 additional copies of the TP53 gene. These additional copies in elephants appear to be retrogenes, which are ancient copies of the original TP53 gene that were incorporated into the elephant genome over evolutionary time. These extra copies of TP53 may play a role in elephant cancer resistance by initiating a hyper-repair or apoptosis response to more efficiently block mutations from propagating throughout cellular generations. To investigate this possibility, African elephant and human radiosensitive peripheral blood mononuclear cells (PBMCs) were exposed to DNA damage by gamma irradiation (IR) and compared in vitro for cell viability, DNA repair, cell cycle regulation, and apoptosis at subsequent time points. This study revealed a significant linear increase in apoptosis in elephant PBMCs exposed to 2 Gray γ-IR compared to human ($p<0.005$) which suggests that the cellular threshold of DNA damage in elephants is lower than in humans. These results indicate that elephant PBMCs preferentially undergo cellular death instead of DNA repair following IR exposure, and do so at a higher rate than humans. This mechanism of action may contribute to elephant cancer resistance by preventing mistakes in replication through rapid elimination of damaged cells which could otherwise lead to cancer.

Rhino Protection Unit and Support Extended by Local Villages to Protect the Remaining Sumatran Rhinos in Bukit Barisan Selatan National Park, Indonesia

Arief Rubianto
Rhino Foundation of Indonesia

The Critically Endangered Sumatran rhinoceros *(Dicerorhinus sumatrensis sumatrensis)* is one the most threatened of all land mammals on Earth. Less than 100 Sumatran rhinos remain, primarily on Indonesia's Sumatra Island, where the population has declined at a rate of 50% over the past 10 years, largely from poaching, deforestation and habitat fragmentation. They live in three important protected areas in Sumatra: Way Kambas National Park (WK) in Southeast Sumatra, Bukit Barisan Selatan National Park (BBS) in Southwest Sumatra and Gunung Leuser National Park (GL) in North Sumatra.

The Rhino Protection Unit (RPU) has been operating in this area since 1997, through intensive anti-poaching and intelligence activities to patrol forests and monitor endangered species, destroy snares and traps, apprehend poachers and prevent encroachment. There are seven units of RPU in BBS operating effectively within South Sumatra. As a result of these operations over the past 11 years, there have been no cases of rhino poaching encountered, and the rhino population seems to be increasing (estimates from 25 to 40) in Bukit Barisan Selatan.

The BBS NP is surrounded by about 12 million people who live in and around forests with a thin buffer zone, and many more are dependent on coastal resources. Forest conversion to agriculture increases the interface between humans and wildlife, which increases the opportunity for conflicts as competition for precious resources escalates. As a result, there is a high level of human-wildlife conflict. The poorest rural people are most dependent on biodiversity and natural habitats for their livelihoods, and they are the ones who suffer most when such habitats are simplified, degraded or otherwise impoverished. These people hold the future in their hands – they will either help save the rhinoceros or help move it toward extinction.

Some villages around NP are recognized as "problematic" as many of the poachers and encroachers operate from these problematic villages. To address the threats posed by some of these areas, the national park authorities supported by some NGOs have conducted several community development programs with no significant success due to difficulty in accessing those areas. However, the RPU has been regularly visiting the areas,staying for some time while performing their activities. Through these interactions, the RPU has earned the respectof the villagers.

Literature studies, surveys and consultations with agriculture experts were conducted by the RPU before they started to facilitate the villagers. The RPU realized that cacao is a more sustainable agriculture crop than coffee (e.g., cacao, has higher yields and brings higher prices). Unlike coffee, cacao is significantly more environmentally friendly, does not require land clearing or massive irrigation, and can be grown as part of a mixed natural forest system in park buffer areas. Before this idea was proposed to the villagers, the RPU conducted some experiments planting cacao in their personal gardens.

As a result of these activities, the local villagers were persuaded to surrender more than 90 illegal arms to RPUs and NP authorities which is indeed an encouraging sign in the effort to reduce wildlife crime. Through community engagement in income generating activities, more than 40% of encroachment in BBS could be eliminated as the farmer's profit increased almost 20% on average, compared to their previous agricultural income. At present many farmer groups and villagers from other areas are seeking support from RPUs to do the same improvement in their villages.

Session VIII: *In situ* Conservation

Control of Invasive Arenga Palm *(Arenga obtusifolia)* in Habitat Suitable for Javan Rhino *(Rhinoceros sondaicus)*, Ujung Kulon National Park, Indonesia

Sectionov Inov
International Rhino Foundation Indonesia Liaison

The Javan Rhino *(Rhinoceros sondaicus)* is Critically Endangered according to the IUCN Red List of Threatened Species (http://www.iucnredlist.org/apps/redlist/details/19495/0). Its population is estimated at 35-44 animals confined to Indonesia's Ujung Kulon National Park, located on the western tip of Java (IUCN 2010). Ujung Kulon National Park is a United Nations World Heritage site and the final stronghold for this species, following its recent extirpation in Vietnam. Unfortunately, suitable Javan rhino habitat within the national park is limited by the spread of an invasive palm *(Arenga obtusifolia)* that threatens to dominate the Ujung Kulon area.

The distribution of *Arenga obtusifolia* in Ujung Kulon renders a significant portion of the tropical forest habitat unsuitable for Javan rhinos. The palm contains sodium oxalate which helps defend it against herbivores such as rhinos, wild cattle and deer. Also, where arenga palm dominates, the growth of other plant species – including many Javan rhino food plants – is suppressed. Currently, an estimated 18,000 hectares (approximately 60% of the park's land area) is covered by the invasive palm (MoF, 2007). Putro (1997a) described the regenerative ability of arenga palm as high; the tree can regenerate via its roots as well as by seed/fruit dispersal.

The multiple aims of current wildlife research and conservation efforts are to: 1) prevent any increase/reduce the distribution of Arenga obtusifolia within Ujung Kulon National Park; 2) increase natural feeding grounds commonly used by Javan rhinos; 3) document Javan rhino habitat utilization pre-and post-clearing of palms on experimental plots; and 4) evaluate the most cost-effective and environmentally-responsible techniques for habitat restoration.

Results obtained to date indicate that the dominant factors affecting palm clearance and regrowth patterns are seasonal weather patterns, light intensity and

methods of seed dispersal. Chemical clearance methods (the injection of glyphosate isoprophylammonium © Roundup), produces relatively rapid palm mortality (three months), produces no detectable negative environmental impacts, and is no more expensive than cutting. By comparison, manual palm clearance (cutting and removing trunks, fronds and fruits) is essentially immediate (about one week to clear one hectare), but requires a larger local work force and thus engages more members of neighboring communities in this wildlife conservation effort.

Preliminary results document a significant rate of plant regrowth on experimental plots, a predominance of rhino food plant species (more than 90%) replacing areas initially covered by *Arenga obtusifolia*, and an apparent increase in restored habitat use by the resident Javan rhino population.

The Role of Standing Sedation in Mitigating Human-elephant Conflict in Sri Lanka

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With the introduction of the commercial dart in 1953, chemical immobilization of wildlife including elephants became a routine management practice. It was in 1967 that the staff of the Department of Wildlife Conservation (DWC) in Sri Lanka was first introduced to the use of the drug M-99 as a means of anaesthetizing elephants by Gray & Nettasinghe (1970). However, such immobilization has its own risks where the elephant can injure itself or die while being anaesthetized. By contrast, standing sedation using xylazine is safer for the elephant and the effect can last longer and be utilized more often than anesthesia. The home-made collar was fastened with just a padlock and chain instead of nuts and bolts for easier and quicker attachment to the elephant tranquilized under standing sedation. The transmitting GPS/GSM unit comprised of 1.6 kg 100 Ah rechargeable battery to signal the location of the elephant once every four minutes. This allowed us to monitor the elephant online in real-time. The software used is quite versatile to establish geo-fencing where e-mail or SMS alerts could be sent to mobile phones. Thus immediate action is possible to chase the elephant back into the forest before any catastrophe occurs. The software also has the capability to monitor remotely the battery level. As the battery is rechargeable, the elephant could be brought under standing sedation to remove the old collar and replace it with a new one for continuous monitoring. Online monitoring also reveals daily behavioral patterns such as patterns of utilization of habitats, the number of attempts the animal makes to raids crops and fine-tuned movement patterns including resting times and the distance traveled each day.

Which Future for Human-Elephant Coexistence in the Boucle du Mouhoun Region (Burkina Faso)?

Julien Marchais
Des Eléphants & des Hommes

In 2002, an aerial count of the elephants of the Boucle du Mouhoun Region, in Burkina Faso, was conducted. The methodology used was a sampling count and it gave the following results: 99 direct observations and an estimate of 541 individuals (+/- 320 - 95% CI). Since then, specialists have discussed these figures and many have thought that the real elephant population was probably closer to the lower limit, including the author of the 2002 count himself.

In 2006, our NGO *"Des Eléphants & des Hommes"* (Elephants & Humans) started a program in this region. The situation was very difficult as most of the elephants' natural habitat had deteriorated and the protected areas were poorly equipped to be efficiently controlled. The presence of cattle was the main pressure on the wildlife habitat. Illegal wood collection, poaching for small and middle sized mammals, uncontrolled fires and land encroachment for agriculture were the other main pressures. The elephants were very difficult to see in the whole of their Mouhoun range, except in the Deux-Balé National Park, the most protected area of the complex. There, elephants were fairly easily observed during the dry season. We started working in this National Park hoping to contribute to the beginning of a rehabilitation process. From 2007 to 2009, we initiated an environmental education program with the main objective of offering a chance for primary school students and their teachers to see their elephant neighbors and natural heritage. It is interesting to note that over 80% of the students had never seen an elephant before!

In 2010, as we hoped, a rehabilitation process was initiated by the government so we adjusted our program to encourage and enable the process. From 2010 to 2013, in partnership with the various stakeholders, we continued the educational program and extended it to 250 primary schools. We also organized over 20 training sessions to develop alternative income generating activities for the communities compatible with the preservation of the elephants and their habitats, we equipped over 150 producers, we offered training and equipment to reduce and avoid human-elephant conflict on the fringe of the Deux-Balé National Park, we supported ranger patrols and provided equipment. We also conducted studies and research.

The idea is to secure Deux-Balé National Park, where elephants will be free to quietly live, and contribute to the prosperity of its periphery so that the human inhabitants can fully benefit from the rehabilitated national park. We are only at the beginning of the process and it is far too early to know if the project will be successful. But time is running out and pressures remain on the other wildlife areas of the complex. Pressures also still remain on the Deux-Balé National Park due to the destructive activities of the neighboring communities. The question is then: are we winning? is it still possible to win? In order to have part of the answer, in 2013 we conducted a new aerial survey of the elephant population. It was led by Dr. Philippe Bouché and the National Office for Protected Areas of Burkina Faso, this time using a total count methodology. The results are frightening…

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The Conservationists' Dilemma: A Need for Pragmastism Regarding Captive Breeding of Exotic Wildlife

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Over this past century, the primary mission of zoos has evolved from collection and exhibition to education and conservation. Strategies for the latter have included captive breeding programs in attempts to protect and expand gene pools of species threatened by inexorable losses of natural habitats and, for rhinoceroses and elephants, by widespread escalation of criminal poaching stimulated by increasing demands for rhinoceros horn and ivory to supply Asian black markets (1).

Advocates for captive breeding of rare species emphasize some secondary benefits: opportunities for more extensive, controlled physiological studies than are feasible in natural habitats, and their potential as reservoirs to replenish wild populations should conditions warrant. The latter possibility remains controversial because integration of naive and native animals carries unpredictable hazards, including maladaptation of the former to the vicissitudes of life in the wild and possible introduction of domestic diseases into indigenous populations (2).

Despite notable successes such as California condors and Arabian oryx, attempts

to breed some species ex situ have had unintended and deleterious consequences. For example, various rhinoceros species often develop novel, sometimes fatal, clinical disorders that are not known to occur in the wild. These include reproductive issues, vulnerability to low-virulence microorganisms, and frequently lethal anemias, vasculopathies, dermatopathies, neural developmental abnormalities, and iron storage disease (ISD).

Iron overloading was first detected in captive African black rhinoceroses by Smith et al. (3), and subsequently found to affect other perissodactyls, Sumatran rhinoceroses (4-6) and tapirs (7), as well as numerous other species of exotic wildlife (8). There is strong evidence that excess iron causes or contributes to many of the aforementioned disorders acquired by browser rhinoceroses under duress of unnatural diets and altered social and environmental conditions(4,5,9).

All rhinoceroses are inherently impaired in their capacities to neutralize ambient oxidants (10-12), so they are highly vulnerable to the toxic effects of hydroxyl and other free radicals that are catalytically generated by elemental iron.

While we have focused on rhinoceroses, it is important to emphasize that progressive iron overburdens develop in many mammalian, avian, marine and other species when extracted from their native environments. ISD therefore represents a global anthropogenic challenge to conservation medicine. Cost/benefit analyses of in situ vs. ex situ conservation programs are now further complicated by this philosophical consideration: Is it ethically justifiable or pragmatically possible to preserve certain wildlife species by dislocating them from their natural habitats even if captive conditions are known unequivocally to induce pathological disorders of high morbidity and mortality? This paradox should be thoughtfully considered along with potential alternatives such as in situ sanctuaries and intensive protection zones (13, 14) in range countries where availability of natural forage and environmental/social conditions might prevent or ameliorate captivity-induced disorders and dyscrasias.

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Trend Analysis of Temporal and Spatial Patterns of Human-Elephant Conflict in Nepal

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This study addresses spatial and temporal patterns of Human Elephant Conflict (HEC) in Nepal. This study is a compilation of available historical records published electronically in 8 daily national newspapers over a 10 year period (2003-2012) of elephant attacks on humans and property and resultant elephant mortality. In addition to direct losses, current mitigation measures are inadequate due to limited resources and are not data-driven. Over the past decade, HEC has caused 103 human deaths, 52 serious human injuries, and 642 cases of extensive property damage; additionally, there have been 18 elephant deaths and 6 severe elephant injuries. Data were analyzed using ANOVA, regression, $\chi 2$ test, correlation, and trend analysis to investigate temporal and spatial patterns of conflict. HEC intensity was high in the migratory route along the Indo-Nepal border region. There was a high correlation between elephant and human casualties ($\varrho = 0.802$, $p < 0.001$). Seasonal variation in HEC was significantly different ($\chi 2 = 117.219$, $p < 0.001$) with greatest conflict occurring during the winter associated with rice harvest. Other seasons with elevated HEC include summer, associated with the harvest of maize, and late winter/early spring, associated with the harvest of wheat. HEC has significantly increased in the number of incidents in more recent years ($\varrho = 0.905$, $p < 0.001$). Most human casualties occurred during the rice harvest period, and were mature males with an age range of 40-70 years. Most elephant casualties occurred during the maize harvest period. As elephant invasion is greatest during harvest of several crops, proper land use planning, and promotion of alternative cropping patterns should be considered for the mitigation of HEC.

Session IX: *In situ* Management of Wildlife and Habitat

The Significance of Pre-Existing Social Bonds in Translocated Black Rhinos

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Black rhinos (Diceros bicornis) are generally assumed to be solitary animals apart from cow-calf pairs and transient mating combinations. This perception induces some rhino management suggestions that may not adequately take rhino social factors into account. For instance, a standard recommendation in rhino metapopulation management is that to counter the loss of genetic diversity through genetic drift, at least one new founder should be introduced into a sub-population every generation. This recommendation implies that a rhino can be taken from one population to another without major social problems. Experiences in translocation operations that the Lowveld Rhino Trust has been involved in suggest that pre-existing social bonds between rhinos are more significant to the success of such operations than has generally been appreciated.

Between 2003 and 2010, 121 black rhinos were introduced into a 1,000 sq mile section of Bubye Valley Conservancy (BVC), Zimbabwe. These translocations have provided a unique opportunity to observe black rhino behaviour as they involved the phased relocation of entire social groups. The translocations were undertaken in response to an expansion, year by year, of human settlement and of poaching pressure within Bubiana Conservancy. The rhino populations in both areas were monitored at the individual level, so associations between individual rhinos were known both before and after the series of translocations. Black rhinos occupy fairly stable home ranges, with dominant bulls overlapping their ranges with those of various cows and sub-dominant animals. Hence rhinos that tend to associate together can be regarded as neighbours.

Not all neighbours were translocated from the one conservancy to the other in the same year, because logistical and political constraints limited the scale of each annual operation. Nor were all rhinos released from the same point in BVC. Nonetheless, a clear tendency was shown by rhinos to re-associate with the same neighbours that they had in Bubiana Conservancy, despite these re-associations requiring some rhinos to move significant distances through unfamiliar territory.

Other observations have been made of rhinos that struggled to fit into the BVC population after being brought in as complete strangers from other populations. Further indications of the significance of pre-existing social bonds in translocation success are shown by the outcome of long-distance translocations of black rhinos from South Africa to North Luangwa National Park in Zambia.

The conclusion from these translocation experiences is that more tranquil re-introduction scenarios (i.e. less dispersion, less fighting) can be achieved by restocking with rhinos that already know each other prior to their translocation.

Boma Adaptation and Development of a Scoring System for Recently Captured White Rhinoceros *(Ceratotherium Simum)* In South Africa

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One hundred nine sub-adult and adult white rhinoceros *(Ceratotherium simum)* were captured between 2009 and 2012 in Kruger National Park and placed in holding bomas prior to translocation to other locations within South Africa. Parameters associated with immobilization, physiological, nutritional and other measurements were recorded at the time of capture and compared between rhinoceroses that adapted to boma conditions and those that were maladapted to determine if there were any predisposing factors. A simple four category system was developed to assess boma adaptation, based on appetite, demeanour, defecation, and activity. Individual and total median scores were used to determine trends and when the animal had successfully adapted to the boma. Twenty-one rhinoceros did not adapt to the boma conditions and were released. Physiological and nutritional measurements were measured at the time of release from the bomas and compared in individual rhinoceros between the time of capture and release as well as between adapted and maladapted groups.

Based on these criteria, 19.3% (21) of rhinoceroses were maladapted with an additional 5.5% (6) having some minor complication which did not require early release. Adapted animals had a mean length of boma confinement of 89.9 days with a range of 39-187 days, while maladapted rhinoceroses had a mean length of boma confinement of 13 days with a range of 8-16 days. The second week in the bomas was a critical period in which statistically significant differences in boma scores were observed between adapted and maladapted groups. Rhinoceros which did not reach a threshold score by the end of the second week or showed a decline in score during this period required early release.

There were no differences in gender or age groupings between adapted and maladapted rhinoceroses. Differences in immobilization protocols, physiological and other capture-related measurements did not appear to be correlated with whether animals adapted to boma conditions. Evaluation of hematologic, biochemical, mineral, and vitamin panels at the time of capture showed no clinically significant differences that could be associated with boma adaptation. However, boma-adapted rhinoceroses did show a few changes between capture and release in values indicating decreased feed intake resulting in mild-moderate negative energy balance. Blood from boma-maladapted rhinoceros demonstrated stress leukocytosis at the time of release. Biochemical values suggested catabolic states due to negative energy balance and was consistent with the large mean weight loss in rhinoceros despite the short time in confinement.

In conclusion, using the scoring system to assess key measures of boma adaptation, a characteristic pattern was documented in rhinoceroses that did not adapt. These patterns became distinct between adapted and maladapted animals by day 8 and typically resulted in release of individuals by day 16. There were significant changes in maladapted rhinoceroses that would likely lead to serious consequences if early and rapid intervention was not undertaken. Application of this newly developed boma scoring system will be a useful tool for scientifically-based decisions for intervention in boma management of white rhinoceros and has potential uses for other species.

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Contact Calls of the Northern and Southern White Rhinoceros: Source of Information on Individual Identity and Species of the Caller?

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Communication of the northern *(Ceratotherium cottoni)* and southern white rhinoceros *(Ceratotherium simum)* has been until now studied only very little. The rhinos have poor eyesight and vocal and olfactory signals are the most important for their communication, however, what information are the rhinos able to transmit and perceive remains unknown. Vocal repertoire of some rhinoceros species has lately been described, nevertheless, studies reporting any information encoded in their calls are completely missing. White rhinos are the most social from all rhino species; well-developed communication system might therefore be especially useful to them. We studied contact call 'pant' of the northern and southern white rhinos, which is formed by a series of inhalations and exhalations and which does not have parallel in any other rhino species. We investigated if pant calls contain information about individual identity and species of the caller. Such ability, in addition to olfactory cues, would allow rhinos to communicate with highly increased accuracy. We recorded and analysed 385 pant calls of six northern and 14 southern white rhinoceroses in several zoological gardens and South African wildlife reserves. Discriminant analysis assigned 86% (77% cross-validated) of pant calls to correct individual, which is significantly more than would be expected by a chance. The most important parameters for distinction between individuals were temporal parameters such as duration of the longest inhalation in call or call duration and ratio of harmonic to nonharmonic energy in an inhalation. Calls of individuals clustered into apparently separated groups according to the species and both species significantly differed in call duration and some frequency parameters of their calls. Our results also suggest the influence of age class and social status on the call structure of males. White rhino pant calls have complex structure and can potentially encode also other information. They might thus represent more sophisticated communication system so far unknown in rhinos. Better knowledge of vocal communication of northern and southern white rhinoceroses might be extremely valuable for improving their management in zoological gardens and wildlife reserves.

The Vanishing Asian Elephant Corridor in the Brahmaputra Valley, Assam: A Threat to Asian Elephant Conservation

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The habitat loss and degradation has been widely recognized as the primary threat to the survival of the endangered (IUCN) and schedule-I (WPA, 1972) Asian elephant *(Elephas maximus)* as the habitat loss directly depresses population size. A problem in dealing with the above threat arises from the fact that, maintaining sufficient contiguous elephant habitat is difficult. The development activities like widening and construction of roads, highways, railway lines on the southern and northern bank of the river Brahmaputra were responsible for fragmentation of the fairly contiguous forest and destruction of corridor responsible for hampering gene flow on the both northern and southern bank of river the Brahmaputra. The river Brahmaputra, National Highways, railway lines may sometimes act as the landscape barrier for gene flow.

Hence, this has been found very much necessary to evaluate the Asian elephant corridor present in both the northern and southern banks of the river Brahmaputra. The main objective of the present study was to study the corridor utilization frequency by elephants in the Brahmaputra valley, Assam with corridor distribution mapping in the valley. Study was done during the year 2012-2013 in the Brahmaputra valley, Assam.

Belt transect method was used to assess the dung density of Asian elephant in the corridor to assess the frequency of utilization of the corridors during the period of the study. DGPS readings of the corridors were recorded with its extent in the forest and non forested area. GIS map were prepared on the distribution and extent of the Asian elephant corridor in the Brahamputra valley by ArcGIS 10 software using the LISS III, FCC image.

During the present study it has been found that, there have been nine Major Elephant Ranges found in the State of Assam (ERs) covering the elephant bearing areas. In the entire the Brahmaputra valley, there were 17 corridors were which were used frequently by the Asian elephant. Amongst the corridors, the Asian elephant used the Daodhara-Bornadi very frequently with relative percentage of use 9.77 %, which was followed by Bornadi-Khalingduar (8.66 %), Kaziranga-Burhapahar (8.32 %), Kaziranga-Panbari (8.14 %), Kukurakata-Bagser (7.7 %), Kalapahar-Doigrung (7.24 %), Kotha-Burhidihing (6.75 %), Ripu-Chirang (6.37 %), Nambor East-Nambor West (5.89 %), Manas-Gabharukhunda (Bhutan) (5.34 %), Dulung-Subansiri (4.87 %), Upperdihing east-Upperdihing West block at Bogapani (4.39 %), Sankosh-Jamduar (4.37 %), Upperdihing east-Upperdihing West block at Golai (3.99 %), Lamding-Amrang RF (3.71 %).

These corridors in the Brahmaputra valley have been found very much important in maintaining the Asian elephant gene flow between different population in both north and south bank of the river Brahmaputra. Current conservation and management strategies have increasingly being designed at the landscape scale, wherein a network of interconnected habitat patches can together support healthy populations, where the role of corridor is very much important. Such an approach can reverse the effects of habitat fragmentation, and can greatly reduce extinction threats faced by an isolated population. Hence, the Asian elephant corridors in the Brahmaputra valley, Assam should be notified with permanent demarcation on ground. All the activities such as developmental activities causing interruption to elephant movement, road construction etc. should be stopped permanently for the conservation of Asian elephant corridor.

Human - Elephant Conflict in the North West Wildlife Zone of Sri Lanka

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Sri Lanka has the second largest wild Asian elephant population and the highest wild elephant density among the 13 range countries. The estimated current population of wild elephants according to the census conducted in August 2011 is around 5879 (Dissanayaka, DWC 2011). According to the Department of Wildlife Conservation (DWC), 553 humans and 1409 elephants have died during the past eight years (2005-2012) due to the conflict (Figure 1). In the year 2011 alone 255 elephants were killed and the main reason of those deaths was gunshot injuries. In the same year 60 people were killed by wild elephants (DWC, 2012).

The North Western Province comprising of two administrative districts, Puttalam (3,013 km2) and Kurunegala (4,813 km2) supports nearly 20% (1189 elephants) of the estimated elephant population of Sri Lanka (DWC, 2011). This elephant population is scattered in small pockets of habitats throughout the NWP as herds and individuals. Highest number of elephant and human deaths were recorded from the North Western region, the most affected area in the county. Location and the number of elephants in two Districts and 14 Divisional Secretariats were identified in conflicted areas of the North West wildlife zone. When analyzing the data of Department of Wildlife Conservation (DWC) for the past eight years (2005-2012); 226 humans and 556 elephants have died in this region (DWC 2012).

Large areas of land have been cleared and extensively planted with crops that are palatable for elephants such as Banana and Paddy. The main objective of the research was to identify human elephant conflict accelerating causes; and identifying the exact locations for development projects, that do not block the natural trails and also that do not harm the natural food sources of the elephants. The next concern was promoting cultivation of crops, changing the possibility of the periods and make.

Elephants are killed due to various reasons. The main reason is human-elephant conflict where elephants are simply killed because they interfere with agriculture and the lives of the villagers. Some of the major causes of elephant mortality include injuries sustained due to gun shot or trap guns, electrocution, poisoning, land mines, accidentally falling into wells and abandoned sand pits, collision with trains, trucks and natural causes. Poaching for ivory though rare is now carried out under the disguise of the conflict and continues to contribute to eliminating the few remaining tuskers.

Managing the conflict is the key to effective elephant management in Sri Lanka. DWC is capable of protecting the elephants within the protected area network, but acknowledges that ensuring the long-term survival of the elephant population outside the protected area network, though difficult, is critical for their long term survival.

Session X: Reproduction II

GnRH Vaccination as a Treatment for Reproductive Tract Pathologies in Female Elephants (Contraception for Post-Reproductive Cows? Why Close the Door After the Cow is Out?)

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As the populations of Asian and African elephants in zoos ages, many females develop ovarian and uterine pathologies, including cystic endometrial hyperplasia and leiomyomas.[1],[5] Though often benign, these lesions can be associated with a bloody discharge, colic, anemia and decreased fertility. It has been suggested that the excessive number of reproductive cycles and steroid hormone exposure experienced by elephants in the captive population is an important factor in the development of these pathologies ("asymmetrical reproductive aging").[5] Wild elephants, by comparison, generally cycle far fewer times in their lives since they are often pregnant or lactating. We hypothesized that cessation of ovarian cyclicity would decrease the development of reproductive tract pathologies and minimize their negative effects by decreasing uterine size and vascularity. GnRH vaccines have been designed to stimulate the production of anti-GnRH antibodies that block the binding of endogenous GnRH to gonadotrope receptors in the pituitary gland. This action inhibits the release of FSH and LH from the anterior pituitary, thereby causing the cessation of ovarian steroidogenic activity and reproductive cyclicity. Our hypothesis was supported by the effectiveness of a GnRH vaccine (Repro-BLOC, Amplicon Vaccine, LLC, Pullman, WA)[4] to suppress ovarian cycle activity and resolve hemorrhage and anemia associated with a suspected vascular reproductive tract tumor in a 59-year-old Asian elephant *(Elephas maximus)*1. Six years after initial vaccination, this elephant continues to lack distinct ovarian cycles and is healthy. Because ReproBloc is not commercially available in the U.S., we initiated a larger study to evaluate the efficacy of Improvest, a GnRH vaccine produced by Pfizer, Inc. (New York, NY) to suppress ovarian cyclicity in non-breeding female elephants that have been diagnosed with reproductive tract pathologies by rectal ultrasound. The goal is to identify an appropriate vaccination protocol to resolve reproductive pathologies in female elephants and determine duration of effect and reversibility. This will be accomplished by monitoring of weekly serum hormone levels (progesterone, FSH, and LH) and GnRH antibody titers, the pituitary response to a GnRH (Cystorelin) challenge, and a follow-up ultrasound[1,2,3]. A total of 7 elephants are at varying stages of the vaccination study protocol, but preliminary hormonal and antibody response data are promising. In addition to resolving reproductive tract problems in older females, there is also interest in the use of GnRH vaccination for contraception to minimize human-elephant conflicts and competition for resources in areas where suitable elephant habitat to sustain large populations is limited.

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Oral Imipramine and Intravenous Xylazine for Pharmacologically-induced Ex Copula Ejaculation in an African Elephant (*Loxodonta africana)*

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Inducing ejaculation ex copula as method for semen collection is a standard approach for semen collection in managed elephants. The current methodologies involved rectal massage to induce ejaculation. While some success has been achieved and successful inseminations have resulted, there is tremendous effort in this and room for improvement. Domestic equids have had pharmacologically-induced ex copula ejaculation with various drugs for decades with varying success. Current recommendations are to incorporate the anti-depressant imipramine at 3mg/kg orally followed in two hours by the alpha-2 agonist xylazine 0.66mg/kg intravenously to facilitate semen collection in equids. An ejaculate is typically produced from 3-20 minutes. This approach was applied to a 23 year old, 4223kg, male African elephant to facilitate an artificial insemination attempt for a multi-parous 28 year old female that was behaviorally incompatible with him.

A trial dose of 12500mg imipramine (mg/kg) po was followed by 100mg xylazine (0.024mg/kg) IV two hours later. There was a very light tranquilization with his penis protruded but after 20 minutes the elephant moved out of the chute and recovered uneventfully. Later a few drops of fluid were reported to be seen dripping from his penis but these were not recovered.

Over the next 6 days, 4 additional trials were made with imipramine ranging from 12500 to 20500 (2.95 – 4.85mg/kg) followed by 170mg xylazine two hours later IV. Sedation was greatly improved and various amounts of fluid and bellowing were exhibited in each session except the final when the initial dose of imipramine was not delivered completely. On the 3rd attempt the bull was given 20500mg imipramine PO and then two hours later 170mg xylazine IV. Over the next half hour no fluid produced but approximate 30 minutes late the staff note a clear fluid from him. This sample had some sperm in it and good motility. The bull was moved back to ERD and a very light rectal massage produced several fractions of ejaculate. The first couple of fractions was clear to slightly cloudy and had a total volume of about 3ml. Little sperm was seen in these samples. The next three tubes were thick white/creamy fluid with a thick coagulum. A total volume of about 4ml of sperm rich fluid was recovered with essentially all alive. They were moving into the coagulum seen, but when diluted with equal volume of Hepes they essentially all (90% or better) had forward motility. This sample was diluted with 12ml of Hepes and utilized for an insemination attempt within 3 hours.

A combination of imipramine followed with xylazine and a light rectal massage may hold some potential for enhanced semen collection in elephants. Samples may be sperm rich without fluid from accessory sex organs as is reported in horses.

Suppression of Testicular Function by Means of a GnRH Vaccine in African Elephant Bulls

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Introduction

In this study on 13 African elephant bulls, we examined the effect of the commercial piglet GnRH vaccine Improvac ® (Pfizer Animal Health) on reproductive organs, hormone levels and sperm production. Aim was to test, if this vaccine is a potential measure for aggression (musth) and fertility control.

Over a two year period, 2 wild and 11 captive African elephant bulls in South Africa were examined every six months to record the changes. After first vaccination and an initial booster 6 weeks later, each bull received a booster vaccination every 5-6 months, followed by a full examination 4 weeks later.

Each bull was injected with 5 ml of Improvac® deeply intramuscular into the gluteal muscle during each vaccination, either by hand injection or dart. Table 1 gives an overview of the males examined.

The examinations took place while the bulls were either fully immobilized (M99/Azaparone) or in a standing sedation (Medetomidin OR Detomidin/Butorphanol) within a chute or pen system (Fig. 1). Blood samples and body measurements were taken, a transrectal ultrasound and a semen collection (either by electro ejaculation or manual prostate massage) were performed.

Fig. 1 semen collection by transrectal massage or by electro ejaculation in lateral recumbency.

Results

During the 2- year study course, each individual received 6 GnRH injections. Serum testosterone levels dropped to non-detectable levels after the second injection in 6/13 bulls and after the 3rd injection in the remainder of the bulls. Similar, at the second examination (6 months after start of treatment), the testicle diameter had already significantly declined. At the end of the study, the testicle size (measured as the area in the 2D ultrasound image) had declined by almost 60% on average, showing the dramatic effect of lacking GnRH stimulation (Table 1).

While the semen collection showed viable spermatozoa in all but two bulls prior to GnRH vaccination (bull#3 was still premature and bull #2 was treated with the vaccine when he was younger), after 3 vaccinations, either no sperm were found anymore or immotile spermatozoa with a large proportion of head and tail separation were detected (Fig. 2). Seminal plasma was still produced, however the volumes also declined. This was reflected by the reduced size and fluid content of the accessory sex glands (seminal vesicles and ampullae) as seen in the ultrasound image.

None of the bulls came into musth, and in the oldest bull of the study (bull #12), who was in musth when he received his first injection, musth ceased immediately and was not observed again.

#	Elephant Name	Age (years)	Status	Height (cm)	Testicle size (area) prior to treatment ($cm^{2)}$	Testicle size (area) at end of study (cm^2)	Testosterone level prior to treatment (nmol/l)	Testosterone level at end of study (nmol/l)
1	Bully	22	captive/wild	281	157,7	66,4	20,5	0,0
2	Mooketsi	19	captive	245	175,4	35,7	0,0	0,0
3	Nduna	8	captive	212	68,9	46,8	0,5	0,0
4	Gambo	10	captive/wild	228	121,0	55,3	22,0	0,0
5	Mukwa	24	captive	307	179,5	81,5	0,7	0,0
6	Thaba	24	captive	309	190,7	83,1	15,2	0,0
7	Duma	24	captive	301	194,7	136,1	4,6	0,0
8	Harry	26	captive	294	177,0	58,5	38,0	0,0
9	Namib	26	captive	285	154,5	70,4	14,1	0,0
10	Clyde	17	captive	256	94,6	40,1	7,4	0,0
11	Shaka	13	captive	243	88,1	28,3	4,2	0,0
12	Jabulani	35	wild	*nm*	272,5	*nm*	117,9	0,0
13	Mashatu	15	wild	*nm*	178,7	58,7	46,8	0,0
	mean	**20,2**		**269,2**	**157,9**	**63,4**	**22,4**	**0,0**

Table 1. Overview of animals examined, showing the sharp decline in testicle size and serum testosterone concentration.

In some animals, behavioral changes, mostly in connection with dominance behaviors amongst other bulls, were observed. Examples include less tension between certain bulls, or mounting of treated bulls by untreated bulls.

In two other cases, bulls that were known to dismantle gates or fences, were not observed to break anything ever since the vaccinations started. However, the general character of the bulls remained and, besides two cases were handlers found their free contact animals more submissive, no changes of behavior towards the keepers were observed. In one case, a bull was even switched from free contact to protected contact during the vaccination trial.

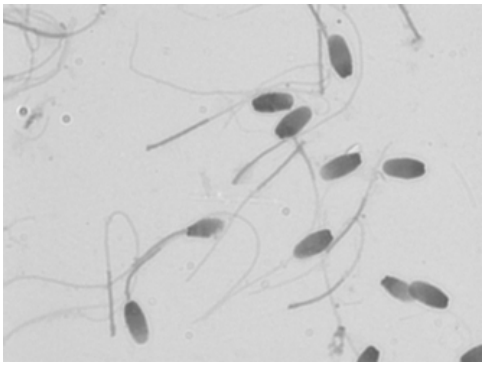

Fig. 2 Typical image of head and tail separation in the ejaculates after 3 vaccinations.

Discussion

So far, our methods have been effective to study the consequences of the GnRH vaccine in elephant bulls. In all animals studied, we have seen a dramatic decrease in testicle size, testosterone level and sperm quality. Therefore, it appears save to assume that the GnRH vaccine is effective as a contraceptive or does at least greatly diminish fertility in elephant bulls.

Although testosterone was suppressed and no musth phases occurred, there were only minor behavioral changes in certain bulls. This shows that by large parts, the character of each animal and the learned behaviors play a more decisive role than the hormone status.

We plan to further monitor these elephants, as we need to look into long term effects as well as how long effects last. The following objectives are still to be tested:

- Long term effects
- Reversibility
- Application in a breeding herd to ultimately proof sterility of vaccinated bulls
- Long term monitoring of behavior

By taking certain elephants off the vaccine, we would gather information on the length of vaccine intervals and time till effects reversed. Currently, we administer the vaccine every six months. But it is probably safe to apply it only once a year after intensive initial treatment.

Acknowledgement

We wish to thank the International Elephant Foundation and the U.S. Fish and Wildlife Service, African elephant fund for funding the research in South Africa and the Birmingham zoo for supporting the hormone analysis.

Pretreatment of Asian Elephant *(Elephas maximus)* Spermatozoa with Cholesterol-loaded Cyclodextrins and Glycerol Addition at 4°C Improves Cryosurvival

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Asian elephant spermatozoa are sensitive to chilling and do not respond well to cryopreservation. The objectives of the present study were to: (1) determine whether cholesterol content can be modified by preincubation of Asian elephant spermatozoa with cholesterol-loaded cyclodextrin (CLC); and (2) assess the effects of CLC concentration(s), temperature at time of glycerol addition (22°C vs 4°C) and dilution medium on post-thaw sperm survival. Spermatozoa incubated with ³1.5 mg CLC exhibited increased ($P < 0.05$) cholesterol concentrations. Pretreatment of spermatozoa with 1.5 mg CLC resulted in improvements ($P < 0.05$) in all post-thaw parameters. Glycerol addition at 4°C also improved all post-thaw parameters compared with 22°C. Dilution of thawed spermatozoa in an egg yolk-based medium improved ($P < 0.05$) motility compared with Ham's F-10 culture medium. In summary, our findings indicate that modifying cholesterol content within the plasma membrane improves the cryosurvival of Asian elephant spermatozoa. The development of an improved cryopreservation method that includes modification of membrane cholesterol and the addition of glycerol at 4°C, as reported in the present study, is an important step towards utilization of cryopreserved spermatozoa in captive management of this species.

Successful Cryopreservation of Asian Elephant *(Elephas maximus)* Semen Using Simple Low-tech Techniques.

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Asian elephant populations are under increasing threats in the wild and the rate of reproduction in zoos is not consistent enough to sustain a genetically diverse population to act as a hedge against extinction. The use of assisted reproductive technologies like artificial insemination combined with cryopreservation of gametes and embryos could be a valuable tool for the conservation of elephants. However, simple low-tech protocols for the cryopreservation of Asian elephant semen are lacking. The purpose of this study was to compare the effect of simple freezing techniques and different thaw temperatures on the post-thaw quality of cryopreserved Asian elephant semen samples. Ejaculates (N = 3) were collected by rectal massage from two bulls. The average initial motility was 75.6% (range 72 – 80%) and the average proportion of intact acrosomes was 76.3% (range 54 – 90%). Semen samples were immediately extended in a lactose/egg-yolk-based extender (BC solution) and then cooled to 4C in an equitainer over 3 hrs. Once cooled, samples were further extended 1:1 with glycerolated extender (14% glycerol for a final concentration of 7%) with 25% of the volume of the glycerolated extender added in 15 minute increments. Samples were then loaded into 0.5 ml straws. Semen samples were cryopreserved by placing straws in LN2 vapour at 5 cm, 2 cm and 1 cm above LN2 as well as by placing straws directly into a charged LN2 dry shipper. After storage in LN2 for at least 48 hrs, straws were thawed at 37C for 30s, 50C for 10s, 50C for 15s or 75C for 6s and diluted 1:4 in non-glycerolated extender. Samples were assessed after cooling (pre-freeze), immediately post-thaw and after incubating thawed samples at 37C for 1 hr. Cooling had no significant effect on acrosomal integrity or motility ($P>0.05$). Only the ejaculate that initially had a low proportion of intact acrosomes lost motility with cooling (initial motility of 72% decreased to 59%) and it had the poorest preservation of pre-freeze motility in all treatment groups. Semen samples cryopreserved by placing straws at 2 cm and 1cm above LN2

or placed directly in the dry shipper had significantly higher post-thaw motility and intact acrosomes than straws placed at 5 cm above LN2 ($P < 0.001$). Thaw temperature had no significant effect on post-thaw motility or acrosomal status ($P>0.05$). Placing straws directly in the dry shipper and thawing them at 75C consistently preserved >60% of the pre-freeze motility (average 82.5%; range 61 – 100%) and acrosomal status (average 87.5%; range 64 – 100%). There was no significant loss of motility observed in the thawed samples after 1hr incubation at 37C. These initial results suggest it is possible to successfully cryopreserve Asian elephant semen using simple low-tech techniques. This study is ongoing with results from additional ejaculates and treatments being assessed and is funded by the International Elephant Foundation.

Session XI: *Ex situ* Reproduction and Management

Relationships Among Birth Presentation, Amniotic Sac Rupture and Stillbirths in Rhinoceros

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In spring 2012, the San Diego Zoo Safari Park experienced a difference between two greater one-horned rhinoceros births. One was a successful live birth, anterior presentation, and the other was a dystocia stillbirth, posterior presentation. Initially the stillbirth was suspected to be due to the posterior presentation at birth. However, years of anecdotal evidence suggested otherwise and a formal investigation was initiated.

Data were gathered by reviewing animal records including behavioral and breeding records for all three rhino species at the San Diego Zoo Safari Park. Data on 173 rhino births between 1970 and 2013 were analyzed for species of rhino, and whether or not the birth was recorded as a live birth or stillbirth. Breeding records indicated that 5.3% ($n = 93$ total births) of the southern white rhinos born at the park were stillborn compared to 0.6% for black rhinos ($n = 15$ total births) and 24.5% for greater one-horned rhinos ($n = 65$ total births). Subsequent analyses of regional studbook records for each of these species through 2010, indicated that stillborn calves account for 7% of southern white rhino, 11.0% of black rhino, and 19.7% of greater one-horned rhino births in North America. This information led to further investigation as to why greater one-horned rhinos have such a high rate of stillborns.

While data are limited, 12 births have been video recorded at the San Diego Zoo Safari Park including 8 greater one-horned, 2 southern white and 2 black rhinos. Seven additional births had written documentation for presentation and are included but are not considered for sac rupture analysis. Of the 19 births, 11 calves were delivered in posterior presentation, 7 were delivered in anterior presentation, and one was unknown. Eight of the 12 births that were recorded were live births, 4 were stillborns. In all four cases of a stillbirth delivery, the amniotic sac ruptured prior to delivery of the stillborn. Three stillborn calves were posterior presentation, and one was unconfirmed. Additionally, the amniotic sac remained intact prior to delivery in all greater one-horned live births recorded. However, one black rhino's amniotic sac ruptured prior to birth but the calf was still viable, so the number of minutes between sac rupture and delivery may be significant.

With the limited results and surrounding questions, rhino births published on Youtube were then incorporated into the data. The caveat for the addition of these recordings is that all births are live births, as no facility would be expected to post video of a stillbirth. Nine Youtube videos of rhinos giving birth were evaluated; all nine rhinos had the amniotic sac intact at time of delivery.

Results of this study indicate that rhinoceros are able to deliver viable calves presented in both anterior and posterior positions, and that premature amniotic sac rupture may be the more significant factor contributing to the delivery of a stillborn calf.

Thermoregulation in the African Elephant and Possible Effects on Fertility.

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As large mammals in hot, arid environments, African elephants may face difficulties losing excess heat gained during the day. They may store heat gained during the day, and release it to the cool night sky through vasodilation in their ears. Three captive African elephants were observed over six nights and their core body, side, and ear temperatures were measured with thermal imaging. Ear and side temperatures decreased faster on cold nights than warm nights. Both ear and side temperatures were more variable on warm nights. Core body temperatures varied by as much as 4 degrees C as measured by thermal imaging of freshly voided urine. Benedict and Lee (1936) showed a strong correlation between core body temperature and urine temperature, it can be assumed that core body temperature is undergoing these same fluctuations. These findings indicate adaptive heterothermy where heat is stored during daylight hours and subsequently lost overnight. Studies in semi-free-ranging African elephants have shown a very mild core temperature fluctuation as measured by differing methods. Data loggers were given to elephants and were retrieved from the faeces. Mean body temperature over the July 2006-November 2007 study period was about 36.4 +/-0.03 degrees C and mean daily amplitude was about 1.2 +/- 0.04 degrees C (Hidden, 2009).

Landscape use (Kinaham 2007) and perhaps even captive diets, with highly fermentable concentrates, may contribute to the measured differences between captive and free-ranging elephants, even given differing methodologies. The measured rises in temperature in captive elephant's raise some questions about possible contributions to infertility in captivity. Heat stress is a well-known cause of both male and female infertility in various livestock

species, mice, and humans. Heat stress has been shown to alter the duration of estrus, colostrum quality, conception rate, uterine function, endocrine status, follicular growth and development, luteolytic mechanisms, early embryonic development, and fetal growth (Jordan 2003). Heat in males will contribute to oxidative stress and decrease in fertility (Tremellen 2008). Environmental factors such as shade, indoor housing overnight, and obesity may contribute to the differences seen between wild and captive elephants in terms of thermoregulation and fertility although the total number of animals both in the wild and captive studies is tantalizingly small. Retrospective analysis of the fertility of the previously studied elephants as well as prospective studies paring thermal imaging with assessments of fertility may prove useful.

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Reproduction and Population Performance in the European Captive Population of Eastern Black Rhinoceros *(Diceros Bicornis Michaeli)*

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With fewer than 5000 black rhinoceros *(Diceros bicornis)* left in the wild, ex situ populations play a vital role in the conservation of this species. However, the European captive population of the eastern subspecies *(D. b. michaeli)* is currently underperforming compared to their in situ counterparts, with annual growth rates of only 1-2%, compared to >5% in situ. In recent years, the primary factor limiting growth of this population has been low rates of reproduction, with only around 11% of adult females breeding each year. Furthermore, approximately 40% of reproductive-age individuals are yet to successfully produce offspring, resulting in high reproductive skew in both males and females. To investigate differences in reproductive success, faecal samples were collected from 23 males and 39 females at 13 institutions across Europe, and used to measure reproductive and adrenal hormones. In females, three-quarters of all oestrus cycles observed were 20-40 days in length, but irregular cyclicity was also apparent in both parous and nulliparous females, with short (<20 days) and extended cycles (>40 days) often exhibited over a 12-month period. Furthermore, within females, these extended cycles were associated with elevated faecal glucocorticoid metabolite concentration compared to other cycle types. In males, differences in faecal testosterone concentration were observed between males that had previously sired offspring, and those that had not, but this was unrelated to faecal glucocorticoid concentration. Potential correlates of the observed differences in reproductive and adrenal hormone concentration are being investigated, in an attempt to minimise reproductive skew and maximise the reproductive output of this population.

Improving the Welfare of Captive Asian Elephants in Kerala, India

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Improving the welfare of captive Asian elephants in India is emerging as an important conservation issue. Improving elephant welfare is one of the research priorities of scientists, policy makers and researchers. This study was done in Kerala state, the southern state of the country where elephants are used widely for festivals. Tuskers are used for processions and festivals in Temples and churches. Recent findings revealed that due to poor elephant management, 400 human lives have been lost during the last three and a half decades in India, of which more than 90 percent of the victims were mahouts. It is clear that poor elephant management practices cause elephant aggression toward mahouts.

This study was conducted in the southern state of India, Kerala which occupies only 1.13 percent of the geographic area of the country. The state has 600 captive Asian elephants. As part of the study, 100 captive Asian elephants were selected to determine the cause of elephant aggression and to formulate measures to improve captive elephant welfare. Variables like breeding, feeding, management, season, age, musth incidence, behavior, transportation and diseases were identified. Major interventions affecting the above variables were also identified and a SWOT analysis was done. Major indicators of poor elephant welfare include few chances for breeding, unscientific feeding and management, prolonged standing in festivals, unscientific musth management, poor musth forecasting system, heat stress, poor transportation and disease management.

Based on the above findings, a system was formulated to improve elephant management after taking into account the major variables identified including best feeding and management practices, musth forecasting system, scientific disease control system and best management practices during transport and festivals. The new scientific management system was administered on 100 captive Asian elephants during the festival season of 2012-2013. During festival season, when the elephants are compelled to stand more than 6 hours, measures were taken to frequently give watery vegetables like cucumber and watermelon. In order to reduce heat stress, the elephants were allowed to walk in shady places and wet gunny bags were placed underneath their feet. Shamianas were made where the elephants stand to protect them from scorching sunlight. A 12 hours rest period was made compulsory for all elephants before moving to the next festival. Mahouts were given training in scientific management practices. As part of transportation norms, elephants are allowed to walk only 20 hours per day during morning and evening hours. For travel of more than 20 km, trucks were made mandatory as per the state's captive elephant management rule.

This study's findings revealed that best captive elephant management practices could reduce 90 percent of the elephant aggression toward humans. In order to reduce stress, scientific feeding and management, disease control and management and attention to musth is required. There is a positive correlation between season and incidence of musth therefore a musth forecasting system will help to reduce elephant aggression toward humans and improve elephant welfare.

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Relationship Between Management, Adrenal Activity and Reproduction in a Captive Group of Female Asian Elephants *(Elephas maximus)*

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Asian elephants in captivity often exhibit low rates of reproduction and can be prone to reproductive problems including acyclicity. In order to maintain a healthy and sustainable population, a better understanding of social and environmental influences on reproductive cyclicity is warranted. Annual routine reproductive monitoring of faecal progesterone of five reproductive-age females revealed that oestrus cycles were highly synchronized among individuals. However, in one year, a 15-week period of acyclicity occurred in three of the five females, which coincided with a number of management changes, and the conception of two of the five females in the group. The aim of this study was therefore to retrospectively investigate whether changes in management factors were associated with increased adrenal activity and subsequent suppression of ovarian activity; or whether the conception of herd-mates might better explain cessation of cyclicity. Faecal samples (n=1032, ~200 samples per individual), collected every other day, were analysed for progesterone and glucocorticoid metabolites (as a reflection of adrenal activity) by enzyme immunoassay over 16-month consecutive period, to cover four potential oestrus cycles, including the observed 15-week period of acyclicity. Concurrently, management factors including training, matriarch presence and husbandry regimes were recorded daily. Data were analysed using General Linear Mixed Models for each individual to investigate whether faecal glucocorticoid metabolites were related to 1) Management [matriarch presence, training and foot care] or 2) Reproduction [individual reproductive status or the presence of other pregnant individuals]. Routine training and foot care were not associated with any change in adrenal activity for any individual; however, intensive foot care and intensive training were associated with an increase in adrenal activity in one female. Matriarch presence influenced adrenal activity in one sub-adult and three adult females, but not the juvenile female. In the three females that exhibited acyclicity there was no consistent relationship between faecal glucocorticoid metabolites and reproductive state; however, the start of the acyclic period coincided with the dominant female's pregnancy and the conception of a second herd-mate. This study provided the physiological evidence (adrenal activity) to support prospective management changes including, no longer separating the matriarch from the group and refurbishing the indoor and outdoor enclosure with sand to improve foot health. This study highlighted how daily zoo management activity records and hormone monitoring can be used to support prospective management changes to improve the care and welfare in a group of Asian elephants.

Social and Reproductive Behaviour of Critically Endangered Northern White Rhinoceros *(Ceratotherium cottoni)* in a Zoological Garden

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The northern and southern white rhinoceroses have very low reproduction in captivity and social interactions between the animals, especially their increased agonistic behaviour, are believed to be one of the possible reasons. Free-ranging white rhinos have a network of social relationships with their conspecifics, which is, however, usually not available to them in captivity. Appropriate group composition and/or a change of social relationships in white rhino herds might therefore have a positive influence and increase the reproductive rate of captive rhinos. However, studies investigating the changes in group structure on the social and reproductive behaviour of captive rhinos are missing. The northern white rhino is currently on the brink of extinction with only seven animals known to survive. We studied the social and reproductive behaviour of a group of northern white rhinoceros (one male, five females) in zoological garden Dvůr Králové in 2005. The most often observed agonistic activities among the animals were threat, snarl and clash of horns. From the total number of agonistic activities in the herd, 73% was directed towards the male. In the middle of our study, one of the females (the oldest one and the only one wild-born) was separated out of the herd. Following her separation, agonistic behaviour among the rhinos significantly increased ($p = 0.04$). In addition, play behaviour, especially between the male and females also increased ($p = 0.04$). Play behaviour is, however, observed in adult male-female interactions in the wild only very rarely. We did not observe any changes in sociopositive behaviour ($p = 0.79$). Social dominance among the females, which might affect their reproduction, was not found. A presence of old and experienced female in the herd might have had a positive influence on the social interactions among other animals. Our results show that a composition of white rhino groups in captivity can have a significant influence on the social interactions among the rhinos. Better knowledge of appropriate composition of their groups in terms of age, sex and wild or zoo origin might therefore improve animals' well-being and increase a chance for their reproduction.

Comparison of Visual Body Condition Scoring Systems in Asian Elephants and Validation By Transcutaneous Ultrasound

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Visual body condition scoring (BCS) is one of the most practical and effective tools for evaluating the general health status of an animal. This tool has been used to maximize reproductive success in production animals and identify health risks in domestic and exotic species (Gearhart et al., 1990; Laflamme, 2005; Lemma et al., 2006; Samarutel et al., 2006; Scarlett and Donoghue, 1998). Several visual BCS systems have been published for the Asian elephant *(Elephas maximus)* (Fernando et al., 2009; Ramanathan and Mallapur, 2008; Ramesh et al., 2011; Wemmer et al., 2006; Wijeyamohan et al., In preparation). Although each system uses a different scale, the relative descriptions are similar.

The Fort Worth Zoo (FWZ) has developed a 9 point BCS scale for the Asian elephant based on successful systems in other species which have been validated against direct measures of fat, correlated to health outcomes and used to manage captive populations. The FWZ system provides descriptions of standardized images (Figure 1) developed from an image database of hundreds of captive and wild Asian elephants in North America, Europe and Asia spanning the full spectrum of body condition (emaciated to extremely obese). These descriptors are being validated using direct measures of fat by transcutaneous ultrasounds collected from over 100 elephants in North America and Asia. Transcutaneous ultrasound is used in many domestic and exotic species to evaluate fatness and has been shown to correlate to visual body condition, although the strength of correlation may depend on ultrasound location and species-specific fat deposition (Domecq et al., 1995; Gentry et al., 2004; Wilkinson and McEwan, 1991). Therefore multiple ultrasound locations were tested for measuring fat in the Asian elephant in this study.

A subset of 12 elephants from 3 institutions in North America were sampled concurrently for visual body condition and transcutaneous ultrasound over rump and ribs (Figure 2). Four FWZ elephants were also sampled longitudinally for evaluation of body condition changes within individuals. Average elephant weight was 3200 kg (range 1491-4242), average BCS was 6.25 (range 4-9) and fat thickness ranged from <1 to more than 5 cm. Body condition score was shown to correlate significantly to ultrasound fat thickness.

Validation of visual BCS in Asian elephants using direct ultrasonic measures of fat supports the use of BCS as a tool for managing the condition of captive elephants and evaluating wild populations. Obesity in captive Asian elephants is suspected to be a significant problem associated with prevalent health concerns such as foot problems and reproductive failure. The combined body of work on Asian elephant body condition to date provides a strong, supported and practical management tool for improved health and success of captive elephant herds.

Figure 1. Standardized photoset for body condition scoring.

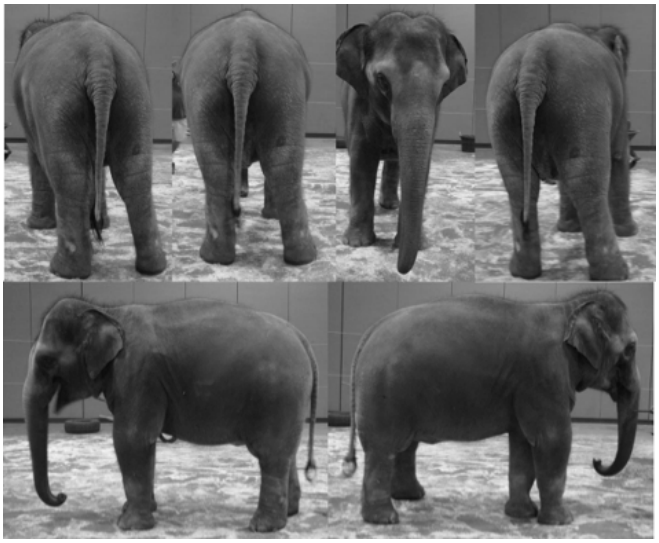
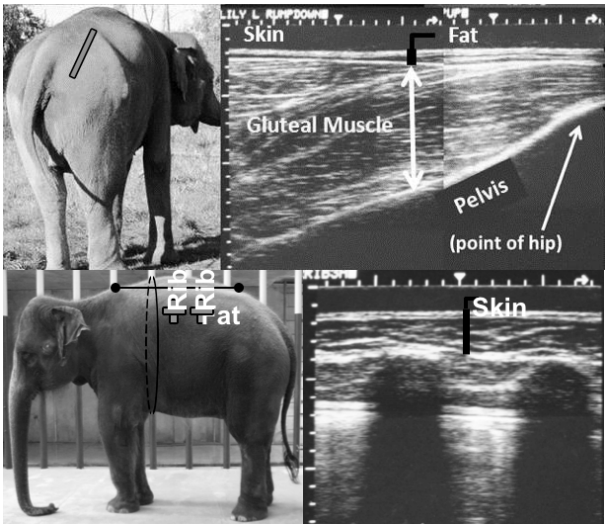


Figure 2. Standardized ultrasound locations over rump (from point of hip parallel to tailhead) and ribs (30% and 50% from point of hip to shoulder peak, down 10% of heartgirth).



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Body Condition Scoring Index for Female African Elephants Validated with Ultrasound Measurements of Subcutaneous Fat

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Several anecdotal reports suggest zoo elephants are obese, and that obesity-related health and reproductive problems are contributing to the non-sustainability of the African elephant *(Loxodonta africana)* population in U.S. zoos. However, a major constraint in screening for obesity is the lack of a practical method to accurately assess body fat in elephants. Body condition scoring (BCS) is the assessment of subcutaneous body fat stores based on visual or tactile evaluation of muscle tone and key skeletal elements, and provides an immediate appraisal of the degree of fatness of an individual. The objective of this study was to develop a visual body condition scoring (BCS) index for female African elephants and validate it using ultrasound measures of subcutaneous fat. To develop the index, standardized photographs were collected from zoo (n=50) and free-ranging (n=57) female African elephants to identify key body regions and anatomical sites that were used to visually assess body fat deposition patterns. The visual BCS method consisted of a list of these body regions and the physical criteria used for obtaining an overall score on a 5-point scale, with 1 being the thinnest and 5 being those with the most body fat. Significant correlations were observed between the visual scores and ultrasound measures of subcutaneous fat thickness at all anatomical sites, but were highest in the pelvic bone ($r = 0.660$, $P < 0.01$) and backbone ($r = 0.664$, $P < 0.01$) regions, indicating that BCS adequately reflects the amount of actual fat reserves. The new BCS index proved to be a reliable and repeatable method, with a high percentage agreement (73 % to 95%) and an overall "substantial" strength of agreement determined by the weighted k statistic ($k_w = 0.62$ to 0.91) between and among three assessors that scored 40 elephants. In comparing photographs of wild vs. captive elephants, the median BCS in the free-ranging individuals (BCS=3, range 1-5) was lower ($P < 0.001$) than that of the zoo population (BCS=4). Results suggest that this new BCS index could be a valuable tool for examining the relationship between body condition and various factors affecting the health and reproduction of zoo and free-ranging elephants.

The Effect of Fresh Forages on the Fat Soluble Vitamin and Lipid Profiles of Greater One-horned Rhinoceros *(Rhinoceros unicornis)*

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The nutritional status of captive rhinoceroses has received many years of dedicated research, especially since nutritional aberrations in this taxonomic group are suspected to be linked to various disease syndromes (Clauss *et al*. 2002, Dierenfeld *et al*. 2005, Ghebremeskel *et al*. 1988). Previous research in the domestic horse has found that green forage is an important source of vitamin A derivatives and that captive diets provide lower amounts of vitamin E than native forage (Mäenpää *et al*. 1998, Dierenfeld *et al*. 1990, Dierenfeld *et al*. 1995, Ghebremeskel *et al*. 1991). Endogenous vitamin E levels have been shown to be affected by the level and type of dietary fat, antagonism of other fat soluble compounds, and additional oxidant stressors (Dierenfeld 1999). In addition, dietary concentration of vitamin E is the single most important variable affecting plasma *a*-tocopherol levels (Dierenfeld *et al*. 1997, Dierenfeld 1999).

'The Wilds' is unique in its ability to provide a semi-free ranging environment for seven months of the year (April to November) for greater one-horned rhinoceroses *(Rhinoceros unicornis)*. During this period, greater one-horned rhinoceroses have free access to fresh grasses and North American browse; with almost no mixed grass hay ingested during this time. During the late fall and winter months (November to April), animals are housed indoors and mixed grass hay forms the majority of their diet with insignificant access to fresh grass and no access to North American browse. A commercial pelleted diet is a part of their daily ration throughout the year.

Given the information presented, there is likely to be a difference in the serum profiles of vitamins A, and E, and lipids between the two management periods. This study aimed to determine the effect of fresh North American browse and grasses on animals' nutritional status, compared with *ad libitum* mixed grass hay. Five animals were included in the study (two sub-adult females, two adult females and one adult male), and blood was collected at the end of winter housing (April) and at the end of the pasture management period (November). Animals were sedated for phlebotomy, reproductive assessment, and ophthalmic examination. Serum values of multiple vitamins and lipids were compared using paired t-tests at a 95% confidence level.

Retinol (a measure of vitamin A status) values (April 0.035µg/ml, November 0.041µg/ml) were similar to those reported by Clauss *et al.* (0.03 ± 0.02µg/ml). No statistically significant differences were noted in retinol and β-carotene between the two sampling times. α-tocopherol measured at both time periods (April 0.40µg/ml, November 0.76µg/ml) was within the range reported by Clauss *et al.* (0.34 ± 0.36µg/ml). α-tocopherol was significantly higher following pasture management ($p = 0.0035$), as was 25-hydroxyvitamin D ($p = 0.017$). Lipids, such as cholesterol, triglycerides and non-esterified fatty acids, were all noted to be statistically higher in November than in April. These results and their implications on the dietary management of captive greater one-horned rhinoceroses will be discussed, emphasizing the importance of access to fresh browse and grass to the nutritional profiles of this species.

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Management of a Breeding Herd of African Elephants *(Loxodonta africana)* on a Predominately Forage Diet

Ray L Ball, DVM & Maura Davis
Tampa's Lowry Park Zoo

The breeding herd of 1.3 African elephants at Tampa's Lowry Park Zoo was transitioned from a traditional zoo diet consisting of large proportions of concentrates to one with predominately forage (hay and browse) with produce and concentrates used only as training items. Body weights were taken on a regular basis and two of the females have conceived while on the diet. Weight gains for the pregnant females have been relatively small, but still positive on this feeding plan. One calf has been delivered and this female has lactated normally. The female calf has shown good weight gain during the first 9 months of life. The second calf is expected mid-August 2013.

Blood samples on the adults were collected weekly for hormones analysis and serum banking. Retrospective analysis of essential fatty acids, minerals, and fat soluble vitamins was conducted on an opportunistic basis as sampling allowed. Comparisons are limited but can be made before and after the diet shift.

Feeding forage only diet to African elephants appears to be beneficial as it has been in gorillas and white rhinos fed in similar fashion. Obvious benefits include weight management but more appropriate EFA may lead to better health of the digital cushion and hence less foot problems and increased feeding times can lead to less stereotypy. Consuming less fermentable feeds (grains) may also lead to less body heat generated during digestion and lessen the potential contribution to excess heat buildup. Less adipose will potentially lessen any effects of adipose derive hormonal affects (leptin) and less heat around gonads will decrease the chance of heat stress contributing to infertility.

Sex Differences in Captive Elephant Calf Social Interactions

Megan L. Houchin, Nicole M. Lyons and Robert H. I. Dale

It is not surprising that male and female elephant calves behave differently as they develop. However, this study took advantage of the opportunity to study a male and a female calf of similar ages (born 5 months apart) interacting in exhibit yards at the Indianapolis Zoo during a period when no other calves were present. In addition, five adult females, but no male elephants, lived at the zoo. This meant that the calves had no opportunity to observe male-female interactions involving older elephants. We observed both similarities and differences in the behaviors of the calves.

Asian Elephant Support: A Year in Review and the Importance of Collaboration

April Yoder & Linda Reifschneider
Asian Elephant Support

Asian Elephant Support (AES) is a registered U.S. non-profit foundation dedicated to the care and conservation of elephants in Asian range countries, and to the people whose lives are intertwined with this magnificent and endangered species. Over the past year, AES has participated in various conservation projects throughout Asia. The AES board of directors would like to share an update on these projects with the symposium participants. The presentation will include updates on the following projects:

- Collaborated with the Elephant Managers Association Conservation Committee to raise funds for the "Hoof Knives for Mahouts- India" program.
- Facilitated a grant from the USFWS to conduct a veterinary workshop entitled "Field course in Emerging Diseases" in India.
- Continued support for ElefantAsia by purchasing a portable scale for the Elephant Hospital in Laos.
- Hosted the 3rd Annual California Pizza Kitchen fundraiser.
- Provided emergency funding for orphaned baby elephants.
- Supported a project to document the relationship between elephants and mahouts.
- Continued support of veterinary workshops in Northern India (Assam).
- Supported EEHV testing in Sumatra, Indonesia.
- Facilitating a grant for a Veterinary Workshop in Myanmar- early 2014.

For more information and a list of the current Directors please visit our website at: www.asianelephantsupport.org.

POSTERS

Allomothering Among Captive African Elephants

Laura Beer, Heather R. Bates and Robert H. I. Dale
Butler University

According to P.C. Lee (1987), interactions between African elephant calves and other elephants are frequent, consisting of friendly, relaxed interactions or of adults providing assistance to calves. When a female elephant other than its mother provides comfort, care or protection a calf, it is called allomothering. Using video recordings of African elephants at the Indianapolis Zoo, we compared the interactions between several calves and Sophi (the "matriarch"/ dominant female) and Tombi (a relatively subordinate adult in the group). Note that allomothering may occur either after an adult approaches a calf or after a calf approaches an adult. We examined the nature of the interactions between the calves and the adults.

Verbal Presentation Title: Sex Differences in Captive Elephant Calf Social Interactions

Megan L. Houchin, Nicole M. Lyons and Robert H. I. Dale
Butler University

It is not surprising that male and female elephant calves behave differently as they develop. However, this study took advantage of the opportunity to study a male and a female calf of similar ages (born 5 months apart) interacting in exhibit yards at the Indianapolis Zoo during a period when no other calves were present. In addition, five adult females, but no male elephants, lived at the zoo. This meant that the calves had no opportunity to observe male-female interactions involving older elephants. We observed both similarities and differences in the behaviors of the calves.

Rhinoceros Horns and Imitations in the Trade

Blount, V. M., B.C. Yates, E.O. Espinoza
National Fish and Wildlife Forensics Laboratory

The illegal trade in rhino horn is a leading contributing factor to the extinction of rhinoceros. Former evidentiary material can be used to further our knowledge base on how the horns are removed, processed, shipped, and modified for sale as whole horns or artifacts. Mechanisms of fakery can be detected using variable light source and low magnification to show how horse hooves and cattle horns are made into rhino horn imitations. Additionally, illegal fakes have been found made from carved elephant toenail. The implications of selling fake rhino horn include continued promulgation of the desire for rhino products and wasted time agencies/organizations spend in monitoring alleged contraband.

Butler Gates

Colin Butler
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All my life I have thought of myself as a problem solver and when the company I consult with was called into solve some issues with an elephant zoo enclosure I became fascinated with the many complex issues involved with keeping large animals safely and effectively in zoos, sanctuaries and game preserves. Learning more about the severe threat for the long-term survival of elephants and rhinos I truly wanted to help this cause. Loving a challenge, seeing the significant need, and after identifying enclosure issues with the keepers, I decided to develop some solutions. Because of my unique background with over 1000 gate systems that I have built and installed (including some very large ones), I have developed and built two scaled models that clearly demonstrate unique solutions for gates, fencing and ERD's for large animals that also protects large animal handlers. The gate equipment is all concealed to be elephant-proof. The gates can move extremely fast in an emergency situation as they are built out of lightweight carbon fiber vs. heavy steel. I also have eliminated tracks in the ground where the animals pass through that presently requires daily maintenance to keep clear. The gate and the fence are built as modular units that get placed in a 2' deep trench and bolted together, which allows for quick and easy installation. The fence, with its patented shallow footing is rated for an impact of 8 tons at 50 mph. The equipment can all be DC power, so that it can be installed in remote areas and run on solar power with a bank of 12V batteries that is mounted high for protection. All aspects of maintenance and installations are thus significantly easier, helping provide viable solutions for zoos, sanctuaries and game preserves.

Elephants for Africa: Conservation Through Research and Education

Kate Evans, Mphoeng Ofithile, and Miguel Cases
Elephants for Africa

Differentiation of *Mycobacterium* Species from Elephant Respiratory Samples in Nepal, Using Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) Analysis

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Tuberculosis caused by Mycobacterium tuberculosis complex (MTBC) species poses a significant threat to the health and welfare of Asian elephants, and to the conservation of the species as a whole (Greenwald et al. 2009). Both *Mycobacterium tuberculosis* (M. tb) and *Mycobacterium bovis* (M. bovis) are endemic to Nepal, yet it is unknown which species are responsible for pulmonary mycobacteriosis in Nepal's elephant population (NETCMAP 2011). Captive elephants interact closely with humans, wildlife and domestic species, increasing the risk for bidirectional transmission of *Mycobacterium* species and raising important public health, economic and conservation concerns (Murphree et al. 2011; Oh et al. 2002). It is widely accepted that, while early detection and management are essential for successful disease control in elephants, key inadequacies exist in current tuberculosis diagnostics (Angkawanish et al. 2010; Montali et al. 1998). Diagnosis relies on trunk wash (TW) culture and serology (Mikota and Maslow 2011). However, TW culture is impractical in many countries and lacks sensitivity (Mikota et al. 2006). Serology, while sensitive, does not differentiate the various *Mycobacterium* species, nor is it useful for monitoring elephants post-treatment (Kay et al 2010). The objective outcome of this research was to minimise the impact of tuberculosis on Asian elephant populations, through development of a novel molecular diagnostic technique, using Nepal's captive elephant population as a model.

A gyrB-based PCR-RFLP assay for the detection of MTBC species was developed, building on preliminary work by Wilson and others (2008) at the Center of Molecular Dynamics Nepal (CMDN). The technique was found to be capable of detecting and differentiating *Mycobacterium tuberculosis and Mycobacterium bovis* DNA from elephant trunk wash and nasal drip samples. Preliminary genetic sequencing confirmed potential for future application for identification of drug resistance. Despite multiple limitations in relation to study protocol, field sampling and in-country laboratory and technical capabilities, it was demonstrated that the PCR-RFLP technique is practical, accessible and relevant to developing Asian elephant range countries such as Nepal, and provides a potentially valuable addition to the current array of diagnostic options. The findings and limitations of this research were collated to provide recommendations for future research and improved management strategies, to minimise the impact of tuberculosis on the wild and captive populations of this endangered species.

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Preliminary Recordings of Wild Asian Elephant *(Elephas maximus)* Vocalizations in Preparation for Playback Experiments

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Communication complexity is thought to increase with social complexity. Asian elephants live in a fission-fusion society and form multiple long-term social relationships, suggesting that their communication system may be particularly complex. They produce at least fourteen distinct call types1, but the meanings of these calls have never been experimentally determined. Growls (a type of low-frequency harmonic call) are individually distinct, but also highly variable within a given individual1. However, it is not known whether there are different structural or functional categories of growls. Combination calls are composed of a broadband segment immediately followed by a low-frequency harmonic segment1. Anecdotal reports suggest that elephants may respond more to combination calls than to low-frequency calls alone. The broadband segment may help call attention to the information contained in the low-frequency segment. Alternatively, the low-frequency segment may allow individual identity to be assigned to a broadband call. In December 2012 and January 2013, we recorded fifty-three calls from wild Asian elephants in Uda Walawe National Park, Sri Lanka, and categorized the vocalizations into basic call types according to de Silva1 by visual inspection of the spectrograms. Thirty-one calls were low-frequency signals with clear harmonics (growls and rumbles), eleven were broadband signals with no clear harmonics (barks, roars, and longroars), and five were combination calls (bark-rumbles). The remainder of the calls consisted of higher frequency sounds (trumpets and squeaks). We will record additional calls and pool our recordings with a pre-existing call library recorded by the Uda Walawe Elephant Research Project. We will then use cluster and discriminant function analysis to determine if growls can be subdivided into discreet structural categories. If structural categories exist, we will use playback experiments to test whether they correspond to functional categories. We will also use playback experiments to test multiple hypotheses for the function of combination calls.

ACKNOWLEDGMENTS

We are deeply grateful to the staff of the Uda Walawe Elephant Research Project, without whom this research would not be possible. We also thank the Sri Lankan Department of Wildlife Conservation for permission to work in Uda Walawe National Park. This project was funded by grants from the Cornell University Graduate School and the Athena Fund of the Cornell Lab of Ornithology.

Confirmation of the First Case of Endotheliotrophic Elephant Herpes Virus (EEHV) Infection in Nepal Using a Real-time Quantitative PCR Assay and DNA Sequence Analysis

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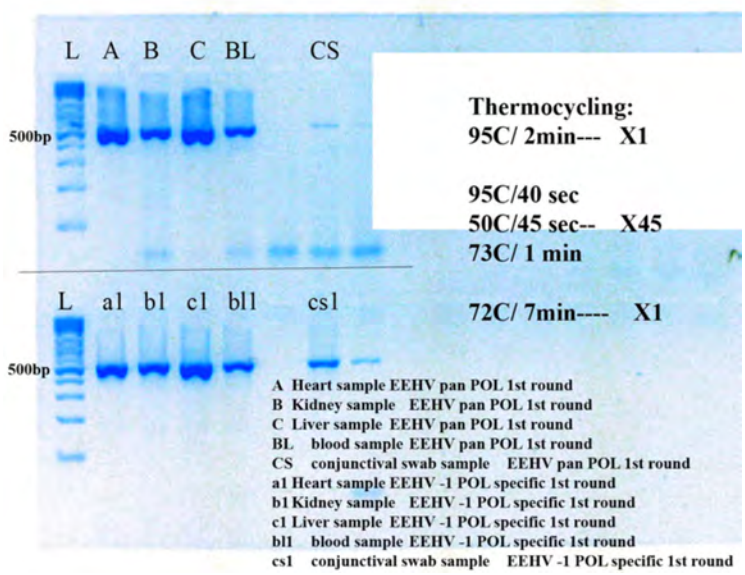
Elephant endotheliotropic herpes virus (EEHV) presents a growing threat to the health and conservation of both captive and wild endangered Asian elephant populations worldwide (Cracknell 2008). In the acute form the disease is characterized by a sudden onset of lethargy, oedema of the head, proboscis and limbs, oral ulcers, and diffuse internal haemorrhaging (Garner et al 2009; Miller and Fowler 2012). In acute cases, death often results within one week following the onset of symptoms, and few cases have survived with intensive treatment (Hayward 2012). There is currently limited knowledge of the prevalence of EEHV in Nepal, a country with both captive and wild Asian elephant populations. However, since 2008, four elephant calves have succumbed to unconfirmed but presumed herpes virus infections (Gairhe 2012). Findings from a study of the Chitwan National Park (CNP) elephant breeding center in mid-2012 suggest the presence of latent EEHV infections in a number of adults within the breeding herd, although PCR and sequencing results were inconclusive (Kaufman et al, unpublished). In December 2012 a juvenile female elephant from the CNP breeding herd presented with ante-mortem and post-mortem changes characteristic of acute EEHV. Conjunctival swabs and heart, liver, kidney and blood samples were collected by the National Trust for Nature Conservation (NTNC) and analysed at the Center for Molecular Dynamics Nepal (CMDN). Samples were analysed using a validated real-time quantitative PCR assay (Hardman et al 2010; Latimer et al 2011), and all samples confirmed to be positive for EEHV. Using DNA sequencing and viral gene sub-typing analysis (Stanton et al 2010; Stanton et al 2012, in review) all samples were subsequently identified as subtype EEHV1A. These results mark the first PCR-confirmed case of EEHV infection in Nepal, and demonstrate the presence of EEHV in the CNP captive elephant population. Such findings support the hypothesis that latently infected individuals reside within the CNP breeding herd, and highlight the urgent need for adaptive management to minimise morbidity and mortality. Further research into EEHV prevalence, epidemiology and dynamics in Nepal are recommended in order to mitigate the impact of this disease on both captive and wild Asian elephant populations.



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Baby Steps

Kathy Suthard and Diane Hagey
Pittsburgh Zoo & PPG Aquarium

The Pittsburgh Zoo & PPG Aquarium celebrated the first black rhino birth in nearly half a century in September of 2012. The calf's dam, Azizi, was born at the Cleveland Metroparks Zoo, and the sire, Jomo, was born at the San Diego Wild Animal Park.

To insure that this calf would grow up self-confident and able to adjust to a variety of situations, the keepers drew from some traditional and not-so-traditional methods for working with rhinos. Initially the focus was on getting the baby familiarized with keepers working around her. Once she was comfortable with people being present, keepers began getting her accustomed to casual physical contact with humans. As she began to enjoy this contact, interactions were expanded to include selected techniques from Parelli™ Natural Horsemanship, Tellington TTouch®, massage therapy, and operant conditioning. This was the beginning of a trusting relationship with this young animal and her keepers.

The successful interactions with the calf would not have been possible without the cooperation of Azizi. The relationship building that began at the Cleveland Metroparks Zoo was instrumental in creating a solid foundation upon which Pittsburgh Zoo keepers could build. Azizi's calm acceptance of her keepers' attentions was and still is a model for her calf.

Notes

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