

2021 Elephant References (alphabetical)  
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Abraham, J. O., E. R. Goldberg, J. Botha and A. C. Staver (2021). "Heterogeneity in African savanna elephant distributions and their impacts on trees in Kruger National Park, South Africa." *Ecol Evol* **11**(10): 5624-5634.

Though elephants are a major cause of savanna tree mortality and threaten vulnerable tree species, managing their impact remains difficult, in part because relatively little is known about how elephant impacts are distributed throughout space. This is exacerbated by uncertainty about what determines the distribution of elephants themselves, as well as whether the distribution of elephants is even informative for understanding the distribution of their impacts. To better understand the factors that underlie elephant impacts, we modeled elephant distributions and their damage to trees with respect to soil properties, water availability, and vegetation in Kruger National Park, South Africa, using structural equation modeling. We found that bull elephants and mixed herds differed markedly in their distributions, with bull elephants concentrating in sparsely treed basaltic sites close to artificial waterholes and mixed herds aggregating around permanent rivers, particularly in areas with little grass. Surprisingly, we also found that the distribution of elephant impacts, while highly heterogeneous, was largely unrelated to the distribution of elephants themselves, with damage concentrated instead in densely treed areas and particularly on basaltic soils. Results underscore the importance of surface water for elephants but suggest that elephant water dependence operates together with other landscape factors, particularly vegetation community composition and historical management interventions, to influence elephant distributions.

Algehani, A. M. G., F. A. Jaber, A. Khan and M. N. Alsulami (2021). "Review on trypanosomiasis and their prevalence in some country on the Red Sea." *Braz J Biol* **83**: e251671.

Trypanosomiasis is a protozoan infection affecting both human and animals in almost all parts of the world. It can affect a very large range of domestic and wild hosts including camelids, equines, cattle, buffaloes, sheep, goats, pigs, dogs and other carnivores, deer, gazelles and elephants. This review paper was designed to address the effect of this economically important disease in countries on the Red Sea, especially in Egypt, Sudan, Somalia, and Saudi Arabia during the period 2010 to 2020. The prevalence of trypanosomiasis is different between these countries due to different types of diagnostic methods (Giemsa-stained blood smears, Hematocrit centrifugation, Serological test, and molecular analysis PCR) used and differential distribution of vector (Tse tse) flies. In current review, retrospective studies of published literature on distribution and prevalence of *Trypanosoma evansi* infection in the Red Sea Countries was conducted

[Google Scholar and PubMed were used to retrieve the published literature from 2000-2020. A total of 77 published articles met the eligibility criteria and were reviewed. A total of 16 reports have been reported on the prevalence and distribution of *Trypanosoma evansi* infection in the Red Sea Countries have been from 2010-2020]. According to the published literature, we can say that trypanosomiasis in camels are more prevalent in Sudan than in other countries, followed by 17% and 51.78% in both clinical and non-clinical cases. Hence, the reliable diagnostic tests should be used for rapid treatment or control of the disease as if not treated appropriately in early-stage, can lead to death of the camels.

Allen, C. R. B., D. P. Croft and L. J. N. Brent (2021). "Reduced older male presence linked to increased rates of aggression to non-conspecific targets in male elephants." *Proc Biol Sci* **288**(1965): 20211374.

Males in many large mammal species spend a considerable portion of their lives in all-male groups segregated from females. In long-lived species, these all-male groups may contain individuals of vastly different ages, providing the possibility that behaviours such as aggression vary with the age demographic of the social environment, as well as an individual's own age. Here, we explore social factors affecting aggression and fear behaviours in non-musth male African elephants (*Loxodonta africana*) aggregating in an all-male area. Adolescent males had greater probabilities of directing aggressive and fearful behaviours to non-elephant targets when alone compared to when with other males. All males, regardless of age, were less aggressive towards non-elephant targets (e.g. vehicles and non-elephant animals) when larger numbers of males from the oldest age cohort were present. The presence of older males did not influence the probability that other males were aggressive to conspecifics or expressed fearful behaviours towards non-elephant targets. Older bulls may police aggression directed towards non-elephant targets or may lower elephants' perception of their current threat level. Our results suggest male elephants may pose an enhanced threat to humans and livestock when adolescents are socially isolated, and when fewer older bulls are nearby.

Allen, C. R. B., D. P. Croft, C. Testard and L. J. N. Brent (2021). "Function of Trunk-Mediated "Greeting" Behaviours between Male African Elephants: Insights from Choice of Partners." *Animals (Basel)* **11**(9).

A common behavioural interaction between male African elephants is for an actor to direct his trunk to contact a same sex conspecific's mouth, temporal gland, or genital region. Such behaviours are often referred to as "greetings". Along with its inherent tactile element, these behaviours also likely provide olfactory information to actors concerning aspects of the target's phenotype, including sexual status, feeding history, individual identity, and emotional state. Here, we explore whether the age and novelty of potential interactors affect the choice of individuals targeted by male African elephants for these trunks to scent emitting organ (SEO) behaviours at social hotspots in a male-dominated area. Male elephants of all ages, except older adolescents aged 16-20 years, preferentially targeted elephants of the same age class for

trunk-to-SEO behaviours. Elephants younger than 26 years did not direct trunk-to-SEO behaviours to mature bulls (26+ years) more than expected by chance, suggesting these behaviours are not primarily used for younger males to establish contact with, or obtain information from or about older, more experienced individuals. We also found no evidence that males directed these behaviours preferentially to new individuals they encountered at male aggregations (compared to those they arrived in groups with), suggesting these behaviours are not primarily employed by males as a reunion display to establish relationships between new individuals or update relationships between familiar individuals separated over time. Age-mates may be preferentially targeted with these behaviours as a means to facilitate further interaction with partners (e.g., for sparring activity), or as a safe way to assess relative dominance rank in similarly aged and hence, size and strength, matched dyads. Our results suggest male African elephants use close contact trunk-to-SEO behaviours continuously over time, to facilitate positive relationships, test willingness to interact, and assess aspects of phenotype, between males occupying the same ecological space.

Allen, W. R. T. and F. J. Stansfield (2021). "Placentation in the African Elephant (*Loxodonta africana*)."  
*Adv Anat Embryol Cell Biol* **234**: 181-204.

The female elephant shows a 3-week "follicular phase" to commence her 16-week estrous cycle at the end of which a second surge in pituitary luteinizing hormone (LH) release matures and ovulates an ovarian follicle in association with estrous behavior and mating, whereas the first LH surge at the start of the follicular phase causes luteinization of 3-5 partially developed follicles. The prolonged pregnancy of 22 months is supported by a zonyary endotheliochorial placenta which secretes placental lactogen (ePL) from around 40 days of gestation in association with replacement of the luminal epithelium of the endometrium by trophoblast and the development of large corpora lutea (CLs) in the maternal ovaries from the previously formed luteinized follicles in response to the first LH peak early in the follicular phase. The zonyary placenta develops above, rather than within, the endometrium. The elephant placenta secretes neither estrogens nor progestagens throughout gestation, as pregnancy maintenance relies on 5 $\alpha$ -dihydroprogesterone and other 5 $\alpha$  reduced progestagens secreted by secondary CLs stimulated by ePL and the stromal tissue of the fetal gonads, which become extremely enlarged during the second half of the 22-month pregnancy. In female fetuses, this ovarian enlargement includes the development and subsequent regression of multiple primary and secondary follicles with a consequent substantial decline in primary follicle numbers at birth. During the next 8-9 years of pre-pubertal life, however, oocyte and primary follicle numbers recover to levels near those found in late gestation, which may be evidence of postnatal oogenesis occurring in the elephant.

Angkwanish, T., H. Vernooij, A. Sirimalaisuwan, P. Charernpan, M. Nielen and V. Rutten (2021). "Prevalence and Demographic Risk Factors of Mycobacterium tuberculosis Infections in Captive Asian Elephants (*Elephas maximus*) Based on Serological Assays." *Front Vet Sci* **8**: 9.

To address putative TB statuses of elephants and to identify and quantify potential demographic risk factors for TB, three ELISAs specific for different mycobacterial antigens (ESAT6, CFP10, MPB83) and the TB Stat-Pak assay were used as surrogate serological markers for TB infection in elephants. In view of the low number of animals of which the infected status could be confirmed (4 out of 708) Latent Class Analyses of TB serology test outcomes was used to predict the putative TB status of each of 708 elephants as positive (17.3%), inconclusive (48.7%), or negative (34%) when assessed on a population basis. Correlation between test performance of the individual assays was high between the ELISAs, but low with that of the TB Stat-Pak assay. Risk factors, assessed based on cut off values for each of the ELISAs determined by ROC analysis, included sex, BCS, age, working time, feed type, management system, camp size and region. Old age elephants were more likely to show a positive TB serology test outcome, than younger ones. Elephants working 7 h per day and the ones in good condition BCS (7-11) were less likely to be positive in TB serology testing. In addition, fewer animals in the large camp size (31-50 elephants) were found to be positive in ELISA tests, compared to elephants in the other camp sizes. In this study, the North region had the lowest percentages of elephants with positive TB test outcome, the West region and to a lesser extend the other regions showed clearly higher percentages of positive animals. Even though assays used in the present study have not been validated yet, results obtained showed promise as diagnostic or screening tests. For the diagnosis of animals suspected to be infected, the ELISA tests, once further optimized for the individual antigens, can be used in parallel. For screening of complete camps for presence or absence of infection, a single optimized ELISA test can be utilized.

Aznar-Cormano, L., J. Bonnald, S. Krief, N. Guma and R. Debruyne (2021).

"Molecular sexing of degraded DNA from elephants and mammoths: a genotyping assay relevant both to conservation biology and to paleogenetics." *Sci Rep* **11**(1): 7227.

It is important to determine the sex of elephants from their samples-faeces from the field or seized ivory-for forensic reasons or to understand population demography and genetic structure. Molecular sexing methods developed in the last two decades have often shown limited efficiency, particularly in terms of sensitivity and specificity, due to the degradation of DNA in these samples. These limitations have also prevented their use with ancient DNA samples of elephants or mammoths. Here we propose a novel TaqMan-MGB qPCR assay to address these difficulties. We designed it specifically to allow the characterization of the genetic sex for highly degraded samples of all elephantine taxa (elephants and mammoths). In vitro experiments demonstrated a high level of sensitivity and low contamination risks. We applied this assay in two actual case studies where it consistently recovered the right genotype for specimens of known sex a priori. In the context of a modern conservation survey of African elephants, it allowed determining the sex for over 99% of fecal samples. In a paleogenetic analysis of woolly mammoths, it produced a robust hypothesis of the sex for

over 65% of the specimens out of three PCR replicates. This simple, rapid, and cost-effective procedure makes it readily applicable to large sample sizes.

Baleka, S., V. L. Herridge, G. Catalano, A. M. Lister, M. R. Dickinson, C. Di Patti, A. Barlow, K. E. H. Penkman, M. Hofreiter and J. L. A. Paijmans (2021). "Estimating the dwarfing rate of an extinct Sicilian elephant." *Curr Biol* **31**(16): 3606-3612.e3607.

Evolution on islands, together with the often extreme phenotypic changes associated with it, has attracted much interest from evolutionary biologists. However, measuring the rate of change of phenotypic traits of extinct animals can be challenging, in part due to the incompleteness of the fossil record. Here, we use combined molecular and fossil evidence to define the minimum and maximum rate of dwarfing in an extinct Mediterranean dwarf elephant from Puntali Cave (Sicily).(1) Despite the challenges associated with recovering ancient DNA from warm climates,(2) we successfully retrieved a mitogenome from a sample with an estimated age between 175,500 and 50,000 years. Our results suggest that this specific Sicilian elephant lineage evolved from one of the largest terrestrial mammals that ever lived(3) to an island species weighing less than 20% of its original mass with an estimated mass reduction between 0.74 and 200.95 kg and height reduction between 0.15 and 41.49 mm per generation. We show that combining ancient DNA with paleontological and geochronological evidence can constrain the timing of phenotypic changes with greater accuracy than could be achieved using any source of evidence in isolation.

Barrett, L. P. and S. Benson-Amram (2021). "Multiple assessments of personality and problem-solving performance in captive Asian elephants (*Elephas maximus*) and African savanna elephants (*Loxodonta africana*)." *J Comp Psychol* **135**(3): 406-419.

Animal personality has been shown to predict many behavioral responses across taxa, but the relationship between personality and performance on cognitive tasks remains unclear. To address this gap, we investigated whether personality predicted problem-solving performance and learning in captive Asian and African savanna elephants. We leveraged 3 novel problem-solving tasks to assess success rate, latency to touch the apparatus, exploratory diversity (the number of different behaviors exhibited toward the task), work time (the proportion of time working on the tasks), and latency to solve. To measure multiple different personality traits, such as boldness, activity, aggressiveness, curiosity, and sociability, across contexts, we carried out novel object presentations, behavioral coding through observations, and trait rating through surveys with zookeepers. We found evidence of personality through behavioral observations and surveys, but not through novel object testing. Aggressiveness and activity were important predictors of problem solving, but this was task-dependent, and the traits we measured did not significantly predict learning. Elephants solved 2 out of 3 tasks faster over time, but they did not vary their latency to touch, exploratory diversity, or work time. We discuss our results in terms of task

difficulty and previous work on personality in elephants. Results from this study lay the foundation for future work connecting individual variation in personality to cognitive performance in elephants. In addition, for zoo-housed animals, individual differences research could inform enrichment and welfare decisions as well as conservation strategies. (PsycInfo Database Record (c) 2021 APA, all rights reserved).

Bartas, M., V. Brázda, A. Volná, J. Červeň, P. Pečinka and J. E. Zawacka-Pankau (2021). "The Changes in the p53 Protein across the Animal Kingdom Point to Its Involvement in Longevity." *Int J Mol Sci* **22**(16).

Recently, the quest for the mythical fountain of youth has produced extensive research programs that aim to extend the healthy lifespan of humans. Despite advances in our understanding of the aging process, the surprisingly extended lifespan and cancer resistance of some animal species remain unexplained. The p53 protein plays a crucial role in tumor suppression, tissue homeostasis, and aging. Long-lived, cancer-free African elephants have 20 copies of the TP53 gene, including 19 retrogenes (38 alleles), which are partially active, whereas humans possess only one copy of TP53 and have an estimated cancer mortality rate of 11-25%. The mechanism through which p53 contributes to the resolution of the Peto's paradox in Animalia remains vague. Thus, in this work, we took advantage of the available datasets and inspected the p53 amino acid sequence of phylogenetically related organisms that show variations in their lifespans. We discovered new correlations between specific amino acid deviations in p53 and the lifespans across different animal species. We found that species with extended lifespans have certain characteristic amino acid substitutions in the p53 DNA-binding domain that alter its function, as depicted from the Phenotypic Annotation of p53 Mutations, using the PROVEAN tool or SWISS-MODEL workflow. In addition, the loop 2 region of the human p53 DNA-binding domain was identified as the longest region that was associated with longevity. The 3D model revealed variations in the loop 2 structure in long-lived species when compared with human p53. Our findings show a direct association between specific amino acid residues in p53 protein, changes in p53 functionality, and the extended animal lifespan, and further highlight the importance of p53 protein in aging.

Bauer, H., A. C. Tehou, M. Gueye, H. Garba, B. Doamba, D. Diouck and C. Sillero-Zubiri (2021). "Ignoring species hybrids in the IUCN Red List assessments for African elephants may bias conservation policy." *Nat Ecol Evol* **5**(8): 1050-1051.

Bechert, U., J. M. Christensen, J. Kottwitz, D. Boothe, S. Alshahrani and S. Mohammed (2021). "PHARMACOKINETICS OF ORALLY ADMINISTERED FLUNIXIN MEGLUMINE IN AFRICAN (LOXODONTA AFRICANA) AND ASIAN (ELEPHAS MAXIMUS) ELEPHANTS." *J Zoo Wildl Med* **51**(4): 905-914.

Flunixin meglumine is the most commonly used nonsteroidal anti-inflammatory drug used to treat elephants; however, no pharmacokinetic study for flunixin has yet been conducted in these species, and dosages used range widely. Pharmacokinetic parameters of flunixin were determined in

African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants after single-dose oral administration of 0.8 and 1.5 mg/kg flunixin paste in each species. Elephant compliance to oral administration of banamine was occasionally challenging, especially among older, female African elephants. After administration of 0.8 mg/kg flunixin, mean serum concentrations peaked in approximately 1.3 hr at  $2.1 \pm 0.8 \mu\text{g/ml}$  for Asian ( $n = 8$ ) and 2.8 hr at  $2.5 \pm 0.7 \mu\text{g/ml}$  for African ( $n = 8$ ) elephants. Dosages of 1.5 mg/kg flunixin resulted in mean serum concentration peaks of  $7.2 \pm 1.5 \mu\text{g/ml}$  in Asian elephants ( $n = 7$ ) and  $4.4 \pm 0.7 \mu\text{g/ml}$  in African elephants ( $n = 6$ ). However, multiple-dose trials using 1.1 mg/kg flunixin resulted in peak serum concentrations that were again less in Asian than African elephants ( $2.7 \mu\text{g/ml}$  versus  $4.4 \mu\text{g/ml}$ , respectively). Asian elephants consistently had lower time to maximal concentration, greater area under the curve, and longer mean residence times compared with African elephants. In other species, flunixin is excreted unchanged primarily via hepatic routes with small amounts in the urine. Asian elephants may engage in some level of enterohepatic recycling of flunixin, as was previously reported for phenylbutazone. This study supports that different oral dosing regimens should be used for Asian (1.0 mg/kg SID) and African (1.2 mg/kg SID) elephants, and oral administration techniques used should ensure complete dosage delivery.

Bechert, U., S. Hixon and D. Schmitt (2021). "Diurnal variation in serum concentrations of cortisol in captive African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants." *Zoo Biol* **40**(5): 458-471.

Cortisol is involved in a broad range of physiological processes and enables animals to adapt to new situations and challenges. Diurnal fluctuations in circulating cortisol concentrations in elephants have been demonstrated based on samples from urine and saliva. The aims of this study were to demonstrate diurnal cortisol fluctuations based on blood samples and compare concentrations between seasons, species, and changes in reproductive hormone concentrations. Nine African (*Loxodonta africana*) and three Asian (*Elephas maximus*) elephants at two facilities in the United States were included in this study. Blood samples were collected every 2-3 h at one location and every 1-6 h at another. Peak serum concentrations of cortisol averaged 28 ng/ml for both African and Asian elephants, and diurnal cycles included a fivefold decrease from morning peak to evening nadir concentrations. Diurnal cortisol profiles varied uniquely among individual elephants. During the winter, nadir concentrations of cortisol were slightly higher, and the timing of peak concentrations was less predictable. There was no correlation between diurnal serum concentrations of progesterone and cortisol; however, a significant correlation ( $p = .02$ ) was identified between serum concentrations of testosterone and cortisol when a time lag of  $\sim 2-3$  h was considered. The physiological significance of the positive correlations between diurnal serum concentrations of cortisol and testosterone in male elephants remains to be determined. If cortisol concentrations are being used to evaluate elephant health or welfare, samples should be obtained at the same time each day to minimize variation

due to diurnal fluctuations, and ideally seasonal variations and individuality in diurnal profiles should also be considered.

Beeck, V. C., G. Heilmann, M. Kerscher and A. S. Stoeger (2021). "A novel theory of Asian elephant high-frequency squeak production." *BMC Biol* **19**(1): 121.

**BACKGROUND:** Anatomical and cognitive adaptations to overcome morpho-mechanical limitations of laryngeal sound production, where body size and the related vocal apparatus dimensions determine the fundamental frequency, increase vocal diversity across taxa. Elephants flexibly use laryngeal and trunk-based vocalizations to form a repertoire ranging from infrasonic rumbles to higher-pitched trumpets. Moreover, they are among the few evolutionarily distantly related animals (humans, pinnipeds, cetaceans, birds) capable of imitating species-atypical sounds. Yet, their vocal plasticity has so far not been related to functions within their natural communicative system, in part because not all call types have been systematically studied. Here, we reveal how Asian elephants (*Elephas maximus*) produce species-specific squeaks (F0 300-2300 Hz) by using acoustic camera recordings to visualize sound emission and examining this alongside acoustic, behavioral, and morphological data across seven captive groups. **RESULTS:** We found that squeaks were emitted through the closed mouth in synchrony with cheek depression and retraction of the labial angles. The simultaneous emission of squeaks with nasal snorts (biphonation) in one individual confirmed that squeak production was independent of nasal passage involvement and this implicated oral sound production. The squeaks' spectral structure is incongruent with laryngeal sound production and aerodynamic whistles, pointing to tissue vibration as the sound source. Anatomical considerations suggest that the longitudinal closed lips function as the vibrators. Acoustic and temporal parameters exhibit high intra- and inter-individual variability that enables individual but no call-subtype classification. Only 19 of 56 study subjects were recorded to squeak, mostly during alarming contexts and social arousal but some also on command. **CONCLUSION:** Our results strongly suggest that Asian elephants force air from the small oral cavity through the tensed lips, inducing self-sustained lip vibration. Besides human brass players, lip buzzing is not described elsewhere in the animal kingdom. Given the complexity of the proposed mechanism, the surprising absence of squeaking in most of the unrelated subjects and the indication for volitional control, we hypothesize that squeak production involves social learning. Our study offers new insights into how vocal and cognitive flexibility enables mammals to overcome size-related limitations of laryngeal sound production. This flexibility enables Asian elephants to exploit a frequency range spanning seven octaves within their communicative system.

Beeck, V. C., G. Heilmann, M. Kerscher and A. S. Stoeger (2021). "Author Correction to: A novel theory of Asian elephant high-frequency squeak production." *BMC Biol* **19**(1): 237.

Beirne, C., T. M. Houslay, P. Morkel, C. J. Clark, M. Fay, J. Okouyi, L. J. T. White



and J. R. Poulsen (2021). "African forest elephant movements depend on time scale and individual behavior." *Sci Rep* **11**(1): 12634.

The critically endangered African forest elephant (*Loxodonta cyclotis*) plays a vital role in maintaining the structure and composition of Afrotropical forests, but basic information is lacking regarding the drivers of elephant movement and behavior at landscape scales. We use GPS location data from 96 individuals throughout Gabon to determine how five movement behaviors vary at different scales, how they are influenced by anthropogenic and environmental covariates, and to assess evidence for behavioral syndromes—elephants which share suites of similar movement traits. Elephants show some evidence of behavioral syndromes along an 'idler' to 'explorer' axis—individuals that move more have larger home ranges and engage in more 'exploratory' movements. However, within these groups, forest elephants express remarkable inter-individual variation in movement behaviours. This variation highlights that no two elephants are the same and creates challenges for practitioners aiming to design conservation initiatives.

Bentley, C. E., J. M. Cracknell, A. C. Kitchener, Y. M. Pereira and R. Pizzi (2021). "IMPROVED DIAGNOSIS OF FOOT OSTEOARTHRITIS IN ELEPHANTS (*ELEPHAS MAXIMUS*, *LOXODONTA AFRICANA*) USING STEREORADIOGRAPHY." *J Zoo Wildl Med* **52**(1): 67-74.

Diagnosis of foot disease in elephants is challenging. Owing to their large size, the available diagnostic tools and the expense of imaging are diagnostically limiting. Stereoradiography is the preparation of paired radiographs that form a three-dimensional (3D) image when viewed stereoscopically. Clinicians and veterinary students graded osteoarthritis in the feet of African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants taken postmortem with standard 2D radiographs, as well as 3D stereoradiographs. These gradings were compared with the actual gross pathology identified in the specimens. Although veterinary students diagnoses were no better than chance from 2D radiographs, 83.6% of the students could correctly differentiate severity between joints on stereoradiography; this is an absolute improvement of 30.1% (95% confidence interval [CI] = 19.6%-40.6%). Overall, participants were 27.4% (95% CI = 18.4%-36.3%) more successful at diagnosing pathology on stereoradiographs. Half of participants were shown standard 2D radiographs first, the others stereoradiographs first, but the difference in gradings between the two groups was not statistically significant. Stereoradiography appears to hold the potential to improve diagnosis of osteoarthritis in elephant feet, particularly by less experienced clinicians, and the technique is low-cost and applicable under field conditions.

Berger, V., S. Reichert, M. Lahdenperä, J. Jackson, W. Htut and V. Lummaa (2021). "The elephant in the family: Costs and benefits of elder siblings on younger offspring life-history trajectory in a matrilineal mammal." *Journal of Animal Ecology* **90**(11): 2663-2677.

Many mammals grow up with siblings, and interactions between them can influence offspring phenotype and fitness. Among these interactions, sibling

competition between different-age offspring should lead to reproductive and survival costs on the younger sibling, while sibling cooperation should improve younger sibling's reproductive potential and survival. However, little is known about the consequences of sibling effects on younger offspring life-history trajectory, especially in long-lived mammals. We take advantage of a large, multigenerational demographic dataset from semi-captive Asian elephants to investigate how the presence and sex of elder siblings influence the sex, survival until 5 years old, body condition, reproductive success (i.e. age at first reproduction and lifetime reproductive success) and long-term survival of subsequent offspring. We find that elder siblings have heterogeneous effects on subsequent offspring life-history traits depending on their presence, their sex and the sex of the subsequent offspring (named focal calf). Overall, the presence of an elder sibling (either sex) strongly increased focal calf long-term survival (either sex) compared to sibling absence. However, elder sisters had higher impact on the focal sibling than elder brothers. Focal females born after a female display higher long-term survival, and decreased age at first reproduction when raised together with an elder sister rather than a brother. Focal males born after a female rather than a male showed lower survival but higher body weight when both were raised together. We did not detect any sibling effects on the sex of the focal calf sex, survival until 5 years old and lifetime reproductive success. Our results highlight the general complexity of sibling effects, but broadly that elder siblings can influence the life-history trajectory of subsequent offspring. We also stress the importance of considering all life stages when evaluating sibling effects on life trajectories. © 2021 The Authors. *Journal of Animal Ecology* published by John Wiley & Sons Ltd on behalf of British Ecological Society

Boehlke, C., S. Schuster, L. Kauthe, O. Zierau and C. Hannig (2021). "Nutritional influences on enzyme activities in saliva of Asian and African elephants." *J Comp Physiol B* **191**(5): 955-970.

Asian and African elephants show morphological adaptations to their ecological niche including the oral cavity. Variety and preferences of forage plants differ between both herbivorous elephant species. Diet can affect salivary enzymes. Asian elephants were shown to have a higher salivary amylase activity than African elephants. Species-specific differences were presumed to be influenced by feeding during collection procedure. This study aimed to determine the influence of feeding on enzyme activities in saliva of both elephant species to differentiate from species-specific effects. Additionally, season and housing conditions on salivary enzyme activities in non-fed elephants of both species were investigated. Salivary amylase (sAA), lysozyme (sLYS) and peroxidase (sPOD) activity were measured photometrically or fluorometrically. Results of this study reinforce previous observations of higher basic sAA activity in Asian elephants compared to African elephants. Salivary LYS and sPOD activity showed neither species-specific nor housing-specific differences. Independent from season, most elephants of both species revealed a lack of or low sPOD activity. Feeding caused a temporary decrease of sAA, sLYS and sPOD activity in both

elephant species kept in four of eight tested zoos. Furthermore, sAA activity in Asian elephants was higher and sLYS activity lower in Spring than in Autumn. This study summarizes that sAA and sLYS are components of Asian and African elephant saliva in an active conformation in contrast to sPOD. Diet varying between season and zoos might influence sAA and sLYS activities primarily in Asian elephants but temporary low effects suggest sufficient buffer capacity of elephant saliva of both species.

Boonprasert, K., Y. Yun, W. Kosaruk, P. Towiboon, P. Tankaew, V. Punyapornwithaya, T. Janyamathakul, P. Muanghong, J. L. Brown, C. Thitaram and C. Somgird (2021). "A Longitudinal Study of Hematology and Stress Biomarker Profiles in Young Asian Elephants (*Elephas Maximus*) in Relation to Elephant Endotheliotropic Herpesvirus (EEHV) in Thailand." *Animals (Basel)* **11**(9).

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD) is a virulent disease that causes severe hemorrhage and sudden death in Asian elephant calves. A change in hematology profiles is one indicator of infection before clinical signs appear; however, to be effective, individual baselines and age-matched reference values are needed. Stress has been speculated to be a factor in clinical EEHV cases, but relationships have not been demonstrated empirically. This study evaluated blood hematology and several stress response markers-salivary cortisol, fecal glucocorticoid metabolites (FGM), salivary Immunoglobulin A (SIgA), and fecal IgA (FIgA) in samples collected for 1 year from three healthy calves with no EEHV history (non-EEHV), and six that had previously been infected, developed clinical signs and survived (prior-EEHV). Hematology values between non-EEHV and prior-EEHV elephants were not different and within published reference ranges. Concentrations of salivary cortisol, FGM, SIgA, and FIgA also were variable and showed seasonal differences, but no relationships to prior EEHV status. One of the prior EEHV calves became re-infected, developed hemorrhagic disease (HD), and died during the study period. That calf exhibited lymphocytopenia, monocytopenia, and thrombocytopenia. Additionally, all stress biomarker concentrations were lower in the 12 days before viremia was observed. Thus, as in other studies, changes in hematology occur with EEHV infection, while preliminary data in one calf suggests that stress-response measures might also be informative and should be studied further.

Bourgeois, S., K. Ouitavon, P. Kongmee, T. Veeramaethaphan, J. Kaden and R. McEwing (2021). "A simple sexing test for elephant species and its application to faecal DNA." *J Appl Genet* **62**(3): 507-509.

We developed a novel real-time PCR assay for rapid sexing in all three elephant species, which amplifies small fragments of the orthologous sexual chromosome zinc finger protein genes ZFX/ZFY (65 bp). This assay is a simple, inexpensive and reliable tool that is suitable for non-invasive DNA samples and can be incorporated into larger SNP panels for individual identification and population genetic studies.

Brenner, E. P., S. A. Hadi, B. Harris, S. Robbe-Austerman and S. Sreevatsan

(2021). "Genome Sequences of Mycobacterium Strains Recovered from Captive Elephants with Tuberculosis." Microbiol Resour Announc **10**(36): e0067121.

Members of the Mycobacterium tuberculosis complex cause tuberculosis, infamous for enormous impacts on human health. As zoonoses, they also threaten endangered species like African/Asian elephants. We report the whole-genome sequences of Mycobacterium tuberculosis bv. tuberculosis and Mycobacterium tuberculosis bv. bovis from two zoo elephants in the United States.

Caballero-Gómez, J., D. Cano Terriza, J. Pujols, E. Martínez-Nevado, M. D. Carbonell, R. Guerra, J. Recuero, P. Soriano, J. Barbero and I. García-Bocanegra (2021). "Monitoring of bluetongue virus in zoo animals in Spain, 2007-2019." Transbound Emerg Dis.

Bluetongue (BT) is an emerging and re-emerging communicable vector-borne disease of animal health concern. A serosurvey was performed to assess exposure to BT virus (BTV) in zoo animals in Spain and to determine the dynamics of seropositivity in longitudinally sampled individuals during the study period. Serum samples were collected from 241 zoo animals belonging to 71 different species in five urban zoos (A-E) in Spain between 2007 and 2019. Twenty-four of these animals were longitudinally surveyed at three of the sampled zoos (zoos B, C and E) during the study period. Anti-BTV antibodies were found in 46 (19.1%; 95% CI: 14.1-24.1) of the 241 captive animals analysed by commercial ELISA. A virus neutralization test confirmed specific antibodies against BTV-1 and BTV-4 in 25 (10.7%; 95% CI: 6.7-14.6) and five (3.0%; 95% CI: 0.3-4.0) animals, respectively. Two of the 24 longitudinally sampled individuals (one African elephant (*Loxodonta africana*) and one aoudad (*Ammotragus lervia*)) showed anti-BTV antibodies at all samplings, whereas seroconversions were detected in one mouflon (*Ovis aries musimon*) in 2016, and one Asian elephant (*Elephas maximus*) in 2019. To the best of the authors' knowledge, this is the first large-scale survey on BTV conducted in both artiodactyl and non-artiodactyl zoo species worldwide. The results confirm BTV exposure in urban zoo parks in Spain, which could be of animal health and conservation concern. Circulation of BTV was detected in yearling animals in years when there were no reports of BTV outbreaks in livestock. Surveillance in artiodactyl and non-artiodactyl zoo species could be a valuable tool for epidemiological monitoring of BTV.

Caballero-Gómez, J., I. García-Bocanegra, N. Navarro, R. Guerra, E. Martínez-Nevado, P. Soriano and D. Cano-Terriza (2021). "Zoo animals as sentinels for Schmallenberg virus monitoring in Spain." Vet Microbiol **252**: 108927.

Schmallenberg virus (SBV) is a newly emerged vector-borne pathogen that affects many domestic and wild animal species. A serosurvey was carried out to assess SBV exposure in zoo animals in Spain and to determine the dynamics of seropositivity in longitudinally sampled individuals. Between 2002 and 2019, sera from 278 animals belonging to 73 different species were collected from five zoos (A-E). Thirty-one of these animals were longitudinally sampled at three of these zoo parks during the study period. Seropositivity was detected in 28 (10.1 %) of 278 animals analyzed by

blocking ELISA. Specific anti-SBV antibodies were confirmed in 20 (7.2 %; 95 %CI: 4.2-10.3) animals of six different species using virus neutralization test (VNT). The multiple logistic regression model showed that "order" (Artiodactyla) and "zoo provenance" (zoo B; southern Spain) were risk factors potentially associated with SBV exposure. Two (8.7 %) of the 31 longitudinally-sampled individuals showed specific antibodies against SBV at all samplings whereas seroconversion was detected in one mouflon (*Ovis aries musimon*) and one Asian elephant (*Elephas maximus*) in 2016 and 2019, respectively. To the best of the author's knowledge, this is the first surveillance conducted on SBV in zoos in Spain. The results confirm SBV exposure in zoo animals in this country and indicate circulation of the virus before the first Schmallenberg disease outbreak was reported in Spain. Surveillance in zoological parks could be a complementary approach to monitoring SBV activity. Further studies are warranted to assess the impact of this virus on the health status of susceptible zoo animals.

Campbell-Staton, S. C., B. J. Arnold, D. Gonçalves, P. Granli, J. Poole, R. A. Long and R. M. Pringle (2021). "Ivory poaching and the rapid evolution of tusklessness in African elephants." *Science* **374**(6566): 483-487.

Understanding the evolutionary consequences of wildlife exploitation is increasingly important as harvesting becomes more efficient. We examined the impacts of ivory poaching during the Mozambican Civil War (1977 to 1992) on the evolution of African savanna elephants (*Loxodonta africana*) in Gorongosa National Park. Poaching resulted in strong selection that favored tusklessness amid a rapid population decline. Survey data revealed tusk-inheritance patterns consistent with an X chromosome-linked dominant, male-lethal trait. Whole-genome scans implicated two candidate genes with known roles in mammalian tooth development (AMELX and MEP1a), including the formation of enamel, dentin, cementum, and the periodontium. One of these loci (AMELX) is associated with an X-linked dominant, male-lethal syndrome in humans that diminishes the growth of maxillary lateral incisors (homologous to elephant tusks). This study provides evidence for rapid, poaching-mediated selection for the loss of a prominent anatomical trait in a keystone species. © 2021 American Association for the Advancement of Science. All rights reserved.

Campos-Arceiz, A., J. A. de la Torre, K. Wei, X. Y. O. Wu, Y. F. Zhu, M. X. Zhao, S. Chen, Y. Bai, R. T. Corlett and F. Chen (2021). "The return of the elephants: How two groups of dispersing elephants attracted the attention of billions and what can we learn from their behavior." *Conservation Letters* **14**(6): 3.

Canney, S. M. (2021). "Making Space for Nature: Elephant Conservation in Mali as a Case Study in Sustainability." *Environment* **63**(2): 4-15.

Carrothers, K. L., L. E. Goodmiller, M. J. McLellan and A. M. Spicer (2021). "A novel approach to combatting proboscidean ivory trafficking using a multiplex High-Resolution Melt (M-HRM) assay." *Forensic Sci Int Genet* **53**: 102511.

To support efforts in prosecuting wildlife crimes, we developed and validated

a multiplex High-Resolution Melt (M-HRM) assay for the identification of proboscidean taxa commonly required to be identified or excluded in ivory seizures and forensic casework: Asian elephant (*Elephas maximus*), African elephant (*Loxodonta spp.*), mammoth (*Mammuthus spp.*), and mastodon (*Mammut spp.*). Five hundred and fifty (550) blood, tissue, and ivory samples from individuals of these 4 proboscidean taxa were used to develop and validate the 2 proboscidean-specific mitochondrial sites targeted by this assay. The 28-basepair (bp) 16S ribosomal RNA (rRNA) and 54-bp cytochrome b (Cytb) gene segments yield a combination of melt peaks that create composite melt profiles unique to each of the 4 proboscidean taxa. Wildlife forensic laboratories can use this sensitive, rapid, and cost-effective assay to assist efforts to combat the unlawful commercialization of proboscidean ivory and to stop the poaching crisis leading to the decline of these ivory-bearing species in the wild.

Chaney, S. B., D. McAloose, R. Greenwald, K. P. Lyashchenko and P. P. Calle (2021). "ASSESSMENT OF MULTIANTIGEN PRINT IMMUNOASSAY AND RAPID LATERAL-FLOW TEST FOR THE DETECTION OF MYCOBACTERIUM BOVIS INFECTION IN MALAYAN TAPIR (*TAPIRUS INDICUS*)."  
*J Zoo Wildl Med* **52**(4): 1257-1262.

A multiantigen print immunoassay (MAPIA) and rapid test (RT) developed and validated for detection of mycobacterial antibodies in elephants (*Elephas maximus* and *Loxodonta africana*) was assessed in Malayan tapir (*Tapirus indicus*). Retrospective analysis of banked serum from one *Mycobacterium bovis* infected and seven presumably uninfected tapir was performed by MAPIA and RT. A sample collected 2 mon prior to the death of a culture-confirmed *M. bovis*-infected tapir served as a positive control. Seroreactivity of this sample was demonstrated via both MAPIA and RT testing. Seven uninfected animals, including four without postmortem evidence of mycobacterial disease and three that remain healthy, were negative controls; none demonstrated seroreactivity to key antigens with either test. These results suggest that MAPIA and RT have potential utility for rapid detection of *M. bovis* infection in Malayan tapir.

Chen, Y., Y. Sun, L. Atzeni, L. Gibson, M. Hua, K. Li, K. Shi and D. Dudgeon (2021). "Anthropogenic pressures increase extinction risk of an isolated Asian elephant (*Elephas maximus*) population in southwestern China, as revealed by a combination of molecular- and landscape-scale approaches." *Integr Zool*.

Identification of the effect of anthropogenic threats on ecosystem is crucial. We used molecular tools and remote sensing to evaluate the population status of an isolated Asian elephant population in southwestern China in response to changes in habitat suitability between 1989 and 2019. A total of 22 unique genotypes were identified from 117 dung samples collected between March and June 2018 using microsatellite DNA analysis, including 13 males and 9 females. Based on the size of fecal boli, 1 animal was a juvenile, 9 were subadults, and 12 were adults, indicating that recruitment was limited. The effective population size was small (15.3) but there was no signature of a recent population bottleneck. We observed a low genetic diversity ( $H(e) = 0.46 \pm 0.05$ ) and a high level of inbreeding ( $F(is) = 0.43 \pm$

0.11), suggesting low population viability and high risk of extinction. In total, these elephants lost nearly two thirds (62%) of their habitat in 3 decades. The expansion of agriculture and rubber plantations followed by an increase in human settlements after 1989 increased the isolation of this population. We recommend that resettlement of 800 inhabitants of 2 villages and the abandonment of associated farmland and rubber plantations would make an additional 20 km<sup>2</sup> of suitable habitat available. This could allow a population increase of 14 elephants, possibly by translocating individuals from elsewhere in China. Our findings can be applied to the management and conservation of other fragmented populations in China or in other range countries of Asian elephants.

Chusyd, D. E., N. L. Ackermans, S. N. Austad, P. R. Hof, M. M. Mielke, C. C. Sherwood and D. B. Allison (2021). "Aging: What We Can Learn From Elephants." Front Aging **2**: 726714.

Elephants are large-brained, social mammals with a long lifespan. Studies of elephants can provide insight into the aging process, which may be relevant to understanding diseases that affect elderly humans because of their shared characteristics that have arisen through independent evolution. Elephants become sexually mature at 12 to 14 years of age and are known to live into, and past, their 7(th) decade of life. Because of their relatively long lifespans, elephants may have evolved mechanisms to counter age-associated morbidities, such as cancer and cognitive decline. Elephants rely heavily on their memory, and engage in multiple levels of competitive and collaborative relationships because they live in a fission-fusion system. Female matrilineal relatives and dependent offspring form tight family units led by an older-aged matriarch, who serves as the primary repository for social and ecological knowledge in the herd. Similar to humans, elephants demonstrate a dependence on social bonds, memory, and cognition to navigate their environment, behaviors that might be associated with specializations of brain anatomy. Compared with other mammals, the elephant hippocampus is proportionally smaller, whereas the temporal lobe is disproportionately large and expands laterally. The elephant cerebellum is also relatively enlarged, and the cerebral cortex is highly convoluted with numerous gyral folds, more than in humans. Last, an interesting characteristic unique to elephants is the presence of at least 20 copies of the TP53 tumor suppressor gene. Humans have only a single copy. TP53 encodes for the p53 protein, which is known to orchestrate cellular response to DNA damage. The effects of these multiple copies of TP53 are still being investigated, but it may be to protect elephants against multiple age-related diseases. For these reasons, among others, studies of elephants would be highly informative for aging research. Elephants present an underappreciated opportunity to explore further common principles of aging in a large-brained mammal with extended longevity. Such research can contribute to contextualizing our knowledge of age-associated morbidities in humans.

Chusyd, D. E., T. R. Nagy, L. Golzarri-Arroyo, S. L. Dickinson, J. R. Speakman, C. Hambly, M. S. Johnson, D. B. Allison and J. L. Brown (2021). "Adiposity,

reproductive and metabolic health, and activity levels in zoo Asian elephant (*Elephas maximus*)." *J Exp Biol* **224**(Pt 2).

Many captive Asian elephant populations are not self-sustaining, possibly due in part to obesity-related health and reproductive issues. This study investigated relationships between estimated body composition and metabolic function, inflammatory markers, ovarian activity (females only) and physical activity levels in 44 Asian elephants (n=35 females, n=9 males). Deuterium dilution was used to measure total body water from which fat mass (FM) and fat-free mass (FFM) could be derived to estimate body composition. Serum was analyzed for progestagens and estradiol (females only), deuterium, glucose, insulin and amyloid A. Physical activity was assessed by an accelerometer placed on the elephant's front leg for at least 2 days. Relative fat mass (RFM) - the amount of fat relative to body mass - was calculated to take differences in body size between elephants into consideration. Body fat percentage ranged from 2.01% to 24.59%. Male elephants were heavier (P=0.043), with more FFM (P=0.049), but not FM (P>0.999), than females. For all elephants, estimated RFM (r=0.45, P=0.004) was positively correlated with insulin. Distance walked was negatively correlated with age (r=-0.46, P=0.007). When adjusted for FFM and age (P<0.001), non-cycling females had less fat compared with cycling females, such that for every 100 kg increase in FM, the odds of cycling were 3 times higher (P<0.001). More work is needed to determine what an unhealthy amount of fat is for elephants; however, our results suggest higher adiposity may contribute to metabolic perturbations.

Colombo, S. A., B. A. Silva, S. Y. M. Gómez, R. d. L. Santos, H. P. Tinoco, C. M. Coelho, J. P. Teixeira and C. A. G. Leal (2021). "Genital infection by *Aerococcus viridans* in a captive african elephant (*Loxodonta africana*)." *Ciência Rural* **51**(1): e20200513-e20200513.

ABSTRACT: *Aerococcus viridans* is an emerging pathogen for humans and livestock animals, mainly associated with genitourinary infections cases. Its occurrence in wild mammals has never been reported. The aim of this study was to determine the etiological agent associated with clinical a case of a genital infection in a female African elephant (*Loxodonta africana*). Phylogenetic analysis and antimicrobial susceptibility profile of the isolate were also addressed. The animal presented frequent cases of genital infection with intermittent white secretion. Purulent secretion was sampled and submitted to bacteriological exam. The isolate obtained was thus identified by phenotypic and molecular methods as *A. viridans* and was found to be similar to human pathogenic isolates in BLASTn and phylogenetic analysis. The isolate was sensitive to almost all antimicrobials evaluated, presenting resistance to ciprofloxacin and norfloxacin. This is the first report of occurrence of *A. viridans* infection in the genital tract of an African elephant. animais de produção, principalmente associado a casos de infecções geniturinárias. Sua ocorrência em mamíferos selvagens nunca foi relatada. O objetivo deste estudo foi determinar o agente etiológico associado a um caso clínico de infecção genital em uma fêmea de elefante africano (*Loxodonta africana*). Análises filogenéticas e perfil de



susceptibilidade antimicrobiana do isolado também foram avaliados. O animal apresentou casos frequentes de infecção genital com eliminação de secreção branca intermitente. A secreção purulenta foi coletada e submetida a exame bacteriológico. O isolado obtido foi identificado por métodos fenotípicos e moleculares como *A. viridans* e apresentou alta similaridade a isolados humanos patogênicos nas análises de BLASTn e filogenética. O isolado foi sensível a quase todos os antimicrobianos avaliados, apresentando resistência à ciprofloxacina e norfloxacina. Este é o primeiro relato de ocorrência de infecção por *A. viridans* no trato genital de elefante africano.

Crawley, J. A. H., O. Liehrmann, D. J. Franco Dos Santos, J. Brown, U. K. Nyein, H. H. Aung, W. Htut, Z. M. Oo, M. W. Seltmann, J. L. Webb, M. Lahdenperä and V. Lummaa (2021). "Influence of handler relationships and experience on health parameters, glucocorticoid responses and behaviour of semi-captive Asian elephants." *Conserv Physiol* **9**(1): coaa116.

Declining wild populations combined with accumulating captive populations of e.g. livestock, pets, draught and zoo animals have resulted in some threatened species with substantial proportions of their populations in captivity. The interactions animals have with humans in captivity depend on handler familiarity and relationship quality and can affect animal health, growth and reproduction with consequences for the success of conservation programmes. However, assessments of how specific human-animal relationships affect a range of physiological and behavioural outcomes are rare. Here, we studied semi-captive Asian elephants with detailed records of elephant-handler (mahout) relationships and veterinary management, allowing assessment of multiple welfare indicators in relation to specific mahout-elephant relationship lengths and mahout experience. These included measures of physiological stress (faecal glucocorticoid metabolite [FGM], heterophil:lymphocyte ratio [H:L]), muscle damage (creatinase kinase [CK]), immunological health (total white blood cell count [TWBC]) and behaviour (response to mahout verbal commands). We found no evidence that FGM or H:L related to aspects of the mahout-elephant relationship. Longer overall mahout experience (i.e. years of being a mahout) was linked to increased muscle damage and inflammation, but the lengths of specific mahout-elephant relationships were inversely associated with muscle damage in working-age elephants. Elephants responded more to familiar mahouts in behavioural tasks and faster to mahouts they had known for longer. In summary, our results found little evidence that the mahout-elephant relationship affects physiological stress in this population based on FGM and H:L, but mahout experience and relationships were linked to other physiological responses (CK, TWBC), and elephants require behavioural adjustment periods following mahout changes.

Dagenais, P., S. Hensman, V. Haechler and M. C. Milinkovitch (2021). "Elephants evolved strategies reducing the biomechanical complexity of their trunk." *Curr Biol*.

The elephant proboscis (trunk), which functions as a muscular hydrostat with a virtually infinite number of degrees of freedom, is a spectacular organ for delicate to heavy object manipulation as well as social and sensory functions.

Using high-resolution motion capture and functional morphology analyses, we show here that elephants evolved strategies that reduce the biomechanical complexity of their trunk. Indeed, our behavioral experiments with objects of various shapes, sizes, and weights indicate that (1) complex behaviors emerge from the combination of a finite set of basic movements; (2) curvature, torsion, and strain provide an appropriate kinematic representation, allowing us to extract motion primitives from the trunk trajectories; (3) transport of objects involves the proximal propagation of an inward curvature front initiated at the tip; (4) the trunk can also form pseudo-joints for point-to-point motion; and (5) the trunk tip velocity obeys a power law with its path curvature, similar to human hand drawing movements. We also reveal with unprecedented precision the functional anatomy of the African and Asian elephant trunks using medical imaging and macro-scale serial sectioning, thus drawing strong connections between motion primitives and muscular synergies. Our study is the first combined quantitative analysis of the mechanical performance, kinematic strategies, and functional morphology of the largest animal muscular hydrostat on Earth. It provides data for developing innovative "soft-robotic" manipulators devoid of articulations, replicating the high compliance, flexibility, and strength of the elephant trunk. VIDEO ABSTRACT.

Dai, Y. (2021). "The overlap of suitable tea plant habitat with Asian elephant (*Elephus maximus*) distribution in southwestern China and its potential impact on species conservation and local economy." Environ Sci Pollut Res Int.

The expansion of land being used for cash crop cultivation has threatened wildlife in recent decades. Tea has become the dominant cash crop in southwestern China. Unfortunately, tea plantations may threaten Asian elephant (*Elephus maximus*) populations via habitat loss and fragmentation. Identifying areas of suitable habitat for tea plant cultivation, and where this habitat overlaps with Asian elephant distribution, is vital for planning land use, managing nature reserves, shaping policy, and maintaining local economies. Here, we assess the potential impact of tea plantations on Asian elephants in southwestern Yunnan province, China. We used MaxEnt modeling with bioclimatic and environmental variables to identify suitable habitat for tea plant cultivation under the current climate scenario, and then overlapped this habitat with 9 known Asian elephant distribution areas (G1-G9) to determine "threatened areas." Our results showed that (1) annual precipitation (48.1% contribution), temperature constancy (29 % contribution), and slope (8.7 % contribution) were key in determining suitable habitat for tea plants; (2) the cumulative area of suitable habitat for tea plants was 13,784.88 km<sup>2</sup>, mainly distributed in Menghai (3934.53 km<sup>2</sup>), Lancang (3198.67 km<sup>2</sup>), and Jinghong (2657.74 km<sup>2</sup>); (3) the distribution area of elephants was 943.75 km<sup>2</sup>, and these areas overlapped with suitable tea plant habitat primarily located in G4 (379.40 km<sup>2</sup>), G3 (251.18), and G7 (168.03 km<sup>2</sup>); and (4) threatened areas in G1 and G7 were predominately located along the periphery of current nature reserves. Win-win solutions that work for elephant conservation and economic development include rescoping nature reserve boundaries, strengthening

management on the periphery of nature reserves, establishing ecological corridors and new nature reserves within regions where elephants are currently distributed, planting alternative cash crops, and financial subsidies to farmers. This study improves understanding of human-elephant coexistence, and will assist in guiding land use policy for the future conservation outcomes seeking to promote responsible and profitable cash crop farming and elephant conservation.

de Flamingh, A., A. Coutu, J. Sealy, S. Chirikure, A. D. S. Bastos, N. M. Libanda-Mubusisi, R. S. Malhi and A. L. Roca (2021). "Sourcing Elephant Ivory from a Sixteenth-Century Portuguese Shipwreck." *Curr Biol* **31**(3): 621-628.e624.

The oldest known shipwreck in southern Africa was found in Namibia in 2008.(1-4) Forty tons of cargo, including gold and silver coins, helped identify the ship as the Bom Jesus, a Portuguese nau (trading vessel) lost in 1533 while headed to India.(4-6) The cargo included >100 elephant tusks,(7) which we examined using paleogenomic and stable isotope analyses. Nuclear DNA identified the ivory source as African forest (*Loxodonta cyclotis*) rather than savanna (*Loxodonta africana*) elephants. Mitochondrial sequences traced them to West and not Central Africa and from  $\geq 17$  herds with distinct haplotypes. Four of the haplotypes are known from modern populations; others were potentially lost to subsequent hunting of elephants for ivory. Stable isotope analyses ( $\delta(13)C$  and  $\delta(15)N$ ) indicated that the elephants were not from deep rainforests but from savanna and mixed habitats. Such habitats surround the Guinean forest block of West Africa(8) and accord with the locations of major historic Portuguese trading ports.(9),(10) West African forest elephants currently range into savanna habitats;(11-13) our findings suggest that this was not consequent to regional decimation of savanna elephants for their ivory in the 19(th) and 20(th) centuries. During the time of the Bom Jesus, ivory was a central driver in the formation of maritime trading systems connecting Europe, Africa, and Asia. Our integration of paleogenomic, archeological, and historical methods to analyze the Bom Jesus ivory provides a framework for examining vast collections of archaeological ivories around the world, in shipwrecks and other contexts.

de Waal, C. R., L. Kleynhans, S. D. C. Parsons, W. J. Goosen, G. Hausler, P. E. Buss, R. M. Warren, P. D. van Helden, J. A. Landolfi, M. A. Miller and T. J. Kerr (2021). "Development of a cytokine gene expression assay for the relative quantification of the African elephant (*Loxodonta africana*) cell-mediated immune responses." *Cytokine* **141**: 155453.

Immunological assays are the basis for many diagnostic tests for infectious diseases in animals and humans. Application in wildlife species, including the African elephant (*Loxodonta africana*), is limited however due to lack of information on immune responses. Since many immunoassays require both identified biomarkers of immune activation as well as species-specific reagents, it is crucial to have knowledge of basic immunological responses in the species of interest. Cytokine gene expression assays (GEAs) used to measure specific immune responses in wildlife have frequently shown that

targeted biomarkers are often species-specific. Therefore, the aim of this study was to identify elephant-specific cytokine biomarkers to detect immune activation and to develop a GEA, using pokeweed mitogen stimulated whole blood from African elephants. This assay will provide the foundation for the development of future cytokine GEAs that can be used to detect antigen specific immune responses and potentially lead to various diagnostic tests for this species.

Deepthi, M. P., P. Kathireswari, J. Rini, K. Saminathan and N. Karmegam (2021). "Vermi transformation of monogastric *Elephas maximus* and ruminant *Bos taurus* excrements into vermicompost using *Eudrilus eugeniae*." Bioresour Technol **320**(Pt A): 124302.

Biotransformation of monogastric (*Elephas maximus*) and ruminant (*Bos taurus*) excrements with *Eudrilus eugeniae* was assessed by establishing five different treatments in triplicate: 100% elephant dung (T1), 50% elephant dung + 50% garden soil (T2), 50% elephant dung + 50% cow dung (T3), 100% cow dung (T4) and 50% cow dung + 50% garden soil (T5) and maintained for 90 days under experimental conditions. An increment of macronutrients and reduction of C/N ratio (<20) in the vermicomposts was recorded, where T3 presented higher NPK contents with the relative nutrient recovery efficiency of 1.65, 2.94 and 1.76, respectively. Cycle I (45 days) endorsed cocoons and juveniles, while Cycle II (90 days) supported sub-adults and adults. Seed germination and 28 days growth studies with *Vigna unguiculata* signified that the vermicomposts were phytotoxicity-free. The binary ratio in T3 (1:1) is suitable for the biotransformation of elephant dung into advantageous vermicompost with *Eudrilus eugeniae*.

Dewolf, A. H., Y. P. Ivanenko, R. M. Mesquita and P. A. Willems (2021). "Postural control in the elephant." J Exp Biol **224**(22).

As the largest extant legged animals, elephants arguably face the most extreme challenge for stable standing. In this study, we investigated the displacement of the centre of pressure of 12 elephants during quiet standing. We found that the average amplitude of the oscillations in the lateral and fore-aft directions was less than 1.5 cm. Such amplitudes for postural oscillation are comparable with those of dogs and other species, suggesting that some aspects of sensorimotor postural control do not scale with size.

Di Minin, E., R. Slotow, C. Fink, H. Bauer and C. Packer (2021). "A pan-African spatial assessment of human conflicts with lions and elephants." Nat Commun **12**(1): 2978.

African lions (*Panthera leo*) and African savanna (*Loxodonta africana*) and forest (*L. cyclotis*) elephants pose threats to people, crops, and livestock, and are themselves threatened with extinction. Here, we map these human-wildlife conflicts across Africa. Eighty-two percent of sites containing lions and elephants are adjacent to areas with considerable human pressure. Areas at severe risk of conflict (defined as high densities of humans, crops, and cattle) comprise 9% of the perimeter of these species' ranges and are found in 18 countries hosting, respectively, ~ 74% and 41% of African lion

and elephant populations. Although a variety of alternative conflict-mitigation strategies could be deployed, we focus on assessing the potential of high-quality mitigation fences. Our spatial and economic assessments suggest that investments in the construction and maintenance of strategically located mitigation fences would be a cost-effective strategy to support local communities, protect people from dangerous wildlife, and prevent further declines in lion and elephant populations.

Dibakou, S. E., U. Maloueki, B. Ngoubangoye, L. Boundenga, S. Ntie, T. A. Tsoumbou, C. Moussadji, R. O. Zang, D. Kombila and D. Basset (2021). "Diversity of gastrointestinal parasites in sympatric mammals in Moukalaba-Doudou National Park, Gabon." *Vet World* **14**(12): 3149-3155.

**BACKGROUND AND AIM:** Gastrointestinal parasites identified in the wild can negatively affect host fitness, lower performance, and growth. On the other side, sympatric mammals that share habitat and resources may also cross-transmit parasites, which are often zoonotic and can contribute to morbidity and mortality. This study aimed to characterize the diversity of gastrointestinal parasites circulating in mammalian hosts in Moukalaba-Doudou National Park. **MATERIALS AND METHODS:** We screened a total of 25 fecal samples collected from nine wild mammalian species, namely, western gorilla (*Gorilla gorilla gorilla*), chimpanzee (*Pan troglodytes*), putty-nosed monkey (*Cercopithecus nictitans*), African forest elephant (*Loxodonta cyclotis*), African buffalo (*Syncerus caffer*), blue duiker (*Philantomba monticola*), bay duiker (*Cephalophus dorsalis*), and red river hog (*Potamochoerus porcus*) as well as people working as trackers (*Homo sapiens*) using direct microscopic observations following a sedimentation technique to concentrate the fecal material. **RESULTS:** Of the total 25 fecal samples screened, 15 (60%) were positive for parasitic gastrointestinal infection. Based on the morphology of parasite eggs and cysts, we identified a rich diversity of nematodes, protozoans, trematodes, and cestodes, including unidentified strongyles (73%), *Oesophagostomum* spp. (53%), *Ancylostoma* spp. (27%), *Trichuris* spp. (13%), *Ascaris* spp. (13%), *Mammomonogamus* spp. (13%), *Strongyloides* spp. (47%), *Balantidium coli* (20%), *Entamoeba coli* (20%), *Endolimax nana* (6%), *Fasciola hepatica* (6%), *Paramphistomum* spp. (13%), and *Taenia* spp. (6%). **CONCLUSION:** All parasites were found at least once in one of the hosts, and most were potentially zoonotic and responsible for several diseases of public health concern. Because of the small sample size, our findings should not be considered conclusive. Nevertheless, they highlight the diversity of gastrointestinal parasites in this area.

Dubost, J. M., P. Kongchack, E. Deharo, P. Sysay, C. Her, L. Vichith, D. Sebastien and S. Krief (2021). "Zootherapeutic uses of animals excreta: the case of elephant dung and urine use in Sayaboury province, Laos." *J Ethnobiol Ethnomed* **17**(1): 18.

**Background** Despite a widespread aversion towards faeces and urine, animal excreta are used in traditional medicine in many countries since centuries, but records are scattered and few therapeutic uses have been accurately documented while in the current context of emerging zoonoses such records

may be of major interest. **Methodology** In this study, we investigated the therapeutic uses that mahouts in Xayaboury province, Lao PDR make of elephant urine and faeces as well as of the brood chamber that beetles (*Heliocopris dominus*) fashion from elephant dung. Semi-structured interviews were conducted with mahouts on elephant diet, health problems and responses to disease, and whether they use elephant products. Data were supplemented by interviews with traditional healers. **Results** Seven respondents reported the use of elephant urine in ethnoveterinary care for elephants and in human medicine in case of diabetes and otitis. 25 respondents reported therapeutic use of elephant faeces (EF) and elephant dung beetle brood chambers. The major indications are gastrointestinal and skin problems. Macerations or decoctions are drunk or used externally as a lotion. The mahouts attribute the therapeutic effectiveness of EFs to their content which includes the remains of many species from the elephant diet which they consider to be medicinal. **Discussion** The indications of these uses are consistent with pharmacological and clinical studies highlighting the properties of different animals' urine and faeces and their curative potential tested *in vivo*. The acknowledgement by the mahouts of medicinal properties of elephant faecal bolus contrasts with the rare justifications of animal material use recorded in zootherapeutic studies, which falls within the symbolic domain. However, numerous studies highlight the preponderant role of the microbiota in physiological processes, raising the hypothesis of a curative action of EF, by rebalancing the user's microbiota. **Conclusion** The therapeutic uses of EF preparations despite their possible curative properties are a potential source of zoonotic transmission from elephants to humans. In the current context of globalisation of trade which favours the emergence of zoonoses and in relation with the issue of One Health, it becomes crucial to further document the zootherapeutic practices to prevent emerging diseases. As elephants and local related ethnoethological knowledge are threatened, documenting them is urgent to contribute to their preservation.

Duporge, I., O. Isupova, S. Reece, D. W. Macdonald and T. Wang (2021). "Using very-high-resolution satellite imagery and deep learning to detect and count African elephants in heterogeneous landscapes." Remote Sensing in Ecology and Conservation **7**(3): 369-381.

Satellites allow large-scale surveys to be conducted in short time periods with repeat surveys possible at intervals of <24 h. Very-high-resolution satellite imagery has been successfully used to detect and count a number of wildlife species in open, homogeneous landscapes and seascapes where target animals have a strong contrast with their environment. However, no research to date has detected animals in complex heterogeneous environments or detected elephants from space using very-high-resolution satellite imagery and deep learning. In this study, we apply a Convolution Neural Network (CNN) model to automatically detect and count African elephants in a woodland savanna ecosystem in South Africa. We use WorldView-3 and 4 satellite data –the highest resolution satellite imagery commercially available. We train and test the model on 11 images from 2014 to 2019. We compare the performance accuracy of the CNN against human

accuracy. Additionally, we apply the model on a coarser resolution satellite image (GeoEye-1) captured in Kenya, without any additional training data, to test if the algorithm can generalize to an elephant population outside of the training area. Our results show that the CNN performs with high accuracy, comparable to human detection capabilities. The detection accuracy (i.e., F2 score) of the CNN models was 0.78 in heterogeneous areas and 0.73 in homogenous areas. This compares with the detection accuracy of the human labels with an averaged F2 score 0.77 in heterogeneous areas and 0.80 in homogenous areas. The CNN model can generalize to detect elephants in a different geographical location and from a lower resolution satellite. Our study demonstrates the feasibility of applying state-of-the-art satellite remote sensing and deep learning technologies for detecting and counting African elephants in heterogeneous landscapes. The study showcases the feasibility of using high resolution satellite imagery as a promising new wildlife surveying technique. Through creation of a customized training dataset and application of a Convolutional Neural Network, we have automated the detection of elephants in satellite imagery with accuracy as high as human detection capabilities. The success of the model to detect elephants outside of the training data site demonstrates the generalizability of the technique. © 2020 The Authors. Remote Sensing in Ecology and Conservation published by John Wiley & Sons Ltd on behalf of Zoological Society of London.

Edwards, B. D., R. Somayaji, D. Fisher and J. C. Chia (2021). "Lymphocutaneous spread of Mycobacterium elephantis in an immunocompetent individual: A case report." *SAGE Open Med Case Rep* **9**: 2050313x211034913.

Mycobacterium elephantis was first described when isolated from an elephant that succumbed to lung abscess. However, despite this namesake, it is not associated with animals and has been described most often as a probable colonizer rather than pathogen in humans with chronic lung disease. In this report, we describe the first case of lymphocutaneous infection from M. elephantis, likely as a result of cutaneous inoculation with contaminated soil. This offers further evidence to its capabilities as a pathogen. We provide a review of the limited prior reports of M. elephantis and outline the available in vitro data on efficacy of various antimycobacterial agents.

Edwards, K. L., E. M. Latimer, J. Siegal-Willott, W. Kiso, L. R. Padilla, C. R. Sanchez, D. Schmitt and J. L. Brown (2021). "Patterns of serum immune biomarkers during elephant endotheliotropic herpesvirus viremia in Asian and African elephants." *PLoS ONE* **16**(11): e0252175.

Hemorrhagic disease (HD) caused by a group of elephant endotheliotropic herpesviruses (EEHV) is one of the leading causes of death for young elephants in human care. These viruses are widespread and typically persist latently in adult elephants with no negative effects; however, in juvenile Asian and more recently young African elephants, the onset of disease can be rapid and the mortality rate high. Measuring biomarkers associated with the immune response could be beneficial to understanding underlying disease processes, as well as the management of infection and HD. The goal of this

study was to measure acute phase proteins and cytokines in serum collected from elephants infected with EEHV (13 Asian and 1 African) and compare concentrations according to presence, severity and outcome of disease. Serum amyloid A (SAA) and haptoglobin (HP) were higher in elephants with EEHV viremia than those without; concentrations increased with increasing viral load, and were higher in fatal cases compared to those that survived. In Asian elephants, SAA was also higher during EEHV1 viremia compared to EEHV5. Cytokine concentrations were typically low, and no statistical differences existed between groups. However, in individuals with detectable levels, longitudinal profiles indicated changes in tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-2 (IL-2) that may reflect an immune response to EEHV infection. However, the overall low concentrations detected using previously validated assays do not support the presence of a 'cytokine storm' and suggest more work is needed to understand if sub-optimal immune responses could be involved in disease progression. These results highlight the potential benefit of measuring circulating biomarker concentrations, such as APPs and cytokines, to improve our understanding of EEHV viremia and HD, assist with monitoring the progression of disease and determining the impact of interventions.

Eren, M. I., D. J. Meltzer, B. Story, B. Buchanan, D. Yeager and M. R. Bebbler (2021). "On the efficacy of Clovis fluted points for hunting proboscideans." Journal of Archaeological Science: Reports **39**.

Clovis fluted points are deemed efficient weapon tips for hunting large game, including Pleistocene proboscideans. However, experimental and archaeological studies cast doubt on their effectiveness as hunting weapons. Owing to the broad and thick tip geometry of Clovis points, their penetration depth into a carcass would have been relatively limited, which would have rendered them unlikely to reach the well-protected vital organs of a proboscidean and inflict lethal wounds. Nor do Clovis points display the types of breakage patterns and impact damage that would be expected were they routinely used as hunting weapons for megafauna, especially when compared with Folsom points found in bison kill sites. Our results question the long-assumed effectiveness of Clovis points for dispatching proboscideans; while these may have on occasion been used as weapon tips on proboscidean prey, they likely had other functions as well. © 2021 The Author(s)

Fayette, M. A., E. E. Brenner, M. M. Garner, M. R. Bowman, E. Latimer and J. S. Proudfoot (2021). "ACUTE HEMORRHAGIC DISEASE DUE TO ELEPHANT ENDOTHELIO-TROPIC HERPESVIRUS 3A INFECTION IN FIVE AFRICAN ELEPHANTS (LOXODONTA AFRICANA) AT ONE NORTH AMERICAN ZOOLOGICAL INSTITUTION." J Zoo Wildl Med **52**(1): 357-365.

Acute hemorrhagic disease caused by elephant endotheliotropic herpesvirus (EEHV) infection is well recognized as a major threat to young Asian elephants (*Elephas maximus*) but has been less frequently documented in African elephants (*Loxodonta africana*). This report describes five sequential cases of EEHV3A infection in African elephants in managed care at one institution. All elephants developed disease within a 4-mo period. The first



two cases were 6.5- and 7.5-yr-old females that presented with depressed mentation, anorexia, hematuria, and diarrhea. Both elephants died within 48-72 hr of the onset of illness despite treatment. Postmortem findings included widespread edema, ascites, and extensive petechiae and ecchymoses on the heart, liver, and spleen and within the gastrointestinal and urogenital tracts. Histologic examination identified disseminated vascular necrosis with edema, hemorrhage, and rare endothelial cell intranuclear inclusions typical of herpesvirus in multiple organs. The third and fourth cases were a 13-yr-old male and a 12-yr-old female that presented with minimal to no clinical signs, but with marked changes in hematologic parameters and high viremia detected by quantitative polymerase chain reaction (qPCR). Both elephants survived the infection with early and aggressive treatment. The fifth case was a 37-yr-old female that presented with lethargy and a decreased appetite. Low viremia was detected by qPCR, and mild to moderate hematologic changes were noted. Early treatment resulted in a successful outcome. This case series documents the first known reports of clinical disease and fatality associated with EEHV3A in African elephants.

Fernandez, E. J., B. Upchurch and N. C. Hawkes (2021). "Public Feeding Interactions as Enrichment for Three Zoo-Housed Elephants." *Animals (Basel)* **11**(6).

The past few decades have seen increased interest in studies examining the welfare of elephants and animal-visitor interactions. One understudied area for both pursuits is the impact of public feeding interactions. Our study examined the effects of public feedings on the general activity of three zoo-housed elephants. Prior to public feedings, we developed and assessed a 21-behavior ethogram split into six classes of behavior. Comparisons between the elephants demonstrated that only one of the elephants engaged in stereotypies with regularity (>30%), and that the stereotypies occurred in place of most foraging. During public feedings, we compared the general activity of each elephant independently and across both public feeding and nonpublic feeding days, as well as the general activity before, during, and after a public feeding. Public feedings increased social activity and decreased stereotypies when compared with nonpublic feeding days for two of the elephants. In addition, all three elephants showed increased foraging and decreased inactivity in the period after a public feeding session. These results demonstrate that public feedings can be a useful tool for enriching the welfare of zoo-housed elephants and are among the first sets of data to demonstrate positive welfare outcomes associated with public feedings.

Fernando, P., M. K. C. R. De Silva, L. K. A. Jayasinghe, H. K. Janaka and J. Pastorini (2021). "First country-wide survey of the Endangered Asian elephant: Towards better conservation and management in Sri Lanka." *Oryx* **55**(1): 46-55.

The Endangered Asian elephant *Elephas maximus* comes into widespread conflict with agrarian communities, necessitating active management. The species' distribution is of primary importance for management planning. However, data-based countrywide distribution maps have not been available

for any of the 13 Asian elephant range states. We conducted a 5 × 5 km grid-based questionnaire survey in Sri Lanka to produce an island-wide elephant distribution map. Elephants occur over 59.9% of Sri Lanka and people are resident in 69.4% of elephant range, indicating the challenge of separating people and elephants at a landscape scale. Elephants in Sri Lanka have lost 16.1% of their range since 1960 but their current distribution remains largely contiguous. We found the range of adult males was 15.1% greater, and less seasonal, than that of herds, possibly because males have a higher tolerance for conflict with people. The distribution of conflict coincided with the co-occurrence of humans and elephants. We conclude that a human-elephant coexistence model is the only viable option for effectively mitigating human-elephant conflict and conserving elephants in Sri Lanka. The findings are currently being used to effect a paradigm change in elephant conservation and management in the country. Copyright © 2019 Fauna & Flora International.

Flower, E. K., G. L. Burns and D. N. Jones (2021). "How Tourist Preference and Satisfaction Can Contribute to Improved Welfare Standards at Elephant Tourism Venues in Thailand." *Animals (Basel)* **11**(4).

Consumer satisfaction and preference can be integral in influencing and solidifying change in user-driven industries such as tourism. High satisfaction rates are imperative to the continual success of a venue as satisfaction determines the likelihood of repeat business and positive recommendations to friends, family and online review forums. Tourist preference for ecocentric tourism venues, over anthropocentric ones, appears to be increasing in elephant tourism venues (ETVs) in Thailand. To explore this, we visited twelve ETVs in Chiang Mai, Thailand, and compared the preferences and satisfaction of tourists who visited riding and non-riding venues toward the use of captive elephants in an entertainment setting. We found that tourists visited riding and non-riding ETVs for similar reasons, primarily due to recommendations from friends and reviews, and because the venue had a good reputation. Tourist preference for higher welfare standards was observed at venues where participants directly observed poor treatment of the elephants. Tourist satisfaction may be impacted by higher elephant welfare standards; therefore, tourists have the ability to influence the elephant tourism industry by demanding better living conditions for elephants and only financially supporting ETVs with higher welfare standards.

Freeman, P. T., E. L. Anderson, K. B. Allen and C. E. O'Connell-Rodwell (2021). "Age-based variation in calf independence, social behavior and play in a captive population of African elephant calves." *Zoo Biol* **40**(5): 376-385.

African elephant calves are highly social and their behavioral development depends heavily on interactions with other elephants. Evaluating early social behaviors offers important information that can inform management decisions and maximize individual- and population-level welfare. We use data collected from the population of elephants at the San Diego Zoo Safari Park in Escondido, CA to evaluate developmental trajectories of spatial independence and social behavior in nine elephant calves across a range of

ages. As calves aged, the probability of being further from mothers also increased. Tactile interactions were common among calves, with all individuals either initiating or receiving physical touches from other elephants in a large proportion of focal scans. While the probability of initiating tactile interactions tended to decline with increases in calf age, the probability of receiving tactile interactions from other elephants remained invariant with regard to this variable. The social play was also common, occurring in a fifth of all focal scans. While there was evidence that social play tended to decline with increases in calf age, results suggest additional factors may be useful in characterizing patterns in play behavior at the individual level. Calves most frequently engaged in play with individuals of similar age but showed substantial variation in play partner choice. Results of this study suggest that maintaining groups of elephants in captivity with diverse age structure positively contribute to their healthy social development.

Fuchs, E., V. C. Beeck, A. Baotic and A. S. Stoeger (2021). "Acoustic structure and information content of trumpets in female Asian elephants (*Elephas maximus*)."  
PLoS ONE **16**(11): e0260284.

Most studies on elephant vocal communication have focused on the low-frequency rumble, with less effort on other vocalization types such as the most characteristic elephant call, the trumpet. Yet, a better and more complete understanding of the elephant vocal system requires investigating other vocalization types and their functioning in more detail as well. We recorded adult female Asian elephants (*Elephas maximus*) at a private facility in Nepal and analyzed 206 trumpets from six individuals regarding their frequency, temporal and contour shape, and related acoustic parameters of the fundamental frequency. We also tested for information content regarding individuality and context. Finally, we recorded the occurrence of non-linear phenomena such as bifurcation, biphonation, subharmonics and deterministic chaos. We documented a mean fundamental frequency  $\pm$  SD of  $474 \pm 70$  Hz and a mean duration  $\pm$  SD of  $1.38 \pm 1.46$  s (Nindiv. = 6, Ncalls = 206). Our study reveals that the contour of the fundamental frequency of trumpets encodes information about individuality, but we found no evidence for trumpet subtypes in greeting versus disturbance contexts. Non-linear phenomena prevailed and varied in abundance among individuals, suggesting that irregularities in trumpets might enhance the potential for individual recognition. We propose that trumpets in adult female Asian elephants serve to convey an individual's identity as well as to signal arousal and excitement to conspecifics.

Fuktong, S., P. Yuttasaen, V. Punyapornwithaya, J. L. Brown, C. Thitaram, N. Luevitoonvechakij and P. Bansiddhi (2021). "A survey of stereotypic behaviors in tourist camp elephants in Chiang Mai, Thailand." Applied Animal Behaviour Science **243**: 5.

Stereotypies are abnormal behaviors found in a wide range of animals that have been used as indicators of poor welfare. Elephants used in tourism have been reported to perform stereotypic behavior, but the occurrence has not been systematically assessed. The aims of this study were to ascertain the

percentage of stereotypic behaviors exhibited by tourist camp elephants and relationship with demographic variables. This study surveyed 283 elephants from 20 elephant camps in Chiang Mai, Thailand. Amounts and types of stereotypic behavior were determined from 15-min direct observations. Additionally, demographic data and occurrence of stereotypic behavior (yes/no) were obtained from mahouts of 181 elephants using a questionnaire. Direct behavioral observations revealed that 57% (N = 161: 44 males and 117 females) of the elephants performed stereotypic behavior, while in mahout interviews, 58% were scored 'yes'. There were no differences in the least-squares mean score of stereotypic behaviors between males and females ( $p = 0.32$ ), whereas there were differences among age groups ( $p < 0.05$ ), with the highest in elephants 4-10 years of age, followed by 11-30 years of age, 31-50 years of age, > 50 years of age. Calves 0-3 years of age displayed the lowest rate of stereotypic behavior, when most were still with their mothers. The most common type of stereotypic behavior was swaying. Our results indicate that scores of stereotypic behaviors in elephants used in tourism differed among age categories. The next step will be to determine how management factors affect stereotypic behavior of elephants in this population and steps to mitigate it.

Glaeser, S. S., D. Shepherdson, K. Lewis, N. Prado, J. L. Brown, B. Lee and N. Wielebnowski (2021). "Supporting Zoo Asian Elephant (*Elephas maximus*) Welfare and Herd Dynamics with a More Complex and Expanded Habitat." *Animals (Basel)* **11**(9).

Ensuring good health and welfare is an increasingly important consideration for conservation of endangered species, whether free-ranging or managed to varying degrees under human care. The welfare-based design of a new habitat for Asian elephants at the Oregon Zoo focused on meeting the elephants' physical, physiological, psychological, and social needs 24 h a day and across life stages. The habitat was designed to encourage activity, promote species-typical behaviors, support changing social dynamics, offer increased opportunities for choice, and provide biologically meaningful challenges. In this 4-year study, we monitored elephant health and welfare indicators throughout the transition and acclimation from the previous habitat to the new habitat. Several welfare indicators obtained through longitudinal hormone analyses, behavior assessments, and GPS measurement of walking distance and space use provided evidence that these goals were achieved. The elephants were more active and walked farther on a daily basis in the new habitat, with an average walking distance of over 15 km per day. A switch from primarily caretaker-delivered food to seeking food on their own indicates that the disbursement of food with less temporal and spatial predictability increased foraging opportunities, which better satisfies appetitive motivations important for psychological well-being. All individuals showed adaptive and normal adrenal responses to change and challenge, with the highest fecal glucocorticoid metabolite (FGM) concentrations and variability during the construction phase, and a return to previous baseline concentrations in the new habitat, suggesting they acclimated well to the new environment. The elephants expressed a diverse

range of species-typical behaviors and demonstrated social dynamics of a healthy herd in both habitats with transitions of individuals through life stages. They exhibited more autonomy in choosing whom to associate with socially and also by choosing different aspects of their environment with regular indoor/outdoor access and extensive resource use in the new habitat. Findings indicate that the complexity and flexibility of the new habitat and habitat management has been effective in improving overall welfare by providing meaningful challenges and the opportunity to express appetitive behaviors, by offering choice in environmental conditions, and by providing the space and resource distribution to support evolving herd dynamics and increased social equity for individuals.

Goldenberg, S. Z., N. Hahn, J. Stacy-Dawes, S. M. Chege, D. Daballen, I. Douglas-Hamilton, R. R. Lendiria, M. J. Lengees, L. S. Loidialo, F. Omengo, F. Pope, C. Thouless, G. Wittemyer and M. A. Owen (2021). "Movement of Rehabilitated African Elephant Calves Following Soft Release Into a Wildlife Sanctuary." Frontiers in Conservation Science **2**: 13.

The ability to locate essential resources is a critical step for wildlife translocated into novel environments. Understanding this process of exploration is highly desirable for management that seeks to resettle wildlife, particularly as translocation projects tend to be expensive and have a high potential for failure. African savannah elephants (*Loxodonta africana*) are very mobile and rely on large areas especially in arid environments, and are translocated for differing management and conservation objectives. Thus, research into how translocated elephants use the landscape when released may both guide elephant managers and be useful for translocations of other species that adjust their movement to social and ecological conditions. In this study, we investigated the movement of eight GPS tracked calves (translocated in three cohorts) following their soft release into a 107 km<sup>2</sup> fenced wildlife sanctuary in northern Kenya and compared their movement with that of five tracked wild elephants in the sanctuary. We describe their exploration of the sanctuary, discovery of water points, and activity budgets during the first seven, 14, and 20 months after release. We explored how patterns are affected by time since release, ecological conditions, and social factors. We found that calves visited new areas of the sanctuary and water points during greener periods and earlier post-release. Social context was associated with exploration, with later release and association with wild elephants predictive of visits to new areas. Wild elephants tended to use a greater number of sites per 14-day period than the released calves. Activity budgets determined from hidden Markov models (including the states directed walk, encamped, and meandering) suggested that released calves differed from wild elephants. The first two cohorts of calves spent a significantly greater proportion of time in the directed walk state and a significantly lower proportion of time in the encamped state relative to the wild elephants. Our results represent a step forward in describing the movements of elephant orphan calves released to the wild following a period of profound social disruption when they lost their natal family and were rehabilitated with other orphan calves under human care. We discuss the

implications of the elephant behavior we observed for improving release procedures and for defining success benchmarks for translocation projects.

Grace, M. K., H. R. Akçakaya, E. L. Bennett, T. M. Brooks, A. Heath, S. Hedges, C. Hilton-Taylor, M. Hoffmann, A. Hochkirch, R. Jenkins, D. A. Keith, B. Long, D. P. Mallon, E. Meijaard, E. J. Milner-Gulland, J. P. Rodriguez, P. J. Stephenson, S. N. Stuart, R. P. Young, P. Acebes, J. Alfaro-Shigueto, S. Alvarez-Clares, R. R. Andriantsimanarilafy, M. Arbetman, C. Azat, G. Bacchetta, R. Badola, L. M. D. Barcelos, J. P. Barreiros, S. Basak, D. J. Berger, S. Bhattacharyya, G. Bino, P. A. V. Borges, R. K. Boughton, H. J. Brockmann, H. L. Buckley, I. J. Burfield, J. Burton, T. Camacho-Badani, L. S. Cano-Alonso, R. H. Carmichael, C. Carrero, J. P. Carroll, G. Catsadorakis, D. G. Chapple, G. Chapron, G. W. Chowdhury, L. Claassens, D. Cogoni, R. Constantine, C. A. Craig, A. A. Cunningham, N. Dahal, J. C. Daltry, G. C. Das, N. Dasgupta, A. Davey, K. Davies, P. Develey, V. Elangovan, D. Fairclough, M. D. Febraro, G. Fenu, F. M. Fernandes, E. P. Fernandez, B. Finucci, R. Földesi, C. M. Foley, M. Ford, M. R. J. Forstner, N. García, R. Garcia-Sandoval, P. C. Gardner, R. Garibay-Orijel, M. Gatan-Balbas, I. Gauto, M. G. U. Ghazi, S. S. Godfrey, M. Gollock, B. A. González, T. D. Grant, T. Gray, A. J. Gregory, R. H. A. van Grunsven, M. Gryzenhout, N. C. Guernsey, G. Gupta, C. Hagen, C. A. Hagen, M. B. Hall, E. Hallerman, K. Hare, T. Hart, R. Hartdegen, Y. Harvey-Brown, R. Hatfield, T. Hawke, C. Hermes, R. Hitchmough, P. M. Hoffmann, C. Howarth, M. A. Hudson, S. A. Hussain, C. Huveneers, H. Jacques, D. Jorgensen, S. Katdare, L. K. D. Katsis, R. Kaul, B. Kaunda-Arara, L. Keith-Diagne, D. T. Kraus, T. M. de Lima, K. Lindeman, J. Linsky, E. Louis, Jr., A. Loy, E. N. Lughadha, J. C. Mangel, P. E. Marinari, G. M. Martin, G. Martinelli, P. J. K. McGowan, A. McInnes, E. Teles Barbosa Mendes, M. J. Millard, C. Mirande, D. Money, J. M. Monks, C. L. Morales, N. N. Mumu, R. Negrao, A. H. Nguyen, M. N. H. Niloy, G. L. Norbury, C. Nordmeyer, D. Norris, M. O'Brien, G. A. Oda, S. Orsenigo, M. E. Outerbridge, S. Pasachnik, J. C. Pérez-Jiménez, C. Pike, F. Pilkington, G. Plumb, R. C. Q. Portela, A. Prohaska, M. G. Quintana, E. F. Rakotondraso, D. H. Ranglack, H. Rankou, A. P. Rawat, J. T. Reardon, M. L. Rheingantz, S. C. Richter, M. C. Rivers, L. R. Rogers, P. da Rosa, P. Rose, E. Royer, C. Ryan, Y. J. S. de Mitcheson, L. Salmon, C. H. Salvador, M. J. Samways, T. Sanjuan, A. Souza Dos Santos, H. Sasaki, E. Schutz, H. A. Scott, R. M. Scott, F. Serena, S. P. Sharma, J. A. Shuey, C. J. P. Silva, J. P. Simaika, D. R. Smith, J. L. Y. Spaet, S. Sultana, B. K. Talukdar, V. Tatayah, P. Thomas, A. Tringali, H. Trinh-Dinh, C. Tuboi, A. A. Usmani, A. M. Vasco-Palacios, J. C. Vié, J. Virens, A. Walker, B. Wallace, L. J. Waller, H. Wang, O. R. Wearn, M. van Weerd, S. Weigmann, D. Willcox, J. Woinarski, J. W. H. Yong and S. Young (2021). "Testing a global standard for quantifying species recovery and assessing conservation impact." *Conserv Biol* **35**(6): 1833-1849.

Recognizing the imperative to evaluate species recovery and conservation impact, in 2012 the International Union for Conservation of Nature (IUCN) called for development of a "Green List of Species" (now the IUCN Green Status of Species). A draft Green Status framework for assessing species' progress toward recovery, published in 2018, proposed 2 separate but interlinked components: a standardized method (i.e., measurement against benchmarks of species' viability, functionality, and preimpact distribution) to determine current species recovery status (herein species recovery score)

and application of that method to estimate past and potential future impacts of conservation based on 4 metrics (conservation legacy, conservation dependence, conservation gain, and recovery potential). We tested the framework with 181 species representing diverse taxa, life histories, biomes, and IUCN Red List categories (extinction risk). Based on the observed distribution of species' recovery scores, we propose the following species recovery categories: fully recovered, slightly depleted, moderately depleted, largely depleted, critically depleted, extinct in the wild, and indeterminate. Fifty-nine percent of tested species were considered largely or critically depleted. Although there was a negative relationship between extinction risk and species recovery score, variation was considerable. Some species in lower risk categories were assessed as farther from recovery than those at higher risk. This emphasizes that species recovery is conceptually different from extinction risk and reinforces the utility of the IUCN Green Status of Species to more fully understand species conservation status. Although extinction risk did not predict conservation legacy, conservation dependence, or conservation gain, it was positively correlated with recovery potential. Only 1.7% of tested species were categorized as zero across all 4 of these conservation impact metrics, indicating that conservation has, or will, play a role in improving or maintaining species status for the vast majority of these species. Based on our results, we devised an updated assessment framework that introduces the option of using a dynamic baseline to assess future impacts of conservation over the short term to avoid misleading results which were generated in a small number of cases, and redefines short term as 10 years to better align with conservation planning. These changes are reflected in the IUCN Green Status of Species Standard.

Graham, V., J. Geldmann, V. M. Adams, A. Grech, S. Deinet and H. C. Chang (2021). "Management resourcing and government transparency are key drivers of biodiversity outcomes in Southeast Asian protected areas." *Biological Conservation* **253**.

Protected areas aim to conserve nature by providing safe havens for biodiversity. However, protection from habitat loss, poaching and other threats, is not guaranteed without adequate investment in their management. Here, we examine the relationship between management effectiveness using the Management Effectiveness Tracking Tool (METT) and trends of 79 populations of mammals and birds in 12 Southeast Asian protected areas from Cambodia, Indonesia, Thailand and Vietnam. Despite the negative influence of corruption on species population change, we find evidence that adequate financial and human resourcing are important determinants in achieving good biodiversity outcomes. Management resourcing, national government transparency and body size collectively explain 29% of the variation in animal population trends in our model. Our paper contributes to a growing evidence base linking management resourcing shortfalls to declining biodiversity populations in protected areas. Our key findings are relevant to international funding agencies, governments and NGOs, to aid decision making around the allocation of conservation resources in Southeast Asia. © 2020 Elsevier Ltd

Grzyb, J. and K. Pawlak (2021). "Staphylococci and fecal bacteria as bioaerosol components in animal housing facilities in the Zoological Garden in Chorzów." Environ Sci Pollut Res Int **28**(40): 56615-56627.

Zoos are places open for a large number of visitors, adults and children, who can admire exotic as well as indigenous animal species. The premises for animals may contain pathogenic microbes, including those exhibiting antibiotic resistance. It poses a threat to people remaining within the zoo premises, both for animal keepers who meet animals on a daily basis and visitors who infrequently have contact with animals. There are almost no studies concerning the presence on the concentration of airborne bacteria, especially staphylococci and fecal bacteria in animal shelters in the zoo. There is no data about antibiotic resistance of staphylococci in these places. The results will enable to determine the scale of the threat that indicator bacteria from the bioaerosol pose to human health within zoo premises. This study conducted in rooms for 5 animals group (giraffes, camels, elephants, kangaroos, and Colobinae (species of monkey)) in the Silesian Zoological Garden in Chorzów (Poland). The bioaerosol samples were collected using a six-stage Andersen cascade impactor to assess the concentrations and size distribution of airborne bacteria. Staphylococci were isolated from bioaerosol and tested for antibiotic resistance. In our study, the highest contamination of staphylococci and fecal bacteria was recorded in rooms for camels and elephants, and the lowest in rooms for Colobinae. At least 2/3 of bacteria in bioaerosol constituted respirable fraction that migrates into the lower respiratory tract of the people. In investigated animal rooms, the greatest bacteria contribution was recorded for bioaerosol fraction sized 1.1-3.3 $\mu$ m. Bacterial concentrations were particularly strong in spring and autumn, what is related to shedding fur by animals. Among the isolated staphylococci which most often occurred were *Staphylococcus succinus*, *S. sciuri*, and *S. vitulinus*. The highest antibiotic resistance was noted in the case of *Staphylococcus epidermidis*, while the lowest for *S. xylosus*. In addition to standard cleaning of animal rooms, periodic disinfection should be considered. Cleaning should be carried out wet, which should reduce dust, and thus the concentrations of bacteria in the air of animal enclosures.

Gulati, S., K. K. Karanth, N. A. Le and F. Noack (2021). "Human casualties are the dominant cost of human-wildlife conflict in India." Proc Natl Acad Sci U S A **118**(8).

Reducing the costs from human-wildlife conflict, mostly borne by marginal rural households, is a priority for conservation. We estimate the mean species-specific cost for households suffering damages from one of 15 major species of wildlife in India. Our data are from a survey of 5,196 households living near 11 wildlife reserves in India, and self-reported annual costs include crop and livestock losses and human casualties (injuries and death). By employing conservative estimates from the literature on the value of a statistical life (VSL), we find that costs from human casualties overwhelm crop and livestock damages for all species associated with fatalities. Farmers experiencing a negative interaction with an elephant over the last year incur damages on average that are 600 and 900 times those incurred by farmers



with negative interactions with the next most costly herbivores: the pig and the nilgai. Similarly, farmers experiencing a negative interaction with a tiger over the last year incur damage that is on average 3 times that inflicted by a leopard and 100 times that from a wolf. These cost differences are largely driven by differences in the incidence of human death and casualties. Our estimate of costs fluctuates across reserves, mostly due to a variation of human casualties. Understanding the drivers of human casualties and reducing their incidence are crucial to reducing the costs from human-wildlife conflict. Most of the tales were about animals, for the Jungle was always at their door. The deer and the pig grubbed up their crops, and now and again the tiger carried off a man at twilight, within sight of the village gates. "Tiger! Tiger!" (Rudyard Kipling, *The Jungle Book*, Collins Classics, 2010).

Guntawang, T., T. Sittisak, V. Kochagul, S. Srivorakul, K. Photichai, K. Boonsri, T. Janyamethakul, K. Boonprasert, W. Langkaphin, C. Thitaram and K. Pringproa (2021). "Pathogenesis of hemorrhagic disease caused by elephant endotheliotropic herpesvirus (EEHV) in Asian elephants (*Elephas maximus*)." *Scientific Reports* **11**(1).

Elephant endotheliotropic herpesvirus-hemorrhagic disease (EEHV-HD) is an acute fatal disease in elephants. Despite the fact that the underlying pathogenesis of EEHV-HD has been proposed, it remains undetermined as to what mechanisms drive these hemorrhagic and edematous lesions. In the present study, we have investigated and explained the pathogenesis of acute EEHV-HD using blood profiles of EEHV-HD and EEHV-infected cases, hematoxylin and eosin (H&E) stain, special stains, immunohistochemistry, quantitative polymerase chain reaction (PCR) and reverse transcriptase polymerase chain reaction (RT-PCR). It was found that EEHV genomes were predominantly detected in various internal organs of EEHV-HD cases. Damage to endothelial cells, vasculitis and vascular thrombosis of the small blood vessels were also predominantly observed. Increases in platelet endothelial cell adhesion molecules-1 (PECAM-1)- and von Willebrand factor (vWF)-immunolabeling positive cells were significantly noticed in injured blood vessels. The expression of pro-inflammatory cytokine mRNA was significantly up-regulated in EEHV-HD cases when compared to EEHV-negative controls. We have hypothesized that this could be attributed to the systemic inflammation and disruption of small blood vessels, followed by the disseminated intravascular coagulopathy that enhanced hemorrhagic and edematous lesions in EEHV-HD cases. Our findings have brought attention to the potential application of effective preventive and therapeutic protocols to treat EEHV infection in Asian elephants. © 2021, The Author(s).

Guy, T. J., M. C. Hutchinson, K. C. R. Baldock, E. Kayser, B. Baiser, P. P. A. Staniczenko, J. R. Goheen, R. M. Pringle and T. M. Palmer (2021). "Large herbivores transform plant-pollinator networks in an African savanna." *Curr Biol* **31**(13): 2964-2971.e2965.

Pollination by animals is a key ecosystem service(1)(,) (2) and interactions between plants and their pollinators are a model system for studying ecological networks,(3)(,) (4) yet plant-pollinator networks are typically

studied in isolation from the broader ecosystems in which they are embedded. The plants visited by pollinators also interact with other consumer guilds that eat stems, leaves, fruits, or seeds. One such guild, large mammalian herbivores, are well-known ecosystem engineers(5-7) and may have substantial impacts on plant-pollinator networks. Although moderate herbivory can sometimes promote plant diversity,(8) potentially benefiting pollinators, large herbivores might alternatively reduce resource availability for pollinators by consuming flowers,(9) reducing plant density,(10) and promoting somatic regrowth over reproduction.(11) The direction and magnitude of such effects may hinge on abiotic context-in particular, rainfall, which modulates the effects of ungulates on vegetation.(12) Using a long-term, large-scale experiment replicated across a rainfall gradient in central Kenya, we show that a diverse assemblage of native large herbivores, ranging from 5-kg antelopes to 4,000-kg African elephants, limited resource availability for pollinators by reducing flower abundance and diversity; this in turn resulted in fewer pollinator visits and lower pollinator diversity. Exclusion of large herbivores increased floral-resource abundance and pollinator-assemblage diversity, rendering plant-pollinator networks larger, more functionally redundant, and less vulnerable to pollinator extinction. Our results show that species extrinsic to plant-pollinator interactions can indirectly and strongly alter network structure. Forecasting the effects of environmental change on pollination services and interaction webs more broadly will require accounting for the effects of extrinsic keystone species.

Hambrecht, S., A. K. Oerke, M. Heistermann, J. Hartig and P. W. Dierkes (2021). "Effects of Positive Reinforcement Training and Novel Object Exposure on Salivary Cortisol Levels under Consideration of Individual Variation in Captive African Elephants (*Loxodonta africana*)." Animals (Basel) **11**(12).

Dealing with potential stress in species that have high husbandry requirements, such as elephants, is a challenge for zoos. The objective of the present study was to determine whether positive reinforcement training (PRT) and exposure to a novel object (NOV) for enrichment induced a salivary cortisol response indicative of activation of the hypothalamic-pituitary-adrenal (HPA) axis and which factors determine individual variation in this regard in captive African elephants. We repeatedly sampled the saliva of ten animals (three zoos) for the analysis of cortisol (SACort) before and up to 60 min (in 10-15 min intervals) after the onset of PRT (three repeats) or NOV (nine repeats), which lasted 10 min. There was considerable individual variation in SACort in response to PRT or NOV. Using mixed models, we were able to control these and to reveal that PRT was associated with high SACort before and relatively low SACort after PRT, while NOV induced a moderate SACort increase. The individual differences in SACort were related to age and sex (NOV), while the effects of zoo, handling method (free vs. protected contact) and reproductive and social status were variable. We conclude that positive affective states, such as anticipation or arousal, should be taken into account when interpreting the differences in the SACort responses between PRT and NOV. In addition, understanding the individuality of stress will

support management decisions aimed at promoting captive elephant welfare.

Hautier, L., R. Tabuce, M. J. Mourlam, K. E. Kassegne, Y. Z. Amoudji, M. Orliac, F. Quillévéré, A. L. Charruault, A. K. C. Johnson and G. Guinot (2021). "New Middle Eocene proboscidean from Togo illuminates the early evolution of the elephantiform-like dental pattern." *Proc Biol Sci* **288**(1960): 20211439.

Africa has played a pivotal role in the evolution of early proboscideans (elephants and their extinct relatives), yet vast temporal and geographical zones remain uncharted on the continent. A long hiatus encompassing most of the Eocene (Ypresian to the Early Priabonian, around 13 Myr timespan) considerably hampers our understanding of the early evolutionary history of the group. It is notably the case with the origin of its most successful members, the Elephantiformes, i.e. all elephant-like proboscideans most closely related to modern elephants. Here, we describe a proboscidean lower molar discovered in Lutetian phosphate deposits from Togo, and name a new genus and species, *Dagbatitherium tassyi*. We show that *Dagbatitherium* displays several elephantiform dental characteristics such as a three-layered Schmelzmuster, the presence of a mesoconid, transversely enlarged buccal cusps and the individualization of a third lophid closely appressed to a minute distal cingulid. *Dagbatitherium* represents a stem Elephantiformes, pushing back the origin of the group by about 10 Myr, i.e. a third of its currently known evolutionary history. More importantly, *Dagbatitherium* potentially unlocks the puzzle of the origin of the unique elephantiform tooth crown organization by bridging a critical temporal and morphological gap between early bunodont incipiently bilophodont proboscidean taxa and more derived elephantiforms.

Hoornweg, T. E., W. Schaftenaar, G. Maurer, P. B. van den Doel, F. M. Molenaar, A. Chamouard-Galante, F. Vercammen, V. Rutten and C. A. M. de Haan (2021). "Elephant Endotheliotropic Herpesvirus Is Omnipresent in Elephants in European Zoos and an Asian Elephant Range Country." *Viruses* **13**(2).

Elephant endotheliotropic herpesviruses (EEHVs) may cause acute, often lethal, hemorrhagic disease (EEHV-HD) in young elephants. Prevalence of EEHV in different elephant populations is still largely unknown. In order to improve diagnostic tools for the detection of EEHV infections and to obtain insight into its spread among elephants, we developed novel ELISAs based on EEHV1A gB and gH/gL. Performance of the ELISAs was assessed using sera from 41 European zoo elephants and 69 semi-captive elephants from Laos, one of the Asian elephant range countries. Sera from all (sub)adult animals tested ( $\geq 5$  years of age) showed high reactivity with both gB and gH/gL, indicating that EEHV prevalence has been highly underestimated so far. Reactivity towards the antigens was generally lower for sera of juvenile animals ( $1 < 5$  years). Only one (juvenile) animal, which was sampled directly after succumbing to EEHV-HD, was found to be seronegative for EEHV. The two other EEHV-HD cases tested showed low antibody levels, suggesting that all three cases died upon a primary EEHV infection. In conclusion, our study suggests that essentially all (semi-)captive (sub)adult elephants in European zoos and in Laos carry EEHV, and that young

elephants with low antibody levels are at risk of dying from EEHV-HD.

Hoorweg, T. E., W. Schaftenaar, G. Maurer, P. B. van den Doel, F. M. Molenaar, A. Chamouard-Galante, F. Vercammen, V. P. M. G. Rutten and C. A. M. de Haan (2021). "Elephant endotheliotropic herpesvirus is omnipresent in elephants in european zoos and an asian elephant range country." *Viruses* **13**(2).

Elephant endotheliotropic herpesviruses (EEHVs) may cause acute, often lethal, hemor-rhagic disease (EEHV-HD) in young elephants. Prevalence of EEHV in different elephant populations is still largely unknown. In order to improve diagnostic tools for the detection of EEHV infections and to obtain insight into its spread among elephants, we developed novel ELISAs based on EEHV1A gB and gH/gL. Performance of the ELISAs was assessed using sera from 41 European zoo elephants and 69 semi-captive elephants from Laos, one of the Asian elephant range countries. Sera from all (sub)adult animals tested ( $\geq 5$  years of age) showed high reactivity with both gB and gH/gL, indicating that EEHV prevalence has been highly underestimated so far. Reactivity towards the antigens was generally lower for sera of juvenile animals ( $1 < 5$  years). Only one (juvenile) animal, which was sampled directly after succumbing to EEHV-HD, was found to be seronegative for EEHV. The two other EEHV-HD cases tested showed low antibody levels, suggesting that all three cases died upon a primary EEHV infection. In conclusion, our study suggests that essentially all (semi-)captive (sub)adult elephants in European zoos and in Laos carry EEHV, and that young elephants with low antibody levels are at risk of dying from EEHV-HD. © 2021 by the authors. Licensee MDPI, Basel, Switzerland.

Hörner, F., A. K. Oerke, D. W. H. Müller, U. Westerhüs, I. Azogu-Sepe, J. Hruby and G. Preisfeld (2021). "Monitoring behaviour in african elephants during introduction into a new group: Differences between related and unrelated animals." *Animals* **11**(10).

The introduction of elephants into new groups is necessary for breeding programmes. However, behavioural studies on the reactions of these animals at first encounters are missing. In the present study, female African elephants (*Loxodonta africana*) living in zoos were observed during unifications with unfamiliar elephants (introduction of two to one females and one to two females;  $n = 6$ ) and reunifications with related elephants (two mother–daughter-pairs;  $n = 4$ ) that were separated for 2 and 12 years, respectively. First encounters of the elephants were observed and recorded by scan sampling. The parameters measured were (a) signs of the characteristic Greeting Ceremony, (b) distance to the fence separating the elephants during first contact, and (c) time until trunks touched for the first time. The data were statistically analysed with SPSS. The results showed that related elephants performed a full Greeting Ceremony on reunifications. Unrelated elephants only expressed a minor greeting. During first encounters, related elephants predominantly showed affiliative behaviour ( $p = 0.001$ ), whilst unrelated elephants expressed more agonistic behaviour ( $p = 0.001$ ). The distance to the fence was significantly smaller for related elephants than for unrelated elephants ( $p = 0.038$ ). first contact of trunks

occurred on average after 3.00 s. in related elephants and 1026.25 s. in unrelated elephants. These findings indicate that related elephants recognise their kin after up to 12 years of separation, meet them with a full Greeting Ceremony during reunification, and seek contact to the related elephant, while unrelated elephants are hesitant during unifications with unfamiliar elephants and express more agonistic behaviour. The results testify that zoo elephants show the same species-specific social behaviour as their conspecifics in the wild. It also confirms the cognitive abilities of elephants and the significance of matrilineal lines for breeding programmes. © 2021 by the authors. Licensee MDPI, Basel, Switzerland.

Hosono, H., T. Asano, J. Takei, M. Sano, T. Tanaka, M. K. Kaneko and Y. Kato (2021). "Development of an Anti-Elephant Podoplanin Monoclonal Antibody PMab-265 for Flow Cytometry." Monoclon Antib Immunodiagn Immunother **40**(3): 141-145.

The development of specific antibodies is essential to understand a wide variety of biological phenomena and pathophysiological analyses. Podoplanin (PDPN), a type I transmembrane glycoprotein, is known as a diagnostic marker. Anti-PDPN monoclonal antibodies (mAbs) against many species, such as human, mouse, rat, rabbit, dog, bovine, cat, tiger, horse, pig, goat, alpaca, Tasmanian devil, bear, whale, and sheep, have been established in recent studies. However, sensitive and specific mAbs against elephant PDPN (elePDPN) have not been established. Thus, this study established a novel mAb against African savanna elephant (*Loxodonta africana*) PDPN using the Cell-Based Immunization and Screening method. elePDPN-overexpressed Chinese hamster ovary-K1 (CHO/elePDPN) cells were immunized, and mAbs were screened against elePDPN using flow cytometry. One of the mAbs, PMab-265 (IgM,  $\kappa$ ), specifically detected CHO/elePDPN cells by flow cytometry. These findings suggested the potential usefulness of PMab-265 for the functional analyses of elePDPN.

Htet, N. N. P., R. Chaiyarat, N. Thongthip, P. Anuracpreeda, N. Youngpoy and P. Chompoopong (2021). "Population and distribution of wild Asian elephants (*Elephas maximus*) in Phu Khieo Wildlife Sanctuary, Thailand." PeerJ **9**: e11896.

**BACKGROUND:** The populations of wild Asian elephants (*Elephas maximus*) have increased recently after a period of worldwide decline in protected areas. It is important to understand the dynamics and distribution of the remaining populations to ensure their conservation and prevent human-elephant conflicts. **METHODS:** We monitored the population distribution of elephants between 2016 and 2019 in the Phu Khieo Wildlife Sanctuary, Thailand. We set one hundred forty-nine camera trap locations; cameras recorded 38,834 photos over 6,896 trap nights. Elephants were captured in 4,319 photographs. The maximum entropy modeling software MaxEnt was used to identify elephants' habitat preferences within 49 of the 149 total camera trap locations according to five environmental factors. **RESULTS:** One hundred fourteen elephants were identified. We identified 30 adult males, 43 adult females, 14 sub-adult males, nine sub-adult females, 11 juveniles, and seven calves. The age structure ratio based on adult females was

0.7:1:0.3:0.2:0.3:0.2, and the ratio of reproductive ability between adult females, juveniles, and calves was 1:0.2:0.1. A suitable elephant habitat was determined to be 1,288.9 km<sup>2</sup> using Area Under the Curve (AUC). An AUC = 0.061 indicated good performance. Our model classified habitat preferences associated with elevation, forests, salt licks, human activity, and slope. CONCLUSIONS: According to our probability map this sanctuary can provide a suitable habitat for elephants. Our results indicate that effective management practices can protect wild Asian elephants in the region and reduce conflict between humans and elephants.

Jacobs, B., H. Rally, C. Doyle, L. O'Brien, M. Tennison and L. Marino (2021). "Putative neural consequences of captivity for elephants and cetaceans." Reviews in the Neurosciences.

The present review assesses the potential neural impact of impoverished, captive environments on large-brained mammals, with a focus on elephants and cetaceans. These species share several characteristics, including being large, wide-ranging, long-lived, cognitively sophisticated, highly social, and large-brained mammals. Although the impact of the captive environment on physical and behavioral health has been well-documented, relatively little attention has been paid to the brain itself. Here, we explore the potential neural consequences of living in captive environments, with a focus on three levels: (1) The effects of environmental impoverishment/enrichment on the brain, emphasizing the negative neural consequences of the captive/impoverished environment; (2) the neural consequences of stress on the brain, with an emphasis on corticolimbic structures; and (3) the neural underpinnings of stereotypies, often observed in captive animals, underscoring dysregulation of the basal ganglia and associated circuitry. To this end, we provide a substantive hypothesis about the negative impact of captivity on the brains of large mammals (e.g., cetaceans and elephants) and how these neural consequences are related to documented evidence for compromised physical and psychological well-being. © 2021 Walter de Gruyter GmbH, Berlin/Boston 2021.

Jansson, T., B. V. Perera, A. Edner and Å. Fahlman (2021). "STANDING SEDATION WITH XYLAZINE AND REVERSAL WITH YOHIMBINE IN JUVENILE ASIAN ELEPHANTS (ELEPHAS MAXIMUS)." J Zoo Wildl Med **52**(2): 437-444.

Evaluation and improvement of immobilization methods are important for wildlife welfare and biodiversity conservation. The sedative and physiological effects of xylazine (50-110 mg per elephant; 0.09-0.15 mg/kg IM) were evaluated in 15 juvenile Asian elephants (*Elephas maximus*) in Sri Lanka. The time from xylazine injection until first sign of sedation, handling, and reversal with yohimbine (0.009-0.03 mg/kg IV) were recorded. Behavioral signs, level of sedation (no effect, light, moderate, or deep) and response to handling were assessed. Rectal temperature, pulse, and respiratory rates were recorded and arterial blood samples were analyzed 30 and 45 min after xylazine injection. The first sign of sedation occurred within 5-18 min. Standing sedation was induced in all elephants, but the level of sedation varied differently over time for each elephant. Twelve elephants remained

standing throughout the sedation period, while 3 elephants became laterally recumbent. Sedative effects included lowered head and trunk, droopy ears, snoring, and penis protrusion. Pulse rate, respiratory rate, and rectal temperature ranged between 30-45 beats/min, 4-12 breaths/min, and 35.6-37.2°C, respectively, at 30 min after xylazine injection, and there were no changes over time. Pulmonary function and acid-base balance were adequate (range partial pressures of arterial oxygen 73-123 mmHg and carbon dioxide 33-52 mmHg, arterial hemoglobin oxygen saturation 96-99%, pH 7.34-7.54, lactate 0.9-2.5 mmol/L). Yohimbine was administered 46-110 min after the injection of xylazine, and the first sign of recovery occurred within 1-4 min. Resedation after reversal with yohimbine was observed in two elephants. In conclusion, xylazine at the doses used induced light to deep sedation with stable physiology and most elephants remained standing.

Jesus, S. A., M. G. Doherr and T. B. Hildebrandt (2021). "Elephant Endotheliotropic Herpesvirus Impact in the European Asian Elephant (*Elephas maximus*) Population: Are Heritability and Zoo-Associated Factors Linked with Mortality?" *Animals (Basel)* **11**(10).

EEHV is a ubiquitous virus, which most likely has co-evolved with elephants and is shed by healthy individuals and maintained in the herds. Yet, the factors determining calf susceptibility to the virus remain unknown. Here, we explored the impact of EEHV-HD in the European captive Asian elephant population in a retrospective statistical study spanning the last 35 years. We show that EEHV-HD was implicated in more than half of all deaths recorded in calves older than one month old. Moreover, the median age across EEHV-HD fatalities was significantly lower compared to other death causes. Finally, we investigated if heredity and zoo-associated factors could be linked to a higher susceptibility of calves to this disease. We used a univariable logistic regression model to evaluate if either fathers, mothers, or zoos could, separately, be considered as risk factors to the development of the disease. Afterwards, we used a two multivariable model, combining: (1) fathers and zoos, and (2) mothers and zoos. Overall, we found that two fathers, one mother, and four zoos had three or more times higher risk of their calves becoming sick when compared to all others, pointing us to the presence of a management or environmental element, which can have paternal and maternal influence and leads to calf susceptibility or resistance to EEHV-HD.

Jia, P., S. Dai, T. Wu and S. Yang (2021). "New Approaches to Anticipate the Risk of Reverse Zoonosis." *Trends in Ecology and Evolution* **36**(7): 580-590.

The coronavirus disease 2019 (COVID-19) pandemic can cause reverse zoonoses (i.e., human-animal transmission of COVID-19). It is vital to utilize up-to-date methods to improve the control, management, and prevention of reverse zoonoses. Awareness of reverse zoonoses should be raised at both individual and regional/national levels for better protection of both humans and animals. © 2021 Elsevier Ltd

Jones, N. (2021). "Ivory hunting drives evolution of tuskless elephants." *Nature*.

Keady, M. M., N. Prado, H. C. Lim, J. Brown, S. Paris and C. R. Muletz-Wolz (2021). "Clinical health issues, reproductive hormones, and metabolic hormones associated with gut microbiome structure in African and Asian elephants." *Anim Microbiome* **3**(1): 85.

**BACKGROUND:** The gut microbiome is important to immune health, metabolism, and hormone regulation. Understanding host-microbiome relationships in captive animals may lead to mediating long term health issues common in captive animals. For instance, zoo managed African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) experience low reproductive rates, high body condition, and gastrointestinal (GI) issues. We leveraged an extensive collection of fecal samples and health records from the Elephant Welfare Study conducted across North American zoos in 2012 to examine the link between gut microbiota and clinical health issues, reproductive hormones, and metabolic hormones in captive elephants. We quantified gut microbiomes of 69 African and 48 Asian elephants from across 50 zoos using Illumina sequencing of the 16S rRNA bacterial gene. **RESULTS:** Elephant species differed in microbiome structure, with African elephants having lower bacterial richness and dissimilar bacterial composition from Asian elephants. In both species, bacterial composition was strongly influenced by zoo facility. Bacterial richness was lower in African elephants with recent GI issues, and richness was positively correlated with metabolic hormone total triiodothyronine (total T3) in Asian elephants. We found species-specific associations between gut microbiome composition and hormones: Asian elephant gut microbiome composition was linked to total T3 and free thyroxine (free T4), while fecal glucocorticoid metabolites (FGM) were linked to African elephant gut microbiome composition. We identified many relationships between bacterial relative abundances and hormone concentrations, including *Prevotella* spp., *Treponema* spp., and *Akkermansia* spp. **CONCLUSIONS:** We present a comprehensive assessment of relationships between the gut microbiome, host species, environment, clinical health issues, and the endocrine system in captive elephants. Our results highlight the combined significance of host species-specific regulation and environmental effects on the gut microbiome between two elephant species and across 50 zoo facilities. We provide evidence of clinical health issues, reproductive hormones, and metabolic hormones associated with the gut microbiome structure of captive elephants. Our findings establish the groundwork for future studies to investigate bacterial function or develop tools (e.g., prebiotics, probiotics, dietary manipulations) suitable for conservation and zoo management.

Keerthipriya, P., S. Nandini and T. N. C. Vidya (2021). "Effects of Male Age and Female Presence on Male Associations in a Large, Polygynous Mammal in Southern India: The Asian Elephant." *Frontiers in Ecology and Evolution* **9**.

We present a detailed study of male associations in the Asian elephant, using 6 years of data on identified, non-musth males. Adult males spent greater proportions of their time solitarily than in mixed-sex or in all-male groups. Old (over 30 years) males were sighted more frequently with their age-peers and less frequently with young (15–30 years) males than expected at



random in all-male groups. Young males were not sighted more frequently with old males than with young males, and did not disproportionately initiate associations with old males. These results suggest that male associations, in the absence of females, may allow for old non-musth males to test strengths against age-peers. Social learning from older individuals did not seem to be important in male associations, unlike that observed in the African savannah elephant. We also found a constraint on the sizes of all-male groups, similar to that seen in female groups in our study population, and all-male groups were rarer and smaller than those in African savannah elephant. Although male associations were weak, most males had a significant top associate, with whom their association was the strongest, in female absence. In mixed-sex groups, male associations occurred at random, suggesting that males were tracking female groups independently. Differences in male social organization from that of the related African savannah elephant that occupies a similar niche possibly arise from differences in ecology. © Copyright © 2021 Keerthipriya, Nandini and Vidya.

Khammesri, S., Y. Mathura, K. Boonprasert, C. Ampasavate, D. Hongwiset, J. L. Brown and C. Thitaram (2021). "Successful treatment of elephant endotheliotropic herpesvirus infection in an Asian elephant (*Elephas maximus*) calf by oral acyclovir medication: Case report." *J Vet Med Sci* **83**(1): 125-129.

Elephant endotheliotropic herpesvirus (EEHV) is a major cause of death in Asian elephant (*Elephas maximus*) calves. A 2-year, 11-month-old female, captive Asian elephant presented with facial edema and a mild fever. Blood samples were collected and showed EEHV1A positivity with a high viral load by real time PCR. Heterophil toxicity also was reported for the first time in this case. The calf was treated orally with acyclovir, 45 mg/kg tid for 28 days, which reduced the EEHV1A viral load to undetectable levels within 9 days and the calf survived. A successful outcome with oral acyclovir administration provides another and affordable option to treat EEHV hemorrhagic disease in Asian elephants, and one that is easier to administer in untrained calves.

Kock, R., A. L. Michel, D. Yeboah-Manu, E. I. Azhar, J. B. Torrelles, S. I. Cadmus, L. Brunton, J. M. Chakaya, B. Marais, L. Mboera, Z. Rahim, N. Haider and A. Zumla (2021). "Zoonotic Tuberculosis – The Changing Landscape." *International Journal of Infectious Diseases* **113**: S68-S72.

Despite slow reductions in the annual burden of active human tuberculosis (TB) cases, zoonotic TB (zTB) remains a poorly monitored and an important unaddressed global problem. There is a higher incidence in some regions and countries, especially where close association exists between growing numbers of cattle (the major source of *Mycobacterium bovis*) and people, many suffering from poverty, and where dairy products are consumed unpasteurised. More attention needs to be focused on possible increased zTB incidence resulting from growth in dairy production globally and increased demand in low income countries in particular. Evidence of new zoonotic mycobacterial strains in South Asia and Africa (e.g. *M. orygis*), warrants urgent assessment of prevalence, potential drivers and risk in order to

develop appropriate interventions. Control of *M. bovis* infection in cattle through detect and cull policies remain the mainstay of reducing zTB risk, whilst in certain circumstances animal vaccination is proving beneficial. New point of care diagnostics will help to detect animal infections and human cases. Given the high burden of human tuberculosis (caused by *M. tuberculosis*) in endemic areas, animals are affected by reverse zoonosis, including multi-drug resistant strains. This, may create drug resistant reservoirs of infection in animals. Like COVID-19, zTB is evolving in an ever-changing global landscape. © 2021 The Author(s)

Köhler, M., V. Herridge, C. Nacarino-Meneses, J. Fortuny, B. Moncunill-Solé, A. Rosso, R. Sanfilippo, M. R. Palombo and S. Moyà-Solà (2021). "Palaeohistology reveals a slow pace of life for the dwarfed Sicilian elephant." *Sci Rep* **11**(1): 22862. The 1-m-tall dwarf elephant *Palaeoloxodon falconeri* from the Pleistocene of Sicily (Italy) is an extreme example of insular dwarfism and epitomizes the Island Rule. Based on scaling of life-history (LH) traits with body mass, *P. falconeri* is widely considered to be 'r-selected' by truncation of the growth period, associated with an early onset of reproduction and an abbreviated lifespan. These conjectures are, however, at odds with predictions from LH models for adaptive shifts in body size on islands. To settle the LH strategy of *P. falconeri*, we used bone, molar, and tusk histology to infer growth rates, age at first reproduction, and longevity. Our results from all approaches are congruent and provide evidence that the insular dwarf elephant grew at very slow rates over an extended period; attained maturity at the age of 15 years; and had a minimum lifespan of 68 years. This surpasses not only the values predicted from body mass but even those of both its giant sister taxon (*P. antiquus*) and its large mainland cousin (*L. africana*). The suite of LH traits of *P. falconeri* is consistent with the LH data hitherto inferred for other dwarfed insular mammals. *P. falconeri*, thus, not only epitomizes the Island Rule but it can also be viewed as a paradigm of evolutionary change towards a slow LH that accompanies the process of dwarfing in insular mammals.

Kongsawasdi, S., J. L. Brown, K. Boonprasert, P. Pongsopawijit, K. Wantanajittikul, S. Khammesri, T. Tajarernduang, N. Thonglorm, R. Kanta-In and C. Thitaram (2021). "Impact of weight carriage on joint kinematics in asian elephants used for riding." *Animals* **11**(8).

Background: Elephants in Thailand have changed their roles from working in the logging industry to tourism over the past two decades. In 2020, there were approximately 2700 captive elephants participating in activities such as riding and trekking. During work hours, riding elephants carry one or two people in a saddle on the back with a mahout on the neck several hours a day and over varying terrain. A concern is that this form of riding can cause serious injuries to the musculoskeletal system, although to date there have been no empirical studies to determine the influence of weight carriage on kinematics in elephants. Methods: Eight Asian elephants from a camp in Chiang Mai Province, Thailand, aged between 21 and 41 years with a mean body mass of  $3265 \pm 140.2$  kg, were evaluated under two conditions:

walking at a normal speed without a saddle and with a 15% body mass load (saddle and two persons plus additional weights). Gait kinematics, including the maximal angles of fore-and hindlimb joints, were determined using a novel three-dimensional inertial measurement system with wireless sensors. Results: There were no statistical differences between movement angles and a range of motion of the fore-and hindlimbs, when an additional 15% of body mass was added. Conclusion: There is no evidence that carrying a 15% body mass load causes significant changes in elephant gait patterns. Thus, carrying two people in a saddle may have minimal effects on musculoskeletal function. More studies are needed to further test longer durations of riding on different types of terrain to develop appropriate working guidelines for captive elephants. Nevertheless, elephants appear capable of carrying significant amounts of weight on the back without showing signs of physical distress. © 2021 by the authors. Licensee MDPI, Basel, Switzerland.

Kongsawasdi, S., K. Wantanajittikul, W. Langkaphin, B. Chuatrakoon, K. Namwongprom, P. Prupetkaew and T. Angkawanish (2021). "Optimal Management to Improve Quality of Life for an Injured Baby Elephant: Thailand Multidisciplinary Care Team." Kafkas Universitesi Veteriner Fakultesi Dergisi **27**(5): 655-659.

This article aimed to report the care, management, and monitoring of an injured female baby elephant. The collaboration among the multidisciplinary team included veterinary medicines, elephant handling, engineering, and physical therapy in the 3-year-old elephant with its left forelimb phalanges amputated through being caught in a snare trap. The management comprised medical wound care, nutrition and applying a prosthetic shoe. The kinematic parameters and vital sign monitoring were analysed. The results show that biomechanics gait analysis and physiological responses revealed promising benefit of the prosthetic shoe by reflecting a greater symmetrical gait pattern without dyspnea and no sign of exertion during daily life activity.

LaDue, C. A., I. Eranda, C. Jayasinghe and R. P. G. Vandercone (2021). "Mortality Patterns of Asian Elephants in a Region of Human-Elephant Conflict." Journal of Wildlife Management **85**(4): 794-802.

Many wildlife species suffer from human-wildlife conflict, especially crop-raiding. Long-term analyses of mortality patterns are needed to assess the efficacy of management strategies that address this issue. We report mortality patterns from necropsies of 498 Asian elephants from 2009-2018 in an area of northwestern Sri Lanka. Deaths were lowest in July and highest in October, a period of peak crop availability. Most (about 70%) deaths were human-related, and males were killed in these incidents more frequently than females. As gunshot deaths decreased, other forms of human-related deaths increased. Additionally, causes of death differed between districts, with more intentional human-related mortality observed in the district with the highest percent of protected land. These results highlight the importance of understanding the long-term spatial and temporal variation in wildlife mortality to effectively address human-wildlife conflict. (c) 2021 The Wildlife Society.

LaDue, C. A., B. A. Schulte, W. K. Kiso and E. W. Freeman (2021). "Musth and sexual selection in elephants: A review of signalling properties and potential fitness consequences." Behaviour.

Sexual selection mediated by multimodal signals is common among polygynous species, including seasonally breeding mammals. Indirect benefit models provide plausible explanations for how and why mate selection can occur in the absence of direct benefits. Musth - an asynchronous reproductive state in male elephants - facilitates both inter- and intrasexual selection via indirect benefits, and it is further communicated through a multimodal signal. In this review, we synthesise existing evidence that supports the hypothesis that musth is a multimodal signal subject to sexual selection and that male elephants increase their direct fitness by propagating this signal while females accrue indirect benefits. Musth is characterised by a suite of physiological and behavioural changes, serving to facilitate copulation between the sexes, and via multisensory modalities musth conveys honest information about the condition of a male. Female elephants mate preferentially with musth males, increasing their own fitness in the absence of direct benefits. In addition, musth resolves dynamic dominance hierarchies among male elephants and often eliminates the need for costly physical combat. Future work in this field should investigate potential postcopulatory selection mechanisms in elephants, including sperm competition and cryptic female choice. These topics join other fundamental questions related to sexual selection, signalling, and indirect benefits that are still unanswered in elephants. © C.A. LADUE ET AL., 2021

Laguardia, A., S. Bourgeois, S. Strindberg, K. S. Gobush, G. Abitsi, H. G. Bikang Bi Ateime, F. Ebouta, J. M. Fay, A. M. Gopalaswamy, F. Maisels, E. L. F. Simira Banga Daouda, L. J. T. White and E. J. Stokes (2021). "Nationwide abundance and distribution of African forest elephants across Gabon using non-invasive SNP genotyping." Global Ecology and Conservation **32**.

Robust monitoring programs are essential for understanding changes in wildlife population dynamics and distribution over time, especially for species of conservation concern. In this study, we applied a rapid non-invasive sampling approach to the Critically Endangered African forest elephant (*Loxodonta cyclotis*), at nationwide scale in its principal remaining population strongholds in Gabon. We used a species-specific customized genetic panel and spatial capture-recapture (SCR) approach, which gave a snapshot of current abundance and density distribution of forest elephants across the country. We estimated mean forest elephant density at 0.38 (95% Confidence Interval 0.24–0.52) per km<sup>2</sup> from 18 surveyed sites. We confirm that Gabon is the main forest elephant stronghold, both in terms of estimated population size: 95,110 (95% CI 58,872–131,349) and spatial distribution (250,782 km<sup>2</sup>). Predicted elephant densities were highest in relatively flat areas with a high proportion of suitable habitat not in proximity to the national border. Protected areas and human pressure were not strong predictors of elephant densities in this study. Our nationwide systematic survey of forest elephants of Gabon serves as a proof-of-concept of application of noninvasive genetic sampling for rigorous population

monitoring at large spatial scales. To our knowledge, it is the first nationwide DNA-based assessment of a free-ranging large mammal in Africa. Our findings offer a useful national baseline and status update for forest elephants in Gabon. It will inform adaptive management and stewardship of elephants and forests in the most important national forest elephant stronghold in Africa. © 2021 The Authors

Landolfi, J. A., P. M. Gaffney, R. McManamon, N. L. Gottdenker, A. E. Ellis, R. R. Rech, S. Han, L. J. Lowenstine, D. Agnew, M. M. Garner, D. McAloose, C. Hollinger, J. St Leger, S. P. Terrell, M. Duncan and A. P. Pessier (2021). "Reproductive tract neoplasia in adult female Asian elephants (*Elephas maximus*)." *Vet Pathol* **58**(6): 1131-1141.

Recent reports have highlighted a lower-than-expected prevalence of neoplasia in elephants and suggested mechanisms for cancer resistance. But despite infrequent reports in the literature, uterine neoplasia is common in managed Asian elephants (*Elephas maximus*). This study is an archival review of reproductive tract neoplasia in 80 adult female Asian elephant mortalities in managed care facilities in the United States from 1988 to 2019. Neoplasms occurred in 64/80 (80%) of cases. Most were in the uterus (63/64; 98%) with only a single case of ovarian neoplasia. Myometrial leiomyomas were present in 57/63 (90%) cases with uterine neoplasia. Uterine adenocarcinoma was present in 8/63 (13%) cases. Remaining cases included endometrial adenoma (2), focal carcinoma in situ in endometrial polyps (1), anaplastic carcinoma (1), endometrial hemangioma (1), primitive neuroectodermal tumor (PNET; 1), and angiosarcoma (1). One case with uterine adenocarcinoma had a separate pelvic mass histologically characterized as an anaplastic sarcoma. Distant metastases were documented in 5/8 (63%) cases of uterine adenocarcinoma, and in the uterine anaplastic carcinoma, PNET, and angiosarcoma. Four uterine adenocarcinomas and one carcinoma in situ were examined immunohistochemically for pan-cytokeratin, vimentin, and estrogen receptor. In all, neoplastic cells were pan-cytokeratin positive and vimentin negative, and in 2 cases were immunoreactive for estrogen receptor. Results show that female reproductive tract neoplasia, particularly of the uterus, is common in Asian elephants and is not limited to leiomyomas. Importantly, uterine neoplasms have the potential to impact fecundity and may represent obstacles to conservation in managed care.

Lasky, M., J. Campbell, J. A. Osborne, E. L. Ivory, J. Lasky and C. J. Kendall (2021). "Increasing browse and social complexity can improve zoo elephant welfare." *Zoo Biol* **40**(1): 9-19.

While recent work has assessed how environmental and managerial changes influence elephant welfare across multiple zoos, few studies have addressed the effects of management changes within a single institution. In this paper, we examine how management changes related to social structure and diet affect the behavior of a group of zoo elephants over a 23-month period while also considering underlying factors, such as time of day, hormonal cycle, and individual differences. We recorded individual behaviors using 2-min scan

samples during 60-min sessions. We analyzed behavioral changes across several study variables using generalized linear mixed models. We found that increasing browse can improve opportunities for foraging throughout the day but may not be sufficient to reduce repetitive behaviors. We observed that increasing group size and integration of bulls with cows can lead to increased social interaction in African elephants. Our results highlight the importance of using multiple management alterations to address elephant welfare, and considering environmental factors, when making management decisions.

Laubscher, L. L., S. Pfitzer, P. S. Rogers, L. L. Wolfe, M. W. Miller, A. Semjonov and J. P. Raath (2021). "EVALUATING THE USE OF A BUTORPHANOL-AZAPERONE-MEDETOMIDINE FIXED-DOSE COMBINATION FOR STANDING SEDATION IN AFRICAN ELEPHANTS (LOXODONTA AFRICANA)." *J Zoo Wildl Med* **52**(1): 287-294.

This study investigated the use of a fixed-dose combination of 30 mg/ml butorphanol, 12 mg/ml azaperone, and 12 mg/ml medetomidine for the standing sedation of captive African elephants (*Loxodonta africana*). In total, seven females (mean age 19.6 yr; range 6-31 yr) and six males (mean age 33.5 yr; range 9-35 yr) were sedated. The estimated dose was  $0.0005 \pm 0.0001$  ml/kg and  $0.006 \pm 0.001$  ml/cm shoulder height, which resulted in a dose of  $0.016 \pm 0.002$  mg/kg or  $0.19 \pm 0.04$  mg/cm shoulder height butorphanol,  $0.006 \pm 0.0008$  mg/kg or  $0.076 \pm 0.015$  mg/cm shoulder height azaperone, and  $0.006 \pm 0.0008$  mg/kg or  $0.076 \pm 0.015$  mg/cm medetomidine. First signs of sedation were observed within 3-10 min (mean  $6 \pm 2$  min) after darting, and monitoring of the animals started on average at  $24 \pm 9$  min after darting. No bradycardia was observed in any of the elephants (mean heart rate  $40.0 \pm 6.55$  beats/min), although all the animals were mildly hypotensive (mean blood pressure 118.5/86 [94.5]). Rectal temperatures fell within acceptable ranges, and respiratory parameters were stable in all the animals throughout sedation and fell within the standard ranges reported for conscious, standing elephants. Only one elephant had clinically significant hypoxemia characterized by a partial pressure of oxygen (PaO<sub>2</sub>) < 60 mm Hg. This elephant was also hypercapnic (PaCO<sub>2</sub>) > 50 mm Hg), although pH and peripheral capillary oxygen saturation fell within acceptable ranges. None of the elephants reacted to moderately painful stimuli while sedated. The combination was reversed with intramuscular injections of naltrexone (1 mg for every 1 mg butorphanol) and atipamezole (5 mg for every 1 mg medetomidine). Recovery was smooth and calm in all the animals. Time from injection of the reversals until the first signs of recovery was  $4.6 \pm 2.01$  min (range 1-8 min).

Lee, M. H., S. Nathan, L. Benedict, P. Nagalingam, E. Latimer, T. Hughes, D. Ramirez and J. R. A. Sukor (2021). "The first reported cases of elephant endotheliotropic herpesvirus infectious haemorrhagic disease in Malaysia: case report." *Virology* **18**(1): 231.

BACKGROUND: Elephant endotheliotropic herpesvirus haemorrhagic disease (EEHV HD) is the leading cause of death in captive Asian elephant calves in Asia, North America, and Europe with a mortality rate of ~65% in calves that are under human care. Although EEHV HD was first found in elephant camps,

more recently it was identified in wild populations which poses a greater threat to the elephant population. Deaths due to EEHV HD have been seen in wild elephants, but the in-situ prevalence and mortality rate is unknown. We report the first EEHV HD cases in Malaysia from 3 wild born endangered Bornean elephant calves from Sabah with known typical clinical signs. CASE PRESENTATION: The first calf died within 24 h of the onset of clinical signs; the second calf died within 12 h of the onset of clinical signs. The third calf succumbed within 72 h. Necropsies revealed that all 3 calves had similar presentations of EEHV HD but in the third calf with less severity. We conducted conventional polymerase chain reaction (cPCR) assays and found EEHV DNA at all 7 loci in the 3 calves; it was identified as EEHV1A, the virus type that has been found in most other reported cases. CONCLUSION: Typical EEHV HD clinical signs and the molecular confirmation of EEHV by cPCR and sequencing point to EEHV as the cause of death. Further genetic investigation of the strain is in progress.

Lekko, Y. M., A. Che-Amat, P. T. Ooi, S. Omar, D. T. Mohd-Hamdan, L. S. Linazah, Z. Zakaria, S. Z. Ramanoon, M. Mazlan, F. F. A. Jesse, M. F. A. Abdul-Razak, S. Jasni and N. Abdul-Hamid (2021). "Detection of Mycobacterium tuberculosis complex antibodies in free-ranged wild boar and wild macaques in selected districts in Selangor and reevaluation of tuberculosis serodetection in captive Asian elephants in Pahang, Peninsular Malaysia." *J Vet Med Sci* **83**(11): 1702-1707.

Tuberculosis (TB) is a chronic inflammatory and zoonotic disease caused by Mycobacterium tuberculosis complex (MTBC) members, affecting several domestic animals, wildlife species and humans. The preliminary investigation was aimed to detect antibody against MTBC among indigenous wildlife which are free-ranged wild boar, free-ranged wild macaques and captive Asian elephants in selected areas of Selangor and elephant conservation centre in Pahang, respectively. The results indicate that MTBC serodetection rate in wild boar was 16.7% (7.3-33.5 at 95% confidence interval (CI)) using an in-house ELISA bPPD IgG and 10% (3.5-25.6 at 95% CI) by DPP(®)VetTB assay, while the wild macaques and Asian elephant were seronegative. The univariate analysis indicates no statistically significant difference in risk factors for sex and age of wild boar but there was a significant positive correlation ( $P < 0.05$ ) between bovine TB in dairy cattle and wild boar seropositivity in the Sepang district.

Leung, J., T. Beths, M. Lynch, S. Frith and S. H. Bauquier (2021). "A simple method to provide positive end expiratory pressure to treat hypoxaemia in an anaesthetised Asian Elephant (*Elephas maximus*)." *J S Afr Vet Assoc* **92**(0): e1-e4.

Hypoxaemia is a common complication in anaesthetised or immobilised elephants. It is presumably because of hypoventilation and ventilation-perfusion mismatch. To prevent hypoxaemia, orotracheal intubation and positive pressure ventilation are recommended. This case report describes a hypoxaemic period despite positive pressure ventilation in a 46-year-old female Asian elephant (*Elephas maximus*) anaesthetised with azaperone-etorphine, medetomidine and an etorphine constant rate infusion in lateral recumbency for a dental procedure. The hypoxaemia was corrected utilising

positive end-expiratory pressure (PEEP) of 5 cm - 10 cm H<sub>2</sub>O, a technique that has not previously been reported in the management of anaesthetised elephants. PEEP decreases atelectasis, shunt fraction, and increases lung compliance. Positive end-expiratory pressure was achieved by partial occlusion of the tailpiece of a manually triggered demand valve ventilator during expiration. This is a simple effective method of generating PEEP and correcting hypoxaemia without the need for any additional specialised equipment. However, PEEP decreased arterial blood pressure and should be implemented with caution if arterial blood pressure is not monitored.

Li, L. L., J. M. Plotnik, S. W. Xia, E. Meaux and R. C. Quan (2021). "Cooperating elephants mitigate competition until the stakes get too high." *PLoS Biology* **19**(9). Cooperation is ubiquitous in the animal kingdom as it aims to maximize benefits through joint action. Selection, however, may also favor competitive behaviors that could violate cooperation. How animals mitigate competition is hotly debated, with particular interest in primates and little attention paid thus far to nonprimates. Using a loose-string pulling apparatus, we explored cooperative and competitive behavior, as well as mitigation of the latter, in semi-wild Asian elephants (*Elephas maximus*). Our results showed that elephants first maintained a very high cooperation rate (average = 80.8% across 45 sessions). Elephants applied "block," "fight back," "leave," "move side," and "submission" as mitigation strategies and adjusted these strategies according to their affiliation and rank difference with competition initiators. They usually applied a "fight back" mitigation strategy as a sanction when competition initiators were low ranking or when they had a close affiliation, but were submissive if the initiators were high ranking or when they were not closely affiliated. However, when the food reward was limited, the costly competitive behaviors ("monopoly" and "fight") increased significantly, leading to a rapid breakdown in cooperation. The instability of elephant cooperation as a result of benefit reduction mirrors that of human society, suggesting that similar fundamental principles may underlie the evolution of cooperation across species. © 2021 Li et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Liehrmann, O., J. A. H. Crawley, M. W. Seltmann, S. Feillet, U. K. Nyein, H. H. Aung, W. Htut, M. Lahdenperä, L. Lansade and V. Lummaa (2021). "Handler familiarity helps to improve working performance during novel situations in semi-captive Asian elephants." *Sci Rep* **11**(1): 15480.

Working animals spend hours each day in close contact with humans and require training to understand commands and fulfil specific tasks. However, factors driving cooperation between humans and animals are still unclear, and novel situations may present challenges that have been little-studied to-date. We investigated factors driving cooperation between humans and animals in a working context through behavioural experiments with 52 working semi-captive Asian elephants. Human-managed Asian elephants constitute approximately a third of the remaining Asian elephants in the



world, the majority of which live in their range countries working alongside traditional handlers. We investigated how the familiarity and experience of the handler as well as the elephant's age and sex affected their responses when asked to perform a basic task and to cross a novel surface. The results highlighted that when novelty is involved in a working context, an elephant's relationship length with their handler can affect their cooperation: elephants who had worked with their handler for over a year were more willing to cross the novel surface than those who had a shorter relationship with their handler. Older animals also tended to refuse to walk on the novel surface more but the sex did not affect their responses. Our study contributes much needed knowledge on human-working animal relationships which should be considered when adjusting training methods and working habits.

Liyanage, D. J., P. Fernando, P. N. Dayawansa, H. K. Janaka and J. Pastorini (2021). "The elephant at the dump: how does garbage consumption impact Asian elephants?" *Mammalian Biology* **101**(6): 1089-1097.

We studied garbage consumption by Asian elephants at the Uddakandara garbage dump in southern Sri Lanka. Garbage at the dump was classified under six categories and quantified using a grid overlay. Elephants visiting the dump were individually identified by morphological criteria and items and quantities consumed by them were determined by focal animal sampling. Dung of elephants that did not consume garbage and those from the dump were compared quantitatively and dung constituents assessed by washing through three layered sieves. A total of 17 individual elephants visited the garbage dump during the study period, all of who were males. The observed sexual bias could be related to behavioural differences between the sexes. Elephants mostly consumed 'fruits and vegetables' and 'prepared food', possibly due to their higher palatability and nutritional value. Ingestion of polythene was incidental and associated with consuming prepared food. Proportions of the six categories in elephant diet and garbage piles were significantly different, indicating that elephants were highly selective when feeding. Elephant arrivals increased in response to unloading of garbage, suggesting attraction to fresh garbage. Dung analysis found that garbage consumption did not change the quantity and constituents of dung, except for the presence of anthropogenic items. As consumed anthropogenic items were regularly excreted, retention and obstruction of the alimentary tract are unlikely in elephants. Elephants feeding on garbage had better body condition than non-garbage consuming elephants, indicating that garbage provided better nutrition than natural food and was not detrimental to their health.

Lo, Y. C. and P. Janta (2021). "Balancing Commercialization and Sustainability in Community-Based Tourism Practices - A Qualitative Study of Factors Affecting Elephant Habitat Communities in Northern Thailand." *Front Psychol* **12**: 685426.

Community-Based Tourism (CBT) offers local residents opportunities to manage local tourism resources while sustaining their lifestyle at the same time. The research objective of the study was to explore the process and experience of communities in Northern Thailand which are known as elephant

habitats, how these communities strive for stimulating the local economy without jeopardizing the way of life. The study was qualitative in nature. Qualitative data collection methods such as field observations and in-depth interviews were employed. The qualitative data were further analyzed with thematic analysis. In practicing CBT, the findings identified positive factors (Establishment of Elephant Camps), negative factors (Waste from Tourism Activity and Effects of Global Crisis on Employment and Local Income), and suggestions (Waste and Environment Management). The study found that the communities took pride in their cultural as well as natural resources and they are willing to commercialize these resources to a certain degree, i.e. founding elephant themed facilities, as has evidently been indicated. Consequently, as many issues factor into the practice of CBT, the study concluded that community participation and government support should have played a crucial role in maintaining new balance of overall local lifestyle sustainability and commercialization during and after the pandemic.

Lueders, I. and C. Stremme (2021). "Construction of a full mouth speculum facilitating oral examinations, bronchoscopy and gastric tubing in elephants." Tierärztliche Praxis Ausgabe G: Grosstiere - Nutztiere.

Objective Here we tested the application of a full mouth speculum to sedated elephants in human care to gain access to the oral cavity, the trachea (bronchi) and esophagus (stomach) and therefore improve diagnostic and therapeutic options in elephant medicine. The construction of this oral speculum for elephants and the procedure are described. Material and methods The oral speculum is a steel construction consisting of 2 bite plates of 0.8×60.0×8.0cm attached between 2 threaded guiding poles (40cm). Through crank handles, the metal plates are dispersed once placed between the elephant's jaws in front of the molars. The oral speculum was applied in 26 elephants (6,16Asian elephants, and 1,3African elephants) during standing sedation. Results All sedated elephants tolerated the positioning of the mouth opener and subsequent manipulations well. The mouth opener was applied for the following procedures: inspection of the oral cavity (n=2), placing a stomach tube (n=16), and/or performing endoscopic examinations such as bronchoscopy (n=20) and/or gastroscopy (n=8). Conclusion This method provides a new possibility to open the jaws to gain access to the molars, larynx and pharynx in captive elephants without full immobilization. Valuable samples for diagnostics may be obtained or animals medicated via stomach tube with this application. Clinical relevance The mouth opener provides veterinarians with a new option to perform necessary diagnostic and therapeutic procedures around the oral cavity, airways and stomach in captive elephants during standing sedation with no need for a full anaesthesia. © 2021 Georg Thieme Verlag. All rights reserved.

M., R., D. P. M., V. K., J. A. and K. K. (2021). "Haemato- biochemical changes in tuberculosis infected and healthy Asian elephants (*Elephas maximus*) from South India." J. Vet. Anim. Sci **52**(4): 345-349.

Tuberculosis is known to be a disease of elephants for the past 2000 years. The main causative agent isolated from reported tuberculosis (TB) cases

were *Mycobacterium tuberculosis*. The study focuses on the haematological and serum biochemical changes in the blood of TB infected Asian elephants (*Elephas maximus*). Twelve apparently healthy elephants and twelve TB infected elephants (confirmed by trunk wash smear positive for acid fast bacilli) were selected for the study. Neonates, pregnant elephants and elephants in musth were not included in the study. The study animals were subjected to haematological and serum biochemical evaluation. The data were analysed statistically. The results showed a significant increase in total leukocyte count, lymphocyte count, monocyte count, thrombocyte count and ESR in TB affected animals compared with apparently healthy animals. Serum creatinine, total bilirubin, direct bilirubin, globulin was significantly high in TB affected animals compared with healthy controls. Assessment of haematological and serum biochemical parameters in TB affected elephants aid in diagnosis and tracking of the infection.

Marshall, M. (2021). "These cellular clocks help explain why elephants are bigger than mice." *Nature* **592**(7856): 682-684.

Miller, M. A., T. J. Kerr, C. R. de Waal, W. J. Goosen, E. M. Streicher, G. Hausler, L. Rossouw, T. Manamela, L. van Schalkwyk, L. Kleynhans, R. Warren, P. van Helden and P. E. Buss (2021). "Mycobacterium bovis infection in free-ranging african elephants." *Emerging Infectious Diseases* **27**(3): 990-992.

*Mycobacterium bovis* infection in wildlife species occurs worldwide. However, few cases of *M. bovis* infection in captive elephants have been reported. We describe 2 incidental cases of bovine tuberculosis in free-ranging African elephants (*Loxodonta africana*) from a tuberculosis-endemic national park in South Africa and the epidemiologic implications of these infections. © 2021 Centers for Disease Control and Prevention (CDC). All rights reserved.

Mortimer, B., J. A. Walker, D. S. Lolchuragi, M. Reinwald and D. Daballen (2021). "Noise matters: Elephants show risk-avoidance behaviour in response to human-generated seismic cues." *Proceedings of the Royal Society B: Biological Sciences* **288**(1953).

African elephants (*Loxodonta africana*) use many sensory modes to gather information about their environment, including the detection of seismic, or ground-based, vibrations. Seismic information is known to include elephant-generated signals, but also potentially encompasses biotic cues that are commonly referred to as 'noise'. To investigate seismic information transfer in elephants beyond communication, here we tested the hypothesis that wild elephants detect and discriminate between seismic vibrations that differ in their noise types, whether elephant- or human-generated. We played three types of seismic vibrations to elephants: seismic recordings of elephants (elephant-generated), white noise (human-generated) and a combined track (elephant- and human-generated). We found evidence of both detection of seismic noise and discrimination between the two treatments containing human-generated noise. In particular, we found evidence of retreat behaviour, where seismic tracks with human-generated noise caused elephants to move further away from the trial location. We conclude that

seismic noise are cues that contain biologically relevant information for elephants that they can associate with risk. This expands our understanding of how elephants use seismic information, with implications for elephant sensory ecology and conservation management. © 2021 The Authors.

Moustafa, M. A. M., H. M. Chel, M. J. Thu, S. Bawm, L. L. Htun, M. M. Win, Z. M. Oo, N. Ohsawa, M. Lahdenperä, W. M. A. Mohamed, K. Ito, N. Nonaka, R. Nakao and K. Katakura (2021). "Anthropogenic interferences lead to gut microbiome dysbiosis in Asian elephants and may alter adaptation processes to surrounding environments." *Sci Rep* **11**(1): 741.

Human activities interfere with wild animals and lead to the loss of many animal populations. Therefore, efforts have been made to understand how wildlife can rebound from anthropogenic disturbances. An essential mechanism to adapt to environmental and social changes is the fluctuations in the host gut microbiome. Here we give a comprehensive description of anthropogenically induced microbiome alterations in Asian elephants (n = 30). We detected gut microbial changes due to overseas translocation, captivity and deworming. We found that microbes belonging to Planococcaceae had the highest contribution in the microbiome alterations after translocation, while Clostridiaceae, Spirochaetaceae and Bacteroidia were the most affected after captivity. However, deworming significantly changed the abundance of Flavobacteriaceae, Sphingobacteriaceae, Xanthomonadaceae, Weeksellaceae and Burkholderiaceae. These findings may provide fundamental ideas to help guide the preservation tactics and probiotic replacement therapies of a dysbiosed gut microbiome in Asian elephants. More generally, these results show the severity of anthropogenic activities at the level of gut microbiome, altering the adaptation processes to new environments and the subsequent capability to maintain normal physiological processes in animals.

Muir, Y. S. S., B. Bryant, M. Campbell-Ward and D. P. Higgins (2021). "Retrospective anti-tetanus antibody responses of zoo-based Asian elephants (*Elephas maximus*) and rhinoceros (*Rhinocerotidae*)." *Dev Comp Immunol* **114**: 103841.

Tetanus toxoids (TT) commercially available for use in horses and livestock are commonly used to vaccinate elephants and rhinoceros that are in human care. Although recommendations for booster intervals have changed in human and horse protocols to reduce the risks associated with hyper-immunity (i.e. B-cell anergy and hypersensitivity reactions) these have generally not been adopted in zoo protocols. Additionally, there is no evidence to demonstrate commercial TT immunogenicity in rhinoceros. In this study, a preliminary analysis of rhinoceros antibody responses to TT was conducted, in addition to an exploration of the impact of various booster frequencies on antibody responses in elephant. Retrospective analysis of archived serum samples was conducted for 9 Asian elephants (*Elephas maximus*), 7 southern black (*Diceros bicornis minor*), one southern white (*Ceratotherium simum simum*), and two greater one-horned (*Rhinoceros unicornis*) rhinoceros. Pre-vaccination (baseline) samples and those following

priming vaccination (rhinoceros only), annual and non-annual boosters were targeted. A commercially available competitive ELISA kit was used to quantify serum anti-TT antibodies. Average baseline and post-vaccination anti-tetanus antibody concentrations were greater in elephant (92 mg/L  $\pm$  42, n = 3, N = 3; 125  $\pm$  76, n = 82, N = 9) than in rhinoceros (47 mg/L  $\pm$  39, n = 8, N = 8; 44 mg/L  $\pm$  37, n = 16, N = 7). Rhinoceros antibody concentrations did not differ markedly following vaccinations from their naturally acquired high pre-vaccination concentrations. Eight elephants demonstrated antibody maintenance for 3-5 years without a tetanus booster. Additionally, although five out of nine elephants developed local reactions consistent with delayed type IV hypersensitivity following some boosters, there was no association between high antibody concentrations and increased incidence of adverse reactions. In addition, no decrease in antibody concentrations was detected as a result of annual vaccination in elephants, though this does not entirely rule out potential for B-cell anergy.

Nair, R. P. and E. A. Jayson (2021). "Estimation of economic loss and identifying the factors affecting the crop raiding behaviour of Asian elephant (*Elephas maximus*) in Nilambur part of the southern Western Ghats, Kerala, India." Current Science **121**(4): 521-528.

The crop damage by the Asian elephant (*Elephas maximus*) on the livelihood of farmers is a major impediment to the conservation of the endangered mammals. The study was carried out in Malappuram district, Kerala, India from January 2013 to May 2016, to estimate the extent of crop damage by Asian elephants and to identify the factors affecting human-elephant conflict. To estimate the monetary loss, the method of running quadrats was employed. The major cash-crops destroyed by the Asian elephant were plantain (*Musa paradisiaca*), rubber (*Hevea brasiliensis*), areca nut (*Areca catechu*) and coconut (*Cocos nucifera*). A potential loss of Rs 5,076,827 (US\$ 72,948) per annum (Rs 2,217,363 (US\$ 31,861) (other crops) + Rs 2859,464 (US\$ 41,087) (rubber)) was estimated. Fifty per cent of the encounters occurred at early midnight. The presence of areca nut cultivation and distance to the Reserve Forest were identified as the two factors affecting crop raiding. The damage to rubber trees by feeding on the bark has also been reported.

Neto de Carvalho, C., Z. Belaústegui, A. Toscano, F. Muñiz, J. Belo, J. M. Galán, P. Gómez, L. M. Cáceres, J. Rodríguez-Vidal, P. P. Cunha, M. Cachão, F. Ruiz, S. Ramirez-Cruzado, F. Giles-Guzmán, G. Finlayson, S. Finlayson and C. Finlayson (2021). "First tracks of newborn straight-tusked elephants (*Palaeoloxodon antiquus*)." Sci Rep **11**(1): 17311.

Tracks and trackways of newborns, calves and juveniles attributed to straight-tusked elephants were found in the MIS 5 site (Upper Pleistocene) known as the Matalascañas Trampled Surface (MTS) at Huelva, SW Spain. Evidence of a snapshot of social behaviour, especially parental care, can be determined from the concentration of elephant tracks and trackways, and especially from apparently contemporaneous converging trackways, of small juvenile and larger, presumably young adult female tracks. The size

frequency of the tracks enabled us to infer body mass and age distribution of the animals that crossed the MTS. Comparisons of the MTS demographic frequency with the morphology of the fore- and hind limbs of extant and fossil proboscideans shed light into the reproductive ecology of the straight-tusked elephant, *Palaeloxodon antiquus*. The interdune pond habitat appeared to have been an important water and food resource for matriarchal herds of straight-tusked elephants and likely functioned as a reproductive habitat, with only the rare presence of adult and older males in the MTS. The preservation of this track record in across a paleosol surface, although heavily trampled by different animals, including Neanderthals, over a short time frame, permitted an exceptional view into short-term intraspecific trophic interactions occurring in the Last Interglacial coastal habitat. Therefore, it is hypothesized that Neanderthals visited MTS for hunting or scavenging on weakened or dead elephants, and more likely calves.

Pabutta, C., N. Bangkaew, P. Inthawong, P. Mahadthai, W. Jairak, N. Soda, M. Sukmak and S. Sripiboon (2021). "The first report on internal transcribed spacer region-based characterization of microfilaria in Asian elephants (*Elephas maximus*) in Thailand." *Vet World* **14**(8): 2260-2266.

**BACKGROUND AND AIM:** Filarial infections can significantly impact the health of both humans and animals. In elephants, filariasis has been associated with cutaneous dermatitis and skin nodules. However, molecular evidence for such infections is limited in Thailand. This study aimed to identify the morphological and molecular characteristics of microfilaria in captive Asian elephants in Thailand. **MATERIALS AND METHODS:** Whole blood collected from the ear vein of 129 captive Asian elephants was hematologically analyzed, and the blood parasites were evaluated using three standard techniques: The microcapillary test, thin blood smears, and polymerase chain reaction (PCR). **RESULTS:** Conventional PCR revealed that approximately 17% (22/129) of the sampled elephants were positive for microfilaria. Microscopy revealed that microfilariae are large, unsheathed, with extended nuclei, a short headspace, and a curved tail tapering at the end. Results of internal transcribed spacer region analysis show that the elephant microfilariae are closely related to *Onchocerca* spp. All of the elephants positive for microfilaria presented with neither skin lesion nor anemic signs. Microfilaria infection was not associated with age; however, microfilariae were more likely to be detected in male elephants due to differences in management systems. **CONCLUSION:** This is the first study to provide both morphological and molecular evidence of microfilaria in Thai elephants. There is an urgent need to investigate the long-term and large-scale effects of microfilaria on the health of elephants.

Parker, J. M., C. T. Webb, D. Daballen, S. Z. Goldenberg, J. Lepirei, D. Letitiya, D. Lolchuragi, C. Leadismo, I. Douglas-Hamilton and G. Wittemyer (2021). "Poaching of African elephants indirectly decreases population growth through lowered orphan survival." *Curr Biol*.

Prolonged maternal care is vital to the well-being of many long-lived mammals.(1) The premature loss of maternal care, i.e., orphaning, can

reduce offspring survival even after weaning is complete.(2-5) However, ecologists have not explicitly assessed how orphaning impacts population growth. We examined the impact of orphaning on population growth in a free-ranging African elephant population, using 19 years of individual-based demographic monitoring data. We compared orphan and nonorphan survival, performed a sensitivity analysis to understand how population growth responds to the probability of being orphaned and orphan survival, and investigated how sensitivity to these orphan parameters changed with level of poaching. Orphans were found to have lower survival compared to nonorphaned age mates, and population growth rate was negatively correlated with orphaning probability and positively correlated with orphan survival. This demonstrates that, in addition to its direct effects, adult elephant death indirectly decreases population growth through orphaning. Population growth rate's sensitivity to orphan survival increased for the analysis parameterized using only data from years of more poaching, indicating orphan survival is more important for population growth as orphaning increases. We conclude that orphaning substantively decreases population growth for elephants and should not be overlooked when quantifying the impacts of poaching. Moreover, we conclude that population models characterizing systems with extensive parental care benefit from explicitly incorporating orphan stages and encourage research into quantifying effects of orphaning in other social mammals of conservation concern.

Paudel, S., E. P. Brenner, S. A. Hadi, Y. Suzuki, C. Nakajima, T. Tsubota, K. P. Gairhe, B. Maharjan and S. Sreevatsan (2021). "Genome Sequences of Two Mycobacterium tuberculosis Isolates from Asian Elephants in Nepal." Microbiol Resour Announc **10**(36): e0061421.

This report describes the genome sequences of two Mycobacterium tuberculosis isolates, S1 and S3, recovered from Asian elephants in Nepal. These genome sequences will enhance our understanding of the genomic epidemiology of Mycobacterium tuberculosis in Asian elephants.

Pearson, V. R., J. B. Bosse, O. O. Koyuncu, J. Scherer, C. Toruno, R. Robinson, L. M. Abegglen, J. D. Schiffman, L. W. Enquist and G. F. Rall (2021). "Identification of African Elephant Polyomavirus in wild elephants and the creation of a vector expressing its viral tumor antigens to transform elephant primary cells." PLoS ONE **16**(2 February).

Wild elephant populations are declining rapidly due to rampant killing for ivory and body parts, range fragmentation, and human-elephant conflict. Wild and captive elephants are further impacted by viruses, including highly pathogenic elephant endotheliotropic herpesviruses. Moreover, while the rich genetic diversity of the ancient elephant lineage is disappearing, elephants, with their low incidence of cancer, have emerged as a surprising resource in human cancer research for understanding the intrinsic cellular response to DNA damage. However, studies on cellular resistance to transformation and herpesvirus reproduction have been severely limited, in part due to the lack of established elephant cell lines to enable in vitro experiments. This report

describes creation of a recombinant plasmid, pAelPyV-1-Tag, derived from a wild isolate of African Elephant Polyomavirus (AelPyV-1), that can be used to create immortalized lines of elephant cells. This isolate was extracted from a trunk nodule biopsy isolated from a wild African elephant, *Loxodonta africana*, in Botswana. The AelPyV-1 genome contains open-reading frames encoding the canonical large (LTag) and small (STag) tumor antigens. We cloned the entire early region spanning the LTag and overlapping STag genes from this isolate into a high-copy vector to construct a recombinant plasmid, pAelPyV-1-Tag, which effectively transformed primary elephant endothelial cells. We expect that the potential of this reagent to transform elephant primary cells will, at a minimum, facilitate study of elephant-specific herpesviruses. © 2021 Pearson et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Perrin, K. L., A. T. Kristensen, M. F. Bertelsen and D. Denk (2021). "Retrospective review of 27 European cases of fatal elephant endotheliotropic herpesvirus-haemorrhagic disease reveals evidence of disseminated intravascular coagulation." *Sci Rep* **11**(1): 14173.

Elephant endotheliotropic herpesvirus haemorrhagic disease (EEHV-HD) is widely acknowledged as the most common cause of mortality in young Asian elephants (*Elephas maximus*) in captivity. The objective of the current study was to perform a blinded, retrospective pathology review of European EEHV-HD fatalities, constituting the largest systematic assessment of EEHV-HD pathology to date. Findings between viral genotypes were compared with the aim to investigate if disseminated intravascular coagulation (DIC) could be substantiated as a significant complicating factor, thereby increasing the understanding of disease pathophysiology. Immunohistochemical staining confirmed endothelial cell (EC) damage and the presence of EC intranuclear inclusion bodies, demonstrating a direct viral cytopathic effect. Microthrombi were observed in 63% of cases in several organs, including lungs, which, together with widespread haemorrhage and thrombocytopenia reported in EEHV-HD case reports, supports the presence of overt DIC as a serious haemostatic complication of active EEHV infection. Death was attributed to widespread vascular damage with multi-organ dysfunction, including severe acute myocardial haemorrhage and subsequent cardiac failure. Systemic inflammation observed in the absence of bacterial infection may be caused by cytokine release syndrome. Findings reinforce the necessity to investigate cytokine responses and haemostatic status during symptomatic and asymptomatic EEHV viraemia, to potentially support the use of anti-inflammatory treatment in conjunction with anti-viral therapy and cardiovascular support.

Perrin, K. L., S. S. Nielsen, T. Martinussen and M. F. Bertelsen (2021). "Quantification and risk factor analysis of elephant endotheliotropic herpesvirus-haemorrhagic disease fatalities in Asian elephants *Elephas maximus* in Europe (1985-2017)." *Journal of Zoo and Aquarium Research* **9**(1): 8-13.



Pokharel, S. S., H. Yoneda, M. Yanagi, R. Sukumar and K. Kinoshita (2021). "The tail-tale of stress: an exploratory analysis of cortisol levels in the tail-hair of captive Asian elephants." *PeerJ* **9**: e10445.

**BACKGROUND:** Assessment of physiological states by measuring biomarkers, such as cortisol, has significantly contributed to the monitoring of health, welfare and management of animals. Immunoreactive cortisol in hair (hC) has been used widely for deciphering 'stressful' past-events in various wild and captive animals. However, no such studies have been done in long-lived mammals. **METHODS:** In this first exploratory study in elephants, we assessed (i) tail-hair growth rate (TGR) and (ii) hC levels in tail-hair samples from six captive Asian elephants from two zoos in Japan for comparing hC levels with zoo-keepers' records of distinct biological events over a c.0.5-2.0-year period. Tail-hair samples were cut into segments (based on monthly growth rate), pulverized or minced and a validated cortisol enzyme-immunoassay employed to measure hC levels. **RESULTS:** When the hC levels of all individuals were compared with the keepers' records, a posteriori, most of the high hC levels were found to be associated with 'stressful' or distinct behavioural events such as pathological (anaemia, colic infection, skin infection, oral sores), psychosocial (reluctance in entering the enclosure, presence of a calf) and husbandry practice-related (contact trials/ space sharing) conditions, indicating that tail-hair indeed can be a potential 'retrospective' calendar of physiological health of an animal. **CONCLUSIONS:** Our observations open up the possibility of using the tail-hair as an alternative matrix to reconstruct the physiological history of elephants.

Prado, N. A., J. L. Brown, J. A. Zoller, A. Haghani, M. Yao, L. R. Bagryanova, M. G. Campana, E. M. J, K. Raj, D. Schmitt, T. R. Robeck and S. Horvath (2021).

"Epigenetic clock and methylation studies in elephants." *Aging Cell* **20**(7): e13414.

Age-associated DNA-methylation profiles have been used successfully to develop highly accurate biomarkers of age ("epigenetic clocks") in humans, mice, dogs, and other species. Here we present epigenetic clocks for African and Asian elephants. These clocks were developed using novel DNA methylation profiles of 140 elephant blood samples of known age, at loci that are highly conserved between mammalian species, using a custom Infinium array (HorvathMammalMethylChip40). We present epigenetic clocks for Asian elephants (*Elephas maximus*), African elephants (*Loxodonta africana*), and both elephant species combined. Two additional human-elephant clocks were constructed by combining human and elephant samples. Epigenome-wide association studies identified elephant age-related CpGs and their proximal genes. The products of these genes play important roles in cellular differentiation, organismal development, metabolism, and circadian rhythms. Intracellular events observed to change with age included the methylation of bivalent chromatin domains, and targets of polycomb repressive complexes. These readily available epigenetic clocks can be used for elephant conservation efforts where accurate estimates of age are needed to predict demographic trends.

Prompiram, P., W. Wiriyarat, B. Bhusri, W. Paungpin, W. Jairak, S. Sripiboon and T. Wongtawan (2021). "The occurrence of elephant endotheliotropic herpesvirus infection in wild and captive Asian elephants in Thailand: Investigation based on viral DNA and host antibody." *Vet World* **14**(2): 545-550.

**BACKGROUND AND AIM:** Elephant endotheliotropic herpesvirus (EEHV) is a serious disease, threatening the life of young elephants. Many elephants have been infected with no clinical signs and may serve as carriers spreading this disease. It is important to monitor the disease through clinical signs and molecular diagnosis. In this study we investigated the occurrence of EEHV and the efficiency of different techniques used to monitor EEHV infection in various samples and populations of Asian elephants. **MATERIALS AND METHODS:** Blood and trunk swabs were collected from live elephants, while visceral organs (lung, digestive tract, spleen, lymph nodes, and kidney) were collected from dead elephants. EEHV was detected by polymerase chain reaction (PCR) in whole blood, trunk swabs, and visceral organs as samples, while elephant anti-EEHV immunoglobulin G (IgG) in serum was detected by enzyme-linked immunosorbent assay (ELISA). A total of 162 samples were analyzed in this study: 129 from healthy, 26 from dead, and 7 from sick elephants. **RESULTS:** The present study showed that the overall incidence of EEHV was 40.1% (n=65/162). Approximately 46.2% (n=12/26) and 85.7% (n=6/7) of dead and sick elephants were positive for EEHV by PCR, respectively. All sick elephants that were young and affected by EEHV clinical disease tested negative for the IgG antibody ELISA, suggesting primary EEHV infection in this group. In addition, 2.3% (n=3/129) of subclinical infections were detected using PCR, and trunk swab samples showed slightly higher sensitivity (5.3%, n=2/38) to detect EEHV than whole blood (1.2%, n=1/84). As many as, 48.4% (n=44/91) of healthy elephants were EEHV seropositive (ELISA-positive), suggesting that many elephants in Thailand had previously been infected. Overall, 30% of dead wild elephants had been infected with EEHV (n=3/10). Moreover, statistical analysis revealed no significant differences in the EEHV detection rate between different age groups or sexes (p>0.05). **CONCLUSION:** PCR is better than ELISA to detect EEHV active infection in dead/sick elephants and to monitor EEHV in young elephants. ELISA is suitable for detecting previous EEHV infection and carriers, particularly adults. Theoretically, we could use both PCR and ELISA to increase the sensitivity of testing, along with observing abnormal behavior to efficiently monitor this disease. Identification of EEHV carriers within elephant populations is important to prevent transmission to healthy individuals, especially young elephants with high mortality from EEHV. This is the first report from Thailand regarding EEHV infection in wild elephants, showing the importance of preventing disease transmission between captive and wild elephants.

Pursell, T., J. L. Spencer Clinton, J. Tan, R. Peng, X. Qin, H. Doddapaneni, V. Menon, Z. Momin, K. Kottapalli, L. Howard, E. Latimer, S. Heaggans, G. S. Hayward and P. D. Ling (2021). "Primary Infection May Be an Underlying Factor Contributing to Lethal Hemorrhagic Disease Caused by Elephant Endotheliotropic Herpesvirus 3 in African Elephants (*Loxodonta africana*)." *Microbiol Spectr* **9**(2): e0098321.

Distinct but related species of elephant endotheliotropic herpesviruses (EEHVs) circulate within Asian and African elephant populations. Primary infection with EEHVs endemic among Asian elephants can cause clinical illness and lethal EEHV hemorrhagic disease (EEHV-HD). The degree to which this occurs among African elephants has not been fully established. Recent cases of EEHV-HD caused by the EEHV3 species in African elephants housed in North American zoos has heightened concern about the susceptibility of this elephant species to EEHV-HD. In this study, we utilize the luciferase immunoprecipitation system (LIPS) to generate a serological assay specific for EEHV3 in African elephants by detecting antibodies against the EEHV3 E34 protein. The results showed that the majority of tested elephants from four separate and genetically unrelated herds, including five elephants that survived clinical illness associated with EEHV3, were positive for prior infection with EEHV3. However, African elephants who succumbed to EEHV3-HD were seronegative for EEHV3 prior to lethal infection. This supports the hypothesis that fatal EEHV-HD caused by EEHV3 is associated with primary infection rather than reactivation of latent virus. Lastly, we observed that African elephants, like Asian elephants, acquire abundant anti-EEHV antibodies prenatally and that anti-EEHV3 specific antibodies were either never detected or declined to undetectable levels in those animals that died from lethal disease following EEHV3 infection. **IMPORTANCE** Prior to 2019, only five cases of clinical disease from EEHV infection among African elephants had been documented. Since 2019, there have been at least seven EEHV-HD cases in North American zoos, resulting in three fatalities, all associated with EEHV3. Evidence is accumulating to suggest that EEHV-associated clinical illness and death among Asian elephants is due to primary infection and may be associated with waning anti-EEHV antibody levels in young elephants. The development of the EEHV3 serological test described in this study enabled us to confirm that similar dynamics may be contributing to EEHV-HD in African elephants. The ability to screen for EEHV immune status in African elephant calves will have a major impact on managing captive African elephant herds and will provide new tools for investigating and understanding EEHV in wild populations.

Rajhans, U., G. Wankhede, B. Ambore, S. Chaudhari, N. Nighot, V. Dhaygude and C. Sonekar (2021). "Sero-diagnosis of Tuberculosis in Elephants in Maharashtra, India." *Journal of Threatened Taxa* **13**(7): 18713-18718.

Tuberculosis is a highly contagious zoonotic disease caused by *Mycobacterium* spp. A study was conducted to detect the presence of *Mycobacterium* in captive elephants. A total of 15 captive elephants were screened from various regions in Maharashtra. The blood and serum samples collected were subjected to rapid test kit, BacT/ALERT 3D system, Ziehl-Neelsen (ZN) staining and PCR. All the samples were found seronegative using rapid test kit and whole blood PCR. Whereas, all samples were signalled culture positive in BacT/ALERT 3D system which were further subjected to PCR, only one amplicon was produced of 176bp of RD4 gene (*Mycobacterium bovis*) and no acid-fast organism was detected upon ZN. Due to the atypical nature of this organism, diagnosis of this disease in

elephants using various tests is complicated unlike the diagnostic tests that are validated in domestic animals. Therefore, many tests have sub-optimal sensitivity and specificity in elephants. As TB is a zoonotic disease, transmission can occur between human-livestock-elephants interface. Therefore, the zoos and state forest authority should inculcate a protocol of periodic TB screening for Mahouts and elephants in captivity along with protocol of elephant-visitor interaction, thus helping in conservation of this endangered species in India. © Rajhans et al. 2021. Creative Commons Attribution 4.0 International License. JoTT allows unrestricted use, reproduction, and distribution of this article in any medium by providing adequate credit to the author(s) and the source of publication.

Ram, A. K., S. Mondol, N. Subedi, B. R. Lamichhane, H. S. Baral, L. Natarajan, R. Amin and B. Pandav (2021). "Patterns and determinants of elephant attacks on humans in Nepal." *Ecol Evol* **11**(17): 11639-11650.

Attacks on humans by Asian elephant (*Elephas maximus*) is an extreme form of human–elephant conflict. It is a serious issue in southern lowland Nepal where elephant-related human fatalities are higher than other wildlife. Detailed understanding of elephant attacks on humans in Nepal is still lacking, hindering to devising appropriate strategies for human–elephant conflict mitigation. This study documented spatiotemporal pattern of elephant attacks on humans, factors associated with the attacks, and human/elephant behavior contributing to deaths of victims when attacked. We compiled all the documented incidences of elephant attacks on humans in Nepal for last 20 years across Terai and Chure region of Nepal. We also visited and interviewed 412 victim families (274 fatalities and 138 injuries) on elephant attacks. Majority of the victims were males (87.86%) and had low level of education. One fourth of the elephant attacks occurred while chasing the elephants. Solitary bulls or group of subadult males were involved in most of the attack. We found higher number of attacks outside the protected area. People who were drunk and chasing elephants using firecrackers were more vulnerable to the fatalities. In contrast, chasing elephants using fire was negatively associated with the fatalities. Elephant attacks were concentrated in proximity of forests primarily affecting the socioeconomically marginalized communities. Integrated settlement, safe housing for marginalized community, and community grain house in the settlement should be promoted to reduce the confrontation between elephants and humans in entire landscape for their long-term survival. © 2021 The Authors. Ecology and Evolution published by John Wiley & Sons Ltd.

Ram, A. K., N. K. Yadav, P. N. Kandel, S. Mondol, B. Pandav, L. Natarajan, N. Subedi, D. Naha, C. S. Reddy and B. R. Lamichhane (2021). "Tracking forest loss and fragmentation between 1930 and 2020 in Asian elephant (*Elephas maximus*) range in Nepal." *Sci Rep* **11**(1): 19514.

Forest cover is the primary determinant of elephant distribution, thus, understanding forest loss and fragmentation is crucial for elephant conservation. We assessed deforestation and patterns of forest

fragmentation between 1930 and 2020 in Chure Terai Madhesh Landscape (CTML) which covers the entire elephant range in Nepal. Forest cover maps and fragmentation matrices were generated using multi-source data (Topographic maps and Landsat satellite images of 1930, 1975, 2000, and 2020) and spatiotemporal change was quantified. At present, 19,069 km<sup>2</sup> forest cover in CTML is available as the elephant habitat in Nepal. Overall, 21.5% of elephant habitat was lost between 1930 and 2020, with a larger (12.3%) forest cover loss between 1930 and 1975. Area of the large forests (Core 3) has decreased by 43.08% whereas smaller patches (Core 2, Core 1, edge and patch forests) has increased multifold between 1930 and 2020. The continued habitat loss and fragmentation probably fragmented elephant populations during the last century and made them insular with long-term ramifications for elephant conservation and human-elephant conflict. Given the substantial loss in forest cover and high levels of fragmentation, improving the resilience of elephant populations in Nepal would urgently require habitat and corridor restoration to enable the movement of elephants.

Reinwald, M., B. Moseley, A. Szenicer, T. Nissen-Meyer, S. Oduor, F. Vollrath, A. Markham and B. Mortimer (2021). "Seismic localization of elephant rumbles as a monitoring approach." *J R Soc Interface* **18**(180): 20210264.

African elephants (*Loxodonta africana*) are sentient and intelligent animals that use a variety of vocalizations to greet, warn or communicate with each other. Their low-frequency rumbles propagate through the air as well as through the ground and the physical properties of both media cause differences in frequency filtering and propagation distances of the respective wave. However, it is not well understood how each mode contributes to the animals' abilities to detect these rumbles and extract behavioural or spatial information. In this study, we recorded seismic and co-generated acoustic rumbles in Kenya and compared their potential use to localize the vocalizing animal using the same multi-lateration algorithms. For our experimental set-up, seismic localization has higher accuracy than acoustic, and bimodal localization does not improve results. We conclude that seismic rumbles can be used to remotely monitor and even decipher elephant social interactions, presenting us with a tool for far-reaching, non-intrusive and surprisingly informative wildlife monitoring.

Romey, A., B. Lamglait, Y. Blanchard, F. Touzain, H. Quenault, A. Relmy, S. Zientara, S. Blaise-Boisseau and L. Bakkali-Kassimi (2021). "Molecular characterization of encephalomyocarditis virus strains isolated from an African elephant and rats in a French zoo." *J Vet Diagn Invest* **33**(2): 313-321.

In November 2013, a fatal encephalomyocarditis virus (EMCV) case in a captive African elephant (*Loxodonta africana*) occurred at the Réserve Africaine de Sigean, a zoo in the south of France. Here we report the molecular characterization of the EMCV strains isolated from samples collected from the dead elephant and from 3 rats (*Rattus rattus*) captured in the zoo at the same time. The EMCV infection was confirmed by reverse-transcription real-time PCR (RT-rtPCR) and genome sequencing. Complete

genome sequencing and sequence alignment indicated that the elephant's EMCV strain was 98.1-99.9% identical to the rat EMCV isolates at the nucleotide sequence level. Phylogenetic analysis of the ORF, P1, VP1, and 3D sequences revealed that the elephant and rat strains clustered into lineage A of the EMCV 1 group. To our knowledge, molecular characterization of EMCV in France and Europe has not been reported previously in a captive elephant. The full genome analyses of EMCV isolated from an elephant and rats in the same outbreak emphasizes the role of rodents in EMCV introduction and circulation in zoos.

Rutherford, L. and L. E. Murray (2021). "Personality and behavioral changes in Asian elephants (*Elephas maximus*) following the death of herd members." Integr Zool **16**(2): 170-188.

Elephants are highly social beings with complex individual personalities. We know that elephants have a general interest in death, investigating carcasses, not just limited to kin; however, research does not explore in depth whether individuals change their behavior or personality following traumatic events, such as the death of a conspecific. Within a captive herd of Asian elephants (*Elephas maximus*) housed at Chester Zoo, UK, we measured social behavior and proximity and personality using the Ten-Item Personality Inventory, and found age-related and relationship-related changes in both behavior and personality following the deaths of herd members. Overall, the herd spent less time socializing and engaging in affiliative behaviors following the death of the adult female when compared to baseline data, yet spent more time engaging in these behaviors after the death of two calves. The death of the central female had a dramatic impact on her infant calf, resulting in increasingly withdrawn behavior, yet had the opposite effect on her adult daughter, who subsequently established a more integrated role within the herd. Emotional Stability fell in the motherless calf but rose in an adult female, who had lost her adult daughter, but had a new calf to care for. We suggest that the greater impact on the behavior and personality of surviving herd members following the deaths of calves, compared to an adult member, attests to the significance of the unifying role played by calves within an elephant herd.

Ryding, S., M. Klaassen, G. J. Tattersall, J. L. Gardner and M. R. E. Symonds (2021). "Shape-shifting: changing animal morphologies as a response to climatic warming." Trends in Ecology and Evolution **36**(11): 1036-1048.

Many animal appendages, such as avian beaks and mammalian ears, can be used to dissipate excess body heat. Allen's rule, wherein animals in warmer climates have larger appendages to facilitate more efficient heat exchange, reflects this. We find that there is widespread evidence of 'shape-shifting' (changes in appendage size) in endotherms in response to climate change and its associated climatic warming. We re-examine studies of morphological change over time within a thermoregulatory context, finding evidence that temperature can be a strong predictor of morphological change independently of, or combined with, other environmental changes. Last, we discuss how Allen's rule, the degree of temperature change, and other

ecological factors facilitate morphological change and make predictions about what animals will show shape-shifting. © 2021 Elsevier Ltd

Sahoo, N., S. K. Sahu, A. K. Das, D. Mohapatra, S. K. Panda, S. K. Gupta, B. K. Behera, A. Pahari and M. Dash (2021). "ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS HEMORRHAGIC DISEASE OUTBREAK IN AN INDIAN ZOO." J Zoo Wildl Med **52**(4): 1286-1297.

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV HD) is an acute viral infection of growing Asian elephants (*Elephas maximus*). Four apparently healthy subadult Asian elephants aged between 6 and 10 yr at Nandankanan Zoological Park (NKZP), India, died of EEHV HD during August-September 2019. All four elephants were rescued from different reserved forests of Odisha state at less than 1 yr of age and hand reared in the NKZP. Elephants exhibited the clinical signs of lethargy, head swelling, fever, loss of appetite, abdominal distension, scant urination and defecation, signs of colic, lameness, trunk discharge, cyanosis/ulceration of tongue, erratic behavior, and recumbence before death. Period of illness varied between 28 and 42 h. Thrombocytopenia was the common significant hematological observation. No significant biochemical alterations were recorded except for higher creatinine concentrations. Analysis of blood samples in RT-PCR assay using two different sets of primers and probes that targeted terminase gene and major DNA-binding protein gene followed by cPCR and sequencing was positive for EEHV-1A in all four animals. Postmortem examination of all four carcasses showed hemorrhages in internal organs, including the hard palate, heart, lungs, stomach, mesenteric lymph nodes, mesentery, colon serosa, spleen, liver, kidney, and meninges. Histopathology showed congestion and/or hemorrhages in heart, lung, brain, kidney, and liver. There was presence of intranuclear inclusion bodies in the sinusoidal epithelial cells. The outbreak of EEHV HD that resulted in the acute death of four juvenile captive Asian elephants within <30 d, the first of its kind documented in India, is increasing the fear of similar outbreaks in the future.

Sampson, C., J. A. Glikman, S. L. Rodriguez, D. Tonkyn, P. Soe, D. O'Connor, A. M. Chit and P. Leimgruber (2021). "Rural and urban views on elephants, conservation and poaching." Oryx.

Successful anti-poaching policies and effective conservation of Asian elephants *Elephas maximus* require input and support from all stakeholders, including the public. But although Myanmar has one of the largest remaining populations of wild Asian elephants, there has been little research on public attitudes there towards elephants and poaching. We developed a questionnaire to assess attitudes of people in rural and urban areas towards elephants and conservation, and their perceptions of and experience with elephant poaching. We conducted 178 interviews across two regions in Myanmar. Although both rural and urban participants supported elephant conservation, people from urban areas expressed more favourable attitudes towards elephants than their rural counterparts. Similarly, conservation priorities differed between rural and urban communities, with rural communities less likely to believe that peaceful human-elephant coexistence

was possible and preferring conservation initiatives that prioritize human activities over elephant conservation. Both groups were familiar with elephant poaching in Myanmar, but rural communities appeared to be better informed regarding the challenges faced by conservation agencies, and were more negatively affected by poaching. Our findings highlight potential areas for intervention by government and conservation agencies to reduce criminal activity and to protect both Myanmar's citizens and its elephants. © The Author(s), 2021. Published by Cambridge University Press on behalf of Fauna & Flora International.

Sampson, C., S. L. Rodriguez, P. Leimgruber, Q. Huang and D. Tonkyn (2021). "A quantitative assessment of the indirect impacts of human-elephant conflict." PLoS ONE **16**(7 July).

Human-wildlife conflict has direct and indirect consequences for human communities. Understanding how both types of conflict affect communities is crucial to developing comprehensive and sustainable mitigation strategies. We conducted an interview survey of 381 participants in two rural areas in Myanmar where communities were exposed to human-elephant conflict (HEC). In addition to documenting and quantifying the types of direct and indirect impacts experienced by participants, we evaluated how HEC influences people's attitudes towards elephant conservation. We found that 99% of participants suffered from some type of indirect impact from HEC, including fear for personal and family safety from elephants and fear that elephants will destroy their home. Despite experiencing moderate levels of indirect impacts from HEC at the community level, participants expressed attitudes consistent with supporting future elephant conservation programs. © This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Scharling, F. S., M. F. Bertelsen, E. Sós and A. M. Bojesen (2021). "PREVALENCE OF SALMONELLA SPECIES, CLOSTRIDIUM PERFRINGENS, AND CLOSTRIDIUM DIFFICILE IN THE FECES OF HEALTHY ELEPHANTS (LOXODONTA SPECIES AND ELEPHAS MAXIMUS) IN EUROPE." J Zoo Wildl Med **51**(4): 752-760.

Pathogenic *Salmonella* spp., *Clostridium perfringens*, and *Clostridium difficile* have been reported to infect and cause severe enteritis and enterotoxemia in African (*Loxodonta* spp.) and Asian elephants (*Elephas maximus*). However, little information exists on whether healthy elephants carry and possibly shed these gastrointestinal organisms. This study was conducted to investigate the prevalence of all three bacteria in feces from healthy elephants in European zoos. Bacterial identification was performed by selective culture on fecal samples and a polymerase chain reaction (PCR) amplification protocol, on the basis of primers targeting the *hilA* gene (*Salmonella* spp.), the *cpa* gene (*C. perfringens*), and the *tpi* gene (*C. difficile*) from deoxyribonucleic acid extracted from elephant feces. The PCR protocol was validated prior to initiation of the investigation. Fecal samples collected from 50 African and 86 Asian elephants originating from 30 European zoologic institutions were



investigated. The PCR validation revealed detection limits ranging from 10(4) to 10(6) colony-forming units per gram of feces of each gene. Only *C. perfringens* (one type A and two type E) was detected in the initial sampling (2.2%, three Asian elephants), whereas no *Salmonella* spp. or *C. difficile* was detected. At a follow-up sampling from *C. perfringens*-positive animals and relatives, 2 mo after the initial sampling, three animals were culture positive for *Salmonella enterica* spp. *enterica*. All positive samples were obtained with bacterial culture, whereas no PCR reactions were positive. Despite carrying these pathogens, all culture-positive animals were clinically healthy and did not develop signs of gastrointestinal disease during the study period. The findings indicate that prevalence of *Salmonella* spp., *C. perfringens*, and *C. difficile* in feces from healthy Asian and African elephants in Europe is very low.

Schulte, B. A. and C. A. LaDue (2021). "The Chemical Ecology of Elephants: 21st Century Additions to Our Understanding and Future Outlooks." *Animals (Basel)* **11**(10).

Chemical signals are the oldest and most ubiquitous means of mediating intra- and interspecific interactions. The three extant species of elephants, the Asian elephant and the two African species, savanna and forest share sociobiological patterns in which chemical signals play a vital role. Elephants emit secretions and excretions and display behaviors that reveal the importance of odors in their interactions. In this review, we begin with a brief introduction of research in elephant chemical ecology leading up to the 21st century, and then we summarize the body of work that has built upon it and occurred in the last c. 20 years. The 21st century has expanded our understanding on elephant chemical ecology, revealing their use of odors to detect potential threats and make dietary choices. Furthermore, complementary in situ and ex situ studies have allowed the careful observations of captive elephants to be extended to fieldwork involving their wild counterparts. While important advances have been made in the 21st century, further work should investigate the roles of chemical signaling in elephants and how these signals interact with other sensory modalities. All three elephant species are threatened with extinction, and we suggest that chemical ecology can be applied for targeted conservation efforts.

Schulz, A. K., J. Ning Wu, S. Y. S. Ha, G. Kim, S. Braccini Slade, S. Rivera, J. S. Reidenberg and D. L. Hu (2021). "Suction feeding by elephants." *J R Soc Interface* **18**(179): 20210215.

Despite having a trunk that weighs over 100 kg, elephants mainly feed on lightweight vegetation. How do elephants manipulate such small items? In this experimental and theoretical investigation, we filmed elephants at Zoo Atlanta showing that they can use suction to grab food, performing a behaviour that was previously thought to be restricted to fishes. We use a mathematical model to show that an elephant's nostril size and lung capacity enables them to grab items using comparable pressures as the human lung. Ultrasonographic imaging of the elephant sucking viscous fluids show that the elephant's nostrils dilate up to  $\left[ \frac{1}{2} \sqrt{\frac{2P}{\rho g}} \right]$  in radius, which

increases the nasal volume by [Formula: see text]. Based on the pressures applied, we estimate that the elephants can inhale at speeds of over 150 m s<sup>-1</sup>), nearly 30 times the speed of a human sneeze. These high air speeds enable the elephant to vacuum up piles of rutabaga cubes as well as fragile tortilla chips. We hope these findings inspire further work in suction-based manipulation in both animals and robots.

Seewald, M., C. Gohl, M. Egerbacher, S. Handschuh and K. Witter (2021). "Endodontic Treatment of a Traumatic Tusk Fracture With Exposed Pulp in an Asian Elephant (*Elephas maximus*)." Journal of Veterinary Dentistry **38**(3): 139-151.

Tusk fracture in elephants is a common incident often resulting in pulp exposure and pulpitis. Extensive lavage, endodontic therapy, direct pulp capping, or extraction are treatment options. In this report, the successful management of a broken tusk of a juvenile male Asian elephant (*Elephas maximus*) including morphological analysis of the tusk tip 2 years after surgery are presented. Treatment was carried out under barn conditions and included antimicrobial photodynamic therapy and partial pulpotomy with direct pulp capping. Immediate pain relief was reached. The fractured tusk was preserved and continued to grow. The therapeutic filling material remained intact for over 1 year but was absent 2 years after treatment. The former pulp cavity of the tusk tip was filled with reparative dentin, osteodentin, and bone, but the seal between these hard tissues and pulp chamber dentin was incomplete. Radiographs obtained 3 years after treatment showed no differences in pulp shape, pulp width, and secondary dentin formation between the treated right and the healthy left tusk. It can be concluded that in case of an emergency, the endodontic therapy of a broken elephant tusk can be attempted under improvised conditions with adequate success. Photodynamic therapy might contribute to prevent infection and inflammation of the pulp. The decision tree published by Steenkamp (2019) provides a valuable tool to make quick decisions regarding a suitable therapy of broken tusks. © The Author(s) 2021.

Shah, Y. and S. Paudel (2021). "Protect elephants from tuberculosis." Science **374**(6569): 832-833.

Siegal-Willott, J. L., P. Anikis, D. L. Neiffer, T. Barthel and L. R. Goodrich (2021). "Use of intracarpal interleukin receptor antagonist protein (IRAP) and hyaluronic acid in a multimodal therapeutic regime for osteoarthritis in an asian elephant (*elephas maximus*)." Journal of Zoo and Wildlife Medicine **52**(1): 401-405.

An approximately 41-yr-old female Asian elephant (*Elephas maximus*) experiencing forelimb stiffness and decreased range of motion was diagnosed with bilateral carpal osteoarthritis (OA). Standing sedation combined with local anesthesia was used to deliver ultrasound-guided carpal articular injections of an autologous conditioned serum product, interleukin receptor antagonist protein, combined with hyaluronic acid. Within 2 mo of completing therapy, improved range and speed of motion were evident. Reduced inflammation was suggested by decreased carpal articular prostaglandin E2 levels. Subjectively improved clinical signs lasted approximately 5-6 mo, at

which point carpal articular injections were repeated. Joint inflammatory markers were useful in gauging response to treatment and may provide guidance in the diagnostic and therapeutic approach to elephant OA. On the basis of the positive response noted, interarticular autologous therapy combined with hyaluronic acid should be considered for carpal OA in elephants. © Copyright 2021 by American Association of Zoo Veterinarians.

Siegel, J. M. (2021). "Memory Consolidation Is Similar in Waking and Sleep." Curr Sleep Med Rep **7**(1): 15-18.

PURPOSE OF REVIEW: I review the current status of the hypothesis that sleep is critically involved in memory consolidation and conclude that there are major methodological problems with the studies used to support this hypothesis. RECENT FINDINGS: Memory consolidation is similar in quiet waking and sleep (Humiston GB, Tucker MA, Summer T, Wamsley EJ. *Sci Rep* 18;9(1):19345, 2019), and suppression of REM sleep for long periods is compatible with learning and highly adaptive behavior (Lyamin OI, Korneva SM, Obukhova ED, Mukhametov LM, Siegel JM. *Dokl Biol Sci* 463:211-4, 2015; Lyamin OI, Kosenko PO, Korneva SM, Vyssotski AL, Mukhametov LM, Siegel JM. *Current Biology* 28(12):2000-5, 2018); despite their considerable abilities to navigate and remember, African elephants have very small amount of sleep, and learning interference effects have not been adequately controlled for in studies purporting to show sleep-dependent memory consolidation (Susic-Vasic Z, Hille K, Kroner J, Spitzer M, Kornmeier J. *Frontiers in psychology* 9:82, 2018; Yonelinas AP, Ranganath C, Ekstrom AD, Wiltgen BJ. *Nat Rev Neurosci* 20(6):364-75, 2019). SUMMARY: Memory consolidation clearly occurs in both sleep and waking. Whether, and the extent to which, consolidation might differ in these two states has not been conclusively determined.

Singh, P. K., S. M. Ali, M. Sethi and D. B. Manohar (2021). "Injuries in survivors of elephant attack - Report of three cases." Chin J Traumatol.

Human-elephant conflict (HEC) in India is becoming a growing health problem causing many fatalities every year. Elephants produce injuries by trampling, stomping, squeezing, tossing in the air, or crushing/targeting the head and chest commonly. The adult elephants are most aggressive in their mating season, leading to maximum incidences of HECs in this period. These attacks are mostly unprovoked, though most HECs are provoked. In this case series, the authors described the injuries sustained by three survivors in a short span of one month due to the sudden and unprovoked elephant attack. All the injuries were mild to moderate in severity and involved the chest in common. Timely rescue and prompt initiation of treatment were pivotal in their survival. The authors also want to create awareness about the mating season of elephants to minimize these unfortunate events in the future.

Snyder, R. J., L. P. Barrett, R. A. Emory and B. M. Perdue (2021). "Performance of Asian elephants (*Elephas maximus*) on a quantity discrimination task is similar to that of African savanna elephants (*Loxodonta africana*)." Anim Cogn **24**(5): 1121-1131.

Using an object-choice task, we measured the relative quantity discrimination ability of Asian elephants. Two zoo-housed elephants were given auditory cues of food being dropped into two containers (Nonvisible condition), and in one condition they could also see the food on top of the containers (Visible condition). Elephants received sets of varying ratios and magnitudes. We found that the elephants chose the greater quantity of food significantly above chance in both the Visible and Nonvisible conditions. Additionally, we found the elephants' ability to discriminate between quantities decreased as the ratio, and not the absolute difference, between the quantities increased, which is predicted by the accumulator model. We also compare our findings to those from a study using the same methods with African savanna elephants and found that the two species performed at similar levels, but given our small sample size it is difficult to make strong species-level conclusions. In discussing our results, we consider differences between the two species' wild environments as well as the types of sensory cues provided in human care, and we provide recommendations for extensions of this work.

Steyrer, C., M. Miller, J. Hewlett, P. Buss and E. H. Hooijberg (2021). "Reference Intervals for Hematology and Clinical Chemistry for the African Elephant (*Loxodonta africana*)." *Front Vet Sci* **8**: 599387.

The African elephant (*Loxodonta africana*) is listed as vulnerable, with wild populations threatened by habitat loss and poaching. Clinical pathology is used to detect and monitor disease and injury, however existing reference interval (RI) studies for this species have been performed with outdated analytical methods, small sample sizes or using only managed animals. The aim of this study was to generate hematology and clinical chemistry RIs, using samples from the free-ranging elephant population in the Kruger National Park, South Africa. Hematology RIs were derived from EDTA whole blood samples automatically analyzed (n = 23); manual PCV measured from 48 samples; and differential cell count results (n = 51) were included. Clinical chemistry RIs were generated from the results of automated analyzers on stored serum samples (n = 50). Reference intervals were generated according to American Society for Veterinary Clinical Pathology guidelines with a strict exclusion of outliers. Hematology RIs were: PCV 34-49%, RBC  $2.80-3.96 \times 10^{12}/L$ , HGB 116-163 g/L, MCV 112-134 fL, MCH 35.5-45.2 pg, MCHC 314-364 g/L, PLT  $182-386 \times 10^9/L$ , WBC  $7.5-15.2 \times 10^9/L$ , segmented heterophils  $1.5-4.0 \times 10^9/L$ , band heterophils  $0.0-0.2 \times 10^9/L$ , total monocytes  $3.6-7.6 \times 10^9/L$  (means for "regular" were 35.2%, bilobed 8.6%, round 3.9% of total leukocytes), lymphocytes  $1.1-5.5 \times 10^9/L$ , eosinophils  $0.0-0.9 \times 10^9/L$ , basophils  $0.0-0.1 \times 10^9/L$ . Clinical chemistry RIs were: albumin 41-55 g/L, ALP 30-122 U/L, AST 9-34 U/L, calcium 2.56-3.02 mmol/L, CK 85-322 U/L, GGT 7-16 U/L, globulin 30-59 g/L, magnesium 1.15-1.70 mmol/L, phosphorus 1.28-2.31 mmol/L, total protein 77-109 g/L, urea 1.2-4.6 mmol/L. Reference intervals were narrower than those reported in other studies. These RI will be helpful in the future management of injured or diseased elephants in national parks and zoological settings.

Stoeger, A. S., A. Baotic and G. Heilmann (2021). "Vocal Creativity in Elephant Sound Production." Biology (Basel) **10**(8).

How do elephants achieve their enormous vocal flexibility when communicating, imitating or creating idiosyncratic sounds? The mechanisms that underpin this trait combine motoric abilities with vocal learning processes. We demonstrate the unusual production techniques used by five African savanna elephants to create idiosyncratic sounds, which they learn to produce on cue by positive reinforcement training. The elephants generate these sounds by applying nasal tissue vibration via an ingressive airflow at the trunk tip, or by contracting defined superficial muscles at the trunk base. While the production mechanisms of the individuals performing the same sound categories are similar, they do vary in fine-tuning, revealing that each individual has its own specific sound-producing strategy. This plasticity reflects the creative and cognitive abilities associated with 'vocal' learning processes. The fact that these sounds were reinforced and cue-stimulated suggests that social feedback and positive reinforcement can facilitate vocal creativity and vocal learning behavior in elephants. Revealing the mechanism and the capacity for vocal learning and sound creativity is fundamental to understanding the eloquence within the elephants' communication system. This also helps to understand the evolution of human language and of open-ended vocal systems, which build upon similar cognitive processes.

Stremme, C., A. Priadi, G. S. Hayward and A. Zachariah (2021). "IDENTIFICATION OF TWO LETHAL CASES OF ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS HEMORRHAGIC DISEASE IN SUMATRAN ELEPHANT CALVES IN INDONESIA." J Zoo Wildl Med **51**(4): 985-993.

As many as a dozen cases of lethal acute hemorrhagic disease (HD) in young captive-born Sumatran sub-species Asian elephant (*Elephas maximus sumatranus roman*) calves raised naturally in camps in Sumatra have been observed in recent years. To address whether these deaths, like many others documented worldwide, might be associated with acute systemic infection by elephant endotheliotropic herpesvirus (EEHV), diagnostic polymerase chain reaction (PCR) tests followed by subtype deoxyribonucleic acid (DNA) sequencing analysis were carried out on pathologic tissue samples from two lethal HD cases that occurred within 6 days of one another in calves at the same camp. Viral DNA from five selected PCR loci was found to be present at high levels in both calves and proved to be the same EEHV1A virus species that has been described most commonly previously in numerous lethal or surviving symptomatic cases in North America, Europe, India, and Thailand. Furthermore, the two cases were identical at all five PCR loci tested (covering a total of 3,050 base pairs) and were therefore likely to have been infected from the same epidemiologic source herd mate. However, the strain involved (which was subtype-D2 in the vGPCR1 locus) differed from all previously characterized EEHV1A strains. In conclusion, these two calves are the first two confirmed HD cases in Sumatra alongside several other suspected HD cases in Sumatra that have succumbed to the same devastating EEHV1A-HD that has afflicted young Asian elephants worldwide over the past 25 yr.

Because the progeny of some of the 1,500 remaining red-listed critically endangered Sumatra subspecies elephants are bred naturally in camps from wild parents it seems very likely that the EEHV1A herpesvirus identified here in these HD camp cases is also present in the free-ranging Sumatran elephant population, and this will have to be taken into account in future wildlife management policies and decisions.

Suga, S., Y. Mukai, S. Ishikawa, S. Yoshida, S. Paudel and T. Wada (2021). "Intensive treatment of a captive bornean elephant (*elephas maximus borneensis*) infected with mycobacterium caprae in Japan." Journal of Zoo and Wildlife Medicine **51**(4): 1062-1066.

In 2015, an estimated 17-year-old female Bornean elephant (*Elephas maximus borneensis*) at Fukuyama Zoo in Japan exhibited anorexia and significant weight loss. Pan-susceptible *Mycobacterium tuberculosis* complex (MTBC) was isolated from vaginal discharge, oral mucus, urine, and fecal samples by culture. The isolate was identified as *Mycobacterium caprae* by genetic analysis. Isoniazid, pyrazinamide, and levofloxacin were administered rectally. Body weight increased to normal, but subsequently decreased again. Elevation of liver enzymes occurred, likely related to the increase in isoniazid dosage. After recovery from side effects, the elephant's weight increased further. However, isoniazid-resistant *M. caprae* was isolated from oral mucus after anti-tuberculosis drug treatment for 9 mo. The regimen was changed to rifampicin, pyrazinamide, ethambutol, and levofloxacin, administered orally or rectally. The 18-mo treatment was completed in October 2018. This elephant has shown no clinical sign since. No MTBC-positive sample had been obtained as of March 2020. © Copyright 2020 by American Association of Zoo Veterinarians.

Sun, Y., Y. Chen, J. J. Díaz-Sacco and K. Shi (2021). "Assessing population structure and body condition to inform conservation strategies for a small isolated Asian elephant (*Elephas maximus*) population in southwest China." PLoS ONE **16**(3): e0248210.

The Asian elephant (*Elephas maximus*) population in Nangunhe National Nature Reserve in China represents a unique evolutionary branch that has been isolated for more than twenty years from neighboring populations in Myanmar. The scarcity of information on population structure, sex ratio, and body condition makes it difficult to develop effective conservation measures for this elephant population. Twelve individuals were identified from 3,860 valid elephant images obtained from February to June 2018 (5,942 sampling effort nights) at 52 camera sites. Three adult females, three adult males, one subadult male, two juvenile females, two juvenile males and one male calf were identified. The ratio of adult females to adult males was 1:1, and the ratio of reproductive ability was 1:0.67, indicating the scarcity of reproductive females as an important limiting factor to population growth. A population density of  $5.32 \pm 1.56$  elephants/100 km<sup>2</sup> was estimated using Spatially Explicit Capture Recapture (SECR) models. The health condition of this elephant population was assessed using an 11-point scale of Body Condition Scoring (BCS). The average BCS was 5.75 (n = 12, range 2-9),

with adult females scoring lower than adult males. This isolated population is extremely small and has an inverted pyramid age structure and therefore is at a high risk of extinction. We propose three plans to improve the survival of this population: improving the quality and quantity of food resources, removing fencing and establishing corridors between the east and wet parts of Nangunhe reserve.

Suwanchatree, N., P. Thanakiatkrai, A. Linacre and T. Kitpipit (2021).

"Discrimination of highly degraded, aged Asian and African elephant ivory using denaturing gradient gel electrophoresis (DGGE)." *Int J Legal Med* **135**(1): 107-115.

**BACKGROUND:** Elephant populations have greatly reduced mainly due to illegal poaching for their ivory. The trade in elephant products is protected by national laws and CITES agreements to prevent them from further decline. For instance, in Thailand, it is illegal to trade ivory from African elephants; however, the law allows possession of ivory from Asian elephants if permission has been obtained from the authorities. As such, means of enforcement of legislation are needed to classify the legal status of seized ivory products. Many DNA-based techniques have been previously reported for this purpose, although all have a limit of detection not suitable for extremely degraded samples. **AIM:** We report an assay based on nested PCR followed by DGGE to confirm the legal or illegal status of seized ivory samples where it is assumed that the DNA will be highly degraded. **METHOD AND RESULTS:** The assay was tested on aged ivory from which the assay was tested for reproducibility, specificity, and, importantly, sensitivity. Blind testing showed 100% identification accuracy. Correct assignment in all 304 samples tested was achieved including confirmation of the legal status of 227 highly degraded, aged ivories, thus underlining the high sensitivity of the assay. **CONCLUSION AND RECOMMENDATION:** The research output will be beneficial to analyze ivory casework samples in wildlife forensic laboratories.

Swadi, T., J. Geoghegan, T. Devine, C. McElnay, J. Sherwood, P. Shoemack, X. Ren, M. Storey, S. Jefferies, E. Smit, J. Hadfield, A. Kenny, L. Jelley, A. Sporle, A. McNeill, G. E. Reynolds, K. Mouldey, L. Lowe, G. Sonder, A. Drummond, S. Huang, D. Welch, E. Holmes, N. French, C. Simpson and J. de Ligt (2021). "Genomic Evidence of In-Flight Transmission of SARS-CoV-2 Despite Predeparture Testing." *Emerging Infectious Disease journal* **27**(3).

Since the first wave of coronavirus disease in March 2020, citizens and permanent residents returning to New Zealand have been required to undergo managed isolation and quarantine (MIQ) for 14 days and mandatory testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of October 20, 2020, of 62,698 arrivals, testing of persons in MIQ had identified 215 cases of SARS-CoV-2 infection. Among 86 passengers on a flight from Dubai, United Arab Emirates, that arrived in New Zealand on September 29, test results were positive for 7 persons in MIQ. These passengers originated from 5 different countries before a layover in Dubai; 5 had negative predeparture SARS-CoV-2 test results. To assess possible points of infection, we analyzed information about their journeys, disease progression, and virus genomic data. All 7 SARS-CoV-2 genomes were

genetically identical, except for a single mutation in 1 sample. Despite predeparture testing, multiple instances of in-flight SARS-CoV-2 transmission are likely.

Syahmi, W. M., M. M. Mafauzy, K. A. Baharuddin, S. M. Ikhwan, K. A. Sayuti and S. Mohd Shukruddeen (2021). "Elephant attack - A rare case of survival." Med J Malaysia **76**(5): 741-743.

Conflict of human-wild elephant is not uncommon in Malaysia. Most of the human victims usually succumb to death due to internal organ injuries. Here we report a case of a woman who was the victim of an elephant attack and successfully survived to share our experience in managing this type of polytrauma.

Thapa, J., S. K. Mikota, K. P. Gairhe, S. Paudel, D. K. Singh, I. P. Dhakal, C. Nakajima and Y. Suzuki (2021). "Tuberculosis seroprevalence and comparison of hematology and biochemistry parameters between seropositive and seronegative captive Asian elephants of Nepal." J Vet Med Sci **83**(8): 1278-1283.

We conducted a tuberculosis (TB) serosurveillance program of captive elephants in Nepal and compared hematology and biochemistry parameters between seropositive and seronegative elephants. A total of 153 elephants (male=20, female=133) from four national parks were tested for TB using the ElephantTB STAT-PAK(®) Assay (ChemBio Diagnostic Systems, Inc., Medford, NY, USA). The mean reported age for 138 elephants was 38.5 years (range 2-71 years). Seroprevalence for TB was 21.56% (33/153). The majority of seropositive elephants were female (n=30) and from Chitwan National Park (n=29). The occurrence of TB seropositive cases in other more remote national parks suggests TB may be widespread among the captive elephant population of Nepal. Hematology and biochemistry analyses were performed on 13 and 22 seropositive elephants, respectively and, nine elephants from a seronegative TB herd for comparison. Hematology parameters (hemoglobin, packed cell volume, platelet, white blood cells, and erythrocyte sedimentation rate) were comparable between the two groups. Total protein, globulin, and lactate dehydrogenase were significantly higher in seronegative elephants, and bilirubin was significantly higher in seropositive elephants whereas blood urea nitrogen, creatinine, glutamic oxaloacetic transaminase/aspartate aminotransferase (GOT/AST), glutamic pyruvic transaminase/alanine aminotransferase (GPT/ALT), gamma glutamyl transferase (GT), and albumin were not significantly different. The range of biochemical parameters that were significantly different between seropositive and seronegative elephants had narrow ranges. Thus, the potential of these parameters as a direct biomarker for TB diagnosis is limited based on the findings in this study. We recommend including blood parameters in future TB surveillance studies.

Titcomb, G., J. N. Mantas, J. Hulke, I. Rodriguez, D. Branch and H. Young (2021). "Water sources aggregate parasites with increasing effects in more arid conditions." Nat Commun **12**(1): 7066.

Shifts in landscape heterogeneity and climate can influence animal



movement in ways that profoundly alter disease transmission. Water sources that are foci of animal activity have great potential to promote disease transmission, but it is unknown how this varies across a range of hosts and climatic contexts. For fecal-oral parasites, water resources can aggregate many different hosts in small areas, concentrate infectious material, and function as disease hotspots. This may be exacerbated where water is scarce and for species requiring frequent water access. Working in an East African savanna, we show via experimental and observational methods that water sources increase the density of wild and domestic herbivore feces and thus, the concentration of fecal-oral parasites in the environment, by up to two orders of magnitude. We show that this effect is amplified in drier areas and drier periods, creating dynamic and heterogeneous disease landscapes across space and time. We also show that herbivore grazing behaviors that expose them to fecal-oral parasites often increase at water sources relative to background sites, increasing potential parasite transmission at these hotspots. Critically, this effect varies by herbivore species, with strongest effects for two animals of concern for conservation and development: elephants and cattle.

Tollis, M., E. Ferris, M. S. Campbell, V. K. Harris, S. M. Rupp, T. M. Harrison, W. K. Kiso, D. L. Schmitt, M. M. Garner, C. A. Aktipis, C. C. Maley, A. M. Boddy, M. Yandell, C. Gregg, J. D. Schiffman and L. M. Abegglen (2021). "Elephant Genomes Reveal Accelerated Evolution in Mechanisms Underlying Disease Defenses." Mol Biol Evol **38**(9): 3606-3620.

Disease susceptibility and resistance are important factors for the conservation of endangered species, including elephants. We analyzed pathology data from 26 zoos and report that Asian elephants have increased neoplasia and malignancy prevalence compared with African bush elephants. This is consistent with observed higher susceptibility to tuberculosis and elephant endotheliotropic herpesvirus (EEHV) in Asian elephants. To investigate genetic mechanisms underlying disease resistance, including differential responses between species, among other elephant traits, we sequenced multiple elephant genomes. We report a draft assembly for an Asian elephant, and defined 862 and 1,017 conserved potential regulatory elements in Asian and African bush elephants, respectively. In the genomes of both elephant species, conserved elements were significantly enriched with genes differentially expressed between the species. In Asian elephants, these putative regulatory regions were involved in immunity pathways including tumor-necrosis factor, which plays an important role in EEHV response. Genomic sequences of African bush, forest, and Asian elephant genomes revealed extensive sequence conservation at TP53 retrogene loci across three species, which may be related to TP53 functionality in elephant cancer resistance. Positive selection scans revealed outlier genes related to additional elephant traits. Our study suggests that gene regulation plays an important role in the differential inflammatory response of Asian and African elephants, leading to increased infectious disease and cancer susceptibility in Asian elephants. These genomic discoveries can inform future functional and translational studies aimed at identifying effective treatment approaches for

ill elephants, which may improve conservation.

Udonsom, R., Y. Nishikawa, R. M. Fereig, T. Topisit, N. Kulkaweewut, S. Chanamrung and C. Jirapattharasate (2021). "Exposure to *Toxoplasma gondii* in Asian Elephants (*Elephas maximus indicus*) in Thailand." *Pathogens* **11**(1).

*Toxoplasma gondii* is the causative agent of toxoplasmosis in humans and various animal species worldwide. In Thailand, seroprevalence studies on *T. gondii* have focused on domestic animals, and information on infections in Asian elephants (*Elephas maximus indicus*) is scarce. This study was conducted to determine the seroprevalence of *T. gondii* infection in archival sera collected from 268 elephants living in Thailand. The serum samples were analyzed for anti-*T. gondii* immunoglobulin G antibodies using the latex agglutination test (LAT) and indirect enzyme-linked immunosorbent assay (iELISA) based on *T. gondii* lysate antigen (TLA-iELISA) and recombinant *T. gondii* dense granular antigen 8 protein (TgGRA8-iELISA). The prevalence of antibodies against *T. gondii* was 45.1% (121/268), 40.7% (109/268), and 44.4% (119/268) using LAT, TLA-iELISA, and TgGRA8-iELISA, respectively. Young elephants had a higher seropositivity rate than elephants aged >40 years (odds ratio = 6.6;  $p < 0.001$ ; 95% confidence interval: 2.9-15.4). When LAT was used as the reference, TLA-iELISA and TgGRA8-iELISA showed a substantial ( $\kappa = 0.69$ ) and moderate ( $\kappa = 0.42$ ) agreement, respectively. Although our findings suggest the widespread exposure of Asian elephants to *T. gondii* in Thailand, the source of infection was not investigated. Therefore, investigation of the predisposing factors associated with toxoplasmosis is necessary to identify the potential risk factors for infection.

van Aarde, R. J., S. L. Pimm, R. Guldemon, R. Huang and C. Maré (2021). "The 2020 elephant die-off in Botswana." *PeerJ* **9**: e10686.

The cause of deaths of 350 elephants in 2020 in a relatively small unprotected area of northern Botswana is unknown, and may never be known. Media speculations about it ignore ecological realities. Worse, they make conjectures that can be detrimental to wildlife and sometimes discredit conservation incentives. A broader understanding of the ecological and conservation issues speaks to elephant management across the Kavango-Zambezi Transfrontier Conservation Area that extends across Botswana, Namibia, Angola, Zambia, and Zimbabwe. Our communication addresses these. Malicious poisoning and poaching are unlikely to have played a role. Other species were unaffected, and elephant carcasses had their tusks intact. Restriction of freshwater supplies that force elephants to use pans as a water source possibly polluted by blue-green algae blooms is a possible cause, but as yet not supported by evidence. No other species were involved. A contagious disease is the more probable one. Fences and a deep channel of water confine these elephants' dispersal. These factors explain the elephants' relatively high population growth rate despite a spell of increased poaching during 2014-2018. While the deaths represent only ~2% of the area's elephants, the additive effects of poaching and stress induced by people protecting their crops cause alarm. Confinement and relatively high densities

probably explain why the die-off occurred only here. It suggests a re-alignment or removal of fences that restrict elephant movements and limits year-round access to freshwater.

Vazquez, J. M. and V. J. Lynch (2021). "Pervasive duplication of tumor suppressors in afrotherians during the evolution of large bodies and reduced cancer risk." *Elife* **10**: 1-45.

The risk of developing cancer is correlated with body size and lifespan within species. Between species, however, there is no correlation between cancer and either body size or lifespan, indicating that large, long-lived species have evolved enhanced cancer protection mechanisms. Elephants and their relatives (Proboscideans) are a particularly interesting lineage for the exploration of mechanisms underlying the evolution of augmented cancer resistance because they evolved large bodies recently within a clade of smaller bodied species (Afrotherians). Here, we explore the contribution of gene duplication to body size and cancer risk in Afrotherians. Unexpectedly, we found that tumor suppressor duplication was pervasive in Afrotherian genomes, rather than restricted to Proboscideans. Proboscideans, however, have duplicates in unique pathways that may underlie some aspects of their remarkable anti-cancer cell biology. These data suggest that duplication of tumor suppressor genes facilitated the evolution of increased body size by compensating for decreasing intrinsic cancer risk. There is an incredible diversity of body sizes and lifespans among living mammals, remarkably even larger mammals lived in the recent past but are now extinct. In living mammals, an individual's body size and lifespan are among the greatest predictors for the likelihood of developing cancer, taller and older humans, for example, have a greater cancer risk than shorter and younger people. Between species, however, body size and lifespan are poor predictors of cancer risk, thus big and long lived species must have evolved ways to reduce their risk of developing cancer. By understanding how big, long-lived species evolved their enhanced tumor suppression mechanisms we can improve our understanding of genes involved in human cancer and inspire new cancer treatments. We tracked how body size and the copy number of most protein coding genes changed elephants and their smaller bodied relatives. We found that as large bodied elephants evolved from smaller bodied ancestors, their cancer risk decreased. While genes involved in tumor suppression were commonly duplicated in elephants and their relatives, elephants have a unique repertoire of tumor suppressor genes that evolved alongside their recent increase in body size. These data show that duplication of tumor suppressor genes facilitated the evolution of large body size by compensating for increasing cancer risk. © 2021, eLife Sciences Publications Ltd. All rights reserved.

Villa, P., G. Boschian, L. Pollarolo, D. Saccà, F. Marra, S. Nomade and A. Pereira (2021). "Elephant bones for the Middle Pleistocene toolmaker." *PLoS ONE* **16**(8): e0256090.

The use of bone as raw material for implements is documented since the Early Pleistocene. Throughout the Early and Middle Pleistocene bone tool

shaping was done by percussion flaking, the same technique used for knapping stone artifacts, although bone shaping was rare compared to stone tool flaking. Until recently the generally accepted idea was that early bone technology was essentially immediate and expedient, based on single-stage operations, using available bone fragments of large to medium size animals. Only Upper Paleolithic bone tools would involve several stages of manufacture with clear evidence of primary flaking or breaking of bone to produce the kind of fragments required for different kinds of tools. Our technological and taphonomic analysis of the bone assemblage of Castel di Guido, a Middle Pleistocene site in Italy, now dated by  $^{40}\text{Ar}/^{39}\text{Ar}$  to about 400 ka, shows that this general idea is inexact. In spite of the fact that the number of bone bifaces at the site had been largely overestimated in previous publications, the number of verified, human-made bone tools is 98. This is the highest number of flaked bone tools made by pre-modern hominids published so far. Moreover the Castel di Guido bone assemblage is characterized by systematic production of standardized blanks (elephant diaphysis fragments) and clear diversity of tool types. Bone smoothers and intermediate pieces prove that some features of Aurignacian technology have roots that go beyond the late Mousterian, back to the Middle Pleistocene. Clearly the Castel di Guido hominids had done the first step in the process of increasing complexity of bone technology. We discuss the reasons why this innovation was not developed. The analysis of the lithic industry is done for comparison with the bone industry.

Vogel, S. M., M. Pasgaard and J. C. Svenning (2021). "Joining forces toward proactive elephant and rhinoceros conservation." Conserv Biol.

Proactive approaches that anticipate the long-term effects of current and future conservation threats could increase the effectiveness and efficiency of biodiversity conservation. However, such approaches can be obstructed by a lack of knowledge of habitat requirements for wildlife. To aggregate and assess the suitability of current information available on habitat requirements needed for proactive conservation, we conducted a systematic review of the literature on elephant and rhinoceros habitat requirements and synthesized data by combining a vote counting assessment with bibliometric and term maps. We contextualized these numeric and terminological results with a narrative review. We mapped current methods, results, terminology, and collaborations of 693 studies. Quantitative evidence for factors that influence the suitability of an area for elephants and rhinoceros was biased toward African savanna elephants and ecological variables. Less than one third of holistic approaches considered equal amounts of ecological and anthropogenic variables in their assessments. There was a general lack of quantitative evidence for direct proxies of anthropogenic variables that were expected to play an important role based on qualitative evidence and policy documents. However, there was evidence for a segregation in conceptual frameworks among countries and species and between science versus policy literature. There was also evidence of unused potential for collaborations among southern hemisphere researchers. Our results indicated that the success of proactive conservation interventions can be increased if ecological

and anthropogenic dimensions are integrated into holistic habitat assessments and holistic carrying capacities and quantitative evidence for anthropogenic variables is improved. To avoid wasting limited resources, it is necessary to form inclusive collaborations within and across networks of researchers studying different species across regional and continental borders and in the science-policy realm.

Wall, J., G. Wittemyer, B. Klinkenberg, V. LeMay, S. Blake, S. Strindberg, M. Henley, F. Vollrath, F. Maisels, J. Ferwerda and I. Douglas-Hamilton (2021). "Human footprint and protected areas shape elephant range across Africa." Current Biology **31**(11): 2437-2445.e2434.

Over the last two millennia, and at an accelerating pace, the African elephant (*Loxodonta* spp. Lin.) has been threatened by human activities across its range.<sup>1–7</sup> We investigate the correlates of elephant home range sizes across diverse biomes. Annual and 16-day elliptical time density home ranges<sup>8</sup> were calculated by using GPS tracking data collected from 229 African savannah and forest elephants (*L. africana* and *L. cyclotis*, respectively) between 1998 and 2013 at 19 sites representing bushveld, savannah, Sahel, and forest biomes. Our analysis considered the relationship between home range area and sex, species, vegetation productivity, tree cover, surface temperature, rainfall, water, slope, aggregate human influence, and protected area use. Irrespective of these environmental conditions, long-term annual ranges were overwhelmingly affected by human influence and protected area use. Only over shorter, 16-day periods did environmental factors, particularly water availability and vegetation productivity, become important in explaining space use. Our work highlights the degree to which the human footprint and existing protected areas now constrain the distribution of the world's largest terrestrial mammal.<sup>9,10</sup> A habitat suitability model, created by evaluating every square kilometer of Africa, predicts that 18,169,219 km<sup>2</sup> would be suitable as elephant habitat—62% of the continent. The current elephant distribution covers just 17% of this potential range of which 57.4% falls outside protected areas. To stem the continued extirpation and to secure the elephants' future, effective and expanded protected areas and improved capacity for coexistence across unprotected range are essential. © 2021 The Authors

Wang, H., P. Wang, X. Zhao, W. Zhang, J. Li, C. Xu and P. Xie (2021). "What triggered the Asian elephant's northward migration across southwestern Yunnan?" Innovation (N Y) **2**(3): 100142.

Wang, H., C. Xu, Y. Liu, E. Jeppesen, J. C. Svenning, J. Wu, W. Zhang, T. Zhou, P. Wang, S. Nangombe, J. Ma, H. Duan, J. Fang and P. Xie (2021). "From unusual suspect to serial killer: Cyanotoxins boosted by climate change may jeopardize megafauna." Innovation (N Y) **2**(2): 100092.

The recent mass mortality event of more than 330 African elephants in Botswana has been attributed to biotoxins produced by cyanobacteria; however, scientific evidence for this is lacking. Here, by synthesizing multiple sources of data, we show that, during the past decades, the widespread

hypertrophic waters in Southern Africa have entailed an extremely high risk and frequent exposure of cyanotoxins to the wildlife within this area, which functions as a hotspot of mammal species richness. The hot and dry climatic extremes have most likely acted as the primary trigger of the recent and perhaps also of prehistoric mass mortality events. As such climate extremes are projected to become more frequent in Southern Africa in the near future, there is a risk that similar tragedies may take place, rendering African megafauna species, especially those that are already endangered, in risk of extinction. Moreover, cyanotoxin poisoning amplified by climate change may have unexpected cascading effects on human societies. Seen within this perspective, the tragic mass death of the world's largest terrestrial mammal species serves as an alarming early warning signal of future environmental catastrophes in Southern Africa. We suggest that systematic, quantitative cyanotoxin risk assessments are made and precautionary actions to mitigate the risks are taken without hesitation to ensure the health and sustainability of the megafauna and human societies within the region.

Weisbrod, T. C., R. Isaza, C. Cray, L. Adler and N. I. Stacy (2021). "The importance of manual white blood cell differential counts and platelet estimates in elephant hematology: blood film review is essential." *Vet Q* **41**(1): 30-35.

Unique features of elephant hematology are known challenges in analytical methodology like two types of monocytes typical for members of the Order Afrotheria and platelet counts of the comparatively small elephant platelet. To investigate WBC differential and platelet data generated by an impedance-based hematology analyzer without availability of validated species-specific software for recognition of elephant WBCs and platelets, compared to manual blood film review. Blood samples preserved in ethylenediaminetetraacetic acid (EDTA) of 50 elephants ( $n = 35$  *Elephas maximus* and  $n = 15$  *Loxodonta africana*) were used. A Mann-Whitney test for independent samples was used to compare parameters between methods and agreement was tested using Bland-Altman bias plots. All hematological variables, including absolute numbers of heterophils, lymphocytes, monocytes, eosinophils, basophils, and platelets, were significantly different ( $p < 0.0001$ ) between both methods of analysis, and there was no agreement using Bland-Altman bias plots. Manual review consistently produced higher heterophil and monocyte counts as well as platelet estimates, while the automated analyzer produced higher lymphocyte, eosinophil, and basophil counts. The hematology analyzer did not properly differentiate elephant lymphocytes and monocytes, and did not accurately count elephant platelets. These findings emphasize the importance of manual blood film review as part of elephant complete blood counts in both clinical and research settings and as a basis for the development of hematological reference intervals.

Weston, M. E., K. E. Mills and M. von Keyserlingk (2021). "Your happiness or mine: Influence of affective states and level of " *Animal Welfare* **30**: 279-293.

Many captive Asian elephants (*Elephas maximus*) in Thailand participate in the tourism industry at attractions known as 'elephant camps.' There has been significant criticism of low welfare venues, where the elephants may

experience injuries, poor nutrition, unnatural social environments and aversive handling. Despite increasing concern for animal welfare, the general public often have difficulty identifying the welfare issues affecting captive animals. The aim of this study was to investigate participants' willingness to support an elephant attraction and their perceived emotional value from the experience, based on the affective state of the captive elephant and their level of contact with it. Participants (n = 590) from the United States were randomly assigned to one of four vignettes (using a 2 × 2 experimental design) that described an elephant attraction, varying the affective state of the elephant (feels excellent, feels terrible) and the level of contact they could have with the elephant (low, high). A mixed methods approach was used, where participants provided answers to Likert-type questions, followed by an open-ended response. Participants showed greater willingness to support the elephant attraction and greater perceived emotional value from the experience when the elephant felt excellent, as opposed to when the elephant felt terrible. There were no significant differences between low and high contact for the measures included in this study. Qualitative responses varied greatly, with participants making many assumptions about the elephant and the attraction, revealing potential misconceptions that they had regarding the welfare of captive elephants. This research may be used to encourage a shift in tourism preferences to venues that reflect positive elephant welfare.

Whitney, M. R., K. D. Angielczyk, B. R. Peacock and C. A. Sidor (2021). "The evolution of the synapsid tusk: Insights from dicynodont therapsid tusk histology." Proceedings of the Royal Society B: Biological Sciences **288**(1961).

The mammalian tusk is a unique and extreme morphotype among modern vertebrate dentitions. Tusks - defined here as ever-growing incisors or canines composed of dentine - evolved independently multiple times within mammals yet have not evolved in other extant vertebrates. This suggests that there is a feature specific to mammals that facilitates the evolution of this specialized dentition. To investigate what may underpin the evolution of tusks, we histologically sampled the tusks of dicynodont therapsids: the earliest iteration of tusk evolution and the only non-mammalian synapsid clade to have acquired such a dentition. We studied the tissue composition, attachment tissues, development and replacement in 10 dicynodont taxa and show multiple developmental pathways for the adult dentitions of dicynodont tusks and tusk-like caniniforms. In a phylogenetic context, these developmental pathways reveal an evolutionary scenario for the acquisition of an ever-growing tusk - an event that occurred convergently, but only in derived members of our sample. We propose that the evolution of an ever-growing dentition, such as a tusk, is predicated on the evolution of significantly reduced tooth replacement and a permanent soft-tissue attachment. Both of these features are fixed in the dentitions of crown-group mammals, which helps to explain why tusks are restricted to this clade among extant vertebrates. © 2021 The Author(s).

Wierucka, K., M. D. Henley and H. S. Mumby (2021). "Acoustic cues to individuality

in wild male adult African savannah elephants (*Loxodonta africana*)."  
PeerJ **9**: e10736.

The ability to recognize conspecifics plays a pivotal role in animal communication systems. It is especially important for establishing and maintaining associations among individuals of social, long-lived species, such as elephants. While research on female elephant sociality and communication is prevalent, until recently male elephants have been considered far less social than females. This resulted in a dearth of information about their communication and recognition abilities. With new knowledge about the intricacies of the male elephant social structure come questions regarding the communication basis that allows for social bonds to be established and maintained. By analyzing the acoustic parameters of social rumbles recorded over 1.5 years from wild, mature, male African savanna elephants (*Loxodonta africana*) we expand current knowledge about the information encoded within these vocalizations and their potential to facilitate individual recognition. We showed that social rumbles are individually distinct and stable over time and therefore provide an acoustic basis for individual recognition. Furthermore, our results revealed that different frequency parameters contribute to individual differences of these vocalizations.

Wood, J., L. J. Minter, D. Bibus, M. K. Stoskopf, V. Fellner and K. Ange-van Heugten (2021). "Comparison of African savanna elephant (*Loxodonta africana*) fatty acid profiles in whole blood, whole blood dried on blood spot cards, serum, and plasma."  
PeerJ **9**: e12650.

**BACKGROUND:** African elephants in managed care have presented differences in the balance between omega-3 and omega-6 fatty acids, a situation primarily thought to be due to dietary differences between the managed animals and their free-ranging counterparts. Because of this, circulating fatty acid status is included in routine monitoring of elephant health. A method of blood collection that requires only a few drops of whole blood, dried on filter paper (DBS) and can be used for analyzing full fatty acid profiles offers advantages in clinical application. **METHODS:** This study compared the use of whole blood, and whole blood DBS, serum or plasma for use in evaluating circulating fatty acid composition in African savannah elephants. Samples from six African elephants (two males and four females) were collected during the same week at the NC Zoo, Asheboro, NC. **RESULTS:** Results found only 2 of 36 individual fatty acids and none of the 10 fatty acid groupings were different when comparing the four blood fraction sample types to each other with Mann-Whitney U-Test pairwise comparisons. Myristic acid (14:0) was lower in the DBS samples than in whole blood, serum, and plasma and pentadecaenoic acid (15:1) was slightly more concentrated in DBS and whole blood. **DISCUSSION:** Results indicate that fatty acid profile of serum, plasma, whole blood, and DBS are comparable in African elephants. The DBS method offers advantages in acquisition and handling and may be preferable to other methods in both routine health assessment of captive animals and field research on free ranging animals.



Wood, M., S. Chamaillé-Jammes, A. Hammerbacher and A. M. Shrader (2021). "African elephants can detect water from natural and artificial sources via olfactory cues." *Anim Cogn.*

Water is vital for mammals. Yet, as ephemeral sources can be difficult to find, it raises the question, how do mammals locate water? Elephants (*Loxodonta africana*) are water-dependent herbivores that possess exceptional olfactory capabilities, and it has been suggested that they may locate water via smell. However, there is no evidence to support this claim. To explore this, we performed two olfactory choice experiments with semi-tame elephants. In the first, we tested whether elephants could locate water using olfactory cues alone. For this, we used water from two natural dams and a drinking trough utilised by the elephants. Distilled water acted as a control. In the second, we explored whether elephants could detect three key volatile organic compounds (VOCs) commonly associated with water (geosmin, 2-methylisoborneol, and dimethyl sulphide). We found that the elephants could locate water olfactorily, but not the distilled water. Moreover, they were also able to detect the three VOCs associated with water. However, these VOCs were not in the odour profiles of the water sources in our experiments. This suggests that the elephants were either able to detect the unique odour profiles of the different water sources or used other VOCs that they associate with water. Ultimately, our findings indicate that elephants can locate water olfactorily at small spatial scales, but the extent to which they, and other mammals, can detect water over larger scales (e.g. km) remains unclear.

Yun, Y., S. Sripiboon, K. Pringproa, P. Chuammitri, V. Punyapornwithaya, K. Boonprasert, P. Tankaew, T. Angkawanish, K. Namwongprom, O. Arjkumpa, J. L. Brown and C. Thitaram (2021). "Clinical characteristics of elephant endotheliotropic herpesvirus (EEHV) cases in Asian elephants (*Elephas maximus*) in Thailand during 2006-2019." *Vet Q* **41**(1): 268-279.

BACKGROUND: Elephant endotheliotropic herpesvirus causes a hemorrhagic disease (EEHV-HD) that is a major cause of death in juvenile Asian elephants with EEHV1 and EEHV4 being the most prevalent. AIM: To perform a retrospective clinical data analysis. METHODS: Records of a total of 103 cases in Thailand confirmed by polymerase chain reaction (PCR) on blood and/or tissue samples. RESULTS: The severity of clinical signs varied among EEHV subtypes. EEHV1A was the most prevalent with 58%, followed by EEHV4 with 34%, EEHV1B with 5.8% and EEHV1&4 co-infection with 1.9%. Overall case fatality rate was 66%. When compared among subtypes, 100% case fatality rate was associated with EEHV1&4 co-infection, 83% with EEHV1B, 75% with EEHV1A, and the lowest at 40% for EEHV4. Calves 2- to 4-year old were in the highest age risk group and exhibited more severe clinical signs with the highest mortality. Majority of cases were found in weaned or trained calves and higher number of cases were observed in rainy season. A gender predilection could not be demonstrated. Severely affected elephants presented with thrombocytopenia, depletion of monocytes, lymphocytes and heterophils, a monocyte:heterophil (M:H) ratio lower than 2.37, hypoproteinemia (both albumin and globulin), severe grade of

heterophil toxicity, and low red blood cell counts and pack cell volumes. Survival was not affected by antiviral drug treatment in the severely compromised animals. CONCLUSION: Early detection by laboratory testing and aggressive application of therapies comprising of supportive and anti-viral treatment can improve survival outcomes of this disease.

Zhou, Y., M. W. Tingley, M. F. Case, C. Coetsee, G. A. Kiker, R. Scholtz, F. J. Venter and A. C. Staver (2021). "Woody encroachment happens via intensification, not extensification, of species ranges in an African savanna." *Ecol Appl* **31**(8): e02437.

Widespread woody encroachment is a prominent concern for savanna systems as it is often accompanied by losses in productivity and biodiversity. Extensive ecosystem-level work has advanced our understanding of its causes and consequences. However, there is still debate over whether local management can override regional and global drivers of woody encroachment, and it remains largely unknown how encroachment influences woody community assemblages. Here, we examined species-level changes in woody plant distributions and size structure from the late 1980s to the late 2000s based on spatially intensive ground-based surveys across Kruger National Park, South Africa. This study region spans broad gradients in rainfall, soil texture, fire frequency, elephant density, and other topographic variables. Species-level changes in frequency of occurrence and size class proportion reflected widespread woody encroachment primarily by *Dichrostachys cinerea* and *Combretum apiculatum*, and a loss of large trees mostly of *Sclerocarya birrea* and *Acacia nigrescens*. Environmental variables determining woody species distributions across Kruger varied among species but did not change substantially between two sampling times, indicating that woody encroachers were thickening within their existing ranges. Overall, more areas across Kruger were found to have an increased number of common woody species through time, which indicated an increase in stem density. These areas were generally associated with decreasing fire frequency and rainfall but increasing elephant density. Our results suggest that woody encroachment is a widespread but highly variable trend across landscapes in Kruger National Park and potentially reflects an erosion of local heterogeneity in woody community assemblages. Many savanna managers, including in Kruger, aim to manage for heterogeneity in order to promote biodiversity, where homogenization of vegetation structure counters this specific goal. Increasing fire frequency has some potential as a local intervention. However, many common species increased in commonness even under near-constant disturbance conditions, which likely limits the potential for managing woody encroachment in the face of drivers beyond the scope of local control. Regular field sampling coupled with targeted fire management will enable more accurate monitoring of the rate of encroachment intensification.