2022 Elephant References (alphabetical)
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Abdullah-Fauzi, N. A. F., K. V. Karuppannan, N. H. S. Mohd-Radzi, M. Gani, A. R. Mohd-Ridwan, N. Othman, H. Haris, N. H. Sariyati, N. R. Aifat, M. A. B. Abdul-Latiff, M. F. A. Abdul-Razak and B. M. Md-Zain (2022). "Determining the Dietary Preferences of Wild Asian Elephants (Elephas maximus) in Taman Negara National Park, Malaysia Based on Sex and Age using trnL DNA Metabarcoding Analysis." Zool Stud **61**: e60.
The world's largest terrestrial mammal, Asian elephants, are known to have enormous feeding needs. Several factors such as season, sex, age, and daily activities influence the amount of food required by an individual. Generally, captive elephants have a limited choice of food on a daily basis compared with that of elephants in the wild. Elephants in captivity are fed according to a prepared feeding schedule, whereas wild elephants are free to choose the type of plants that they consume in their natural habitat. In the past, ecological observations have been widely used to determine the diet of wild elephants. However, the molecular approach has never been carried out. In the present study, we aimed to; 1) identify the plant diet of wild Asian elephants in Taman Negara National Park (TNNP) according to their sex and age using high-throughput DNA metabarcoding; and 2) determine the dietary formulation of captive elephants based on the generated plant metabarcoding database. DNA was extracted from 24 individual fecal samples collected using noninvasive sampling techniques from TNNP and the National Elephant Conservation Centre (NECC) Kuala Gandah. Seven pooled samples from male adult, female adult, male subadult, female subadult, male juvenile, female juvenile, and captive elephants were amplified and sequenced targeting the trnL region (50-150 base pairs). The CLC Genomic Workbench and PAST 4.02 software were used for data analysis. In total, 24 orders, 41 families, 233 genera, and 306 species of plants were successfully detected in the diet of the Asian elephants. The most abundant plant genera consumed were Sporobolus (21.88%), Musa (21.48%), and Ficus (10.80%). Plant variation was lower in samples from male elephants than in those from female elephants. The plant species identified were correlated with the nutrient benefits required by elephants. Adults and subadults consumed more plant species than were consumed by juvenile elephants. However, there was no significant difference between ages and sexes. The findings of this study can be used as guidance by the Department of Wildlife and National Parks for the management of captive elephants, especially in NECC Kuala Gandah.

Abegglen, L. M., T. M. Harrison, A. Moresco, J. S. Fowles, B. V. Troan, W. K. Kiso, D. Schmitt, A. M. Boddy and J. D. Schiffman (2022). "Of Elephants and Other Mammals: A Comparative Review of Reproductive Tumors and Potential Impact on

Conservation." Animals (Basel) **12**(15).

Reproductive tumors can impact conception, pregnancy, and birth in mammals. These impacts are well documented in humans, while data in other mammals are limited. An urgent need exists to understand the reproductive impact of these lesions in endangered species, because some endangered species have a documented high prevalence of reproductive tumors. This article documents that the prevalence of both benign and malignant neoplasia differs between African and Asian elephants, with Asian elephants more frequently diagnosed and negatively affected by both. The prevalence of these tumors across mammalian species is compared, and impact plus treatment options in human medicine are reviewed to inform decision making in elephants. Evidence suggests that reproductive tumors can negatively impact elephant conservation. Future studies that document reproductive outcomes, including the success of various treatment approaches in elephants with tumors will benefit conservation efforts.

Ahmed, R. and A. Saikia (2022). "Pandora's Box: A spatiotemporal assessment of elephant-train casualties in Assam, India." PLoS ONE **17**(7): e0271416.

Railways are an indispensable component of sustainable transportation systems, but also exact a toll on wildlife. Wild Asian elephants are often killed by trains in Assam, India, where we assess temporal variations in the occurrences of elephant-train collisions (ETCs) and casualties during 1990-2018. This study also assesses spatially varying relationships between elephant-train collision (ETC) rates and elephant and train densities in the adjoining 10 km2 grid cells of 11 prioritized railroad segments using ordinary least squares (OLS) and geographically weighted regression (GWR) models. The temporal analysis indicated that ETCs spiked at certain hours and months. The adult and calf elephant casualties on the railroads were found to be two to fivefold high during the post monsoon season compared to other seasons. During the operation period of meter gauge railroads (1990-1997), the proportions of ETCs and casualties were only 15.6% and 8.7% respectively. However, these increased substantially to 84.4% and 91.3% respectively during the operation of broad gauge railroads (1998-2018). The OLS model indicated that both elephant and train densities explained 37% of the variance of ETC rate, while GWR model showed 83% of the variance of ETC rate. The local coefficient values of GWR indicated that both the predictor variables interplayed significantly and positively to determine ETC rates in the Mariani-Nakachari and Khatkhati-Dimapur railroad segments. However, the relationship between ETC rate and elephant density is significantly negative in the Habaipur-Diphu railroad, implying that the elephant population along this railroad stretch is significantly affected by railways through large scale ETCs. Hence, there is an urgent need to address long-term mitigation strategies so that elephants can be conserved by providing safe passages and survival resources along railway lines.

Baral, K., S. Bhandari, B. Adhikari, R. M. Kunwar, H. P. Sharma, A. Aryal and W. Ji (2022). "Anthropogenic mortality of large mammals and trends of conflict over two decades in Nepal." Ecol Evol **12**(10): e9381.

Wildlife conservation in human-dominated landscapes faces increased challenges due to rising conflicts between humans and wildlife. We investigated the human and wildlife loss rates due to human-wildlife conflict between 2000 and 2020 in Nepal. We concentrated on Asian elephant (Elephas maximus), greater one-horned rhino (Rhinoceros unicornis), tiger (Panthera tigirs), and leopard (Panthera pardus) mortality, as well as human mortality caused by these species. Over the 21-year period, we recorded 1139 cases of wildlife mortality and 887 cases of human mortality. Leopard mortality was the highest, followed by that of greater one-horned rhinos, tigers, and Asian elephants. Overall, the rate of wildlife mortality has been increasing over the years. Asian elephants were found to be more responsible for crop damage than greater one-horned rhinos, while leopards were found to be more responsible for livestock depredation than tigers. The generalized linear model indicated that the mortality of wildlife in the districts is best predicted by the additive effect of human mortality, the proportion of agricultural land, and the literacy rate of the districts. Retaliatory wildlife mortality was the most challenging issue for wildlife conservation, especially for the large mammals. Findings from this study are important for mitigation of human-wildlife conflicts, controlling retaliatory killing, and conserving these threatened large mammals.

Bargues, M. D., A. Halajian, P. Artigas, W. J. Luus-Powell, M. A. Valero and S. Mas-Coma (2022). "Paleobiogeographical origins of Fasciola hepatica and F. gigantica in light of new DNA sequence characteristics of F. nyanzae from hippopotamus." Front Vet Sci **9**: 990872.

Fascioliasis is a highly pathogenic disease affecting humans and livestock worldwide. It is caused by the liver flukes Fasciola hepatica transmitted by Galba/Fossaria lymnaeid snails in Europe, Asia, Africa, the Americas and Oceania, and F. gigantica transmitted by Radix lymnaeids in Africa and Asia. An evident founder effect appears in genetic studies as the consequence of their spread by human-guided movements of domestic ruminants, equines and Old World camelids in the post-domestication period from the beginning of the Neolithic. Establishing the geographical origins of fasciolid expansion is multidisciplinary crucial for disease assessment. Sequencing of selected nuclear ribosomal and mitochondrial DNA markers of F. nyanzae infecting hippopotamuses (Hippopotamus amphibius) in South Africa and their comparative analyses with F. hepatica and F. gigantica, and the two Fascioloides species, Fs. jacksoni from Asian elephants and Fs. magna from Holarctic cervids, allow to draw a tuned-up evolutionary scenario during the pre-domestication period. Close sequence similarities indicate a direct derivation of F. hepatica and F. gigantica from F. nyanzae by speciation after host capture phenomena. Phylogenetic reconstruction, genetic distances and divergence estimates fully fit fossil knowledge, past interconnecting bridges between continents, present fasciolid infection in the wild fauna, and lymnaeid distribution. The paleobiogeographical analyses suggest an origin for F. gigantica by transfer from primitive hippopotamuses to grazing bovid ancestors of Reduncinae, Bovinae and Alcelaphinae, by keeping the same vector Radix natalensis in warm lowlands of southeastern Africa in the mid-

Miocene, around 13.5 mya. The origin of F. hepatica should have occurred after capture from primitive, less amphibious Hexaprotodon hippopotamuses to mid-sized ovicaprines as the wild bezoar Capra aegagrus and the wild mouflon Ovis gmelini, and from R. natalensis to Galba truncatula in cooler areas and mountainous foothills of Asian Near East in the latest Miocene to Early Pliocene, around 6.0 to 4.0 mya and perhaps shortly afterwards.

Beeck, V. C., G. Heilmann, M. Kerscher and A. S. Stoeger (2022). "Sound Visualization Demonstrates Velopharyngeal Coupling and Complex Spectral Variability in Asian Elephants." Animals (Basel) **12**(16).

Sound production mechanisms set the parameter space available for transmitting biologically relevant information in vocal signals. Low-frequency rumbles play a crucial role in coordinating social interactions in elephants' complex fission-fusion societies. By emitting rumbles through either the oral or the three-times longer nasal vocal tract, African elephants alter their spectral shape significantly. In this study, we used an acoustic camera to visualize the sound emission of rumbles in Asian elephants, which have received far less research attention than African elephants. We recorded nine adult captive females and analyzed the spectral parameters of 203 calls, including vocal tract resonances (formants). We found that the majority of rumbles (64%) were nasally emitted, 21% orally, and 13% simultaneously through the mouth and trunk, demonstrating velopharyngeal coupling. Some of the rumbles were combined with orally emitted roars. The nasal rumbles concentrated most spectral energy in lower frequencies exhibiting two formants, whereas the oral and mixed rumbles contained higher formants, higher spectral energy concentrations and were louder. The roars were the loudest, highest and broadest in frequency. This study is the first to demonstrate velopharyngeal coupling in a non-human animal. Our findings provide a foundation for future research into the adaptive functions of the elephant acoustic variability for information coding, localizability or sound transmission, as well as vocal flexibility across species.

Benitez, L., J. W. Kilian, G. Wittemyer, L. F. Hughey, C. H. Fleming, P. Leimgruber, P. du Preez and J. A. Stabach (2022). "Precipitation, vegetation productivity, and human impacts control home range size of elephants in dryland systems in northern Namibia." Ecol Evol **12**(9): e9288.

Climatic variability, resource availability, and anthropogenic impacts heavily influence an animal's home range. This makes home range size an effective metric for understanding how variation in environmental factors alter the behavior and spatial distribution of animals. In this study, we estimated home range size of African elephants (Loxodonta africana) across four sites in Namibia, along a gradient of precipitation and human impact, and investigated how these gradients influence the home range size on regional and site scales. Additionally, we estimated the time individuals spent within protected area boundaries. The mean 50% autocorrelated kernel density estimate for home range was 2200 km(2) [95% CI:1500-3100 km(2)]. Regionally, precipitation and vegetation were the strongest predictors of home range size, accounting for a combined 53% of observed variation.

However, different environmental covariates explained home range variation at each site. Precipitation predicted most variation (up to 74%) in home range sizes (n = 66) in the drier western sites, while human impacts explained 71% of the variation in home range sizes (n = 10) in Namibia's portion of the Kavango-Zambezi Transfrontier Conservation Area. Elephants in all study areas maintained high fidelity to protected areas, spending an average of 85% of time tracked on protected lands. These results suggest that while most elephant space use in Namibia is driven by natural dynamics, some elephants are experiencing changes in space use due to human modification.

Birgfellner, C. M. V., J. T. Soley, E. Polsterer, G. Forstenpointner and G. E. Weissengruber (2022). "The graviportal spine: Epaxial muscles of the African savanna elephant (Loxodonta africana)." Anat Histol Embryol.

In this study, we present not only a new and detailed anatomical description of the epaxial muscles and adjacent ligamentous and fascial structures in the African savanna elephant but also a structural and functional comparison with other Afrotherian mammals and some domestic quadrupeds. All structures were examined by means of standard anatomical techniques. The back of the largest land mammal is a crucial part of trunk construction according to the bow and string concept, which is applied also in other quadrupedal animals. The epaxial muscles of the African savanna elephant play an important role in the biomechanical properties of the entire back and in supporting and moving the heavy head. Situated in the short cervical region of the African savanna elephant is a large mass comprised of numerous muscle individuals together with a well-developed ligamentum nuchae. Parts of the mm. interansversarii ventralis cervicis form a strong muscle belly, which was named the m. intertransversarius longus. Whereas the head is held in a high or extended position most of the time during locomotion, the head and neck are highly mobile while the animal is foraging or socially interacting. Movements between the elements of the thoracic and lumbar spine are likely to be very limited due to the obvious rigidity of the bony vertebral column. Aponeuroses surrounding long epaxial muscles could contribute to an energy-saving mechanism, which is active during both stance and locomotion. The well-developed m. serratus dorsalis cranialis helps in facilitating effective breathing in an animal, which is equipped with an unusual pleural structure.

Buranapim, N., P. Kulnanan, K. Chingpathomkul, T. Angkawanish, S. Chansitthiwet, W. Langkaphin, P. Sombutputorn, N. Monchaivanakit, K. Kasemjai, K. Namwongprom, K. Boonprasert, P. Bansiddhi, N. Thitaram, P. Sharp, C. Pacharinsak and C. Thitaram (2022). "Dexmedetomidine Effectively Sedates Asian Elephants (Elephas maximus)." Animals (Basel) **12**(20).

This study investigated the sedative effects of dexmedetomidine in Asian elephants. We hypothesized that 2 μg/kg dexmedetomidine would provide sufficient standing sedation. A crossover design study was performed in three Asian elephants. Each elephant was assigned to 1 of 3 treatment groups-1 (D1), 1.5 (D1.5) or 2 (D2) μg/kg dexmedetomidine (intramuscular injection,

IM) with a two-week 'washout period' between doses. Elephants were monitored for 120 min. At 120 min (Ta), atipamezole was administered IM. Sedation and responsiveness scores were evaluated. Physiological parameters (pulse rate, respiratory rate, and %SpO(2)) and clinical observations were monitored during the study and for 3 days post drug administration. D2 provided the longest sedation (approximately 70 min), compared to D1 and D1.5. After Ta, each elephant's sedative stage lessened within 10-15 min without complications. No significant abnormal clinical observations were noted throughout and during the 3-days post study period. These data suggest that a single 2 μg/kg IM dexmedetomidine injection provides sufficient standing sedation for approximately 70 min in Asian elephants.

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 (2022). "Monitoring of bluetongue virus in zoo animals in Spain, 2007-2019." Transbound Emerg Dis **69**(4): 1739-1747.

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 (Loxodanta 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.

Campbell, K. M., J. A. Wilson and K. A. Morfeld (2022). "Predictors of testosterone in zoo-managed male African elephants (Loxodonta Africana)." Zoo Biol.

Reproductive complications for both male and female zoo-managed African elephants (Loxodonta africana) contribute to the rapidly declining population. In zoo-managed bull elephants, few studies have explored the potential physiological, physical, social, and environmental factors that influence bull fertility, particularly, androgen production. Testosterone is the essential

steroid hormone for male sexual maturation and inadequate concentrations can be detrimental for spermatogenesis. Testosterone, fecal glucocorticoid metabolites, leptin, glucose, insulin, and triglycerides were analyzed from weekly fecal and blood serum samples taken over 6 months from six zoo-managed African elephant bulls (10-19 years of age). Testosterone levels were compared to endocrine factors, weekly social and environmental variables, daily musth signs, and body condition scores (BCS). The glucose-to-insulin ratio (G:I) was the only physiological biomarker found to be positively associated with testosterone. Predictive physical variables included Musth Score (+) and Moderate Exercise (+). Bulls with BCS signifying overweight (BCS 4) had lower testosterone (36.6 ± 1.6 ng/g fecal extraction [FE]) than bulls with healthy BCS 3; 51.2 ± 4.9 ng/g FE). Numerous social variables influenced testosterone concentrations, including Total Contact Day (+), Female Interaction Day (+), Indirect Contact Day (+), Indirect Contact Night (+) and Total No Contact (-). Both percentage of Time Outdoor and Time Mixed positively influenced testosterone, whereas testosterone decreased for percentage of Time Indoors. Each additional daily browse opportunity increased testosterone by approximately 7 ng/g FE. In managed care, the emphasis should be placed on optimizing these predictors of testosterone production to promote bull reproductive health.

Chan, A. N., G. Wittemyer, J. McEvoy, A. C. Williams, N. Cox, P. Soe, M. Grindley, N. M. Shwe, A. M. Chit, Z. M. Oo and P. Leimgruber (2022). "Landscape characteristics influence ranging behavior of Asian elephants at the human-wildlands interface in Myanmar." Mov Ecol **10**(1): 6.

CONTEXT: Asian elephant numbers are declining across much of their range driven largely by serious threats from land use change resulting in habitat loss and fragmentation. Myanmar, holding critical range for the species, is undergoing major developments due to recent sociopolitical changes. To effectively manage and conserve the remaining populations of endangered elephants in the country, it is crucial to understand their ranging behavior. OBJECTIVES: Our objectives were to (1) estimate the sizes of dry, wet, and annual ranges of wild elephants in Myanmar; and quantify the relationship between dry season (the period when human-elephant interactions are the most likely to occur) range size and configurations of agriculture and natural vegetation within the range, and (2) evaluate how percentage of agriculture within dry core range (50% AKDE range) of elephants relates to their daily distance traveled. METHODS: We used autocorrelated kernel density estimator (AKDE) based on a continuous-time movement modeling (ctmm) framework to estimate dry season (26 ranges from 22 different individuals), wet season (12 ranges from 10 different individuals), and annual range sizes (8 individuals), and reported the 95%, 50% AKDE, and 95% Minimum Convex Polygon (MCP) range sizes. We assessed how landscape characteristics influenced range size based on a broad array of 48 landscape metrics characterizing aspects of vegetation, water, and human features and their juxtaposition in the study areas. To identify the most relevant landscape metrics and simplify our candidate set of informative metrics, we relied on exploratory factor analysis and Spearman's rank correlation coefficient.

Based on this analysis we adopted a final set of metrics into our regression analysis. In a multiple regression framework, we developed candidate models to explain the variation in AKDE dry season range sizes based on the previously identified, salient metrics of landscape composition. RESULTS: Elephant dry season ranges were highly variable averaging 792.0 km(2) and 184.2 km(2) for the 95% and 50% AKDE home ranges, respectively. We found both the shape and spatial configuration of agriculture and natural vegetation patches within an individual elephant's range play a significant role in determining the size of its range. We also found that elephants are moving more (larger energy expenditure) in ranges with higher percentages of agricultural area. CONCLUSION: Our results provide baseline information on elephant spatial requirements and the factors affecting them in Myanmar. This information is important for advancing future land use planning that takes into account space-use requirements for elephants. Failing to do so may further endanger already declining elephant populations in Myanmar and across the species' range.

Chel, H. M., S. Bawm, L. L. Htun, M. A. Masum, O. Ichii, N. Nonaka, R. Nakao and K. Katakura (2022). "Scanning electron microscopy of Quilonia renniei from Asian elephants revealing variation in coronal leaflet number." Parasitology **149**(4): 529-533.

Although parasitic nematodes in the genera Murshidia and Quilonia (family Strongylidae) are recognized as major gastrointestinal parasites in Asian elephants, they have been poorly studied. Recently, light micrographs of these parasites in Myanmar have been presented, almost 100 years after the original drawings. However, the number of coronal leaflets, a key taxonomic feature of Quilonia species, has not been precisely determined based on light microscopy. The current study aimed to determine the exact number of coronal leaflets in Quilonia renniei specimens from Asian elephants in Myanmar. On the basis of scanning electron micrographs, leaflet number in females (19–20, average 19.7, n = 9) was significantly higher ($P < 0.005$) than that in males (16–19, average 18.1, n = 8). This compares with 18 coronal leaflets indicated in the original species description. Specimens bearing 19 coronal leaflets were most numerous, followed by those with 20 leaflets. Median-joining network analysis of mitochondrial cytochrome c oxidase subunit I gene sequences with 16 haplotypes from 19 individuals revealed no clear association between parasite populations and the number of coronal leaflets. These results highlight the importance of determining the number of coronal leaflets in the taxonomy of Q. renniei and other related Quilonia species infecting Asian elephants.

Chelopo, N. D., P. E. Buss, M. A. Miller and G. E. Zeiler (2022). "Cardiopulmonary responses of free-ranging African elephant (Loxodonta africana) bulls immobilized with a thiafentanil-azaperone combination." Vet Anaesth Analg **49**(3): 291-298.

OBJECTIVE: To determine the time course and certain cardiopulmonary effects of trunk-breathing elephants immobilized with thiafentanil-azaperone. STUDY DESIGN: Prospective descriptive study. ANIMALS: A convenience sample of 10 free-ranging African elephant bulls (estimated weight range:

3000-6000 kg). METHODS: Elephants were immobilized using thiafentanil (15-18 mg) and azaperone (75-90 mg) administered by dart. Once recumbent, the respiratory rate, minute ventilation (V˙e), end-tidal carbon dioxide (Pe'CO(2)), arterial blood pressure and heart rate were recorded immediately after instrumentation and at 5 minute intervals until 20 minutes. Arterial blood gases were analysed at the time of initial instrumentation and at 20 minutes. On completion of data collection, thiafentanil was antagonized using naltrexone (10 mg mg(-1) thiafentanil; administered intravenously). A stopwatch was used to record time to recumbency (dart placement to recumbency) and time to recovery (administration of antagonist to standing). Data were compared using a one-way anova. Data are presented as mean ± standard deviation. RESULTS: All elephants were successfully immobilized, and there were no significant changes in cardiopulmonary variables over the monitoring period. Average time to recumbency was 12.5 (± 3.9) minutes. The measured V˙e was 103 (± 30) L minute(-1). The average heart and respiratory rates over the 20 minute immobilization were steady at 49 (± 6) beats minute(-1) and 5 (± 1) breaths minute(-1), respectively. The mean arterial blood pressure was 153 (± 31) mmHg. The elephants were acidaemic (pH: 7.18 ± 0.06), mildly hypoxaemic (PaO(2): 68 ± 15 mmHg; 9.1 ± 2.0 kPa) and hypercapnic (PaCO(2): 52 ± 7 mmHg; 6.9 ± 0.9 kPa). Average time to recovery was 2.2 ± 0.5 minutes. CONCLUSION AND CLINICAL RELEVANCE: African elephant bulls can be successfully immobilized using thiafentanil-azaperone. Recumbency was rapid, the cardiopulmonary variables were stable over time, and recovery was rapid and complete. Mild hypoxaemia and hypercapnia were evident.

Chen, X. M., D. F. An, S. R. He, S. J. Yang, Z. Z. Yang, L. S. Xiong, G. D. Li, M. G. Jiang, C. L. Jiang and Y. Jiang (2022). "Acinetobacter faecalis Sp. Nov., Isolated from Elephant Faeces." Curr Microbiol **80**(1): 21.

A Gram-negative coccobacillus, YIM 103518(T), isolated from wild elephant feces in Xishuangbanna, Yunnan Province, West China, was characterized and identified using a polyphasic taxonomic approach. The strain was strictly aerobic, non-motile, catalase-positive and oxidase-negative, colonies were round, convex, smooth, and pale yellow. The strain growth at 4-40 ℃ (optimum, 28 ℃), pH 6.0-10.0 (optimum, pH 7.0) and 0-4% NaCl (optimum, 0%) in culture medium YIM 38. The major fatty acids of strain YIM 103518(T) were summed feature 3 (C(16:1) ω6c/C(16:1) ω7c), C(16:0), and C(18:1) ω9c. The predominant ubiquinone was Q-9. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and phospholipids. The 16S rRNA gene sequence showed moderate level of similarity with Acinetobacter portensis AC 877(T) (98.7%), Acinetobacter sichuanensis CCTCC AB 2018118(T) (97.1%), and Acinetobacter cumulans CCTCC AB 2018119(T) (97.1%). The G+C content of the genomic DNA was 36.5 mol%. Strain YIM 103518(T) showed an average nucleotide identity value of 86.6%, 77.3% and 78.5%, a digital DNA-DNA hybridizations value of 31.2%, 21.9% and 23.0% with the type strain of A. portensis, A. sichuanensis and A. cumulans based on draft genome sequences, respectively. The results of the

phenotypic, chemotaxonomic and phylogenetic analyses, showed that strain YIM 103518(T) represents a novel species of the genus Acinetobacter, for which the name Acinetobacter faecalis sp. nov. is proposed. The type strain is YIM 103518(T) (=CCTCC AB 2019201(T) = NBRC 114057(T)).

Chen, Y., Y. Sun, M. Hua, K. Shi and D. Dudgeon (2022). "Using genetic tools to inform conservation of fragmented populations of Asian elephants (Elephas maximus) across their range in China." Integr Zool.

A herd of 15 Chinese elephants attracted international attention during their 2021 northward trek, motivating the government to propose establishment of an Asian elephant national park. However, planning is hampered by a lack of genetic information on the remaining populations in China. We collected DNA from 497 dung samples from all 5 populations encompassing the entire range of elephants in China and used mitochondrial and microsatellite markers to investigate their genetic and demographic structure. We identified 237 unique genotypes (153 females, 84 males), representing 81% of the known population. However, the effective population size was small (28, range 25-32). Historic demographic contraction appeared to account for low haplotype diversity (H(d) = 0.235), but moderate nucleotide and nuclear diversity (π = 0.6%, H(e) = 0.55) was attributable to post-bottleneck recovery involving recent population expansion plus historical gene exchange with elephants in Myanmar, Lao PDR, and Vietnam. The 5 populations fell into 3 clusters, with Nangunhe elephants differing consistently from the other 4 populations (F(ST) = 0.23); elephants from Mengyang, Simao, and Jiangcheng belonged to a single population (henceforth, MSJ), and differed from the Shangyong population (F(ST) = 0.11). Interpopulation genetic variation reflected isolation by distance and female-biased dispersal. Chinese elephants should be managed as 2 distinct units: Nangunhe and another combining Shangyong and MSJ; their long-term viability will require restoring gene flow between Shangyong and MSJ, and between elephants in China and neighboring countries. Our results have the potential to inform conservation planning for an iconic megafaunal species.

Chu, P. C., K. Wierucka, D. Murphy, H. B. Tilley and H. S. Mumby (2022). "Human interventions in a behavioural experiment for Asian Elephants (Elephas maximus)." Anim Cogn: 1-12.

Experiments are widely used to investigate the behaviour and cognition of animals. While the automation of experiments to avoid potential experimenter bias is sometimes possible, not all experiments can be conducted without human presence. This is particularly true for large animals in captivity, which are often managed by professional handlers. For the safety of the animals and experimenters, a handler must be present during behavioural studies with certain species. It is not always clear to what extent cues provided by handlers affect the animals, and therefore the experimental results. In this study, we investigate handler interventions during the training process for a behavioural experiment with Asian elephants (Elephas maximus) in Nepal. We show that elephant handlers (mahouts) intervened to guide elephants in performing the learning task using vocal and behavioural

cues, despite experimenters requesting minimal intervention. We found that although the frequency of mahout interventions did not decrease as the training progressed, the nature of their interventions changed. We also found more non-verbal than verbal cues across the training. Our results suggest that guidance from handlers may be common in behavioural studies, and continued consideration should be put into experimental design to reduce or account for cues that animals may receive from humans. This study also emphasises the need to take into account the presence of humans in interpreting the results of animal behavioural experiments, which not only presents challenges to behavioural research, but also represents opportunities for further study.

Common, S. M., Y. Yun, A. Silva-Fletcher, C. Thitaram, T. Janyamethakul, S. Khammesri and F. M. Molenaar (2022). "Developing a non-invasive method of detecting elephant endotheliotropic herpesvirus infections using faecal samples." Vet Rec **190**(2): e833.

BACKGROUND: Elephant endotheliotropic herpesvirus (EEHV)-associated haemorrhagic disease (EEHV-HD) is a leading cause of death in Asian elephant calves across the world. Cases of EEHV-HD have been detected in free-living calves through post-mortem examination (PME) indicating the presence of the virus in the wild. In the absence of a non-invasive sampling method, little research into free-living populations has been possible. This study aimed to provide evidence that faeces can be used as a non-invasive sampling method for the detection of EEHV excretion using quantitative polymerase chain reaction. METHODS: Serial saliva swabs and faecal samples were taken from five captive Asian elephants in Thailand over 12 weeks. To ensure the presence of detectable elephant DNA within the sample, qPCR was run for amplification of the Asian elephant tumour necrosis factor (TNF-α) gene, EEHV1 and EEHV4. RESULTS: Of 28 sample pairs, seven saliva samples were positive for EEHV, of which two had paired positive faecal samples. CONCLUSIONS: This study presents the first evidence that EEHV is excreted in faeces at detectable levels. This method may in future be used for improved understanding of the epidemiology of EEHV in free-living elephant populations, as well as detection of EEHV excretion in captive herds.

Costa, T., G. Rocchigiani, F. Zendri, G. Drake, J. Lopez, J. Chantrey and E. Ricci (2022). "Elephant Endotheliotropic Herpesvirus 4 and Clostridium perfringens Type C Fatal Co-Infection in an Adult Asian Elephant (Elephas maximus)." Animals (Basel) **12**(3).

Elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD) is an acute, often fatal, multisystemic hemorrhagic disease and one of the most significant causes of mortality of Asian elephants in captivity. Most fatal cases of EEHV-HD are associated with EEHV1A and EEHV1B in juveniles. This case report describes the clinical and pathological features of a fatal co-infection of Clostridium perfringens type C and EEHV-HD, caused by EEHV4, in an adult female Asian elephant. Although fatal clostridial enterotoxemia has been occasionally reported in elephants, this report highlights the importance of having both EEHV-HD and clostridial enterotoxemia as

potential differential diagnoses in cases of widespread tissue necrosis and internal hemorrhage in elephants, regardless of the animal age group, due to their macroscopic similarities, frequent co-occurrence and cumulative morbid potential.

Coughlin, L. L., C. R. Sanchez, M. I. Monti, J. A. Griffioen, F. B. Nutter and G. L. Beamer (2022). "Potential diagnostic biomarkers for pulmonary tuberculosis in humans are not elevated in Mycobacterium tuberculosis culture-positive Asian elephants (Elephas maximus)." Am J Vet Res **83**(8).

OBJECTIVE: To determine (1) if chemokine (C-X-C motif) ligand 1 (CXCL1), matrix metalloproteinase 8 (MMP8), interleukin-10 (IL-10), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) can be detected in serum from Asian elephants, and (2) if their concentrations are significantly elevated in Mycobacterium tuberculosis (M.tb) culture-positive elephants compared to -negative elephants. CXCL1, MMP8, IL-10, IFN-γ, and TNF-α were recently identified as potential diagnostic biomarkers for pulmonary tuberculosis in experimental studies in animals and humans. Therefore, we hypothesized that they would be detectable and significantly elevated in M.tb culture-positive elephants compared to M.tb culture-negative elephants. SAMPLE: 101 Asian elephant serum samples, including 91 samples from 6 M.tb-negative elephants and 10 samples from 5 M.tb-positive elephants (none of which exhibited clinical signs of disease). M.tb status was determined by trunk wash culture. PROCEDURES: Commercially available ELISA kits were used to determine the concentrations of each biomarker in serum samples. RESULTS: Biomarker concentrations were below the limit of detection for the assay in 100/101 (99%) samples for CXCL1, 98/101 (97%) samples for MMP8, 85/101 (84%) samples for IL-10, 75/101 (74%) samples for IFN-γ, and 45/101 (45%) samples for TNF-α. Multiple M.tb culture-positive elephants did not have detectable levels of any of the 5 biomarkers. CLINICAL RELEVANCE: CXCL1, MMP8, IL-10, IFN-γ, and TNF-α were not elevated in M.tb culture-positive Asian elephants compared to M.tb culture-negative Asian elephants. This may be related to disease state (ie, clinically asymptomatic). More sensitive assays are needed to better understand the role of these biomarkers in M.tb infection in Asian elephants.

Dai, Y. (2022). "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 **29**(4): 5960-5970.

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(2), mainly distributed in Menghai (3934.53 km(2)), Lancang (3198.67 km(2)), and Jinghong (2657.74 km(2)); (3) the distribution area of elephants was 943.75 km(2), and these areas overlapped with suitable tea plant habitat primarily located in G4 (379.40 km(2)), G3 (251.18), and G7 (168.03 km(2)); 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.

Das, P., A. Kshettry and H. N. Kumara (2022). "Trunk picking from a truncating menu: Dry season forage selection by Asian elephant in a multi-use landscape." PLoS ONE **17**(7): e0271052.

Elephants show a strong selection towards areas with high foraging opportunities at the landscape level making top-down decisions by first selecting patch types within landscapes and finally species within them. Understanding forage selection in a multi-use landscape is critical for prioritising patches for habitat management, ensuring availability of selected forage, helping in minimizing pressure on food crops and subsequent negative interactions with people. We assessed dry season forage selection in a multi-use landscape of West Bengal state, India. Relative forage use and relative plant species availability ratio were calculated to assess forage selection in a multi-use landscape comprising of the forest, tea estates, agricultural land, and human settlement. Forage use was assessed using the opportunistic feeding trail observation method (150.01 km). Stratified random sampling was used to assess plant species availability using the quadrat method (123 plots of 0.1 ha each). Among 286 plant species recorded, 132 plant species were consumed by elephants. A majority (80.21%) of plant species were consumed more than the proportional availability thereby showing selective foraging during the dry season in the study area. From forest to semi-open forest and open forest, canopy layer tree density and the total number of species decreased whereas invasive species density increased. This indicates the high impact on the forage species availability for elephants and the requirement of appropriate habitat management strategies. The presence of 32.14% of the selected forage

species in human-use landscape alone demands the development of conservation interventions. This is the first study to assess forage selection by elephants in a multi-use landscape and used to prioritise conservation and management strategies at a landscape level.

de Flamingh, A., Y. Ishida, P. Pečnerová, S. Vilchis, H. R. Siegismund, R. J. van Aarde, R. S. Malhi and A. L. Roca (2022). "Combining methods for non-invasive fecal DNA enables whole genome and metagenomic analyses in wildlife biology." Front Genet **13**: 1021004.

Non-invasive biological samples benefit studies that investigate rare, elusive, endangered, or dangerous species. Integrating genomic techniques that use non-invasive biological sampling with advances in computational approaches can benefit and inform wildlife conservation and management. Here, we used non-invasive fecal DNA samples to generate low- to medium-coverage genomes (e.g., >90% of the complete nuclear genome at six X-fold coverage) and metagenomic sequences, combining widely available and accessible DNA collection cards with commonly used DNA extraction and library building approaches. DNA preservation cards are easy to transport and can be stored non-refrigerated, avoiding cumbersome or costly sample methods. The genomic library construction and shotgun sequencing approach did not require enrichment or targeted DNA amplification. The utility and potential of the data generated was demonstrated through genome scale and metagenomic analyses of zoo and free-ranging African savanna elephants (Loxodonta africana). Fecal samples collected from free-ranging individuals contained an average of 12.41% (5.54-21.65%) endogenous elephant DNA. Clustering of these elephants with others from the same geographic region was demonstrated by a principal component analysis of genetic variation using nuclear genome-wide SNPs. Metagenomic analyses identified taxa that included Loxodonta, green plants, fungi, arthropods, bacteria, viruses and archaea, showcasing the utility of this approach for addressing complementary questions based on host-associated DNA, e.g., pathogen and parasite identification. The molecular and bioinformatic analyses presented here contributes towards the expansion and application of genomic techniques to conservation science and practice.

de Jonge, N., B. Carlsen, M. H. Christensen, C. Pertoldi and J. L. Nielsen (2022). "The Gut Microbiome of 54 Mammalian Species." Front Microbiol **13**: 886252.

The gut microbiome plays a critical role in many aspects of host life, and the microbial community composition is heavily influenced by the prevailing conditions in the gut environment. Community composition has been suggested to have large implications for conservation efforts, and gut health has become of interest for optimizing animal care in captivity. In this study, we explore the gut microbiome of a wide range of animals in the context of conservation biology. The composition of the gut microbial community of 54 mammalian animal species was investigated using 16S rRNA gene amplicon sequencing. The composition of the gut microbiota clearly reflects diet and the structure of the gastrointestinal system, and it is to a certain degree more similar between closely related animals. Specific clusters of taxa were

observed across animals of the same species, diet, and gut morphology. The microbiota retained regardless of captivity status is hypothesized to cover important symbiotic relationships with the host, while the remaining part reflects the artificial living conditions and can therefore be used as a future tool for conservation biologists. For five animal species (giraffes, horses, baboons, elephants, and zebras), it was possible to compare the microbiota of wild and captive individuals. Differences were observed in the proportion of microbiota detected between wild and captive specimens of the same animal species. We propose that the gut microbiota harbours important species, which can potentially serve as indicators for the well-being of the animal and the effect of living in captivity.

Devi, A., S. A. Hussain, M. Sharma, G. V. Gopi and R. Badola (2022). "Seasonal pattern of food habits of large herbivores in riverine alluvial grasslands of Brahmaputra floodplains, Assam." Sci Rep **12**(1): 482.

Jarman-Bell (1974) hypothesized that in the dry savanna of Africa, small-bodied herbivores tend to browse more on forage with high protein and low fibre content. This implies browsing on high nutritive forage by meso-herbivores, and grazing and mixed feeding on coarse forage by mega-herbivores. We tested this hypothesis in the riverine alluvial grasslands of the Kaziranga National Park (KNP), where seasonal flood and fire play an important role in shaping the vegetation structure. We analyzed the feeding habits and quality of major forage species consumed by three mega-herbivores, viz. greater one-horned rhino, Asian elephant, and Asiatic wild buffalo, and three meso-herbivores, viz. swamp deer, hog deer, and sambar. We found that both mega and meso-herbivores were grazers and mixed feeders. Overall, 25 forage plants constituted more than 70% of their diet. Among monocots, family Poaceae with Saccharum spp. (contributing > 9% of the diet), and, among dicots, family Rhamnaceae with Ziziphus jujuba (contributing > 4% of the diet) fulfilled the dietary needs. In the dry season, the concentration of crude protein, neutral detergent fibre, calcium, sodium, and phosphorous varied significantly between monocots and dicots, whereas only calcium and sodium concentrations varied significantly in the wet season. Dicots were found to be more nutritious throughout the year. Compared to the dry season, the monocots, viz. Alpinia nigra, Carex vesicaria, Cynodon dactylon, Echinochloa crus-galli, Hemarthria compressa, Imperata cylindrica, and Saccharum spp., with their significantly high crude protein, were more nutritious during the wet season. Possibly due to the availability of higher quality monocots in the wet season, both mega and meso-herbivores consume it in high proportion. We concluded that the Jarman-Bell principle does not apply to riverine alluvial grasslands as body size did not explain the interspecific dietary patterns of the mega and meso-herbivores. This can be attributed to seasonal floods, habitat and forage availability, predation risk, and management practices such as controlled burning of the grasslands. The ongoing succession and invasion processes, anthropogenic pressures, and lack of grassland conservation policy are expected to affect the availability of the principal forage and suitable habitat of large herbivores in the Brahmaputra floodplains, which necessitates wet

grassland-based management interventions for the continued co-existence of large herbivores in such habitats.

Dominguez-Oliva, A., M. D. Ghezzi, P. Mora-Medina, I. Hernandez-Avalos, J. Jacome, A. Castellon, I. Falcon, F. Resendiz, N. Romero, R. Ponce and D. Mota-Rojas (2022). "Anatomical, physiological, and behavioral mechanisms of thermoregulation in elephants." Journal of Animal Behaviour and Biometeorology **10**(4): 1-13.

Elephants use different thermoregulatory mechanisms that depend on the anatomical and morphological characteristics of the species. The crevices and wrinkles of the skin enhance the water-retention capacity of the epidermis. The highly vascularized ear is another region of particular interest, as its movement and vasomotor changes promote heat dissipation. Generally, these mechanisms are modulated by the hypothalamic thermoregulatory center and by the peripheral response of animals. Nonetheless, elephants are currently exposed to alterations in their habitats, such as global warming and climatic changes, which challenge their homeothermy. This article aims to discuss the thermoregulation mechanisms of African (Loxodonta africana) and Asian (Elephas maximus) elephants from an anatomical, physiological, and behavioral basis. The practical implications of these elements will be analyzed to implement tools, such as infrared thermography, or environmental enrichment, as strategies to promote the thermal balance of elephants.

Duangurai, T., A. Rungruengkitkul, T. Kong-Ngoen, W. Tunyong, N. Kosoltanapiwat, P. Adisakwattana, M. Vanaporn, N. Indrawattana and P. Pumirat (2022). "Phylogenetic analysis and antibiotic resistance of Escherichia coli isolated from wild and domestic animals at an agricultural land interface area of Salaphra wildlife sanctuary, Thailand." Vet World **15**(12): 2800-2809.

BACKGROUND AND AIM: Domestic and wild animals are important reservoirs for antibiotic-resistant bacteria. This study aimed to isolate Escherichia coli from feces of domestic and wild animals at an agricultural land interface area of Salaphra Wildlife Sanctuary, Thailand, and study the phylogenic characteristics and antibiotic resistance in these isolates. MATERIALS AND METHODS: In this cross-sectional, descriptive study, we randomly collected ground feces from free-ranging wild animals (deer and elephants) and domestic animals (cattle and goats). All fecal samples were inoculated onto MacConkey agar plates, and lactose-fermenting colonies were identified as E. coli. Antibiotic susceptibility of the E. coli isolates was determined using the disc diffusion method. Polymerase chain reaction assays were used to detect antibiotic resistance and virulence genes. RESULTS: We obtained 362 E. coli isolates from the collected fecal samples. The E. coli isolates were categorized into four phylogenetic groups according to the virulence genes (chuA, vjaA, and TspE4C2). Phylogenetic Group D was predominant in the deer (41.67%) and elephants (63.29%), whereas phylogenetic Group B1 was predominant in the cattle (62.31%), and phylogenetic Groups A (36.36%) and B2 (33.33%) were predominant in the goats. Antibiotic susceptibility testing revealed that most antibiotic-resistant E. coli were isolated from

domestic goats (96.96%). Among the 362 E. coli isolates, 38 (10.5%) were resistant to at least one antibiotic, 21 (5.8%) were resistant to two antibiotics, and 6 (1.66%) were resistant to three or more antibiotics. Ampicillin (AMP) was the most common antibiotic (48.48%) to which the E. coli were resistant, followed by tetracycline (TET) (45.45%) and trimethoprim-sulfamethoxazole (3.03%). One isolate from an elephant was resistant to five antibiotics: AMP, amoxicillin, sulfisoxazole, TET, and ciprofloxacin. Determination of antibiotic resistance genes confirmed that E. coli isolates carried antibiotic resistance genes associated with phenotypic resistance to antibiotics. Most antibiotic-resistant E. coli belonged to phylogenic Groups A and B1, and most non-resistant E. coli belonged to phylogenic Groups B2 and D. CONCLUSION: Monitoring E. coli isolates from wild and domestic animals showed that all four phylogenic groups of E. coli have developed antibiotic resistance and are potential sources of multidrug resistance. High levels of antibiotic resistance have been linked to domestic animals. Our results support strengthening surveillance to monitor the emergence and effects of antibiotic-resistant microorganisms in animals.

Garai, M. E., T. Roos, T. Eggeling, A. Ganswindt, Y. Pretorius and M. Henley (2022). "Developing welfare parameters for African elephants (Loxodonta africana) in fenced reserves in South Africa." PLoS ONE **17**(3): e0264931.

South Africa has many fenced reserves harbouring small to medium sized populations of African elephant (Loxodonta africana), most of which have been translocated. Elephants on fenced reserves may be exposed to various management interventions and practices (translocation, hunting, darting, high tourism impact, contraception programs, disruption due to infrastructure maintenance, etc.). These factors may impact the welfare of elephants. Poor elephant welfare may have serious consequences such as increased inter- and intra-species aggression that could result in fatalities. This is the first study to attempt to define behavioural and physiological welfare parameters for free-ranging elephants on small to medium sized reserves. The eight study sites incorporated reserves with different social structure combinations, elephant life-histories, reserve sizes, habitat, management, and tourism intensity. Data collection consisted of behavioural observations (10-minute videos) as well as faecal samples. By incorporating both behavioural and physiological (faecal glucocorticoid metabolite (fGCM) concentration) parameters, we aimed to investigate whether the two parameters showed similar trends. Five behavioural categories were identified (Arousal, Assessing, Ambivalent, Ambivalent/ Body care, and Frustrated behaviour), with various detailed behaviours demonstrated by the elephants that may indicate the influence of anthropogenic disturbance and possibly impact on animal welfare. The study showed significant differences between the selected detailed behaviours, behavioural categories and fGCM concentrations of elephants across the eight reserves. History seemed to be a decisive factor, as reserves with predominantly ex-captive elephants showed higher frequencies of certain behaviours as well as higher fGCM concentrations. Age, sex, reserve size and season were also found to contribute to our defined welfare indices and fGCM concentrations. This

indicates that behavioural parameters, indicative of certain behavioural states, are valuable indicators of welfare, as supported by the physiological response of the elephants. The results also highlight the importance of taking multiple specified behaviours from a category into consideration when evaluating the welfare of elephants, to account for individual variation.

Goosen, W. J., L. Kleynhans, T. J. Kerr, P. D. van Helden, P. Buss, R. M. Warren and M. A. Miller (2022). "Improved detection of Mycobacterium tuberculosis and M. bovis in African wildlife samples using cationic peptide decontamination and mycobacterial culture supplementation." J Vet Diagn Invest **34**(1): 61-67.

In South Africa, mycobacterial culture is regarded as the gold standard for the detection of Mycobacterium tuberculosis complex (MTBC) infection in wildlife even though it is regarded as "imperfect." We compared a novel decontamination and mycobacterial culture technique (TiKa) to the conventional mycobacterium growth indicator tube (MGIT) system using known amounts of bacilli and clinical samples from MTBC-infected African buffaloes (Syncerus caffer), white rhinoceros (Ceratotherium simum), and African elephants (Loxodonta africana). Use of the TiKa-KiC decontamination agent on samples spiked with 10,000 to 10 colony forming units (cfu) of M. bovis (SB0121) and M. tuberculosis (H37Rv) had no effect on isolate recovery in culture. In contrast, decontamination with MGIT MycoPrep resulted in no growth of M. bovis samples at concentrations < 1,000 cfu and M. tuberculosis samples < 100 cfu. Subsequently, we used the TiKa system with stored clinical samples (various lymphatic tissues) collected from wildlife and paucibacillary bronchoalveolar lavage fluid, trunk washes, and endotracheal tube washes from 3 species with known MTBC infections. Overall, MTBC recovery by culture was improved significantly ($p < 0.01$) by using TiKa compared to conventional MGIT, with 54 of 57 positive specimens versus 25 of 57 positive specimens, respectively. The TiKa mycobacterial growth system appears to significantly enhance the recovery of MTBC members from tissue and paucibacillary respiratory samples collected from African buffaloes, African elephants, and white rhinoceros. Moreover, the TiKa system may improve success of MTBC culture from various sample types previously deemed unculturable from other species.

Guntawang, T., T. Sittisak, P. Tankaew, C. Thitaram, V. Langkapin, T. Angkawanish, T. Singhla, N. Sthitmatee, W. L. Hsu, R. Thanawongnuwech and K. Pringproa (2022). "Development of Nonstructural Protein-Based Indirect ELISA to Identify Elephant Endotheliotropic Herpesvirus (EEHV) Infection in Asian Elephants (Elephas maximus)." Animals (Basel) **12**(14).

Disease caused by elephant endotheliotropic herpesvirus (EEHV) infection is the most highly fatal hemorrhagic disease in Asian elephant calves worldwide. To date, adult elephants that have been infected with EEHV have predominantly displayed mild clinical signs, while they are believed to serve as EEHV shedders to other elephants. Hence, the diagnostic tools employed for monitoring EEHV-active infection are considered vitally important. In this study, partial EEHV-DNA polymerase (DNApol) nonstructural proteins (NSPs) were used to detect anti-EEHV antibodies through the use of an in-house

indirect enzyme-linked immunosorbent assay (ELISA). The results were then compared to those obtained from a PCR test. In this study, a total of 175 serum samples were collected from Asian elephants living in elephant camps located in Chiang Mai and Lampang Provinces, Thailand. The elephants were aged between 2 and 80 years old. The overall percentages of positive samples by the PCR and EEHV-DNApol ELISA tests were 4% (21/175) and 12% (21/175), respectively. The ELISAs demonstrated values of 77.9% (95% posterior probability interval (PPI) = 52.5-95%) sensitivity and 87.7% (PPI = 82.5-91.9%) specificity, respectively. Accordingly, the sera obtained from the elephants exhibiting no clinical signs of EEHV infection, and those who were negative according to PCR tests, revealed a value of 14% seropositivity for EEHV-DNApol. Our results indicate that these asymptomatic, active EEHV-infected elephants could likely serve as a source of EEHV shedding within elephant herds. Consequently, the developed EEHV-DNApol NSPs-based ELISA test employed in the present study may be of use for routine monitoring and identification of EEHV shedders in elephant herds, and could be an alternative diagnostic tool for EEHV detection in Asian elephants.

Gupta, S. K. (2022). "Zona pellucida glycoproteins: Relevance in fertility and development of contraceptive vaccines." Am J Reprod Immunol: e13535.

Mammalian zona pellucida (ZP) is composed of three to four glycoproteins, which plays an important role during fertilization. Mutations in the genes encoding zona proteins are reported in women with empty follicle syndrome, degenerated oocytes and those with an abnormal or no ZP further emphasizing their relevance during fertility. Immunization with either native or recombinant ZP glycoproteins/proteins leads to curtailment of fertility in various animal species. Observed infertility is frequently associated with ovarian pathology characterized by follicular atresia and degenerative changes in ZP, which may be due to oophoritogenic T cell epitope(s) within ZP glycoproteins. To avoid ovarian dystrophy, B cell epitopes of ZP glycoproteins have been mapped by using bio-effective monoclonal antibodies. Immunization with the immunogens encompassing the mapped B cell epitopes by and large led to amelioration of follicular atresia. However, their use for human application will require more rigorous research to establish their safety and reversibility of the contraceptive effect. Nonetheless, to minimize human-animal conflicts, ZP-based contraceptive vaccines have been used successfully in the population management of free-ranging animal species such as feral horses, white-tailed deer and elephants. To control zoonotic diseases, attempts are also underway to control the population of other animal species including stray dogs, which acts as one of the major vectors for the rabies virus.

Hahn, N. R., J. Wall, K. Denninger-Snyder, M. Goss, W. Sairowua, N. Mbise, A. B. Estes, S. Ndambuki, E. E. Mjingo, I. Douglas-Hamiliton and G. Wittemyer (2022). "Risk perception and tolerance shape variation in agricultural use for a transboundary elephant population." J Anim Ecol **91**(1): 112-123.

To conserve wide-ranging species in human-modified landscapes, it is

essential to understand how animals selectively use or avoid cultivated areas. Use of agriculture leads to human-wildlife conflict, but evidence suggests that individuals may differ in their tendency to be involved in conflict. This is particularly relevant to wild elephant populations. We analysed GPS data of 66 free-ranging elephants in the Serengeti-Mara ecosystem to quantify their use of agriculture. We then examined factors influencing the level of agricultural use, individual change in use across years and differences in activity budgets associated with use. Using clustering methods, our data grouped into four agricultural use tactics: rare (<0.6% time in agriculture; 26% of population), sporadic (0.6%-3.8%; 34%), seasonal (3.9%-12.8%; 31%) and habitual (>12.8%; 9%). Sporadic and seasonal individuals represented two-thirds (67%) of recorded GPS fixes in agriculture, compared to 32% from habitual individuals. Increased agricultural use was associated with higher daily distance travelled and larger home range size, but not with age or sex. Individual tactic change was prevalent and the habitual tactic was maintained in consecutive years by only five elephants. Across tactics, individuals switched from diurnal to nocturnal activity during agricultural use, interpreted as representing similar risk perception of cultivated areas. Conversely, tactic choice appeared to be associated with differences in risk tolerance between individuals. Together, our results suggest that elephants are balancing the costs and benefits of crop usage at both fine (e.g. crop raid events) and long (e.g. yearly tactic change) temporal scales. The high proportion of sporadic and seasonal tactics also highlights the importance of mitigation strategies that address conflict arising from many animals, rather than targeted management of habitual crop raiders. Our approach can be applied to other species and systems to characterize individual variation in human resource use and inform mitigations for human-wildlife coexistence.

Hartono, R., A. H. Iswanto, E. Herawati, R. E. Suramana, J. Sutiawan, Y. Amin and I. Sumardi (2022). "The Improvement of Sumatran Elephant (Elephas maximussumatranus) Dung Particleboard Characteristics Using Bamboo Layering." Polymers (Basel) **14**(16).

The use of natural fibers or particles as alternative raw materials for particleboard production is essential due to the shrinking forest area. Currently, dung waste from the Sumatran elephant (Elephas maximus sumatranus) is being used as a raw material for particleboard due to its high fiber content. Although the product still has inferior mechanical and physical characteristics, it can be improved by layering bamboo. Therefore, this study aimed to enhance the mechanical and physical qualities of elephant dung particleboard by adding layers of bamboo. The particleboard constructed had three layers; namely, the face and back in the form of a bamboo layers, as well as the core, which was in the form of elephant dung. The elephant dung was evenly mixed with isocyanate adhesive using a spray gun, and the bamboo layers were coated with adhesive on one side of the surface. The sample was subjected to a hot press at a temperature of 150 °C and 30 kg/cm(2) pressure for 10 min. Generally, JIS A 5908-2003 is the specification used to test the physical and mechanical properties of particleboard. During the experiment, the characteristics examined include

density, moisture content, water absorption, thickness swelling, modulus of elasticity, modulus of rupture, and internal bonding, which were enhanced by using layers of bamboo. The results showed that the physical properties of the particleboard with bamboo layers were a density of 0.62-0.69 g/cm(3), a moisture content of 7.87-10.35%, water absorption of 38.27-68.58%, and a thickness swelling of 10.87-30.00%, which met the minimum standards of JIS A 5908-2003. The mechanical characteristics had values for the modulus of elasticity of 1952-7282 MPa, the modulus of rupture of 20.44-68.27 MPa, and the internal bonding of 0.16-0.38 MPa, which met the JIS A 5908-2003 standard. Based on these results, the particleboard with Belangke bamboo layers was the best in this study.

Hayward, P. T., S. Liu, A. P. Thigpen and L. A. Hart (2022). "Animal Tourism: Thai Caregivers' Perspectives on Their Relationships with Elephants and Tigers." Animals (Basel) **12**(6).

This study explored the perspectives of elephant mahouts (n = 55) and tiger caregivers (n = 18) working in 4 private or 2 government facilities in Thailand to learn their experiences and viewpoints pertaining to use of animals in tourism. Interviews were conducted in Thailand at facilities in four cities. Mahouts working in private tourism facilities used one-to-one management and were significantly younger and more poorly compensated than those working at government-funded zoos, where some had shifted to group management. Tiger caregivers in tourism had direct contact with young tigers, with group management; these caregivers also were significantly younger than in government zoos, and with fewer benefits. Mahouts and tiger caregivers differed in how they viewed their relationships with their animals. Most mahouts considered their elephants as family members; a slight majority of these questioned the ethics of use of elephants in tourism. Tiger caregivers classified tigers as family or friend equally often; one-third of tiger caregivers declined answering on their approval of using tigers in tourism. What to do with aging tigers is a problem; this may explain some tiger caregivers' reticence to answer questions about using young tigers in tourism. While solving some problems, animal tourism creates several challenges.

Hewavithana, D. K., M. R. Wijesinghe and P. V. Udagama (2022). "Gastrointestinal parasites of six large mammals in the Wasgomuwa National Park, Sri Lanka." Int J Parasitol Parasites Wildl **17**: 1-6.

Gastrointestinal (GI) parasites may impose detrimental consequences on wildlife populations due to their capacity to cause mortality and reduce fitness. Additionally, wild animals play an important role in the transmission of zoonoses. Despite this importance, information on GI parasites of tropical wild mammals is critically lacking. The present study aimed to document GI parasites of six wild-dwelling large mammal taxa in Sri Lanka: Asian elephant (Elephas maximus), Sloth bear (Melursus ursinus), civet (Paradoxurus sp.), Leopard (Panthera pardus), Grey langur (Semnopithecus priam) and buffalo (Bubalus sp). Fresh faecal samples (n = 56) collected from the Wasgomuwa National Park, Sri Lanka were subjected to coprological examination using

faecal smears, and the brine floatation technique followed by microscopic identification; quantitative data were accrued using the formol-ether method. The survey revealed a high prevalence of GI parasites, where 86% (48/56) of faecal samples screened positive for parasitic infections. Faecal samples of the civet, buffalo and Leopard recorded 100% prevalence, while the lowest (40%) was recorded for the Grey langur. Eight types of GI parasites were documented: protozoan cysts, platyhelminth ova (three types of digenean and a single cyclophillidean type), nematode ova (strongyle, strongyloid, ascarid, and trichuroid types) and rhabditiform larvae. The buffaloes and civets had a comparatively high number and diversity of GI parasites (buffalo: 7 types, H' = 1.02; civet: 6 types, H' = 1.52), whilst only a single type (digenean) was detected in the Grey langur. Likewise, parasite loads were also highly variable; highest in the bear (486 per g faeces) and lowest in the monkey (10 per g faeces). The outcome of this survey is important on two accounts; i) to fill the knowledge gap on GI parasites of tropical wild mammals, and ii) the revelation of many first-time parasite-host records for some of the threatened wild-dwelling large mammals in Sri Lanka.

Hoornweg, T. E., V. P. Perera, R. N. S. Karunarathne, W. Schaftenaar, T. A. N. Mahakapuge, A. W. Kalupahana, V. Rutten and C. A. M. de Haan (2022). "Young elephants in a large herd maintain high levels of elephant endotheliotropic herpesvirus-specific antibodies and do not succumb to fatal haemorrhagic disease." Transboundary and Emerging Diseases **69**(5): E3379-E3385.

Elephant endotheliotropic herpesviruses (EEHVs) have co-existed with elephants for millions of years, yet may cause fatal haemorrhagic disease (EEHV-HD), typically in elephants between 1 and 10 years of age. EEHV is omnipresent in (sub)adult elephants, and young elephants with low EEHV-specific antibody levels are at risk for EEHV-HD, suggesting that fatal disease may occur due to an insufficiently controlled primary infection. To further address this hypothesis, sera of three large elephant cohorts were subjected to a multiple EEHV species ELISA: (I) 96 Asian elephants between 0 and 57 years, including 13 EEHV-HD fatalities, from European zoo herds typically sized five to six elephants, (II) a herd of 64 orphaned elephants aged 0-15 years at the Elephant Transit Home in Sri Lanka and (III) 31 elephants aged 8-63 years, part of a large herd of 93 elephants at Pinnawala Elephant Orphanage, Sri Lanka. All Sri Lankan elephants showed high EEHV-specific antibody levels regardless of their age. While antibody levels of most European zoo elephants were comparable to those of Sri Lankan elephants, the average antibody level of the European juveniles (1-5 years of age) was significantly lower than those of age-matched Sri Lankan individuals. Moreover, the European juveniles showed a gradual decrease between 1 and 4 years of age, to be attributed to waning maternal antibodies. Maintenance of high levels of antibodies in spite of waning maternal antibodies in young Sri Lankan elephants is likely due to the larger herd size that increases the likelihood of contact with EEHV-shedding elephants. Together with the observation that low levels of EEHV-specific antibodies correlate with increased numbers of EEHV-HD fatalities, these results suggest that infection in presence of high maternal antibody levels may protect calves from

developing EEHV-HD, while at the same time activating an immune response protective in future encounters with this virus.

Horrell, H. D., A. Lindeque, A. P. Farrell, R. S. Seymour, C. R. White, K. M. Kruger and E. P. Snelling (2022). "Relationship between capillaries, mitochondria and maximum power of the heart: a meta-study from shrew to elephant." Proc Biol Sci **289**(1968): 20212461.

This meta-study uses phylogenetic scaling models across more than 30 species, spanning five orders of magnitude in body mass, to show that cardiac capillary numerical density and mitochondrial volume density decrease with body mass raised to the -0.07 ± 0.03 and -0.04 ± 0.01 exponents, respectively. Thus, while an average 10 g mammal has a cardiac capillary density of approximately 4150 mm(-2) and a mitochondrial density of 33%, a 1 t mammal has considerably lower corresponding values of 1850 mm(-2) and 21%. These similar scaling trajectories suggest quantitative matching for the primary oxygen supply and oxygen consuming structures of the heart, supporting economic design at the cellular level of the oxygen cascade in this aerobic organ. These scaling trajectories are nonetheless somewhat shallower than the exponent of -0.11 calculated for the maximum external mechanical power of the cardiac tissue, under conditions of heavy exercise, when oxygen flow between capillaries and mitochondria is probably fully exploited. This mismatch, if substantiated, implies a declining external mechanical efficiency of the heart with increasing body mass, whereby larger individuals put more energy in but get less energy out, a scenario with implications for cardiovascular design, aerobic capacity and limits of body size.

Hota, S. R., S. K. Padhi, A. Pahari, B. K. Behera, B. Panda, S. K. Mor, V. K. Singh, S. M. Goyal and N. Sahoo (2022). "Characterization and Whole Genome Sequencing of Chromobacterium violaceum OUAT_2017: A Zoonotic Pathogen Found Fatal to a Wild Asiatic Elephant." Indian J Microbiol **62**(4): 627-633.

This study reports a rare fatal case of Chromobacterium violeceum OUAT_2017 strain infection in an Asiatic elephant calf in India. Necropsy revealed pus-filled nodules in liver, spleen, and lungs. Nutrient broth cultures of nodule content showed sediment of violet pigment whereas smooth, non-diffusible, violet-pigmented, homogeneous colonies appeared on nutrient agar. The organism was found to be non-haemolytic and resistant to 8 of the 24 antibiotics tested in vitro. Partial 16S rRNA gene sequence measuring 1410 bp revealed 97% homology with C. violeceum. The bacterial genome composed of 64.87% of G + C content with total size of 4,681,202 bp. The genome annotation has 42 genes responsible for multidrug antibiotic resistance with the presence of Aminoglycoside-modifying enzymes (AAC (6')) that targets streptomycin and spectinomycin. Our findings corroborated the lethal effect of C. violeceum in a new host (elephant) that enriched scientific information on epidemiological picture and whole genome sequencing as well. SUPPLEMENTARY INFORMATION: The online version contains supplementary material available at 10.1007/s12088-022-01047-4.

Huang, R. M., R. J. van Aarde, S. L. Pimm, M. J. Chase and K. Leggett (2022). "Mapping potential connections between Southern Africa's elephant populations." PLoS ONE **17**(10): e0275791.

Southern Africa spans nearly 7 million km2 and contains approximately 80% of the world's savannah elephants (Loxodonta africana) mostly living in isolated protected areas. Here we ask what are the prospects for improving the connections between these populations? We combine 1.2 million telemetry observations from 254 elephants with spatial data on environmental factors and human land use across eight southern African countries. Telemetry data show what natural features limit elephant movement and what human factors, including fencing, further prevent or restrict dispersal. The resulting intersection of geospatial data and elephant presences provides a map of suitable landscapes that are environmentally appropriate for elephants and where humans allow elephants to occupy. We explore the environmental and anthropogenic constraints in detail using five case studies. Lastly, we review all the major potential connections that may remain to connect a fragmented elephant metapopulation and document connections that are no longer feasible.

Huffman, M. A. (2022). "Folklore, Animal Self-Medication, and Phytotherapy-Something Old, Something New, Something Borrowed, Some Things True." Planta Med **88**(3-04): 187-199.

The use of medicines was long considered by Western schools of thought to be a a domain unique to humans; however, folklore/Traditional Ecological Knowledge (TEK) from around the world suggests that animals have also long provided inspiration for the discovery of some medicinal plants used to treat humans and their livestock. Searching for medicinal knowledge from animals depends on the recognition of their ability to select and effectively use medicinal plants to prevent or actively ameliorate disease and other homeostatic imbalances. The interdisciplinary field of animal self-medication is providing scientific evidence for this ability in species across the animal kingdom and lends support to animal-origin medicinal plant folklore and recent ethnomedicinal information. Here, 14 case studies of purported animal-inspired plant medicines used by cultures around the world are presented together with ethnomedicinal and pharmacological evidence. Based on this evidence, the diversity and potential mode of self-medicative behaviors are considered. Over 20 animal species, including llama, sloth and jaguar in South America, reindeer and yak in Eurasia, langur and macaque in Asia, and chimpanzee, wild boar, porcupine and elephant in Africa, are linked to these case studies, representing a variety of potential preventative or therapeutic self-medicative behaviors. These examples provide an important perspective on what is likely to have been a much wider practice in the development of human traditional medicine. A role for animal self-medication research in the rejuvenation of old therapies and possible new discoveries of phytotherapies for human and livestock health is encouraged.

Ishikawa, S., Y. Ozeki, S. Suga, Y. Mukai, H. Kobayashi, E. Inouchi, S. A. Kaboso, G. Gebretsadik, D. Dewi, A. Nishiyama, Y. Tateishi, H. Takihara, S. Okuda, S.

Yoshida, N. Misawa and S. Matsumoto (2022). "Monitoring IgG against Mycobacterium tuberculosis " Sci Rep **12**(1): 4310.

Tuberculosis (TB) is fatal in elephants, hence protecting elephants from TB is key not only in the conservation of this endangered animal, but also to prevent TB transmission from elephants to humans. Most human TB cases arise from long-term asymptomatic infections. Significant diagnostic challenges remain in the detection of both infection and disease development from latency in elephants due to their huge bodies. In this study, we assessed cryopreserved sera collected for over 16 years, from the first Japanese treatment case of elephant TB. Semi-quantification of IgG levels to 11 proteins showed high detection levels of 3 proteins, namely ESAT6/CFP10, MPB83 and Ag85B. The level of IgG specific to these 3 antigens was measured longitudinally, revealing high and stable ESAT6/CFP10 IgG levels regardless of onset or treatment. Ag85B-specifc IgG levels were largely responsive to onset or treatment, while those of MPB83 showed intermediate responses. These results suggest that ESAT6/CFP10 is immunodominant in both asymptomatic and symptomatic phases, making it useful in the detection of infection. On the other hand, Ag85B has the potential to be a marker for the prediction of disease onset and in the evaluation of treatment effectiveness in elephants.

Ishikawa, S., Y. Ozeki, S. Suga, Y. Mukai, H. Kobayashi, E. Inouchi, S. A. Kaboso, G. Gebretsadik, D. N. S. S. Dewi, A. Nishiyama, Y. Tateishi, H. Takihara, S. Okuda, S. Yoshida, N. Misawa and S. Matsumoto (2022). "Monitoring IgG against Mycobacterium tuberculosis proteins in an Asian elephant cured of tuberculosis that developed from long-term latency." Scientific Reports **12**(1).

Iyer, M. L., C. M. Molter, J. P. Flanagan, K. L. Bauer, R. Bernardy, D. Hoffman, L. Parkinson, B. M. Brainard, T. S. Evans, T. Pursell and P. D. Ling (2022). "NOVEL DIAGNOSTIC AND THERAPEUTIC APPROACHES TO ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS 1A HEMORRHAGIC DISEASE IN A CAPTIVE JUVENILE ASIAN ELEPHANT (ELEPHAS MAXIMUS)." J Zoo Wildl Med **53**(1): 232-240.

Novel diagnostic and therapeutic methods were utilized in the successful management of severe elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD) in a 1.9-yr-old captive Asian elephant (Elephas maximus). High levels of EEHV1A viremia were detected for 12 d. In addition to established EEHV treatments, therapies included famciclovir-fortified elephant whole blood and plasma, mesenchymal stem cells harvested from elephant umbilical tissue, and aminocaproic acid. Testing conducted to examine the effects of EEHV infection on hemostasis suggested marked intravascular coagulation with decreased plasminogen activity and increased D-dimer concentrations. Thromboelastography was used to assess the efficacy of aminocaproic acid and demonstrated hypofibrinolysis on samples taken after drug administration, as compared with samples from healthy adult Asian elephants. A serological assay for a novel EEHV1A-specific antibody marker (E52) was developed due to lack of seroconversion to a previously established EEHV1A-specific antibody marker (ORFQ) and showed a sustained increase after EEHV-HD illness.

Jacobs, B., H. Rally, C. Doyle, L. O'Brien, M. Tennison and L. Marino (2022). "Putative neural consequences of captivity for elephants and cetaceans." Rev Neurosci **33**(4): 439-465.

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.

Jacobson, S. L., A. Puitiza, R. J. Snyder, A. Sheppard and J. M. Plotnik (2022). "Persistence is key: investigating innovative problem solving by Asian elephants using a novel multi-access box." Anim Cogn **25**(3): 657-669.

Innovative problem solving is considered a hallmark measure of behavioral flexibility as it describes behavior by which an animal manipulates its environment in a novel way to reach a goal. Elephants are a highly social taxa that have demonstrated a remarkable capacity for adapting to changing environments. To understand how individual differences in behavior impact expressions of innovation, we used a novel extractive foraging device comprised of three compartments to evaluate innovation in 14 captive Asian elephants. In the first phase of testing, elephants had an opportunity to learn one solution, while the second phase gave them an opportunity to innovate to open two other compartments with different solutions. We measured the behavioral traits of neophilia, persistence, motivation, and exploratory diversity, and hypothesized that higher levels of each would be associated with more success in the second phase. Eight elephants innovated to solve three compartments, three solved two, and two solved only one. Consistent with studies in other species, we found that higher success was associated with greater persistence, but not with any other behavioral traits when analyzed per test session. Greater persistence and, unexpectedly, lower exploratory diversity, were associated with success when analyzed at the level of each individual door. Further work is needed to understand how innovation varies both within and between species, with particular attention to the potential impact of anthropogenic changes in wild environments.

Jamieson, C. A., S. L. Baillie and J. P. Johnson (2022). "Blood Transfusion in Equids-A Practical Approach and Review." Animals **12**(17): 16.

Simple Summary Transfusion medicine is an accessible, technically simple, and often lifesaving tool that can be used in both field and hospital settings, in cases of significant bleeding or anemia. A thorough understanding of the indications, methodology and complications of blood transfusion allows the practitioner to identify cases where administration of whole blood is necessary, and how to safely perform the transfusion. This review collects the current literature surrounding blood transfusion into one readily accessible document to allow clinicians a comprehensive understanding of all aspects of equine blood transfusion, while serving as a reference for performing these procedures. Transfusion medicine is a crucial part of equine intensive and critical care. Blood transfusions can save lives in both acute and chronic cases of anemia, hemorrhage, and hemolysis. It is vital to have a comprehensive theoretical and practical understanding of the techniques, implications, risks, and complications. This review covers the physiology and pathophysiology of conditions requiring transfusion, as well as step by step guidance for practitioners of all experience levels. This review is designed to serve as a practical reference for those who are treating horses in either the field or hospital setting. It aims to provide both theoretical background and easy to locate formulae with guidance that is easy to refer to in a critical situation. When risks and benefits are well understood, these techniques can be confidently employed in critical situations to improve outcomes and save lives.

Jesus, S. A., A. Schmidt, J. Fickel, M. G. Doherr, K. Boonprasert, C. Thitaram, L. Sariya, P. Ratanakron and T. B. Hildebrandt (2022). "Assessing Coagulation Parameters in Healthy Asian Elephants (Elephas maximus) from European and Thai Populations." Animals (Basel) **12**(3).

The Asian elephant population is continuously declining due to several extrinsic reasons in their range countries, but also due to diseases in captive populations worldwide. One of these diseases, the elephant endotheliotropic herpesvirus (EEHV) hemorrhagic disease, is very impactful because it particularly affects Asian elephant calves. It is commonly fatal and presents as an acute and generalized hemorrhagic syndrome. Therefore, having reference values of coagulation parameters, and obtaining such values for diseased animals in a very short time, is of great importance. We analyzed prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen concentrations using a portable and fast point-of-care analyzer (VetScan Pro) in 127 Asian elephants from Thai camps and European captive herds. We found significantly different PT and aPTT coagulation times between elephants from the two regions, as well as clear differences in fibrinogen concentration. Nevertheless, these alterations were not expected to have biological or clinical implications. We have also sequenced the coagulation factor VII gene of 141 animals to assess the presence of a previously reported hereditary coagulation disorder in Asian elephants and to investigate the presence of other mutations. We did not find the previously reported mutation in our study population. Instead, we discovered the

presence of several new single nucleotide polymorphisms, two of them being considered as deleterious by effect prediction software.

Jones, S. A., M. B. Whitcomb, B. Vaughan, G. Goorchenko, R. Busch, I. Kilcoyne and M. Spriet (2022). "Ultrasonographic diagnosis of femoral fractures in large animals." J Am Vet Med Assoc **260**(13): 1675-1682.

OBJECTIVE: Femoral fractures are often catastrophic in large animals. Radiographic diagnosis is limited by patient size and feasibility, especially in ambulatory settings. Ultrasonography is widely available and may provide an alternative to radiography for definitive diagnosis. ANIMALS: 12 large animals (6 horses, 5 cattle, and 1 elephant). PROCEDURES: Retrospective analysis of large animal patients diagnosed with femoral fracture by use of femoropelvic ultrasonography (2000 to 2019). RESULTS: 5 of 12 cases were ≤ 1 year of age. The remaining 7 cases were 2 to 33 years of age (median, 13 years). All patients developed severe acute lameness after falling (n = 4), limb entrapment (2), dystocia (1), vehicular collision (1), ipsilateral full limb casting (1), or unknown events (3). All were non-weight-bearing or lame at the walk, including 2 recumbent cattle. Ten cases showed upper limb swelling that was variable in location, and 3 had nonspecific upper limb crepitus. Ultrasonography revealed evidence of diaphyseal (n = 6), greater trochanteric (2), capital physeal (2), and distal femoral (2) fractures. Fracture movement during limb manipulation or weight shifting was sonographically visualized in 5 animals. Radiography confirmed fractures in 3 of 8 animals: 2 bovines with distal femoral fractures detected on standing projections and 1 capital physeal fracture that required ventrodorsal projections under general anesthesia. All animals were euthanized (11) or slaughtered (1 bovine). Postmortem examination confirmed ultrasonographic findings in 10 of 10 necropsied animals. CLINICAL RELEVANCE: Femoral fractures were not localized nor confirmed in any case prior to ultrasonography. Study findings supported the use of ultrasonography for rapid patient-side diagnosis, prognostication, and decision-making in suspect cases.

Kaufmann, L. V., U. Schneeweiß, E. Maier, T. Hildebrandt and M. Brecht (2022). "Elephant facial motor control." Sci Adv **8**(43): eabq2789.

We studied facial motor control in elephants, animals with muscular dexterous trunks. Facial nucleus neurons (~54,000 in Asian elephants, ~63,000 in African elephants) outnumbered those of other land-living mammals. The large-eared African elephants had more medial facial subnucleus neurons than Asian elephants, reflecting a numerically more extensive ear-motor control. Elephant dorsal and lateral facial subnuclei were unusual in elongation, neuron numerosity, and a proximal-to-distal neuron size increase. We suggest that this subnucleus organization is related to trunk representation, with the huge distal neurons innervating the trunk tip with long axons. African elephants pinch objects with two trunk tip fingers, whereas Asian elephants grasp/wrap objects with larger parts of their trunk. Finger "motor foveae" and a positional bias of neurons toward the trunk tip representation in African elephant facial nuclei reflect their motor strategy.

Thus, elephant brains reveal neural adaptations to facial morphology, body size, and dexterity.

Khammesri, S., C. Ampasavate, D. Hongwiset, R. Mektrirat, S. Sangsrijan, J. L. Brown and C. Thitaram (2022). "Pharmacokinetics and analytical determination of acyclovir in Asian elephant calves (Elephas maximus)." Vet Anim Sci **15**: 100227.

A therapeutic regimen that includes antiviral drugs is critical for the survival of Asian elephant (Elephas maximus) calves infected with elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD), with acyclovir showing considerable promise. The purpose of this study was to determine the pharmacokinetics and bioavailability of acyclovir following intravenous (IV) and oral (PO) administration in Asian elephants. A single dose of acyclovir (15 mg/kg, IV or 45 mg/kg, PO) was administered to four healthy elephant calves, with a minimum 2-week washout period between treatments. Serial plasma samples were collected after each injection for acyclovir analysis using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique. Maximum plasma acyclovir concentrations were 27.02 ± 6.79 μg/mL at 0.94 ± 0.31 h after IV administration, and 1.45 ± 0.20 μg/mL at 3.00 ± 0.70 h after PO administration. The half-life of the elimination phase (T(1/2)) was 5.84 ± 0.74 and 8.74 ± 2.47 h after IV and PO administration, respectively. After IV administration, acyclovir concentrations were higher than the half-maximal inhibitory concentration (IC(50)) of those found for herpes simplex virus (HSV) 1 and 2 in humans, and equid alpha herpesvirus-1 (EHV-1) for at least 12 h. By contrast, the bioavailability of oral administration was low, only 6.03 ± 0.87%, so higher doses by that route likely are needed to be effective. Due to the high concentration of plasma acyclovir after IV administration, the dose may need to be adjusted to prevent any negative side effects.

Kislaya, I., E. F. Rodrigues, V. Borges, J. Gomes, C. Sousa, J. Almeida, A. Peralta-Santos and B. Nunes (2022). "Comparative Effectiveness of Coronavirus Vaccine in Preventing Breakthrough Infections among Vaccinated Persons Infected with Delta and Alpha Variants." Emerging Infectious Disease journal **28**(2).

We developed a case–case study to compare mRNA vaccine effectiveness against Delta versus Alpha coronavirus variants. We used data on 2,097 case-patients with PCR-positive severe acute respiratory syndrome coronavirus 2 infections reported in Portugal during May–July 2021. We estimated the odds of vaccine breakthrough infection in Delta-infected versus Alpha-infected patients by using conditional logistic regression adjusted for age group and sex and matched by the week of diagnosis. We compared reverse-transcription PCR cycle threshold values by vaccination status and variant as an indirect measure of viral load. We found significantly higher odds of vaccine breakthrough infection in Delta-infected patients than in Alpha-infected patients (odds ratio 1.96 [95% CI 1.22–3.14]), suggesting lower effectiveness of the mRNA vaccines in preventing infection with the Delta variant. We estimated lower mean cycle threshold values for the Delta cases (mean difference −2.10 [95% CI −2.74 to −1.47]), suggesting higher

infectiousness than the Alpha variant.

Kottwitz, J. J., W. Kiso, D. M. Boothe and D. Schmitt (2022). "Administration of Altrenogest to Maintain Pregnancy in Asian Elephants (Elephas maximus)." Animals (Basel) **12**(14).

Progesterone and progesterone derivatives are key hormones in pregnancy maintenance in mammalian species. Cessation of pregnancy, including birth or miscarriage, is certain if levels of these hormones drop below a given species-specific requirement necessary to maintain pregnancy. The synthetic progestin, altrenogest, is FDA-approved in the United States for suppression of estrus or synchronization and is administered extra-label to multiple species to maintain pregnancies in cases of luteal deficiency or otherwise abnormally low progesterone levels. Three pregnant Asian elephants received altrenogest from 41 to 131 days during the final trimester of pregnancy, with parturition occurring from 15 to 31 days after altrenogest administration stopped. A single dose of 0.2 mg/kg altrenogest administered to two nonpregnant Asian elephants provided pilot pharmacokinetic data. Serum samples from two of the three clinical cases and the two pilot study elephants were analyzed using Ultra Performance Liquid chromatography coupled to a triple quadruple mass spectrometer (UPLC-MS). Small sample numbers limited analysis; however, the following were determined: AUC∞ of 635.4 ± 73.8 ng*h/mL, Cmax of 30.2 ± 14.4 ng/mL at a Tmax of 4 ± 2.8 h, terminal T1/2 of 47.5 ± 3.0 h, MRT of 36.0 + 3.4 h and Vd/F of 1243.8 + 275.0 L/kg. These data and the three described cases serve as an indication that altrenogest can be administered to Asian elephants as an exogenous progestin to support pregnancy in elephant pregnancies with low endogenous progestin levels.

Kudo, Y., H. Suzuki, M. K. Kaneko and Y. Kato (2022). "Development of a Monoclonal Antibody PMab-295 Against Elephant Podoplanin." Monoclon Antib Immunodiagn Immunother **41**(4): 194-201.

Podoplanin (PDPN) is an essential marker of lung type I alveolar cells, kidney podocytes, and lymphatic endothelial cells. Monoclonal antibodies (mAbs) that can specifically recognize PDPN in immunohistochemistry are important to analyze the development of tissues and the pathogenesis of diseases, including cancers. We have developed anti-PDPN mAbs against many animal species; however, mAbs that can recognize elephant-derived membrane proteins and distinguish the specific cell types in immunohistochemistry are limited. In this study, a novel anti-elephant PDPN (elePDPN) mAb, PMab-295 (IgG(1), kappa), was established using the peptide immunization method. PMab-295 recognized both elePDPN-overexpressed Chinese hamster ovary (CHO)-K1 cells and endogenous elePDPN-expressed LACF-NaNaI cells by flow cytometry and western blotting. Kinetic analyses using flow cytometry showed that the K(D) of PMab-295 for CHO/elePDPN was 1.5 × 10(-8) M. Furthermore, PMab-295 detected elePDPN-expressing cells using immunohistochemistry. These results showed the usefulness of PMab-295 to investigate the molecular function of elePDPN and the pathogenesis of diseases.

LaDue, C. A., R. P. G. Vandercone, W. K. Kiso and E. W. Freeman (2022). "Social Behavior and Group Formation in Male Asian Elephants (Elephas maximus): The Effects of Age and Musth in Wild and Zoo-Housed Animals." Animals (Basel) **12**(9).

Asian elephants are endangered, and the long-term viability of the species depends on integrative approaches to address the sustainability of in-situ and ex-situ populations. Growing evidence shows that male elephants exhibit extensive and flexible social behavior that rivals the complexity of that of females. Male elephant sociality is expected to change dramatically around the unique sexual state of musth. However, data related to male Asian elephant sociality is lacking. Here, we conducted complementary observations in Wasgamuwa National Park, Sri Lanka, and North American zoos of male Asian elephant social behavior. Age and musth status, along with other factors, were associated with variation in social behavior and group formation of males. In wild male elephants, both musth status and age impacted elephant associations within all-male and mixed-sex groups: non-musth elephants were generally sighted less often in mixed-sex groups as they aged, while the inverse occurred with musth elephants. Musth status interacted with age to predict the number of conspecifics with which a wild male elephant associated: younger males were observed with more females during non-musth (but the opposite was true during musth), and male elephants between 20 and 30 years were observed with the highest number of male conspecifics except during musth. Finally, we found variation in aggression, prosocial behavior, and submissive behavior was influenced by intrinsic (age and musth status) and extrinsic factors (group size and type) in similar ways in both populations; prosocial behavior was most common and was influenced by the number of conspecifics present (both populations), and age, group type, and musth status (zoo population), while aggression was rare, especially among older elephants. We suggest that longitudinal studies of this threatened species will be particularly helpful to promote the reproduction and conservation of Asian elephants in in-situ and ex-situ environments.

Lalande, L. D., V. Lummaa, H. H. Aung, W. Htut, U. K. Nyein, V. Berger and M. Briga (2022). "Sex-specific body mass ageing trajectories in adult Asian elephants." J Evol Biol **35**(5): 752-762.

In species with marked sexual dimorphism, the classic prediction is that the sex which undergoes stronger intrasexual competition ages earlier or quicker. However, more recently, alternative hypotheses have been put forward, showing that this association can be disrupted. Here, we utilize a unique, longitudinal data set of a semi-captive population of Asian elephants (Elephas maximus), a species with marked male-biased intrasexual competition, with males being larger and having shorter lifespans, and investigate whether males show earlier and/or faster body mass ageing than females. We found evidence of sex-specific body mass ageing trajectories: adult males gained weight up to the age of 48 years old, followed by a decrease in body mass until natural death. In contrast, adult females gained body mass with age until a body mass decline in the last year of life. Our

study shows sex-specific ageing patterns, with an earlier onset of body mass declines in males than females, which is consistent with the predictions of the classical theory of ageing.

Lefeuvre, M., P. Gouat, B. Mulot, R. Cornette and E. Pouydebat (2022). "Analogous laterality in trunk movements in captive African elephants: A pilot study." Laterality **27**(1): 101-126.

Lateralization of hand use in primates has been extensively studied in a variety of contexts, and starts to be investigated in other species and organs in order to understand the evolution of the laterality according to different tasks. In elephants, the orientation of the movements of the trunk has been observed mainly in feeding and social contexts, in free conditions. However, little is known about the influence of task complexity on trunk laterality. In this study, we compared the lateralization of the trunk in two conditions: standardized and free. We offered granules to six African elephants on each side of an opened trapdoor to create a constraining environment and reported the different behaviours employed and their orientation. In addition, we observed the same individuals in free conditions and noted the lateralization of the use of their trunk. We revealed a common right side preference in all our elephants, both in standardized and free conditions. This side bias was stronger in our constraining task, adding evidence for the task complexity theory. We finally described laterality in new behaviours in the literature on elephants, such as pinching, gathering or exploration with the trunk.

Lesku, J. A. and N. C. Rattenborg (2022). "The missing cost of ecological sleep loss." Sleep Adv **3**(1): zpac036.

Sleep serves many important functions. And yet, emerging studies over the last decade indicate that some species routinely sleep little, or can temporarily restrict their sleep to low levels, seemingly without cost. Taken together, these systems challenge the prevalent view of sleep as an essential state on which waking performance depends. Here, we review diverse case-studies, including elephant matriarchs, post-partum cetaceans, seawater sleeping fur seals, soaring seabirds, birds breeding in the high Arctic, captive cavefish, and sexually aroused fruit flies. We evaluate the likelihood of mechanisms that might allow more sleep than is presently appreciated. But even then, it appears these species are indeed performing well on little sleep. The costs, if any, remain unclear. Either these species have evolved a (yet undescribed) ability to supplant sleep needs, or they endure a (yet undescribed) cost. In both cases, there is urgent need for the study of non-traditional species so we can fully appreciate the extent, causes, and consequences of ecological sleep loss.

Li, G., Y. Jiang, Q. Li, D. An, M. Bao, L. Lang, L. Han, X. Huang and C. Jiang (2022). "Comparative and functional analyses of fecal microbiome in Asian elephants." Antonie Van Leeuwenhoek **115**(9): 1187-1202.

Asian elephant is large herbivorous animal with elongated hindgut. To explore fecal microbial community composition with various ages, sex and

diets, and their role in plant biomass degrading and nutrition conversation. We generated 119 Gb by metagenome sequencing from 10 different individual feces and identified 5.3 million non-redundant genes. The comprehensive analysis established that the Bacteroidetes, Firmicutes and Proteobacteria constituted the most dominant phyla in overall fecal samples. In different individuals, the alpha diversity of the fecal microbiota in female was lower than male, and the alpha diversity of the fecal microbiota in older was higher than younger, and the fecal microbial diversity was the most complex in wild elephant. But the predominant population compositions were similar to each other. Moreover, the newborn infant elephant feces assembled and maintained a diverse but host-specific fecal microbial population. The discovery speculated that Asian elephant maybe have start to building microbial populations before birth. Meanwhile, these results illustrated that host phylogeny, diets, ages and sex are significant factors for fecal microbial community composition. Therefore, we put forward the process of Asian elephant fecal microbial community composition that the dominant populations were built first under the guidance of phylogeny, and then shaped gradually a unique and flexible gut microbial community structure under the influences of diet, age and sex. This study found also that the Bacteroidetes were presumably the main drivers of plant fiber-degradation. A large of secondary metabolite biosynthetic gene clusters, and genes related to enediyne biosynthesis were found and showed that the Asian elephant fecal microbiome harbored a diverse and abundant genetic resource. A picture of antibiotic resistance genes (ARGs) reservoirs of fecal microbiota in Asian elephants was provided. Surprisingly, there was such wide range of ARGs in newborn infant elephant. Further strengthening our speculation that the fetus of Asian elephant has colonized prototypical fecal microbiota before birth. However, it is necessary to point out that the data give a first inside into the gut microbiota of Asian elephants but too few individuals were studied to draw general conclusions for differences among wild and captured elephants, female and male or different ages. Further studies are required. Additionally, the cultured actinomycetes from Asian elephant feces also were investigated, which the feces of Asian elephants could be an important source of actinomycetes.

Loeb, J. (2022). "Defra's captive elephant report delayed." Vet Rec **191**(2): 57.

Lueders, I. and C. Stremme (2022). "Construction of a full mouth speculum facilitating oral examinations, bronchoscopy and gastric tubing in elephants." Tierarztl Prax Ausg G Grosstiere Nutztiere **50**(2): 86-90.

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 × 8.0 cm attached between 2 threaded guiding poles (40 cm). 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 (6,16 Asian elephants, and 1,3 African 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 veterinarains 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.

Luo, L., X. Wang, H. Guo, L. Zhu, Y. Ma, R. Yang, S. Wang, G. Wang, M. Wang, J. Shao and C. Liu (2022). "Eighteen years (2001-2018) of forest habitat loss across the Asian elephant's range and its drivers." Sci Bull (Beijing) **67**(15): 1513-1516.

Madsen, A. E., C. Minge, T. V. Pushpakumara, U. S. Weerathunga, U. K. Padmalal, D. K. Weerakoon and S. de Silva (2022). "Strategies of protected area use by Asian elephants in relation to motivational state and social affiliations." Sci Rep **12**(1): 18490.

Animals' space requirements may vary according to life-history and social considerations. We observed 516 wild adult Asian elephants from both sexes, over 9 years, to investigate how life-history traits and social behavior influence protected-area (PA) use at Udawalawe National Park, Sri Lanka. Male PA-use, quantified in terms of average between-sightings-interval (BSI), was significantly influenced by the interaction of age class and motivational state (i.e. reproduction vs. foraging). Musth lengthened with age, with a median of 24.5 days for ages 21-30, 32.5 days for ages 31-40, and 45 days for those > 40. A minority (11%) used it exclusively during musth, while others used it exclusively for foraging (44%) or both (45%). Males using it in both states and older musth-only males were more likely to be seen across years. There were 16 social communities containing between 2-22 adult females. Females' BSI was significantly influenced by social ties, but this relationship was weak, because members of social communities do not necessarily disperse together, resulting in high individual variation in space-use. Inter-annual variability in sightings among individuals of both sexes indicates that around ¾ of the population is likely non-residential across years, challenging the prevailing fortress-conservation paradigm of wildlife management.

Makecha, R. N., S. Phalke and Y. Nakai (2022). "Assessing the Effects of a Cognition-Based Education Program on Attitudes of Villagers Toward Asian Elephants (Elephas maximus) in Conflict-Prone Areas." J Appl Anim Welf Sci **25**(4): 368-381.

A vital role in mitigating human-elephant conflict (HEC) involves conservation education programs in local communities. It is therefore important to assess the types of information that make conservation education programs effective. Given the public's fascination with animal minds, the elephant being a cognitively complex species, and the high occurrence of HEC surrounding Asian elephants, the current research assessed whether using information on elephant cognition in a conservation education program increased positive attitudes toward elephants/elephant conservation in Bannerghatta National Park (BNP). BNP, located in Karnataka, India, is an area reporting high HEC. Results indicated no significant difference in adult male villagers' attitudes toward elephants/elephant conservation when exposed to one of two educational programs, one of which included information on elephant cognition. However, a significant difference in attitudes between the two programs and a control group was discovered, suggesting the importance of an educational intervention in the communities surrounding BNP.

Makumbe, P., S. Mapurazi, S. Jaravani and I. Matsilele (2022). "Human-Wildlife Conflict in Save Valley Conservancy: Residents' Attitude Toward Wildlife Conservation." Scientifica (Cairo) **2022**: 2107711.

Human settlement in protected areas (PAs) is a major conservation concern in developing nations as it fuels human-wildlife conflicts (HWCs). The objectives of this study were to (i) determine the key wildlife species causing conflict, (ii) assess the perceptions of residents toward the major causes of conflict with wildlife, and (iii) evaluate the attitudes of residents toward problem animals. We conducted face-to-face semistructured interviews and two reconnaissance field surveys with 290 respondents residing in Save Valley Conservancy (SVC), in Southeast Lowveld Zimbabwe from January 2014 to June 2014. Results showed that lions (Panthera leo), spotted hyenas (Crocuta crocuta), elephants (Loxodonta africana), and Nile crocodiles (Crocodylus niloticus) were the major animals involved in the conflict. Our results also showed that the land-use change from wildlife ranching to farming and contested land ownership were perceived as the major causes of HWCs. Respondents who had lived in the area longer were more likely to agree that change in land use (Ordinal logistic regression: B = 1.32, Odds Ratio (OR) = 3.74) and contested land ownership (B = .67, OR = 1.95) were major sources of conflict. In addition, increased encounters between people and wildlife triggered mixed attitudes toward problem animals. For example, males were less likely to have a negative attitude toward problem animals compared to females (Multinomial logistic regression: B = -1.39; OR = .25). Residents who had stayed for less than five years were more likely to have a negative attitude toward problem animals than those who had stayed longer (B = 3.6; OR = 36.71). These results suggest that there is a need to relook at the resettlement pattern because coordinating HWCs and implementing sustainable conservation objectives are easy in a well-planned settlement. Stakeholders need to come together and create awareness of the use of HWCs mitigations measures.

Mills, G. (2022). "Should elephants be kept in zoos?" Vet Rec **190**(10): 396-397.
Georgina Mills reflects on a recent report from Born Free which calls for an end to keeping elephants in captivity.

Min, J., P. Kim, S. Yun, M. Hong and W. Park (2022). "Zoo animal manure as an overlooked reservoir of antibiotic resistance genes and multidrug-resistant bacteria." Environ Sci Pollut Res Int.
Animal fecal samples collected in the summer and winter from 11 herbivorous animals, including sable antelope (SA), long-tailed goral (LTG), and common eland (CE), at a public zoo were examined for the presence of antibiotic resistance genes (ARGs). Seven antibiotics, including meropenem and azithromycin, were used to isolate culturable multidrug-resistant (MDR) strains. The manures from three animals (SA, LTG, and CE) contained 10(4)-fold higher culturable MDR bacteria, including Chryseobacterium, Sphingobacterium, and Stenotrophomonas species, while fewer MDR bacteria were isolated from manure from water buffalo, rhinoceros, and elephant against all tested antibiotics. Three MDR bacteria-rich samples along with composite samples were further analyzed using nanopore-based technology. ARGs including lnu(C), tet(Q), and mef(A) were common and often associated with transposons in all tested samples, suggesting that transposons carrying ARGs may play an important role for the dissemination of ARGs in our tested animals. Although several copies of ARGs such as aph(3')-IIc, bla(L1), bla(IND-3), and tet(42) were found in the sequenced genomes of the nine MDR bacteria, the numbers and types of ARGs appeared to be less than expected in zoo animal manure, suggesting that MDR bacteria in the gut of the tested animals had intrinsic resistant phenotypes in the absence of ARGs.

Mohd-Radzi, N. H. S., K. V. Karuppannan, N. A. F. Abdullah-Fauzi, A. R. Mohd-Ridwan, N. Othman, A. L. Muhammad Abu Bakar, M. Gani, M. F. A. Abdul-Razak and B. M. Md-Zain (2022). "Determining the diet of wild Asian elephants (Elephasmaximus) at human-elephant conflict areas in Peninsular Malaysia using DNA metabarcoding." Biodivers Data J **10**: e89752.
Human-elephant conflict (HEC) contributes to the increasing death of Asian elephants due to road accidents, retaliatory killings and fatal infections from being trapped in snares. Understanding the diet of elephants throughout Peninsular Malaysia remains crucial to improve their habitat quality and reduce scenarios of HEC. DNA metabarcoding allows investigating the diet of animals without direct observation, especially in risky conflict areas. The aim of this study was to determine: i) the diet of wild Asian elephants from HEC areas in Peninsular Malaysia using DNA metabarcoding and ii) the influence of distinct environmental parameters at HEC locations on their feeding patterns. DNA was extracted from 39 faecal samples and pooled into 12 groups representing the different sample locations: Kuala Koh, Kenyir, Ulu Muda, Sira Batu, Kupang-Grik, Bumbun Tahan, Belum-Temengor, Grik, Kampung Pagi, Kampung Kuala Balah, Aring 10 and the National Elephant Conservation Centre, which served as a positive control for this study. DNA amplification and sequencing targeted the ribulose-bisphosphate carboxylase

gene using the next-generation sequencing Illumina iSeq100 platform. Overall, we identified 35 orders, 88 families, 196 genera and 237 species of plants in the diet of the Asian elephants at HEC hotspots. Ficus (Moraceae), Curcuma (Zingiberaceae), Phoenix (Arecaceae), Maackia (Fabaceae), Garcinia (Clusiaceae) and Dichapetalum (Dichapetalaceae) were the highly abundant dietary plants. The plants successfully identified in this study could be used by the Department of Wildlife and National Parks (PERHILITAN) to create buffer zones by planting the recommended dietary plants around HEC locations and trails of elephants within Central Forest Spine (CFS) landscape.

Molenaar, F. M. and P. Silvestre (2022). "Clinical approach to colic and collapse in an Asian elephant (Elephas maximus) with Salmonella saintpaul septicaemia and subsequent ileus." Veterinary Record Case Reports **10**(1): 7.

An adult female Asian elephant (Elephas maximus) presented with clinical signs of colic unresponsive to analgesia, which progressed to hypothermia and collapse within 48 hours. Repeated sedations using butorphanol and detomidine were performed for initial diagnostic sampling, first aid and subsequent treatment. Initial haematology showed evidence of septicaemia and disseminated intravascular coagulation; urine analysis was consistent with metabolic acidosis. The initial treatment focused on rectal administration of enrofloxacin, metronidazole and fluids. By Day 7, the immune system was recovering as demonstrated by blood parameters but ileus had developed. Sedation interventions were discontinued and treatment consisted of oral ranitidine, fibre provision and rehydration. Salmonella saintpaul was cultured from the faeces and a disease risk analysis identified a possible infection route through food contamination. Serial haematology provided direction in clinical decision making throughout this challenging case.

Montero Botey, M., M. Soliño, R. Perea and M. Martínez-Jauregui (2022). "Let Us Give Voice to Local Farmers: Preferences for Farm-Based Strategies to Enhance Human-Elephant Coexistence in Africa." Animals (Basel) **12**(14).

Local communities surrounding wildlife corridors and natural reserves often face challenges related to human-wildlife coexistence. To mitigate the challenges and ensure the long-term conservation of wildlife, it is important to engage local communities in the design of conservation strategies. By conducting 480 face-to-face interviews in 30 villages along and adjacent to the Selous-Niassa Wildlife Corridor (Tanzania), we quantified farmers' preferences for farm-based measures to mitigate African elephant damage using choice experiments. Results show that farmers considered no action the least preferred option, revealing that they are open to trying different measures. The most preferred management strategy matched with the preferences of wildlife rangers in the area, suggesting low concern about the potential conflicts between stakeholders. However, a latent class model suggests that there are significant differences among responses triggered by farmers' previous experience with elephants, the intensity of the elephant damage, and the socioeconomic situation of the farmer. Results show a marked spatial distribution among respondents, highlighting the benefits of zone management as conflicts were found to be highly context dependent.

Understanding the human dimension of conservation is essential for the successful planification and implementation of conservation strategies. Therefore, the development and broad utilization of methodologies to gather specific context information should be encouraged.

Neupane, D., S. Baral, T. S. Risch and A. Campos-Arceiz (2022). "Broad scale functional connectivity for Asian elephants in the Nepal-India transboundary region." J Environ Manage **321**: 115921.

The Nepal-India transboundary region hosts one of Asia's most complex large mammal assemblages, including a small (but growing) population of Asian elephants (Elephas maximus). These elephants occur in four widespread and geographically disjunct subpopulations, and some of them undergo seasonal transboundary movements. We conducted a broad-scale evaluation of the amount and quality of elephant habitat available in the region and of functional landscape connectivity between and within subpopulations using Maxent, circuit theory, and least-cost path analysis. Habitat suitability was highly influenced by abiotic geographical factors (altitude and precipitation) and less by ecological factors (habitat heterogeneity, plant productivity) and human disturbance (distance to settlements). The region had a relatively small amount of high and optimal suitability habitat (12.6% out of 93,700 km(2)) but all subpopulations seem to be far from carrying capacity, suggesting ample potential for further population growth. Landscape connectivity was higher between and within the west and far-west subpopulations, which should be considered a single subpopulation. The central and ea st subpopulations, however, had low to very low between-subpopulation connectivity. Conservation priorities include maintaining the current connectivity in the west subpopulation and across the border in the east, and protecting high-quality habitats in eastern Nepal. Restoring connectivity between the central and other subpopulations is possible if the number of elephants continues growing, and it should be a long-term conservation aspiration. Maintaining and enhancing landscape connectivity in this region requires transboundary cooperation and coordination between Nepali and Indian authorities. If successful, it will bring considerable benefits for the conservation of elephants and other wildlife.

Nunney, L. (2022). "Cancer suppression and the evolution of multiple retrogene copies of TP53 in elephants: A re-evaluation." Evol Appl **15**(5): 891-901.

Evolving to become bigger and/or longer lived should increase cancer susceptibility, but this predicted increase is not observed, a contradiction named Peto's paradox. A solution is that cancer suppression evolves to minimize cancer susceptibility, and the discovery of 19 retrogene (RTG) copies of the tumor suppressor gene TP53 in the African elephant (Loxodonta africana) is increasingly cited as a classic example of such adaptive suppression. However, classic examples need rigorous evaluation and an alternative hypothesis is that the RTGs spread by genetic drift. This study shows that before its duplication, the ancestral elephant RTG was already truncated from 390 amino acids to 157 by a frameshift mutation, and that 14 of the 19 copies are now truncated to ≤88 amino acids. There was no

compelling evidence of either positive or negative selection acting on these 88 codons, and the pattern of RTG accumulation fits a neutral model with a duplication rate of ~10(-6) per generation. It is concluded that there is no evidence supporting the hypothesis that the 19 elephant RTGs spread to fixation by selection; instead, the evidence indicates that these RTGs accumulated primarily by segmental duplication and drift. It is shown that the evolutionary multistage model of carcinogenesis (EMMC) predicts the recruitment of 1-2 independently acting tumor suppressor genes to suppress the increased cancer risk in elephants, so it is possible that one or a few RTGs may have been favored by selection resulting in the known enhanced sensitivity of elephant cells to DNA damage. However, the analysis does not provide any support for either a direct (via conserved TP53 activity) or indirect (via supporting canonical TP53 function) role of the RTGs sequences, so that the presence of multiple copies of TP53 retrogenes in elephants needs to be further justified before being used as a classic example of tumor suppression in large-bodied animals.

O'Connell-Rodwell, C. E., M. N. Sandri, J. L. Berezin, J. M. Munevar, C. Kinzley, J. D. Wood, M. Wiśniewska and J. W. Kilian (2022). "Male African Elephant (Loxodonta africana) Behavioral Responses to Estrous Call Playbacks May Inform Conservation Management Tools." Animals (Basel) **12**(9).

Driven by reproductive motives, male African elephants (Loxodonta africana) in musth often expand their home ranges to locate estrous females. This extended range, coupled with heightened aggression often observed in musth males, can be particularly problematic in regions where human-modified landscapes and elephant territories increasingly overlap. Several mitigation tools have been tested to resolve a wide range of human-elephant conflicts with varying degrees of success due to geographical disparities and habituation. We present findings on the potential application of estrous call playbacks in manipulating the behavior and movement of male elephants non-invasively, particularly mature musth adults and younger post-dispersal males, in Etosha National Park. Estrous vocalizations were presented across 26 experimental trials to mature musth adults (n = 5), mature non-musth adults (n = 6), and non-musth males belonging to younger, post-dispersal age classes (n = 8), with behavioral responses scored on a gradient scale from 0-1. Both mature musth adults and younger non-musth elephants were significantly more likely to respond with the highest intensity by approaching the acoustic source compared to mature non-musth adults that avoided the call. However, younger males tested in the presence of an older, higher-ranking male tended to react with a lower intensity than those tested alone. This result likely demonstrates the influence of social hierarchy and associations on male elephant behavior. We also observed a significant increase in physiological response, measured by defecation rate, across all male groups in response to the estrous call playbacks. Our findings suggest that using estrous calls as acoustic deterrents may effectively and non-invasively aid in reducing tension at the human-elephant interface, depending on the age, social context, and reproductive status of the male elephant.

Okada, Y., H. Suzuki, M. K. Kaneko and Y. Kato (2022). "Epitope Mapping of an Anti-elephant Podoplanin Monoclonal Antibody (PMab-295) Using Enzyme-Linked Immunosorbent Assay." Monoclon Antib Immunodiagn Immunother **41**(4): 221-227.

Podoplanin (PDPN) is a marker of lung type I alveolar cells, kidney podocytes, and lymphatic endothelial cells. The overexpression of PDPN contributes to the malignant progression of tumors. Therefore, the development of anti-PDPN monoclonal antibodies (mAbs) to animals is essential to evaluate the pathogenesis and cellular functions. Using peptide immunization, we previously developed an anti-elephant PDPN (elePDPN) mAb, PMab-295, which is useful for flow cytometry, Western blotting, and immunohistochemistry. In this study, we determined the critical epitope of PMab-295 by enzyme-linked immunosorbent assay (ELISA). We performed ELISA with the alanine-substituted peptides of elePDPN extracellular domain (amino acids 38-51), and found that PMab-295 did not recognize the alanine-substituted peptides of M41A, P44A, and E47A. Furthermore, these peptides could not inhibit the recognition of PMab-295 to elePDPN-expressing cells by flow cytometry and immunohistochemistry. The results indicate that the binding epitope of PMab-295 includes Met41, Pro44, and Glu47 of elePDPN.

Padariya, M., M. L. Jooste, T. Hupp, R. Fåhraeus, B. Vojtesek, F. Vollrath, U. Kalathiya and K. Karakostis (2022). "The Elephant Evolved p53 Isoforms that Escape MDM2-Mediated Repression and Cancer." Mol Biol Evol **39**(7).

The p53 tumor suppressor is a transcription factor with roles in cell development, apoptosis, oncogenesis, aging, and homeostasis in response to stresses and infections. p53 is tightly regulated by the MDM2 E3 ubiquitin ligase. The p53-MDM2 pathway has coevolved, with MDM2 remaining largely conserved, whereas the TP53 gene morphed into various isoforms. Studies on prevertebrate ancestral homologs revealed the transition from an environmentally induced mechanism activating p53 to a tightly regulated system involving cell signaling. The evolution of this mechanism depends on structural changes in the interacting protein motifs. Elephants such as Loxodonta africana constitute ideal models to investigate this coevolution as they are large and long-living as well as having 20 copies of TP53 isoformic sequences expressing a variety of BOX-I MDM2-binding motifs. Collectively, these isoforms would enhance sensitivity to cellular stresses, such as DNA damage, presumably accounting for strong cancer defenses and other adaptations favoring healthy aging. Here we investigate the molecular evolution of the p53-MDM2 system by combining in silico modeling and in vitro assays to explore structural and functional aspects of p53 isoforms retaining the MDM2 interaction, whereas forming distinct pools of cell signaling. The methodology used demonstrates, for the first time that in silico docking simulations can be used to explore functional aspects of elephant p53 isoforms. Our observations elucidate structural and mechanistic aspects of p53 regulation, facilitate understanding of complex cell signaling, and suggest testable hypotheses of p53 evolution referencing Peto's Paradox.

Pandraud, A., A. M. Shrader, A. Tshipa, N. Ngwenya and S. Chamaillé-Jammes (2022). "Cueing on distant conditions before migrating does not prevent false starts: a case study with African elephants." Oecologia.

Migratory animals often use environmental cues to time their seasonal migrations. Local conditions may, however, differ from distant ones, and current conditions may poorly predict future conditions. This may be particularly true for early wet season conditions in tropical systems, as storms and associated rainfall events are generally not predictable at the scale of weeks or days and are heterogeneously distributed even at the scale of a few kilometres. How migratory animals cope with such challenges, and the consequences they may have, remain poorly known. We used time-to-event models based on GPS data from 19 African elephant herds from Hwange National Park (Zimbabwe) to study the effect of local and distant rainfall events on the elephants' decision to initiate their wet season migration. Elephants relied more on distant rainfall events occurring along the future migration route than on local events when initiating their migration. Such ability to use distant cues does not, however, ensure an immediate migration success. In over 30% of the cases, the elephants came back to their dry season range, sometimes after having travelled > 80% of the expected migration distance. This happened particularly when there was little additional rain falling during the migration. All elephants successfully migrated later in the season. Our study improves the understanding of the migratory ecology of elephants. More broadly, it raises questions about the reliability of rainfall as a migratory cue in tropical systems, and shed light on one of its potential consequences, the poorly quantified phenomenon of migration false starts.

Parker, J. M., J. L. Brown, N. T. Hobbs, N. P. Boisseau, D. Letitiya, I. Douglas-Hamilton and G. Wittemyer (2022). "Social support correlates with glucocorticoid concentrations in wild African elephant orphans." Commun Biol **5**(1): 630.

Social relationships have physiological impacts. Here, we investigate whether loss of the mother/offspring relationship has lasting effects on fecal glucocorticoid metabolite (fGCM) concentrations in wild African elephant orphans several years following their mothers' deaths. We find no difference in fGCM concentrations between orphans and nonorphans, but find lower fGCM concentrations in elephants with more age mates in their family. We also unexpectedly identify lower concentrations in orphans without their natal family versus nonorphans and natal orphans, which we speculate may be due to the development of hypocortisolism following a prolonged period without familial support. An index of plant productivity (i.e. food) shows the largest correlation with fGCM concentrations. Our findings indicate no lasting differences in glucocorticoid concentrations of surviving orphan elephants who are with their family, suggest the presence of age mates may reduce glucocorticoid concentrations in elephants, and emphasize that basic survival needs are the primary regulators of the stress response.

Parker, J. M. and G. Wittemyer (2022). "Orphaning stunts growth in wild African elephants." Conserv Physiol **10**(1): coac053.

Orphans of several species suffer social and physiological consequences such as receiving more aggression from conspecifics and lower survival. One physiological consequence of orphaning, stunted growth, has been identified in both humans and chimpanzees, but has not been assessed in a non-primate species. Here, we tested whether wild African elephant orphans show evidence of stunted growth. We measured individually known female elephants in the Samburu and Buffalo Springs National Reserves of Kenya, with a rangefinder capable of calculating height, to estimate a von Bertalanffy growth curve for female elephants of the study population. We then compared measurements of known orphans and non-orphans of various ages, using a Bayesian analysis to assess variation around the derived growth curve. We found that orphans are shorter for their age than non-orphans. However, results suggest orphans may partially compensate for stunting through later growth, as orphans who had spent a longer time without their mother had heights more similar to non-orphans. More age mates in an individual's family were associated with taller height, suggesting social support from peers may contribute to increased growth. Conversely, more adult females in an individual's family were associated with shorter height, suggesting within-group competition for resources with older individuals may reduce juvenile growth. Finally, we found a counterintuitive result that less rainfall in the first 6 years of life was correlated with taller height, potentially reflecting the unavoidable bias of measuring individuals who were fit enough to survive conditions of low rainfall as young calves. Reduced growth of individuals has been shown to reduce survival and reproduction in other species. As such, stunting in wildlife orphans may negatively affect fitness and represents an indirect effect of ivory poaching on African elephants.

Pokharel, S. S., N. Sharma and R. Sukumar (2022). "Viewing the rare through public lenses: insights into dead calf carrying and other thanatological responses in Asian elephants using YouTube videos." R Soc Open Sci **9**(5): 211740.

Documenting the behavioural repertoire of an animal species is important for understanding that species' natural history. Many behaviours such as mating, parturition and death may be observed only rarely in the wild due to the low frequency of occurrence, short duration and the species' elusiveness. Opportunistic documentation of rare behaviours is therefore valuable for deciphering the behavioural complexity in a species. In this context, digital platforms may serve as useful data sources for studying rare behaviours in animals. Using videos uploaded on YouTube, we document and construct a tentative repertoire of thanatological responses (death-related behaviours) in Asian elephants (Elephas maximus). The most frequently observed thanatological responses included postural changes, guarding/keeping vigil, touching, investigating the carcass, epimeletic behaviours and vocalizations. We also describe some infrequently observed behaviours, including carrying dead calves by adult females, re-assurance-like behaviours and attempts to support dying or dead conspecifics, some of which were only known anecdotally in Asian elephants. Our observations indicate the significance of open-source video data on digital platforms for gaining insights into rarely

observed behaviours and support the accumulating evidence for higher cognitive abilities of Asian elephants in the context of comparative thanatology.

Purkart, L., J. M. Tuff, M. Shah, L. V. Kaufmann, C. Altringer, E. Maier, U. Schneeweiß, E. Tunckol, L. Eigen, S. Holtze, G. Fritsch, T. Hildebrandt and M. Brecht (2022). "Trigeminal ganglion and sensory nerves suggest tactile specialization of elephants." Curr Biol **32**(4): 904-910.e903.

Sensory nerves are information bottlenecks giving rise to distinct sensory worlds across animal species.(1) Here, we investigate trigeminal ganglion(2)(,)(3) and sensory nerves(4) of elephants. The elephant trigeminal ganglion is very large. Its maxillary branch, which gives rise to the infraorbital nerve innervating the trunk, has a larger diameter than the animal's spinal cord, i.e., trunk innervation is more substantive than connections of the brain to the rest of the body. Hundreds of satellite cells surround each trigeminal neuron, an indication of exceptional glial support to these large projection neurons.(5-7) Fiber counts of Asian elephant infraorbital nerves of averaged 4,00,000 axons. The infraorbital nerve consists of axons that are ~10 μm thick and it has a large diameter of 17 mm, roughly 3 times as thick as the optic and 6 times as thick as the vestibulocochlear nerve. In most mammals (including tactile specialists) optic nerve fibers(8-10) greatly outnumber infraorbital nerve fibers,(11)(,)(12) but in elephants the infraorbital nerve fiber count is only slightly lower than the optic nerve fiber count. Trunk innervation (nerves and ganglia) weighs ~1.5 kg in elephant cows. Our findings characterize the elephant trigeminal ganglion as one of the largest known primary sensory structures and point to a high degree of tactile specialization in elephants.

Qiu, J. (2022). "Asian elephants mostly roam outside protected areas - and it's a problem." Nature.

Rajbhandari, R. M., J. de la Fuente, D. Karmacharya, S. Mathema, B. Maharjan, S. M. Dixit, N. Shrestha, J. Queirós, C. Gortázar and P. C. Alves (2022). "Understanding Mycobacterium tuberculosis complex in elephants through a One Health approach: a systematic review." BMC Vet Res **18**(1): 262.

BACKGROUND: Mycobacterium tuberculosis complex (MTC) that causes the chronic infectious disease- tuberculosis (TB), often presents with a complicated epidemiological pattern where the transmission chain may include humans, domestic animals and wildlife, including elephants. TB has been reported globally in both captive and wild elephants. The One Health approach might be the most effective way of understanding the shared MTC infection dynamics in captive and wild animals like Asian elephants. This systematic review accumulates evidence on occurrence, transmission pathways, and preventive measures of TB in elephants from a One Health perspective. RESULTS: The prevalence of TB reported in elephant populations ranges from 0 to 23.33% and high prevalence's are reported for elephants that are in close proximity to infected humans. The risk of elephant to human infection transmission increased significantly with exposure duration and

contact with infected elephants. Some studies described the plausible TB transmission to captive elephants from other animals (wild and domestic), suggesting inter- and intra-species transmission. The results of this systematic review based on 27 relevant published works, suggest three overarching interrelated transmission pathways for M. tuberculosis infections in Asian elephants- i) humans and elephants, ii) other animals (wild or domestic) and elephants and iii) unclear sources of infection. CONCLUSIONS: The progress made with new TB diagnostic tools provides multiple methods to choose from. However, lack of harmonization of TB testing in elephants and their human contacts remains a challenge to prevent TB in those animals. Routine TB screening among elephants and caretakers by setting up an occupational health program for early diagnosis of infection through combined efforts of public health, veterinary medicine, and occupational health experts is suggested. This implies the need for a One Health approach to elephant TB control. This review reveals the need for more research on Mycobacterium tuberculosis complex transmission pathways at the human-animal interface.

Reichert, S., V. Berger, D. J. F. Dos Santos, M. Lahdenperä, U. K. Nyein, W. Htut and V. Lummaa (2022). "Age related variation of health markers in Asian elephants." Exp Gerontol **157**: 111629.

Although senescence is often observed in the wild, its underlying mechanistic causes can rarely be studied alongside its consequences, because data on health, molecular and physiological measures of senescence are rare. Documenting how different age-related changes in health accelerate ageing at a mechanistic level is key if we are to better understand the ageing process. Nevertheless, very few studies, particularly on natural populations of long-lived animals, have investigated age-related variation in biological markers of health and sex differences therein. Using blood samples collected from semi-captive Asian elephants, we show that pronounced differences in haematology, blood chemistry, immune, and liver functions among age classes are also evident under natural conditions in this extremely long-lived mammal. We provide strong support that overall health declined with age, with progressive declines in immune and liver functions similarly in both males and females. These changes parallel those mainly observed to-date in humans and laboratory mammals, and suggest a certain ubiquity in the ageing patterns.

Rose, J. B., A. Leeds, L. M. Yang, R. LeMont, M. A. Fayette, J. S. Proudfoot, M. R. Bowman, A. Woody, J. Oosterhuis and D. A. Fagan (2022). "Treatment and Outcomes of Tusk Fractures in Managed African Savanna and Asian Elephants (Loxodonta africana and Elephas maximus) across Five Continents." Animals (Basel) **12**(9).

Elephant tusk fractures are a clinical challenge that can impact the overall health of the animal, particularly when they result in pulp exposure. An international survey was sent to veterinarians to understand individual fracture characteristics and management strategies as they relate to outcomes, with the goal of better informing treatment procedures. The data

set consisted of 79 fractures from 64 elephants (including Asian and African males and females), 44.3% of which were Class III fractures with pulpal involvement. Of this subset, pulp canal exposures of >0.5 cm were 23.8-fold more likely to develop pulpitis than fractures with <0.5 cm exposed, though canal size did not impact healing versus extraction outcome. Odds ratios showed that treatments including endodontics were 12.0-fold more likely to heal than tusks treated exclusively with medical management, though no association was observed in reducing the risk of pulpitis. Further, pulpitis was 7.58-fold more likely to develop when tap water was used to rinse exposed pulpal tissue; a finding that merits further investigation. The use of endodontic treatment versus medical management alone was significantly associated with improved recovery outcomes (i.e., reduced risk of extraction) in tusk fractures with pulpal involvement.

Ryding, S., M. Klaassen, G. J. Tattersall, J. L. Gardner and M. R. E. Symonds (2022). "Erratum: Shape-shifting: changing animal morphologies as a response to climatic warming: (Trends in Ecology and Evolution 36, 1036–1048; 2021) (Trends in Ecology & Evolution (2021) 36(11) (1036–1048), (S016953472100197X), (10.1016/j.tree.2021.07.006))." Trends in Ecology and Evolution **37**(1): 106.

A reference was omitted from the article 'Shape-shifting: changing animal morphologies as a response to climatic warming' when it was published. The corrected text, reference citation, and reference details appear below. The authors apologise profusely for this oversight. On page 1039, paragraph 1, line 1: 'Studies investigating this link often use museum specimens to assess morphological change over the past decades or century (e.g., [37–39]) and some complement this with long-term field studies (e.g., [40,41,100]).' And on page 1040, Table 1: 'Wood mice (Apodemus sylvaticus) – Ear length increase [40,100]' © 2021 Elsevier Ltd

Sach, F., L. Fields, S. Chenery, L. Yon, M. D. Henley, P. Buss, E. S. Dierenfeld, S. C. Langley-Evans and M. J. Watts (2022). "Method development to characterise elephant tail hairs by LA-ICP-MS to reflect changes in elemental chemistry." Environ Geochem Health.

This paper evaluated analytical methods used to generate time-series data from elephant tail hairs, which can be used to reflect changing exposure to environmental geochemistry. Elephant tail hairs were analysed by three methods sequentially, each providing data to inform subsequent analysis. Scanning Electron Microscopy (SEM) and X-ray Microanalysis visually showed the structure of the hair, specific structures such as tubules, and the mineral crusting around the edge of the hair, informing targeting of subsequent analysis by Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). LA-ICP-MS generated time-series data which informed sectioning of the tail hairs for subsequent quantitative analysis for potentially toxic elements and micronutrients using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) of dissolved tail hairs. This novel approach to characterise the tail hair enabled time-series analysis to reflect changes in environmental exposure which may result from seasonal or geochemical spatial variation and could inform elephant movement patterns. The seasonal

change between wet and dry seasons was reflected down the length of the hair. Correlations were seen between LA-ICP-MS data and ICP-MS data in several elements including Mg, P, Ca, Fe, Na, Mn and U. This study provided time-series data for the analysis of elephant tail hairs by evaluating analytical challenges to obtaining quantitative data, such as improving protocols to ensure removal of extraneous material, determining where to section the tail hairs to best reflect environmental changes/exposure and ensuring representative analyses. A protocol was established to determine mineral status across a 12-18 month time period utilizing single elephant tail hairs.

Sarma, R. K., A. Gohain, T. H. Ahmed, A. Yadav and R. Saikia (2022). "An environment-benign approach of bamboo pulp bleaching using extracellular xylanase of strain Bacillus stratosphericus EB-11 isolated from elephant dung." Folia Microbiol (Praha).

The use of microbial enzymes is highly encouraged in paper and pulp industries to reduce the excessive use of hazardous chemicals. During the study, xylanase of Bacillus stratosphericus EB-11 was characterized for pulp bleaching applications. The extracellular xylanase was produced under submerged fermentation using bamboo waste as a natural carbon source. There was fast cell division and enzyme production under optimized fermentation conditions in the bioreactor. The highest activity was 91,200U after 30 h of growth with Km and Vmax of 3.52 mg/mL and 391.5 µmol/min per mg respectively. The purified enzyme with molecular mass ~ 60 kDa had conferred positive activity on native PAGE. The strong inhibition by ethylenediaminetetraacetate and SDS showed the metallo-xylanase nature of the purified enzyme. The bacterial xylanase reduces the use of hydrogen peroxide by 0.4%. Similarly, biological oxygen demand and chemical oxygen demand were reduced by 42.6 and 35.2%. The xylanase-hydrogen peroxide combined treatment and conventional chlorine dioxide-alkaline (CDE(1)D(1)D(2)) bleaching showed almost similar improvement in physicochemical properties of bamboo pulp. Xylanase-peroxide bleaching reduces the lignin content to 4.95% from 13.32% unbleached pulp. This content after CDE(1)D(1)D(2)treatment was 4.21%. The kappa number decreased from 15.2 to 9.46 with increasing the burst factor (15.51), crystallinity index (60.25%), viscosity (20.1 cp), and brightness (65.4%). The overall finding will encourage the development of new cleaner methods of bleaching in the paper and pulp industry.

Scherer, L., L. Bingaman Lackey, M. Clauss, K. Gries, D. Hagan, A. Lawrenz, D. W. H. Müller, M. Roller, C. Schiffmann and A. K. Oerke (2022). "The historical development of zoo elephant survivorship." Zoo Biol.

In the discussion about zoo elephant husbandry, the report of Clubb et al. (2008, Science 322: 1649) that zoo elephants had a "compromised survivorship" compared to certain non-zoo populations is a grave argument, and was possibly one of the triggers of a large variety of investigations into zoo elephant welfare, and changes in zoo elephant management. A side observation of that report was that whereas survivorship in African elephants (Loxodonta africana) improved since 1960, this was not the case in Asian

elephants (Elephas maximus). We used historical data (based on the Species360 database) to revisit this aspect, including recent developments since 2008. Assessing the North American and European populations from 1910 until today, there were significant improvements of adult (≥10 years) survivorship in both species. For the period from 1960 until today, survivorship improvement was significant for African elephants and close to a significant improvement in Asian elephants; Asian elephants generally had a higher survivorship than Africans. Juvenile (<10 years) survivorship did not change significantly since 1960 and was higher in African elephants, most likely due to the effect of elephant herpes virus on Asian elephants. Current zoo elephant survivorship is higher than some, and lower than some other non-zoo populations. We discuss that in our view, the shape of the survivorship curve, and its change over time, are more relevant than comparisons with specific populations. Zoo elephant survivorship should be monitored continuously, and the expectation of a continuous trend towards improvement should be met.

Schulz, A. K., M. Boyle, C. Boyle, S. Sordilla, C. Rincon, S. Hooper, C. Aubuchon, J. S. Reidenberg, C. Higgins and D. L. Hu (2022). "Skin wrinkles and folds enable asymmetric stretch in the elephant trunk." Proc Natl Acad Sci U S A **119**(31): e2122563119.

The elephant's trunk is multifunctional: It must be flexible to wrap around vegetation, but tough to knock down trees and resist attack. How can one appendage satisfy both constraints? In this combined experimental and theoretical study, we challenged African elephants to reach far-away objects with only horizontal extensions of their trunk. Surprisingly, the trunk does not extend uniformly, but instead exhibits a dorsal "joint" that stretches 15% more than the corresponding ventral section. Using material testing with the skin of a deceased elephant, we show that the asymmetry is due in part to patterns of the skin. The dorsal skin is folded and 15% more pliable than the wrinkled ventral skin. Skin folds protect the dorsal section and stretch to facilitate downward wrapping, the most common gripping style when picking up items. The elephant's skin is also sufficiently stiff to influence its mechanics: At the joint, the skin requires 13 times more energy to stretch than the corresponding length of muscle. The use of wrinkles and folds to modulate stiffness may provide a valuable concept for both biology and soft robotics.

Seltmann, M. W., J. Jackson, E. Lynch, J. L. Brown, W. Htut, M. Lahdenperä and V. Lummaa (2022). "Sex-specific links between the social landscape and faecal glucocorticoid metabolites in semi-captive Asian elephants." Gen Comp Endocrinol **319**: 113990.

Although social behaviour is common in group-living mammals, our understanding of its mechanisms in long-lived animals is largely based on studies in human and non-human primates. There are health and fitness benefits associated with strong social ties, including increased life span, reproductive success, and lower disease risk, which are attributed to the proximate effects of lowered circulating glucocorticoid hormones. However,

to deepen our understanding of health-social dynamics, we must explore species beyond the primate order. Here, using Asian elephants as a model species, we combine social data generated from semi-captive timber elephants in Myanmar with measurements of faecal glucocorticoid metabolite (FGM) concentrations. These data enable a "natural experiment" because individuals live in work groups with different demographic compositions. We examine sex-specific FGM concentrations for four different aspects of an individuals' social world: general sociality, work group size, sex ratio and the presence of immatures (<5 years) within the work group. Males experienced lower FGM concentrations when engaged in more social behaviours and residing in female-biased work groups. Surprisingly, females only exhibited lower FGM concentrations when residing with calves. Together, our findings highlight the importance of sociality on individual physiological function among elephants, which may have broad implications for the benefits of social interactions among mammals.

Shah, Y., S. Paudel, K. Pandey, G. P. Gupta, E. S. Solo, J. Joshi, D. K. Pant and B. D. Pandey (2022). "Insights into transmission dynamics of Mycobacterium tuberculosis complex in Nepal." Tropical Medicine and Health **50**(1).

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis complex (MTBC) in humans and animals. Numbers of multi drug resistance TB (MDR-TB), extrapulmonary TB (EPTB) and zoonotic TB cases are increasingly being reported every year in Nepal posing a major public health problem. Therefore, the Government of Nepal should act immediately to strengthen the screening facilities across the country to be able to identify and treat the TB infected patients as well as detect zoonotic TB in animal species. Endorsement of One Health Act by the Government of Nepal is an opportunity to initiate the joint programs for TB surveillance among human and animal species using one health approach to reduce the TB burden in Nepal. © 2022, The Author(s).

Sharma, M., M. Karikalan, P. Dandapat, M. A. Kumar, V. Beena, S. C. Mohan, S. Ilayaraja, A. Mathur, A. Bhawal, A. M. Pawde and A. K. Sharma (2022). "Tuberculosis in free-ranging and captive wild animals: Pathological and molecular diagnosis with histomorphological differentiation of granulomatous lesions." Microbial Pathogenesis **172**: 10.

Tuberculosis (TB) is a serious zoonotic threat, impacting the human-livestock-wildlife interface globally. Here, we evaluated the status and histomorphological differentiation of TB lesions in 89 morbid cases of wild animals (bovids, cervids, carnivores, non-human primates, and pachyderms) in India. Histomorphological and molecular studies were done using Ziehl-Neelsen (ZN) staining, immunohistochemistry, and polymerase chain reaction (PCR), whereas cultural isolation was performed on selected samples. A total of 32 (35.95%) cases were confirmed as TB, comprising of 12 carnivores, 09 bovids, 06 cervids, 04 non-human primates, and a pachyderm. The TB lesions in the lungs, liver, and lymph nodes varied from the large-sized caseous nodules filled with dry cheesy material in bovids and cervids to variable-sized cavitations containing liquefied caseum in

carnivores' lungs. The lungs, livers, and spleens of non-human primates exhibited small to medium-sized nodules. Histo-logically, lesions were divided into four categories (Types I, II, III, and IV) based on the extent of necrosis, the presence of mineralization, giant cells, and fibrous encapsulation. Extensive caseous necrosis with calcification, abundant giant cells, and thick fibroblastic encapsulation were consistent findings in the lungs, livers, and lymph nodes of bovids and cervids, whereas airway impaction with cellular exudate containing a teeming number of acid-fast bacilli and, at times, alveolar rupture leading to cavity formation was present in the lungs of carnivores. Absence of calcification and fibrous encapsulation was recorded in lungs of non-human primates. Immunohis-tochemical labelling with anti-early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) antibodies showed mild, moderate, and intense positive reactions in type II and III, type I, and type IV granulomatous lesions, respectively. Molecular detection by PCR revealed Mycobacterium tuberculosis (12 carnivores, 02 non-human primates and 01 pachyderm), M. bovis (02 cervids and 01 bovid) and M. orygis (02 cervids and 01 bovid). Cultural isolation confirmed M. tuberculosis in 03 carnivores and M. orygis in 02 cervids and 01 bovid. Our findings imply that TB is quite prevalent in the wildlife of India and there are considerable differences in the histomorphological lesions induced by distinct Mycobacterium species in different wild animals. The circulation of TB organisms in wild animals warrants a strict surveillance programme to identify the carrier status of these animals so that effective TB control strategies can be formulated to prevent spillover and spillback incidences at the human-livestock-wildlife interface.

Sharma, M., M. Karikalan, M. A. Kumar, P. S. Lakshmi, K. Sharma, S. Ilayaraja, A. Mathur and A. M. Pawde (2022). "A study on clinical diagnosis of tuberculosis in free ranging and captive wild animals of India." Iranian Journal of Veterinary Research **23**(4): 7.

Background: Tuberculosis (TB) is a disease of paramount importance at the wildlife-livestock-human interface. Aims: To study the occurrence and Mycobacterium (M) species involved in the TB of free-ranging and captive wild animals in various Indian states. Methods: A total of 396 clinical samples from 207 different wild animal species from various Indian national parks, zoological gardens, etc., were analyzed by lateral flow assay (LFA), Ziehl-Neelsen (ZN) staining, and PCR. Clinical samples include blood (n=156), faecal swabs (n=103), serum (n=73), and nasal swabs or trunk wash fluids (n=64). Results: Clinical signs of TB were absent in 202 animals, although 21 wild animals were seropositive for pathogenic Mycobacterium antigens by LFA. Clinical signs like progressive weight loss, and respiratory distress were exhibited by 4 sloth bears (Melursus ursinus) and an elephant (Elephas maximus), which were also found positive for LFA, PCR, and ZN staining. ZN staining showed positivity for acid-fast bacilli (AFB) in 9 (8.74%) faecal and 9 (14.06%) nasal swabs or trunk wash fluids of sloth bears (7 samples) and elephants (2 samples). M. tuberculosis was detected in 7 sloth bears and 2 elephants, whereas M. bovis was found in a spotted deer (Axis axis) by species-specific PCR. Conclusion: The circulation of TB organisms in wild

animals warrants a strict surveillance programme to identify the carrier status of these animals so that effective TB control strategies can be formulated.

Steyrer, C., M. Miller, J. Hewlett, P. Buss and E. H. Hooijberg (2022). "Markers of inflammation in free-living African elephants (Loxodonta africana): Reference intervals and diagnostic performance of acute phase reactants." Vet Clin Pathol.

INTRODUCTION: Acute phase reactants (APRs) have not been investigated in free-living African elephants (Loxodonta africana), and there is little information about negative APRs albumin and serum iron in elephants. OBJECTIVES: We aimed to generate reference intervals (RIs) for APRs for free-living African elephants, and to determine the diagnostic performance of APRs in apparently healthy elephants and elephants with inflammatory lesions. METHODS: Stored serum samples from 49 apparently healthy and 16 injured free-living elephants were used. The following APRs and methods were included: albumin, bromocresol green; haptoglobin, colorimetric assay; serum amyloid A (SAA), multispecies immunoturbidometric assay, and serum iron with ferrozine method. Reference intervals were generated using the nonparametric method. Indices of diagnostic accuracy were determined by receiver-operator characteristic (ROC) curve analysis. RESULTS: Reference intervals were: albumin 41-55 g/L, haptoglobin 0.16-3.51 g/L, SAA < 10 mg/L, and serum iron 8.60-16.99 μmol/L. Serum iron and albumin concentrations were lower and haptoglobin and SAA concentrations were higher in the injured group. Serum iron had the best ability to predict health or inflammation, followed by haptoglobin, SAA, and albumin, with the area under the ROC curve ranging from 0.88-0.93. CONCLUSIONS: SAA concentrations were lower in healthy African vs Asian elephants, and species-specific RIs should be used. Serum iron was determined to be a diagnostically useful negative APR which should be added to APR panels for elephants.

Supanta, J., J. L. Brown, P. Bansiddhi, C. Thitaram, V. Punyapornwithaya and J. Khonmee (2022). "Effect of the COVID-19 pandemic and international travel ban on elephant tourist camp management in northern Thailand." Front Vet Sci **9**: 1038855.

The COVID-19 pandemic has had a significant impact on the tourism industry, especially in Thailand. Starting in April 2020, the Thai government banned international travel and all elephant tourist camps closed. A wide variety of management changes were implemented because of the lack of income from tourists. This study surveyed 30 camps that cared for >400 elephants in northern Thailand to obtain information on camp, elephant, and mahout management during the COVID-19 pandemic from April 2020 to 2022 compared to the year before. The survey consisted of questionnaires that interviewed elephant camp owners, managers, veterinarians, and mahouts, and captured information on changes in camp operations, including numbers of tourists, elephants and mahouts, elephant and mahout activities, and veterinary care. Results revealed significant changes in camp structure, elephant work activities and general care. Staff layoffs led to a decrease in

the ratio of mahouts to elephants from 1:1 to 1:2. Elephant activities, distance walked, and amounts of food were reduced when compared to pre-COVID-19, while chain hours were increased due to reduced activity. Overall, the COVID-19 crisis altered elephant management significantly, potentially affecting animal welfare resulting from changes in nutrition, health, exercise, and numbers of mahouts. We hope to use these data to develop better management plans and guidelines for elephant camps in Thailand so they can cope with the current and potential imminent pandemics that result in decreased tourism income. A follow-up study will measure health and welfare markers in relation to COVID-19 induced changes to determine if any camps adapted management to still meet elephant health and welfare needs, and could serve as models for responding to future pandemics.

Szydlowski, M. (2022). "Elephants in Nepal: Correlating disease, tourism, and welfare." Journal of Applied Animal Welfare Science.

Asian elephants and humans have long shared their lives, but recent changes in human perspectives on animal use have created ripples through the small country of Nepal. Captive elephants are caught in the crossfire between local communities, elephant owners, mahouts, and NGOs in debates over their treatment, health, welfare and use in tourism. In addition, zoonotic disease, natural disasters and political strife affect the lives of captive elephants and mahouts. For example, during the COVID-19 pandemic, elephants, caregivers and owners found themselves facing income loss, decreased welfare from housing and husbandry issues, and food shortages. Many owners sold elephants, fired mahouts, and "quit" the tourism industry. Others sought help from outside organizations, community members, and governmental agencies to retain ownership of what they viewed as valuable commodities. NGOs and grassroots organizations assisted in the hopes of keeping elephants in Nepal, thus preventing them from long, treacherous walks across the border and into situations where they might face further welfare decreases. This article combines elephant stable visits and interviews with mahouts, owners, NGO, and government staff between January 2019 and December 2021. It highlights the ongoing health and welfare challenges faced by elephants and mahouts in Nepal.

Tang, Z., Z. H. Liu, W. Chen, C. Wang, Y. J. Wu, H. Wang, Z. Dang and Y. Liu (2022). "Twelve natural estrogens in urines of six threatened or endangered mammalian species in Zoo Park: implications and their potential risk." Environ Sci Pollut Res Int **29**(32): 49404-49410.

This work was the first to report twelve natural estrogens (NEs) in the urines of six threatened or endangered mammalians in a Zoo Park of Guangzhou (i.e., panda, gorilla, elephant, African lion, jaguar, and leopard). Ten out of twelve NEs were detected at least in one urine sample of the six mammalians studied, including the four major NEs (i.e., estrone (E1), 17β-estradiol (E2), 17α-estradiol (αE2), estriol (E3)), and six other NEs (i.e., 4-hydroxyestrone (4OHE1), 2-hydroxyestradiol (2OHE2), 4-hydroxyestradiol (4OHE2), 16α-hydroxyestrone (16α-OHE1), 16ketoestradiol (16ketoE2), and 17epiestriol

(17epiE3)). The six studied mammalians, ranked in the order of high to low urinary concentration of total NEs, were jaguar, African lion, gorilla, elephant, panda, and leopard, with respective urinary concentrations of 110.4, 86.4, 71.4, 66.0, 55.9, and 52.8 ng/mL. According to the average urinary concentration of NE in the six mammalians ranked from high to low, the top five NEs detected were 16α-OHE1, 4OHE1, E1, E3, and 17epiE3, respectively. These clearly indicated the occurrence of NEs other than the four major types in urines of animals in a Zoo Park. Moreover, the daily excretion rates of the five detected NEs by one elephant ranged from 1162-2254 μg/d with a total daily excretion rate of 8260 μg/d, suggesting that the total urinary excretion of NEs by one adult elephant was equivalent to that by 170 premenopausal women or 506 adult men. Consequently, it appears from this study that NEs in the urines of zoo animals should be considered an emerging source of NEs.

Tangyuenyong, S., P. Kongdang, N. Sirikaew and S. Ongchai (2022). "First study on the effect of transforming growth factor beta 1 and insulin-like growth factor 1 on the chondrogenesis of elephant articular chondrocytes in a scaffold-based 3D culture model." Vet World **15**(7): 1869-1879.

BACKGROUND AND AIM: Osteoarthritis (OA) is recognized as a degenerative joint disease that leads to chronic pain and low quality of life in animals. Captive elephants, the largest land mammals with a long lifespan, are more prone to develop OA due to restricted spaces and insufficient physical activity. This study aimed to investigate the effect of transforming growth factor-β1 (TGF-β1) and insulin-like growth factor 1 (IGF-1) on elephant chondrogenesis in a scaffold culture of articular chondrocytes. MATERIALS AND METHODS: Elephant chondrocytes-seeded gelatin scaffolds were cultured in chondrogenic media with or without 10 ng/mL of TGF-β1 or IGF-1 alone or 5-10 ng/mL of their combination for up to 21 days. The mRNA expression of cartilage-specific anabolic genes, ACAN and COL2A1, was analyzed using a real-time reverse transcription-polymerase chain reaction. The amounts of sulfated glycosaminoglycans (sGAGs) in conditioned media and contents in cultured scaffolds were determined through dimethylmethylene blue assay. Cell morphology, accumulation of proteoglycans, and details of the cultured scaffolds were determined using hematoxylin-eosin staining, safranin O staining, and scanning electron microscopy (SEM), respectively. RESULTS: TGF-β1 alone significantly upregulated ACAN gene expression but not COL2A1, while IGF-1 alone did not enhance both ACAN and COL2A1 genes. The combination significantly upregulated both mRNA expression levels of ACAN and COL2A1 gene at day 14. The sGAGs accumulation and contents in the treatment groups, except IGF-1 tended to be higher than the controls, concomitantly with the production of the extracellular matrix, showed the formation of a cartilage-like tissue through histological and SEM analyses. CONCLUSION: Together, our results suggest that the single treatment of TGF-β1 has a selective effect on ACAN gene, while the combined growth factors seem to be an advantage on elephant chondrogenesis. This three-dimensional culture model is probably helpful for developing cartilage regeneration in vitro and is further applied in tissue engineering for OA treatment in vivo.

Taylor, N. (2022). "Should we be keeping elephants in captivity?" Vet Rec **191**(3): 129.

Thapa, J., S. V. Gordon, C. Nakajima and Y. Suzuki (2022). "Threat from Mycobacterium orygis-associated tuberculosis in south Asia." Lancet Microbe **3**(9): E641-E642.

Titus, S. E., S. Patterson, J. Prince-Wright, A. Dastjerdi and F. M. Molenaar (2022). "Effects of between and within Herd Moves on Elephant Endotheliotropic Herpesvirus (EEHV) Recrudescence and Shedding in Captive Asian Elephants (Elephas maximus)." Viruses **14**(2).

Haemorrhagic disease associated with elephant endotheliotropic herpesvirus (Elephantid herpesvirus, EEHV) infections is the leading cause of death for Asian elephant (Elephas maximus) calves. This study assessed the effect of captive herd management on EEHV shedding, as evidence of latent infection reactivation, focusing on: (1) the influence of social change on the odds of recrudescence; (2) the respective effects of between and within herd moves; and (3) characteristics of recrudescent viral shedding. Trunk and conjunctival swabs (n = 165) were obtained from six elephants at an EAZA-accredited zoo, collected during a period of social stability, and at times of social change. Longitudinal sampling took place at times of moving two bulls out of the collection and one new bull into an adjacent enclosure to the cow herd (between herd moves), and during a period of mixing this new bull with the cow herd to facilitate mating (within herd moves). Quantitative PCR was employed to detect EEHV 1a/b, 4a/b, and EF-1-α (housekeeping gene). Generalised estimating equations determined EEHV recrudescence odds ratios (OR) and relative viral DNA load. Sixteen EEHV 1a/b shedding events occurred, but no EEHV 4a/b was detected. All management-derived social changes promoted recrudescence (social change OR = 3.27, 95% CI = 0.412-26, p = 0.262; and between herd moves OR = 1.6, 95% CI = 0.178-14.4, p = 0.675), though within herd movements posed the most significant increase of EEHV reactivation odds (OR = 6.86, 95% CI = 0.823-57.1, p = 0.075) and demonstrated the strongest relative influence (post hoc Tukey test p = 0.0425). Shedding onset and magnitude ranged from six to 54 days and from 3.59 to 11.09 ΔCts. Differing challenges are associated with between and within herd movements, which can promote recrudescence and should be considered an exposure risk to naïve elephants.

Towiboon, P., K. Saenphet, C. Tayapiwattana, S. Tangyuenyong, G. Watanabe, S. Mahasawangkul, J. L. Brown and C. Thitaram (2022). "Relationship among Serum Progestagens, Cortisol, and Prolactin in Pregnant and Cycling Asian Elephants in Thailand." Vet Sci **9**(5).

The aim of this study was to examine relationships among serum progestagens, cortisol, and prolactin in pregnant and normal cycling Asian elephants living in tourist camps in northern Thailand. Samples were collected twice a month for 22 months from nine elephants. Of those, four were pregnant (24.3 ± 2.9 years of age; range 21-28 years) and five (20.2

± 9.6 years; range 8-34 years) exhibited normal ovarian cycles based on serum progestagen analyses. Gestation was divided into three periods: 1st (week 1-31), 2nd (week 32-62), and 3rd (week 63 to parturition), while the estrous cycle was divided into the follicular and luteal phases. Serum progestagens were higher during the luteal phase of the cycle (p < 0.003), whereas cortisol and prolactin were similar. In pregnant elephants, there were no differences in serum progestagens or cortisol concentrations across the three gestational periods, whereas prolactin concentrations increased significantly during the 2nd and 3rd periods (p < 0.0001). By contrast, prolactin concentrations in nonpregnant elephants were consistently low throughout the ovarian cycle. In one cycling female, prolactin concentrations were similar to pregnant elephants, perhaps because she was an allomother to two calves. Another cycling female exhibited consistently elevated cortisol concentrations, 5 to 10 times higher than the other elephants. There were no correlations between serum progestagens, cortisol, and prolactin throughout gestation; however, serum progestagens and cortisol were positively related in cycling elephants (r = 0.386, p < 0.001). From our results, there were a number of individual differences in reproductive hormonal patterns, so it is important to develop personalized monitoring programs for each elephant to enhance breeding success and create sustaining captive populations of elephants in Asia.

Valášek, V., K. Pachnerová Brabcová, J. Kufnerová, M. Molnár and I. Světlík (2022). "REFINING RADIOCARBON DATING OF IVORY." Radiat Prot Dosimetry **198**(9-11): 675-680.

Elephants are on the verge of extinction due to extensive poaching to obtain ivory and illegal income. According to international law, the trade in ivory of African elephants is outlawed, with a few exceptions, as for example in European Union for antique ivory obtained before 1947. There is basically only one physical method for determining the age of ivory and that is radiocarbon dating. The method uses artificially temporarily enhanced 14C content in the atmosphere to date relevant samples with high resolution. Since this advantage is slowly fading, the question arises of how to fill in the loss of the resolution. One possibility is exploiting the chronology of ivory. This work studies a whole elephant tusk and uses radiocarbon dating of samples obtained longitudinally and transversely from along the tusk to analyse the growth rate.

Veerman, J., A. Kumar and D. R. Mishra (2022). "Exceptional landscape-wide cyanobacteria bloom in Okavango Delta, Botswana in 2020 coincided with a mass elephant die-off event." Harmful Algae **111**: 102145.

In 2020, nearly 400 elephants died within the Okavango Delta region in Botswana, creating the worst-ever elephant mass die-off event in history. This catastrophic event was widely blamed on toxic cyanobacterial blooms after lab results showed the presence of toxin-forming cyanobacteria in inland waters of the Delta. However, it did not explain why we saw this mass die-off of elephants in 2020 and not in previous years. We conducted a landscape-wide time-series analysis using freely available European Space

Agency's Sentinel-2 and NASA's Landsat-8 satellite data. We used existing bio-optical models, Normalized Difference Chlorophyll Index and Green Line Height, as proxies for chlorophyll-a and phycocyanin (cyanobacteria) concentrations. We found that 2020 was an exceptional year for cyanobacteria blooms in the Okavango Delta region compared to the past three years (2017-2019). Bloom phenology indicated that the cyanobacteria blooms initiated in September-October 2019, experienced an exponential growth reaching peak in January-February 2020, and eventually senescing in June 2020. This being a notoriously data-scarce region of the world, we did not have any means to perform site-specific validation of the models. Although magnitude and timeline of the blooms coincided with the timeline of elephant death reports, our study do not confirm it to be the trigger. For the first time, we show the widespread nature of these blooms across the landscape, which may have increased the toxin exposure for elephants. We theorize that 2020 might have been the first year for such a mass die-off event, but it will certainly not be the last because warming trends under changing climate are creating increasingly suitable conditions for these blooms to be pervasive and ubiquitous. Through this preliminary study, we demonstrate the critical need for frequent and comprehensive monitoring of toxic cyanobacterial blooms in the Delta to avoid another such event in the future.

Villar, M., R. M. Rajbhandari, S. Artigas-Jeronimo, M. Contreras, A. Sadaula, D. Karmacharya, P. C. Alves, C. Gortazar and J. de la Fuente (2022). "Differentially Represented Proteins in Response to Infection with Mycobacterium tuberculosis Identified by Quantitative Serum Proteomics in Asian Elephants." Pathogens **11**(9).

Tuberculosis is a major global concern. Tuberculosis in wildlife is a risk for zoonotic transmission and becoming one of the challenges for conservation globally. In elephants, the number of cases is likely rising. The aim of this study was to identify proteins related to tuberculosis infection in elephants, which could then be used for the development of diagnostic tools and/or vaccines. A serum proteomics approach was used to characterize differentially represented proteins in response to Mycobacterium tuberculosis in Asian elephants (Elaphas maximus). Blood samples were collected from eight elephants, four of which were antibody positive for tuberculosis and four were antibody negative. Proteomics analysis identified 26 significantly dysregulated proteins in response to tuberculosis. Of these, 10 (38%) were identified as immunoglobulin and 16 (62%) as non-immunoglobulin proteins. The results provided new information on the antibody response to mycobacterial infection and biomarkers associated with tuberculosis and protective response to mycobacteria in Asian elephants. Protective mechanisms included defense against infection (Alpha- 1-B glycoprotein A1BG, Serpin family A member 1 SERPINA1, Transthyretin TTR), neuroprotection (TTR), and reduced risks of inflammation, infections, and cancer (SERPINA1, Keratin 10 KRT10). Using a translational biotechnology approach, the results provided information for the identification of candidate diagnostic, prognostic, and protective antigens for monitoring and control of tuberculosis in Asian elephants.

Villar, M., R. M. Rajbhandari, S. Artigas-Jerónimo, M. Contreras, A. Sadaula, D. Karmacharya, P. C. Alves, C. Gortázar and J. de la Fuente (2022). "Differentially Represented Proteins in Response to Infection with Mycobacterium tuberculosis Identified by Quantitative Serum Proteomics in Asian Elephants." Pathogens **11**(9).

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Vivash-Jones, B. (2022). "Elephants in captivity." Vet Rec **191**(5): 222-223.

von Dürckheim, K. E. M., L. C. Hoffman, C. Poblete-Echeverría, J. M. Bishop, T. E. Goodwin, B. A. Schulte and A. Leslie (2022). "A pachyderm perfume: odour encodes identity and group membership in African elephants." Sci Rep **12**(1): 16768.

Group-living animals that live in complex social systems require effective modes of communication to maintain social cohesion, and several acoustic, olfactory and visual signaling systems have been described. Individuals need to discriminate between in- and out-group odour to both avoid inbreeding and to identify recipients for reciprocal behaviour. The presence of a unique group odour, identified in several social mammals, is a proposed mechanism whereby conspecifics can distinguish group from non-group members. African elephants (Loxodonta africana) live in stable, socially complex, multi-female, fission-fusion groups, characterized by female philopatry, male dispersal and linear dominance hierarchies. Elephant social behaviour suggests that individuals use odour to monitor the sex, reproductive status, location, health, identity and social status of conspecifics. To date, it is not clear what fixed or variable information is contained in African elephant secretions, and whether odour encodes kinship or group membership

information. Here we use SPME GC-MS generated semiochemical profiles for temporal, buccal and genital secretions for 113 wild African elephants and test their relationship with measures of genetic relatedness. Our results reveal the existence of individual identity odour profiles in African elephants as well as a signature for age encoded in temporal gland and buccal secretions. Olfactory signatures for genetic relatedness were found in labial secretions of adult sisters. While group odour was not correlated with group genetic relatedness, our analysis identified "group membership" as a significant factor explaining chemical differences between social groups. Saturated and short-chain fatty acids (SCFAs), derived from key volatile compounds from bacterial fermentation, were identified in temporal, buccal and genital secretions suggesting that group odour in African elephants may be the result of bacterial elements of the gut microbiome. The frequent affiliative behavior of African elephants is posited as a likely mechanism for bacterial transmission. Our findings favour flexible group-specific bacterial odours, which have already been proposed for other social mammals and present a useful form of olfactory communication that promotes bond group cohesion among non-relatives in fission-fusion mammals.

Wantanajittikul, K., C. Thitaram, S. Khammesri and S. Kongsawasdi (2022). "Development of a Protocol for Biomechanical Gait Analysis in Asian Elephants Using the Triaxial Inertial Measurement Unit (IMU)." Vet Sci **9**(8).

Gait analysis is a method of gathering quantitative information to assist in determining the cause of abnormal gait for the purpose of making treatment decisions in veterinary medicine. Recent technology has offered the wearable wireless sensor of an inertial measurement unit (IMU) for determining gait parameters. This study proposed the use of a triaxial IMU, comprising an accelerometer, a gyroscope, and a magnetometer, for detecting three-dimensional limb segment motion (XYZ axis) during the gait cycle in Asian elephants. A new algorithm was developed to estimate the kinematic parameter that represents each limb segment of the forelimbs and hindlimbs while walking at a comfortable speed. For future use, this study aimed to create a new prototype of the IMU with a configuration that is tailored to the elephant and apply machine learning in an effort to achieve greater precision.

Wasser, S. K., C. J. Wolock, M. K. Kuhner, J. E. Brown, 3rd, C. Morris, R. J. Horwitz, A. Wong, C. J. Fernandez, M. Y. Otiende, Y. Hoareau, Z. A. Kaliszewska, E. Jeon, K. L. Han and B. S. Weir (2022). "Elephant genotypes reveal the size and connectivity of transnational ivory traffickers." Nat Hum Behav **6**(3): 371-382.

Transnational ivory traffickers continue to smuggle large shipments of elephant ivory out of Africa, yet prosecutions and convictions remain few. We identify trafficking networks on the basis of genetic matching of tusks from the same individual or close relatives in separate shipments. Analyses are drawn from 4,320 savannah (Loxodonta africana) and forest (L. cyclotis) elephant tusks, sampled from 49 large ivory seizures totalling 111 t, shipped out of Africa between 2002 and 2019. Network analyses reveal a repeating pattern wherein tusks from the same individual or close relatives are found in separate seizures that were containerized in, and transited through, common

African ports. Results suggest that individual traffickers are exporting dozens of shipments, with considerable connectivity between traffickers operating in different ports. These tools provide a framework to combine evidence from multiple investigations, strengthen prosecutions and support indictment and prosecution of transnational ivory traffickers for the totality of their crimes.

Williams, E., N. Clark, J. Rendle-Worthington and L. Yon (2022). "Behaviour and Welfare Impacts of Releasing Elephants from Overnight Tethers: A Zimbabwean Case Study." Animals (Basel) **12**(15).

Within the southern African elephant tourism industry, chaining or tethering elephants is still a relatively routine practice, despite the known negative impacts. Cited reasons for chaining include fear of aggressive interactions between elephants when handlers are absent, or a general increase in expression of aggressive behaviours (both to other elephants and to their human handlers). In Zimbabwe, concerns expressed include the danger of elephants escaping and entering human-inhabited areas. Four male semi-captive elephants at a Zimbabwe tourist facility were taken off overnight (~12 h) tethers and were placed in small pens ('bomas'), approximate sizes from 110 m(2) to 310 m(2)), as part of a strategy to improve elephant welfare. Behavioural data were collected from overnight videos from December 2019 to March 2020, between 18:00 to 06:00, using focal, instantaneous sampling (5-min interval). Data were collected for three nights at three time periods: (i) Tethered; (ii) approximately four weeks post-release; (iii) approximately eight weeks post-release. Behavioural change over these time points was analysed using general linear models with quasibinomial error structures. Behavioural changes indicative of improved welfare were observed following these management changes, and no significant increases in aggression were observed either between elephants, or towards their human handlers. Proportion of time engaging in lying rest was higher in the first month after release from tethering (mean ± SD, 50 ± 14%) than when elephants were tethered (20 ± 18%) (p < 0.05). Additionally, although not statistically significant, stereotypies were reduced when elephants were no longer tethered (4 ± 6% observations tethered compared to 2 ± 2% off tethers), and positive social behaviour also increased (1 ± 1% on tethers, 2 ± 2% off tethers), with the greatest improvements seen in the pair-housed elephants. To improve elephant welfare in southern African tourism facilities we strongly advocate that less restrictive management practices which enable greater choice and freedom of movement overnight are implemented.

Wiśniewska, M., I. Puga-Gonzalez, P. Lee, C. Moss, G. Russell, S. Garnier and C. Sueur (2022). "Simulated poaching affects global connectivity and efficiency in social networks of African savanna elephants-An exemplar of how human disturbance impacts group-living species." PLoS Comput Biol **18**(1): e1009792.

Selective harvest, such as poaching, impacts group-living animals directly through mortality of individuals with desirable traits, and indirectly by altering the structure of their social networks. Understanding the relationship between disturbance-induced, structural network changes and group

performance in wild animals remains an outstanding problem. To address this problem, we evaluated the immediate effect of disturbance on group sociality in African savanna elephants-an example, group-living species threatened by poaching. Drawing on static association data from ten free-ranging groups, we constructed one empirically based, population-wide network and 100 virtual networks; performed a series of experiments 'poaching' the oldest, socially central or random individuals; and quantified the immediate change in the theoretical indices of network connectivity and efficiency of social diffusion. Although the social networks never broke down, targeted elimination of the socially central conspecifics, regardless of age, decreased network connectivity and efficiency. These findings hint at the need to further study resilience by modeling network reorganization and interaction-mediated socioecological learning, empirical data permitting. The main contribution of our work is in quantifying connectivity together with global efficiency in multiple social networks that feature the sociodemographic diversity likely found in wild elephant populations. The basic design of our simulation makes it adaptable for hypothesis testing about the consequences of anthropogenic disturbance or lethal management on social interactions in a variety of group-living species with limited, real-world data.

Wood, J., D. R. Morgan, K. Ange-van Heugten, M. Serrano, L. J. Minter, V. Fellner and M. K. Stoskopf (2022). "Observable Metabolites and Metabolomic Sampling Protocols for Managed African Savanna Elephant (Loxodonta africana) Whole Blood Using H-NMR Spectroscopy." Metabolites **12**(5).

We used nuclear magnetic spectroscopy (NMR) to evaluate the metabolomics of heparinized whole blood drawn from six African savanna elephants (Loxodonta africana) maintained on a well characterized diet. Whole blood samples obtained under behavioral restraint, then quickly frozen in liquid nitrogen, were stored at -80 °C until analysis. Frozen samples were thawed under controlled conditions and extracted with methanol and chloroform to separate the polar and non-polar metabolites. We identified 18 polar metabolites and 14 non-polar lipids using one-dimensional (1D) and two-dimensional (2D) NMR spectra. Despite unexpected rouleaux formation in the thawed frozen samples, spectra were consistent among animals and did not vary dramatically with age or the sex of the animal.

Wood, M., S. Chamaillé-Jammes, A. Hammerbacher and A. M. Shrader (2022). "African elephants can detect water from natural and artificial sources via olfactory cues." Anim Cogn **25**(1): 53-61.

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.

Yang, N., M. Bao, B. Zhu, Q. Shen, X. Guo, W. Li, R. Tang, D. Zhu, Y. Tang, D. N. Phalen and L. Zhang (2022). "Elephant Endotheliotropic Herpesvirus 1, 4 and 5 in China: Occurrence in Multiple Sample Types and Implications for Wild and Captive Population Surveillance." Viruses **14**(2).

Elephant endotheliotropic herpesviruses (EEHVs) are important causes of death in both captive and wild Asian elephants (Elephas maximus). Nothing is known about the prevalence of EEHVs in wild or domestic elephants in China. To determine if EEHVs are present in elephants in China, 126 wild elephants from three populations and 202 captive individuals from zoos (n = 155) and the Wild Elephant Valley (n = 47) were screened using semi-nested polymerase chain reaction assays with EEHV-redundant and EEHV1/4/5-specific primers. EEHV1B and EEHV4 were detected in samples from both wild (EEHV1B:8/126; EEHV4:2/126) and captive (EEHV1B:5/155; EEHV4:9/155) elephants, while EEHV1A (six cases) and EEHV5 (one case) were only present in the captive elephants from the Wild Elephant Valley. EEHV1 was detected in blood and trunk and oral swabs; EEHV4 was detected in trunk and oral swabs as well as feces; EEHV5 was found in trunk and oral swabs. No significant age or sex association with EEHV1A, EEHV1B, or EEHV5 positivity was observed. An age association with EEHV4 positivity was found, with all unweaned elephants being EEHV4 positive, but an association with the sex of the elephant was not observed. These findings represent the first documentation of EEHV presence in captive and wild elephants in China. These findings also document EEHV1B and EEHV4 shedding in feces and demonstrate the utility of fecal screening as a tool for investigating EEHV4 infection in wild populations of elephants. It is recommended that EEHV testing be included in surveillance programs for captive and wild elephants in China.

Zehtabvar, O., A. R. Vajhi, H. A. Akbarein, F. S. Ahmadian, M. Khanamooeiashi, R. Soflaei and F. Borgheie (2022). "CT anatomy of cervical vertebrae of Asian elephant (Elephas maximus)." Vet Med Sci **8**(4): 1750-1768.

BACKGROUND: Elephants are currently the largest mammals on earth. A comprehensive examination of the anatomy of this animal to diagnose various disorders is required. In addition, due to the heavy head of these

animals, adaptations have been made in the anatomical structure of the neck that is worth studying. OBJECTIVE: This study aimed to investigate a standard morphologic and morphometric description of the elephant cervical spine. Another aim of this study was to compare the changes in the cervical skeleton of elephants with horses and cattle. METHODS: For this study, the cervical vertebrae of the Asian elephant, cattle and horse were examined. CT Images were obtained using Somatom Spirit II CT Machine. Statistical analysis was done by SPSS 24 software. RESULTS: Two dorsal tubercles and a groove between them were observed on the dorsal arch of the atlas vertebra of the Asian elephant. In elephant samples, the variation of vertebral body height, spinous process height, transverse process width, vertebral body length and vertebral foramen volume indices were statistically significant. The volume of the vertebral foramen in the elephant decreases in the second vertebra compared to the first vertebra, decreases in the third vertebra, decreases in the fourth, increases in the fifth, decreases in the sixth and increases in the seventh. CONCLUSIONS: In this study, the structure of the cervical vertebrae of the Asian elephant was examined, and certain features were observed. One of the main features was the reduction of the length of the vertebrae, which leads to the decrease of the ratio of neck length to the size of the body. This condition can be due to the high weight of the head in the elephant. To maintain this weight, it is necessary to reduce the length of the neck and confer less mobility.