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RESEARCH ARTICLE

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Abstract

This study documents the introduction process of eight unrelated captive male Asian elephants in a free-contact management setting in Laos, utilizing a two-phase introduction process comprising limited tactile contact followed by full physical introduction. Behavioral data were collected using all-occurrence and focal-animal sampling, while fecal samples were analyzed for glucocorticoid (fGCM) and androgen (fAM) metabolite concentrations to assess physiological responses. Results indicated a prevalence of affiliative over aggressive or submissive behaviors throughout both introduction phases, supporting the idea that unrelated males can create social bonds without excessive aggression in captive settings. Affiliative behaviors declined over time during the limited tactile contact phase, then stabilized during physical introductions. Aggressive and submissive behaviors were consistently low throughout the study. Individual variations in behavior were observed, highlighting the importance of considering temperament in elephant introductions. No significant differences were found in fGCM concentrations before or after social introductions among the males, suggesting that the process did not cause substantial physiological stress. Only one male exhibited decreased fAM concentrations after social interactions, which could indicate testosterone suppression from more dominant males. During the limited contact period, aggressive interactions were positively associated with fGCM concentrations, whereas a longer duration since first introduction was associated with a decrease. In the physical introduction step, the number of days since the first introduction positively predicted an increase in fGCM concentrations, while the fAM concentration before social interactions negatively predicted fGCM. In addition, age was significantly positively predictive of fAM concentrations. These findings challenge traditional views on male elephant sociality under captive conditions and suggest that, with proper management, forming all-male groups can be a viable option for conservation and *ex situ* management programs. This study emphasizes

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the importance of gradual introduction processes, individual monitoring, and long-term behavioral observations in the successful introduction of unrelated captive male Asian elephants.

Introduction

Asian elephant (*Elephas maximus*) population numbers continue to decline and have been reduced by at least 50% across all 13 range countries in the last three generations [1]. Of the estimated 35,000–50,000 extant Asian elephants, approximately 15,000 live under human care, participating in tourism, logging, and religious activities, with fewer than 2,000 in zoos [2]. If managed appropriately, captive populations, especially in range countries, can play a vital role in conservation through captive breeding for purposes such as reintroduction, public education, and scientific research. Given the critical state of Asian elephant populations and the significant number of individuals living under human care, it is crucial to implement effective conservation and management strategies. One key aspect is understanding elephant behavior and social dynamics in the wild, the lack of which could be detrimental to the proper management of captive counterparts [3,4]. Bridging insights from wild elephant behavior to captive management is crucial to ensure that captive populations effectively support elephant conservation.

Consideration of species-typical social behavior in captive settings has improved welfare across taxa. In cotton-top tamarins *(Saguinus oedipus*), resemblance to wild-type groups led to increased breeding success, higher infant survival, and lower incidences of abortion, stillbirths, and parental neglect [5]. In male baboons (*Papio anubis*), social integration was associated with lower basal cortisol levels, suggesting reduced stress in socially integrated individuals [6]. Providing chimpanzees (*Pan troglodytes*) with the opportunity to engage in fission-fusion dynamics, akin to those found in wild populations, led to lower rates of aggression [7]. Finally, Schapiro (2002) [8] found that species-typical behavior in rhesus macaques (Macaca mulatta) was increased, and abnormal behavior was decreased by social rather than single housing. In Asian elephants, animals managed in social groups show lower rates of stereotypic behavior than those housed in isolation [9]. Additionally, herd stability significantly influenced the survival of calves among both Asian and African savanna elephants (Loxodonta africana) in European zoos [10]. Recent research has further illuminated the complex social behaviors and relationships that elephants exhibit, even in captive settings. For instance, Williams et al. [3] found that social interactions among zoo-housed elephants were influenced by factors such as age, personality, and the presence of calves within the herd, not unlike those in the wild. Moreover, Asian elephant females can maintain strong friendships even after long separations [11], underscoring the importance of considering social dynamics in captive management.

While the importance of female sociality is well-established, there is a growing recognition that male elephant welfare can also be enhanced by managing them

socially, not only with females but also with other males. Although elephant males were historically considered primarily solitary [12,13], the richness and complexity of male-male social relationships are now widely acknowledged. For example, male elephants in Kenya were observed in all-male groups 46% of the time [14], while in Botswana, older males were shown to play a central role in male society, particularly in guiding younger, inexperienced males to critical resources [15]. Among Asian elephants, a study conducted in southern India concluded that male elephants spent 43% of their time in mixed-sex groups, 34% alone, and 23% in all-male groups ranging from 2 to 12 individuals [16]. In Sri Lanka, male elephants congregate in small groups of up to seven animals, with approximately 20% of elephant sightings comprising all-male groups [17]. These findings support the idea that adult males alternate time spent alone, in mixed-sex groups, and in male groups, albeit with weaker social bonds than those of females [18–20].

The amount of time that wild males spend alone or with conspecifics depends on various factors, including age and reproductive state [18,19,21,22], forage abundance, kin relationships [14], and human activities [16]. Consequently, housing male elephants singly in captivity, with limited to no opportunity to interact with other elephants, fails to provide the social interactions they would naturally seek in the wild—interactions that are key to their development and social competence, potentially compromising their well-being. Recognizing this, western zoos have begun integrating males into female groups or creating bachelor groups [23–26]. However, in range countries, males are still primarily housed individually due to concerns about aggression, particularly during musth periods [27]. Furthermore, regional guidelines for elephant care and husbandry often overlook the social needs of male elephants [28–30], and there is a lack of clear guidance on introducing unrelated male elephants into all-male groups or female herds. Various approaches have been used to introduce unrelated animals, ranging from immediate co-housing [31] to a gradual introduction through sensory and limited tactile contact before sharing physical space [26,32,33]. While introductions can succeed without rigorous protocols, Burks et al. [23] found that a systematic method—progressing from sensory access to restricted physical access and finally unrestricted physical access—effectively managed aggression during female African elephant introductions.

Introductions of new individuals can be stressful; therefore, glucocorticoid concentrations are commonly used as stress indicators for welfare management [34]. Glucocorticoid measures have also been used to monitor social introductions in zoo female elephants [35,23]. In addition, the initial months of an introduction are crucial for establishing social hierarchies through dominance interactions (López Pérez et al. in prep), which may be influenced by testosterone. Testosterone has been proposed to facilitate socioemotional mechanisms that enhance an individual's ability to achieve and maintain social dominance [36]. Furthermore, across the animal kingdom, testosterone concentrations fluctuate rapidly in the context of competitive interactions, with winners demonstrating increased testosterone concentrations relative to losers [37]. Therefore, testosterone may mediate social interactions of male elephants during introduction periods.

The goal of this study was to assess the integration of eight unrelated males at the Elephant Conservation Center (ECC) in Lao PDR by combining measures of behavior and endocrine responses as guides. Specifically, we investigated whether 1) social behaviors (affiliative, aggressive, and submissive) change as a function of time, and 2) how fecal glucocorticoid (fGCM) and fecal androgen (fAM) metabolite concentrations were related to any changes in male-male social interactions. Understanding how the introduction process affects behavior and physiological function can provide valuable insights to improve management standards for and enhance the welfare of captive male elephants, potentially contributing to conservation efforts and the recovery of this endangered species.

Materials and methods

Animal ethical consent

This study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand (FVM-ACUC, permit number (S5/2564).

Study animals

The study was conducted at the Elephant Conservation Center (ECC) in Sayaboury province, Laos PDR (Latitude, 19°14'0.29"N; Longitude, 101°39'12.06"E; Elevation 301.752 m – 393.192 m) from February 2020 to June 2022. The ECC was founded in 2011 and, at the time of the study, was home to 30 elephants (20 females and 10 males). The ECC serves dual purposes: as an ecotourism destination and a conservation center dedicated to preserving Lao elephants through established research, breeding, and reintroduction programs. Of the elephants at the ECC, 18 were owned by the center, one was rented, and 11 were confiscated by the government and placed under ECC care. All elephants resided within a 530-hectare forest concession year-round, with access to specific areas changing daily. During the day, movements were based on several factors, including food availability, social interactions, health status, and seasonal conditions. At night, all elephants were restrained with 30–40 m chains in different locations throughout the forest. While elephants had limited access to certain parts of the forest on any given day, over the course of the year, they had access to the entire 530-hectare area.

Eight male Asian elephants (age range: 9–60 years at study start) were the subjects of this study (Table 1). These males were captive-born in Laos and privately owned by locals before being obtained by the ECC. Seven were separated from their mothers at the age of 3 years, as is typical for captive elephants in Laos, and grew up with little to no opportunity to freely socialize with conspecifics. Only the youngest male, Suriya—who arrived at the ECC with his mother when he was less than a year old—was not separated and grew up in a female herd, having daily social interactions with other elephants. At the ECC, males are handled by mahouts (i.e., keepers) in a traditional free-contact management system

Table 1. Male elephant subjects included in the study.

Subject	Year of birth	Previous job	Social background before arriving to the ECC	Arrival at the ECC	Social background at the ECC
Thong Khoun (TK)	1960	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Limited physical interactions with BP, XY, PKS, and DKS during walks. • Visual, auditory, and olfactory contact with BB, S, and JB. Died the 24th of April 2022. • Temporary encounters with female elephants for breeding purposes.
Xaiyo (XY)	1984	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Limited physical interactions with BP, XY, PKS, and DKS during walks. • Visual, auditory, and olfactory contact with BB, S, and JB. • Temporary encounters with female elephants for breeding purposes. • Left the ECC the 8th of May 2021.
Bua Ban (BB)	1970	Logging	Little to no opportunity to freely socialize with conspecifics.	2017	• Visual, auditory, and olfactory contact with all the males included in the study. • Temporary encounters with female elephants for breeding purposes.
Joun Ban (JB)	1987	Logging/ tourism	Little to no opportunity to freely socialize with conspecifics. Daily contact through a barrier with a female elephant from 2017 to 2019.	2019	• Visual, auditory, and olfactory contact with all the males included in the study. • Temporary encounters with female elephants for breeding purposes.
Boun Pheng (BP)	1992	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Limited physical interactions with TK, XY, PKS, and DKS during walks. • Visual, auditory, and olfactory contact with BB, S, JB. • Temporary encounters with female elephants for breeding purposes.
Pai Kham Sing (PKS)	2001	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Limited physical interactions with TK, XY, BP, and DKS during walks. • Visual, auditory, and olfactory contact with BB, S, and JB. • Temporary encounters with female elephants for breeding purposes.
Do Kham Son (DKS)	2003	Circus shows	Little to no opportunity to freely socialize with conspecifics.	2018	• Limited physical interactions with TK, XY, BP, and PKS during walks. • Visual, auditory, and olfactory contact with BB, S, and JB.
Suriya (S)	2011	NA	Daily social interactions with females.	2011	• Visual, auditory, and olfactory contact with all the males included in the study. • Daily interaction with female herd.

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[38]. Before physical introductions, some males had limited direct contact with other males during weekly walks (2–3 times/week) or temporary social interactions with females for breeding purposes. Otherwise, males were alone foraging in the forest. Males in musth were isolated from other elephants and tethered in the forest using a 20–25 m chain within reach of natural food and water until it was safe to reintegrate them.

Introduction phases and behavioral data collection

Burks et al. [23] described three sequential phases to introduce unrelated captive elephants and effectively manage potential aggression: visual contact (consisting of visual, auditory, and olfactory contact with conspecifics); limited tactile contact (individuals were allowed limited contact through separating adjacent stalls or yards); and physical introduction (individuals were placed in the same physical space with no barriers). In this study, all males had visual, auditory, and olfactory contact with each other for at least 1 year by sharing feeding and bathing grounds during daily routines. For that reason, the introduction protocol at ECC was reduced to the last two phases described by Burks et al. [23]: limited tactile contact and physical introduction (Table 2). At the beginning of the limited contact step, mahouts allowed male elephants to interact for only short periods (10–15 minutes) due to concerns about potential aggressive interactions. Because it was not feasible to conduct focal sampling during these times, all-occurrence sampling was used [39] to collect behavioral data from all males. During the physical contact step, elephants were placed together for longer periods (up to 3 hours), making all-occurrence sampling impractical; therefore, behavioral data collection switched to focal sampling [39]. Since all-occurrence sampling is a focal animal sampling technique for an entire group at once, the behaviors collected should be comparable between the two methods [39].

Introductions were not attempted if the weather was extreme (i.e., heavy rain, agricultural fires), elephants were sick and/or needed to rest, or males were in musth.

Limited tactile contact

The ECC does not have barriers or fences strong enough to stop aggression between males, so the limited tactile contact phase was accomplished through walks around the ECC concession (S1 Fig), which occurred over 3 consecutive days per week, lasting 2–4 hours, depending on weather conditions and elephant behavior. During these walks, males walked in a single-file line with mahouts riding on their necks. This arrangement allowed the elephants to become familiar with each other in a controlled manner and mahouts to manage any potential aggression. The order of elephants in the line was varied daily to ensure all males had opportunities to interact throughout this phase.

During walks, elephants and mahouts took periodic breaks in open, flat areas, which allowed elephants to freely interact with each other while mahouts remained nearby to supervise behavior. After each introduction session, the collected data were reviewed by the observer and mahouts, specifically, rates of aggressive and affiliative behaviors exhibited by the group, as well as the context in which aggression occurred. If, for 3 consecutive days, the rate of affiliative behaviors exceeded the rate of aggressive behaviors, and any aggression observed did not require intervention by the mahouts (i.e., was not considered a risk to the elephant or mahout), the introduction process was progressed the following week. This progression consisted of increasing the duration of walk breaks and allowing a greater distance between the mahouts and

Table 2. Behavioral data collection summary.

Introduction type	Time period	Data collection type	Observations	Total hours observed
Limited tactile contact	13-Feb-2020 to 9-Jul-2020	All occurrence sampling	83	34
Physical introduction	15-Jul-2020 to 16-Jun-2022	Focal-animal sampling	1107	351

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elephants during rest periods. Initially, the breaks were short (10–15 minutes) but gradually increased to 90 minutes over a period of 104 days. When 90-minute breaks were reached and the rate of aggressive behaviors remained consistently low, without requiring intervention, the introduction was moved to the physical introduction step.

Based on protocols developed for this study following Burks et al. [23], dyads exhibiting high rates of affiliative behaviors and low rates of aggression were considered suitable for physical introduction. By contrast, dyads showing high rates of aggression and low rates of affiliative behavior would be further evaluated. If the majority of aggressive behaviors within the dyad were non-contact aggressive behaviors (i.e., did not involve physical confrontation), the pair would be considered suitable to move to the physical contact phase. However, if the majority were contact-aggression, those individuals would not be introduced until aggressive rates were reduced. In this study, no dyads were deemed unsuitable, allowing us to proceed with introducing all eight males together during the physical contact phase.

Physical introduction

During the physical introduction phase, all males were placed together 3 consecutive days per week for 1.5–3 hours (between 0900 and 1200 hours) in large patches of open forest (3–11 ha). The mahouts selected the areas based on the amount of natural food, shade, and water points. When food, shelter, and water became scarce, mahouts selected a new location to minimize male competition. Once the area was chosen, males were released, allowing them to freely interact with other males or remain alone.

Mahouts remained at least 30 m from the elephants during observations. They intervened only if elephants moved too far away (>150–200 m) or were involved in dangerous situations that needed immediate intervention, such as approaching agricultural areas or engaging in aggressive fights.

Data collection

An ethogram was developed using descriptions of social behaviors drawn from prior studies (Table 3) [3,23,40–42]. The selected behaviors were then grouped into categories considered functionally similar [26,23,43–45] and were defined as aggressive, affiliative, submissive, and other. The aggressive category was subdivided into aggressive contact and aggressive non-contact as previously described [26,23,44,45]. Data on social behavior was collected from each male between 0900 and 1200 hours, 3 consecutive days per week throughout the study period (February 2020 to June 2022). Elephants were observed for at least 90 minutes each day from a minimum distance of 30 m so that the observer would not interfere with their behavior. The lead author collected all behavioral observation data. Throughout the limited tactile contact period, all occurrence sampling [39] recorded behaviors during breaks until the group resumed walking. For safety reasons, only non-musth males were present during walks. The start and end times, the initiator/receiver of each interaction, the individuals present in the group during the observation, and the temperature were also recorded.

During physical introductions, 20-minute focal-animal sampling [39] was used to assess male behavior using the same ethogram as above. For safety reasons, only non-musth males were placed with other males during this period. Start and end times, the initiator/receiver of each interaction, the individuals present in the group during the observation, and the temperature were also recorded [23].

Fecal sample collection

Fresh fecal samples were collected between 0800 and 1200 hours before social interactions and then 48 hours after the 3 consecutive days of social interactions to account for fecal excretion lag times. Approximately 50 g of fecal material was collected from at least three parts of the central portion of the fecal bolus and placed in ziplock bags. Gloves were worn during collection to prevent sample contamination. The bags were labeled with the name of the elephant, collection time, and date, and were immediately placed in a Styrofoam cooler with ice. The samples were kept in a cooler filled with ice for 6–7 hours until transported to the ECC endocrine laboratory, where they were immediately frozen at −20ºC.

Table 3. Elephant ethogram grouped by behavioral category.

Behavior category		Behaviors	Definition
Aggressive	Contact	Push	Initiator uses head to forceful push the recipient elephant, resulting in the target elephant moving. Pushes during play may be less intense and followed by affiliative behaviors.
		Drive	Initiator uses head, tusks or trunk to push the rear of another elephant, maintaining contact while both elephants move at least one body length. Drives during play may be less intense and followed by affiliative behaviors.
		Kick	Initiator strikes at recipient with rear front foot or rear limb. Kicks during play may be less intense and followed by affiliative behaviors.
		Spar dominant	Initiator engages in a strong head-to-head contact, pushing trunks, tusking, shoving, wrestling or trunk entwining with another elephant.
		Trunk over back	Initiator places at least two-thirds of his trunk over the recipient back.
		Trunk over head	Initiator places the trunk or head on top of the recipient elephant head or neck.
	Non-contact	Approach head high	Initiator moves toward recipient with head above shoulders and ears extended.
		Ears extended	Initiator fully extends his ears while facing another elephant.
		Charge	Initiator rapidly approaches another elephant with a rapid gait towards a stationary conspecific without making contact.
		Chase	Initiator rapidly pursues the receiver who is moving away from the initiator.
		Displace	Initiator approaches the recipient who moves away from his position without physical contact.
Affiliative	Spar play (escalated spar play)		Initiator pushes trunks, tusks, shoves, wrestles with recipient elephant. This is associated with relaxed contact including trunks entwined, trunk touching to each other's bodies, especially to mouths, head low, and ears drooped.
	Head butt play		Initiator pushes the butt of the recipient. The recipient responded with an affiliative behavior.
	Push play		Initiator pushes head-to-head, head-to-body or body-to-body of the recipient with another elephant in play.
	Drive play		Initiator uses head, tusks or trunk to push the rear of another elephant, maintaining contact while both elephants move at least one body length. The recipient responded with an affiliative behavior.
	Kneel play		Initiator kneel on the forelegs or rear legs in response to affiliative behaviors of another elephant.
	Palatal pit area		Initiator uses trunk to touch or to place the tip of the trunk inside the recipient elephant's mouth.
	Anal touch		Initiator uses trunk to touch recipient elephant's anus.
	Genital touch		Initiator uses trunk to touch another elephant's genitals.
	Non-aggressive approach		Initiator calmly moves toward the recipient with head low and flapping ears.
	Follow		Initiator walks calmly behind another elephant withing a few body lengths.
	Body touch		Initiator uses head or body to contact the recipient elephant.
	Trunk touch		Initiator touches with trunk to another elephant's head, trunk, body, front legs, or mammary glands.
	Lie down play		Initiator lay on one side while interacting with the recipient elephant in affiliative context.
Submissive	Scream		Initiator performs a high frequency vocalization emitted from open mouth.
	Back up		Initiator walks backward into the recipient elephant.
	Back away		Initiator walks backward away from recipient elephant while facing recipient.
	Present back		Initiator presents their back when the recipient elephant approaches.
	Run away		Initiator flees from recipient elephant in response to aggressive contact, display, or approach.
	Blow air		Initiator expels air audibly from his trunk.
	Trunk ground		Initiator hits the ground with the trunk making an audible noise while blowing air.
	Trumpet		Loud, high-pitched, resonant vocalization.
Other	Sniff		Initiator extends trunk and sniffs toward the recipient elephant without touching.
	Stereotypy		Initiator performs stereotypic behavior, including head-bobbing or pacing.
	Other		Any other behavior not described in the ethogram. Describe the behavior and classify it into one of the categories: other aggressive contact, other aggressive non-contact, other submissive, and other affiliative.

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Hormone processing and extraction

Fecal samples were extracted as described by Norkaew et al. [46]. Briefly, wet samples were dried in a conventional oven at 60ºC for ~48 hours. Dry samples were pulverized and sifted using a steel mesh strainer to remove fibrous material. The resulting fecal powder was stored at −20ºC until extraction. The frozen dried fecal powder was thawed at room temperature, mixed well, and 0.1 g (± 0.003) of powdered feces placed in a 16 x 135 mm glass tube. Samples were extracted in 90% ethanol by boiling in a water bath (95ºC) for 20 minutes and adding 100% ethanol as needed to keep from boiling dry. Samples were centrifuged at 1500 x g for 20 minutes. The extracts were decanted into a second set of tubes, 95% ethanol was added to the fecal pellets, and the tubes were centrifuged at 1500 x g for another 20 minutes. Extracts were pooled and then dried under air in a 50ºC water bath. Dried extracts were reconstituted in 1 ml assay buffer and stored at –20ºC until enzyme immunoassay (EIA) analysis [46].

Hormone analyses

Concentrations of fGCM were determined using a double-antibody EIA with a polyclonal rabbit anti-corticosterone antibody (CJM006) validated for Asian elephants [47] and described by Norkaew et al. [46]. The absorbance was measured at 450 nm by a microplate reader (TECAN, Sunrise microplate reader, Salzburg, Austria). Assay sensitivity (based on 90% binding) was 0.14 ng/ml. Samples (25 µl) were diluted 1:6 or 1:8 in assay buffer for analysis in duplicates. The inter-assay CV for high (30% binding) and low (70% binding) control samples was < 15%. Samples were reanalyzed if the duplicate CV was > 10%; thus, intra-assay CVs were <10%.

Concentrations of fAM were quantified by a double-antibody EIA validated for Asian elephants [48] utilizing an anti-testosterone antibody (R156/7). Fecal extracts (25 µl) were diluted 1:16 in assay buffer, and absorbance was measured at 450 nm. Assay sensitivity (based on 90% binding) was 0.04 ng/ml, and the intra- and inter-assay CVs were <10% and <15%, respectively. Fecal data are expressed as ng/g dried feces.

Statistical analysis

Analyses were based on 385 hours of behavioral observations and 1090 fecal samples (545 samples before social interactions and 545 samples 48 hours after social interactions) from the eight males during the two phases of the introduction process.

Rates of initiated behaviors per minute were calculated for each behavioral category and for each individual by dividing the total number of initiations by the total observation time. These rates were computed separately for the limited tactile contact and physical introduction phases. Rates of affiliative, aggressive, and submissive behavior were plotted against the number of days since the first introduction to visualize whether behavioral rates increased or decreased over time. A Spearman correlation test was used to determine if the observed trend over time was statistically significant.

A Wilcoxon signed-rank test was used to compare population-level fAM and fGCM concentrations before and after social interactions. Since social interactions occurred over 3 consecutive days, the counts of behaviors were calculated by summing all observed behaviors within each category during these 3-day periods. Similarly, the total observation time was calculated by summing the time the elephants were observed over these 3 days. Observation times were tallied separately for each individual male, as not all males participated in every possible interaction day, resulting in variable observation durations across individuals. Once behavior rates were calculated for each 3-day period, corresponding pre- and post-interaction fAM and fGCM concentrations were assigned to match the timing of each rate.

In addition to assessing whether all before-interaction fGCM and fAM values were significantly different from all after-interaction values as described above, we assessed differences at the individual pairwise level (i.e., whether a male's endocrine values increased from their starting point before the interaction period). To determine whether male-male interactions influenced fAM and fGCM concentrations, we calculated the change in fAM and fGCM concentrations for each male

after each social interaction period. This was done by subtracting the fAM and fGCM concentrations for a given time period (after minus before social interactions). Because these values were continuous and could be positive or negative, we then constructed Gaussian error distribution models with "after minus before" concentration differences as response variables in the glmmTMB package [49] using R Statistical Software v 4.1.2 [50]. The covariates considered for all models included days since first introduction, mean temperature over the 3 days, male age, counts of affiliative interactions, and counts of aggressive interactions. For the fGCM models, we also included fAM concentration before the interaction period as a covariate because elevated fAM in males is known to influence social interactions [51,52]. Variables were scaled prior to inclusion in models. Male ID was used as a random effect to account for individual variations and to avoid pseudo-replication. We conducted these models separately for the limited tactile contact and physical introduction phases and assessed models fit using the DHARMa package in R [53]. However, these models did not meet diagnostic criteria, so we converted the response variable "after minus before" values for fAM and fGCM into a binary variable to simplify the models (1 = fAM and fGCM increased within the male after an interaction period; 0 = fAM and fGCM decreased within the male following an interaction period). We then ran logistic regression models with these binary variables as the response variables for fAM and fGCM models, respectively, using the lme4 package in R and the same model features described above [54]. Diagnostic assessments for these models indicated good fit, and we therefore present results from logistic regression models.

Results

Affiliative behaviors were the predominant form of interaction throughout both the limited tactile contact and physical introduction phases, consistently occurring at higher rates per minute than either aggressive or submissive behaviors over time (Fig 1). Analysis using the Spearman correlation test revealed a significant decrease in affiliative behavior rates over time during the limited tactile contact period (rho = −0.54, p = 0.0014), whereas affiliative behaviors remained relatively stable during the physical introduction phase, showing no significant temporal change (rho = −0.09, p = 0.19). By contrast, aggressive behaviors were notably absent during the initial days of the limited tactile contact phase, not appearing until day 13, albeit at low rates throughout, with no discernible trend over time (rho = 0.01, p = 0.97). In contrast, during the physical introduction period, aggressive behaviors began at similarly low rates but declined as time progressed (rho = −0.19, p = 0.004). Submissive behaviors were consistently low across both phases, with rates that remained unchanged over time, as reflected in non-significant correlations during both the limited tactile contact (rho = 0.08, p = 0.68) and physical introduction steps (rho = 0.02, p = 0.76).

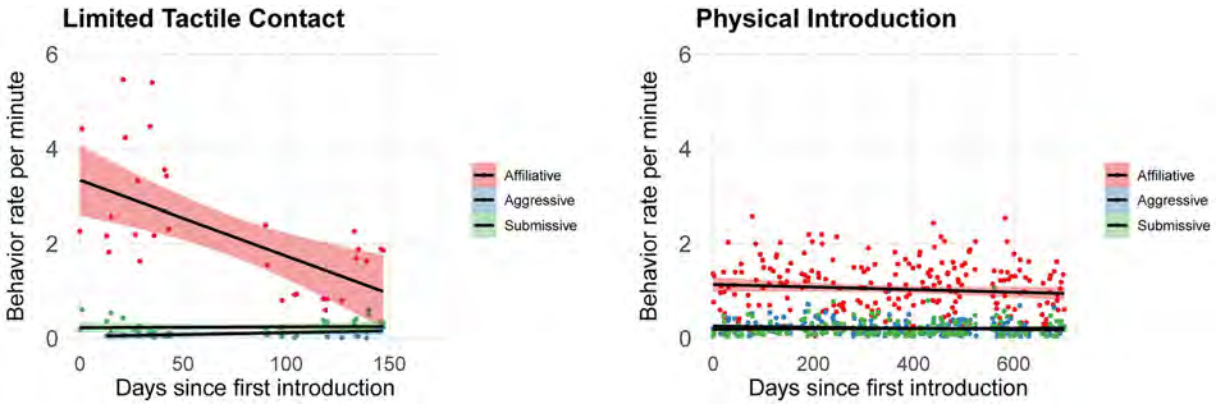

Fig 1. Overall temporal changes in behavioral rates during introduction phases. Scatter plots with trend lines showing the temporal progression of three behavioral categories (affiliative, aggressive, and submissive) during the limited tactile contact and the physical introduction.

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Behavior rates plotted over time by individual elephant confirmed the trends described above (Fig 2). Notably, none of the males initiated aggressive behavior during the first days of the limited-contact phase, while two individuals (DKS and PKS) never initiated aggressive behaviors during the entire period. The main trend observed by four of the males (BB, DKS, S, and TK) was that affiliative behavior decreased over time, while aggressive behaviors were performed at low but consistent rates throughout the two introduction periods. However, the other four males (PKS, XY, BP, and JB) showed an increase in affiliation rate over time during the full physical introduction steps, but this trend was only significant for PKS.

Considerable variability was observed in both fAM and fGCM concentrations across elephants in the study (S2 Table). Despite this high variability, changes in fGCM concentrations before and after interactions were not significant in any of the males (S3 Table, Fig 3). Similarly, changes in fAM concentrations before and after social interactions were not significant for all males with the exception of PKS, whose fAM decreased significantly after the social interactions (S4 Table, Fig 4).

The logistic regression model for differences in fGCM before and after introductions was a good fit for the data for the limited and full contact periods (S5 Fig). For the limited contact period model, the number of aggressive interactions

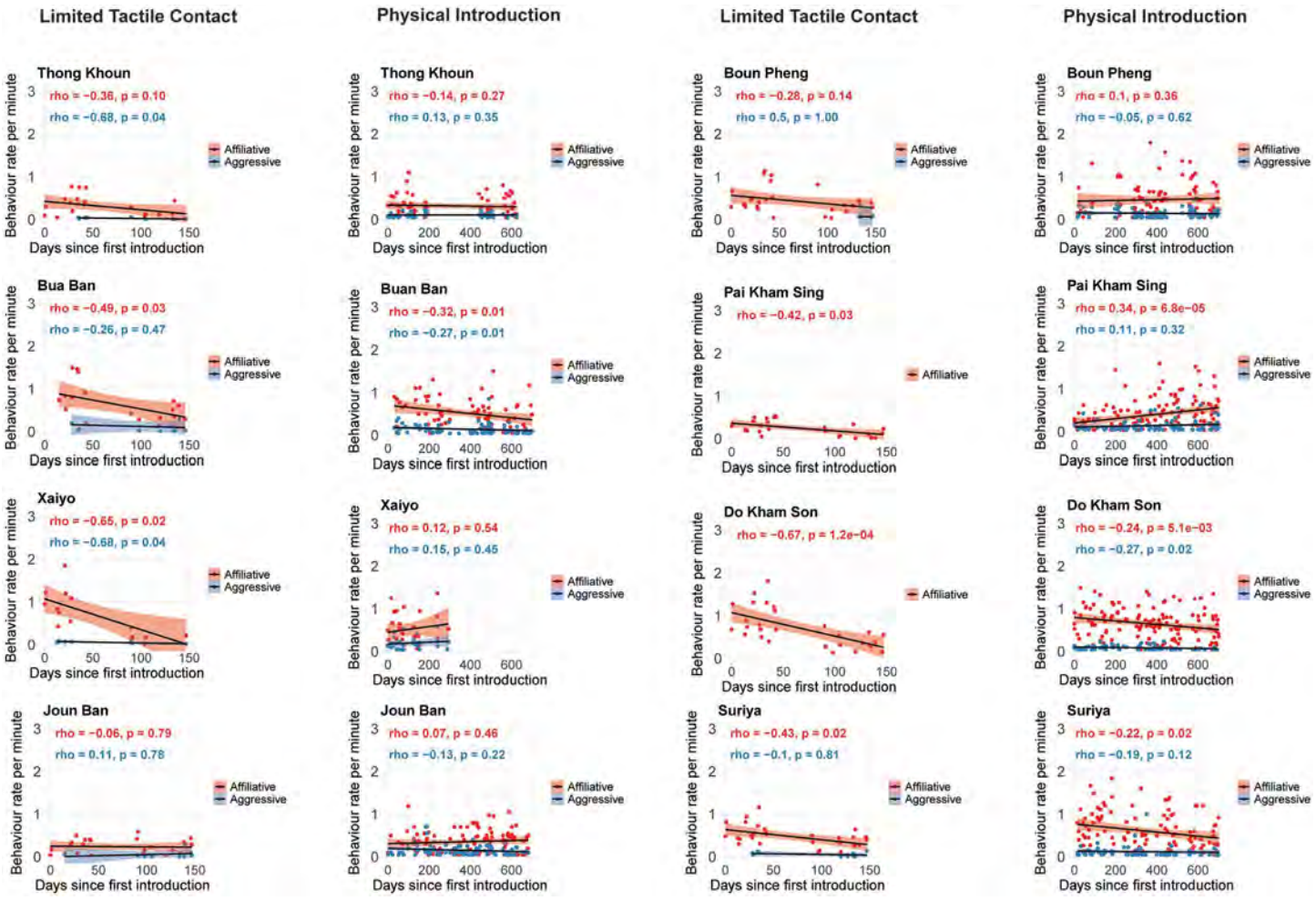

Fig 2. Temporal changes in behavioral rates during introduction phases per individual. Scatter plots with trend lines of each male in the study (n = 8) showing the temporal progression of two behavioral categories (affiliative and aggressive) during the limited tactile contact and the full physical introduction phases. The strength and significance of behavioral rate changes over time were assessed using Spearman's rank correlation analyses.

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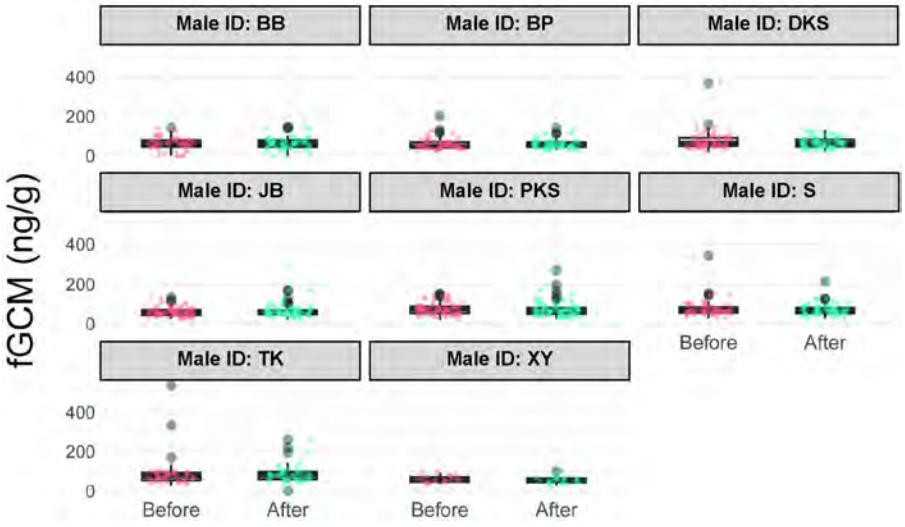

Fig 3. Fecal glucocorticoid metabolite (fGCM) concentrations before and 48 hours after social interactions for each male. Each plot shows the median (horizontal line), 25 and 75% quartile ranges, and minimum and maximum ranges (whiskers). Before = fecal samples collected before social interactions; After = fecal samples collected 48 hours after social interactions.

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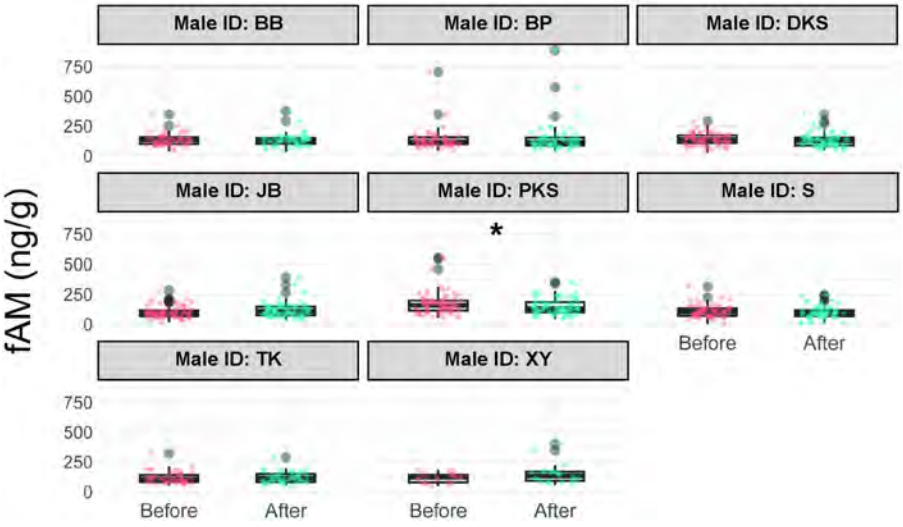

Fig 4. Fecal androgen metabolite (fAM) concentrations before and 48 hours after social interactions for each male. Each plot shows the median (horizontal line), 25 and 75% quartile ranges, and minimum and maximum ranges (whiskers). Before = fecal samples collected before social interactions; After = fecal samples collected 48 hours after social interactions. The * symbol denotes statistical significance ($p < 0.05$).

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during an interaction period was positively predictive of an increase in fGCM following the interaction period. Days since first introduction was negatively predictive of an increase in fGCM following the interaction period (Fig 5). No other covariates were significant. For the full contact period model, days since introduction was significantly positively predictive of an increase in fGCM, while fAM before the interaction period was significantly negatively predictive of an increase in fGCM.

The logistic regression model for the difference in fAM before and after introductions was a good fit for the data for the limited and full contact periods (S6 Fig). For the limited contact period model, none of the covariates were significant



Fig 5. Odds ratios for covariates for fecal glucocorticoid metabolite (fGCM) logistic regression models. Blue color indicates positive effect, while red color indicates negative effect between the covariate and fGCM. (*) p-value<0.01; (**) p-value< 0.001.

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(Fig 6). For the full contact period model, age was significantly positively predictive of an increase in fAM. The rest of the covariates were not significant.

Discussion

This study documented, for the first time, how the integration and socialization of unrelated male Asian elephants in a free-contact management setting affected behavior and hormonal responses. In stark contrast to their reputation as being highly aggressive [55], this study revealed a consistent trend of higher rates of affiliative behavior per minute over aggressive or submissive behaviors over time during both the limited tactile contact and full physical introduction phases in all individuals, regardless of age. Affiliative behaviors gradually decreased over time during the limited tactile contact phase and remained constant over time during the physical introduction step, as males habituated to the social situation. This pattern suggests a stabilization of relationships followed by an initial period of social exploration. Affiliation was also the most common type of social behavior observed in the wild (72.5%) and in all-male groups of Asian elephants housed in zoos (68.5%), indicating that the study animals exhibited behavior consistent with other populations [56]. The low rates of aggressive and submissive behaviors observed suggest that elephants maintained relatively stable interactions throughout the introduction process. These findings closely align with those described by Burks et al. [23] for female elephants,

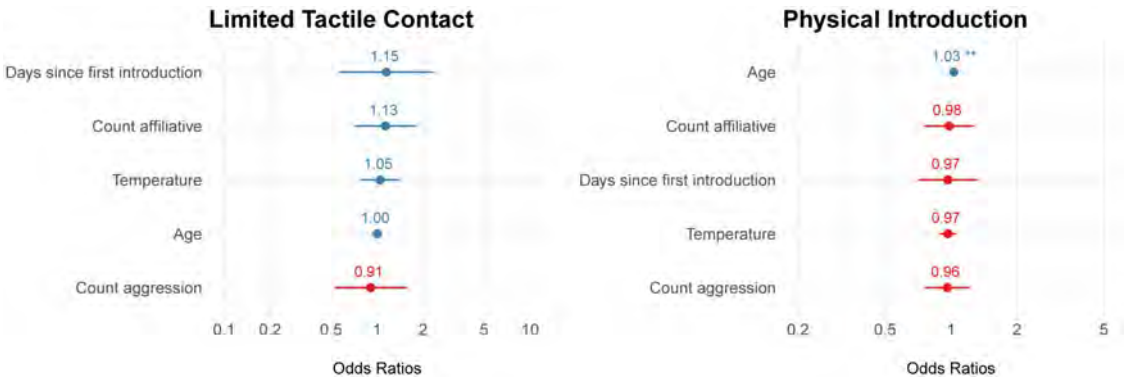

Fig 6. Odds ratios for covariates of the fecal androgen metabolite (fAM) logistic regression models. Blue color indicates a positive effect, while red color indicates a negative effect between the covariate and fAM. (*) p-value<0.01; (**) p-value< 0.001.

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indicating that sequential introduction methods can successfully manage aggression while forming unrelated elephant groups. They also indicate that male elephants can form social bonds even in artificial social settings. Schreier et al. [26] reported that the proportion of aggressive behaviors was initially high during a 5-month introduction period for male zoo Asian elephants but diminished and became more affiliative over time. In this study, contrary to expectations, none of the males exhibited aggressive behaviors during the first days of introduction. As the process progressed, each male began displaying aggressive behaviors at different times and rates, revealing individual variation. The study revealed distinct behavioral patterns for the affiliative category. Four individuals—PKS, XY, BP, and JB—exhibited an increase in affiliative behaviors over time during the full physical introduction phase. Notably, PKS, characterized by its mahout as "shy" and intimidated by other males, required additional time to develop comfort within the social group, demonstrating increased affiliative behaviors later than its counterparts. He was also a juvenile and demonstrated significantly lower fAM concentrations following social interaction periods. Previous studies have reported that high-ranking musth males can suppress musth in lower-ranking males [21,57,58]. Musth, a phenomenon unique to African and Asian elephants, is typically observed in sexually mature males in good physical condition [22,59] and is associated with increased androgen production [60,61]. Although none of the male elephants in this study were in musth, the results suggest that submissive males may exhibit testosterone suppression when close to more dominant males, even outside of musth periods. JB and BP, considered shy and nondominant, also exhibited increased rates of affiliative behavior over time, although the trends were not statistically significant. By contrast, XY, labeled as an "aggressive male" by its mahout, displayed high rates of affiliative behavior, especially in the first days of introduction, which was contrary to expectations. This variation in individual responses highlights the complexity of elephant social behavior and underscores the importance of considering individual temperament when managing introductions. Research across various zoo species has demonstrated that animals exhibit diverse behavioral traits that influence their responses to management practices [62,63]. Consequently, future elephant introductions should adopt a personalized approach, accounting for individual personalities rather than applying a "one size fits all" strategy.

The observed negative effect of fAM concentrations before social interactions on fGCM could be explained by a dual-hormone regulation through cortisol's associations with stress and social dominance [64–66]. Higher fAM may be positively related to dominance when cortisol is low, as that allows for the overt expression of dominant behaviors. Conversely, when cortisol is high, higher testosterone may actually decrease dominance and motivate lower status-seeking behavior [67]. Given the growing body of literature on the context-dependent effects of hormones, future research should continue to measure multiple hormones in conjunction with behavior to better understand these complex interactions.

In the logistic regression model, age emerged as a significant predictor of increased fAM during the full contact period. This finding suggests that older male elephants were more likely to experience elevated androgen levels following social interactions than their younger counterparts. In the context of male elephant society, age is often correlated with social status, with older males generally occupying more dominant positions in the hierarchy [21,68]. However, it is important to note that dominance status in male elephants is not static and can fluctuate over time, particularly in relation to musth [69]. This dynamic nature of elephant social hierarchies adds complexity to their social interactions and hormonal responses.

Maintaining a high-status position within such a fluid social structure requires heightened vigilance towards potential threats, particularly those that might challenge an individual's social standing [70]. Testosterone plays a crucial role in these processes, influencing a wide range of behaviors and physiological responses. Specifically, elevated testosterone levels are associated with increased motivational drive, reduced fearfulness, and enhanced stress resilience [36,67]. In elephant society, these hormonal dynamics may help explain why older, typically more dominant males show increased androgen responses following social interactions. This heightened hormonal reactivity may be a physiological mechanism that supports their ability to gain or maintain high-status positions within the dynamic social hierarchy of male elephants [60,71].

When comparing fGCM concentrations before and after social introductions, no significant differences were found for any of the males. However, a closer examination of before-after data revealed the influence of covariates on changes in endocrine concentrations following interaction days. The fewer days since an introduction, the more likely males were to have higher fGCM concentrations following interactions during the limited contact period. This finding is consistent with the notion that during the initial exploration period, interactions may have elicited a stress response in males, as observed in other studies [26,35].

During the limited tactile contact phase, an increase in fGCM concentrations was positively predicted by the number of aggressive behaviors and negatively predicted by the number of days since first introduction. These findings suggest that aggressive behaviors may trigger an adrenal response during this period, which then diminishes over time. During the limited tactile contact period, elephants began to familiarize themselves with one another and establish a new social dominance hierarchy—a process that can be inherently stressful for the individuals involved. Previous studies in non-human primates have shown that group formation triggers the HPA axis, with glucocorticoid concentrations typically normalizing once social hierarchies stabilize [72]. A similar HPA axis response likely occurred in the ECC elephants during group formation and hierarchy establishment, which could explain why fGCM increases were negatively associated with time as social dynamics stabilized. The decrease of fGCM concentrations over time predicted by the model could also be related to the introduction process, as mahouts remained close to the males during the initial days to quickly mitigate any perceived aggressions. Mahouts stayed further away from the males as the introductions progressed over time. The close presence of the mahouts could have potentially triggered certain stress responses in the elephants during the initial days of the introduction, responses that decreased over time as the mahouts increased their observation distance. Numerous factors can influence glucocorticoid concentrations. While aversive or stressful events often trigger elevated levels, increases can also occur in response to neutral or even positive stimuli. Since rates of aggressive behavior were performed at a higher rate than aggressive or submissive behavior over time during the full physical introduction step, the increased effect observed over time on fGCM could be related to engaging in a social group. This fits with the hypothesis of a cortisol "boost," suggesting that cortisol levels can temporarily rise during stimulating and rewarding social experiences, not just in response to stress. The increase may serve an adaptive function by enhancing alertness, engagement, and social bonding, possibly due to direct effects on metabolic processes, such as increased blood glucose levels [73]. However, further investigation is needed to support this hypothesis in Asian elephants. Another explanation for the increase in fGCM concentrations during the full contact phase may be related to the change in group composition following XY's departure from the ECC in May 2021 and TK's death in April 2022. The change in group composition and the re-established relationships after the departure of these males could have led to an increase in fGCM. Wild elephants have been observed showing awareness and interest in dying and deceased conspecifics [74,75], suggesting that the death of conspecifics, in addition to disrupting social groups, can be emotionally distressing for individual elephants, at least in the short term. Similarly, disruption in group composition from poaching in wild female African elephants has been associated with increased fGCM concentrations [76]. Transportation and relocation, which often result in the dissolution of social bonds or require the formation of new ones, can also trigger increased glucocorticoid excretion in elephants [77,78]. Therefore, further studies that help to understand the impact of life events on perceived stressors would be beneficial in determining the welfare needs of individuals and groups. Captive elephants could play a crucial role in the long-term preservation of the species through reintroduction efforts, breeding programs, and research initiatives. To effectively support future conservation strategies, it is crucial that the remaining captive elephant populations are managed according to the highest welfare standards.

Conclusions

This study provides valuable insights into the social behavior and physiological responses of male Asian elephants during group formation. The prevalence of affiliative behaviors over time and the absence of significant stress responses suggest

that, with proper management, all-male groups can be a viable option for elephant conservation and captive management programs. The success of the two-phase introduction process, involving limited tactile contact followed by physical introduction, indicates that a gradual approach can effectively promote positive social interactions and manage aggression during the introduction of male captive Asian elephants**.** Behavioral data collection proved to be a crucial tool in decision-making, allowing animal managers to obtain an instantaneous and objective assessment of each male's behavior. Notably, affiliative behavior was more indicative of overall behavior patterns than expected, highlighting the importance of measuring these behaviors during introductions rather than focusing solely on aggressive interactions. The non-significant differences in hormone concentrations observed before and after social interactions indicate that, when properly managed, the introduction process may not be overly stressful for the elephants. However, it is crucial to note that these recommendations must be carefully tailored to each facility, taking into account available space, individual elephant temperaments, staff proficiency in interpreting behavior, and the time required to adequately monitor the introductions. Our findings, although informative, are based on a specific context, which involved managing elephants in a free-contact facility with expansive spaces that allow animals to avoid one another as needed. Therefore, conditions at the ECC may not fully capture the diversity of experiences across different facilities. This highlights the need for further research, particularly studies that examine the dynamics of all-male social groups in various settings. By questioning traditional assumptions and providing evidence-based recommendations for group formation, our study contributes to the ongoing development of best practices in elephant conservation and captive management, while also highlighting the need for continued investigation in this area. As our understanding of the social needs and behaviors of male elephants grows, we will be better equipped to design living environments and social structures that truly enhance their well-being and quality of life.

Supporting information

S1 Fig. ECC map.
(PDF)

S2 Table. Descriptive statistics.
(DOCX)

S3 Table. Wilcoxon Signed-Rank Test Summary (fGCM).
(DOCX)

S4 Table. Wilcoxon Signed-Rank Test Summary (fAM).
(DOCX)

S5 Fig. Diagnostic plots generated by the DHARMa package for fGCM models.
(DOCX)

S6 Fig. Diagnostic plots generated by the DHARMa package for fAM models.
(DOCX)

S1 File. Data set.
(XLSX)

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Social dynamics and network structure of a group of captive male Asian elephants (*Elephas maximus*) in Lao PDR

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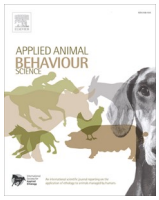



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ABSTRACT

Male Asian elephants under human care are frequently managed in isolation due to concerns about aggression, despite increasing evidence that they can form compatible social relationships. This study investigated behavioral dynamics of eight unrelated male Asian elephants at the Elephant Conservation Center in Lao PDR that were socialized over a 2-year period. Behavioral data were collected using a combination of focal animal and instantaneous scan sampling. Rates of affiliative, aggressive, and submissive behaviors, along with proximity data, were used to construct social and proximity networks for each time period. Network analyses included calculations of centrality metrics (In-Strength, Out-Strength, Betweenness), coefficients of variation (CV) to evaluate partner selectivity, and Quadratic Assignment Procedure correlations to assess network stability. The results revealed that in affiliative networks, interactions were significantly more frequent and better connected than in the aggressive or submissive networks. Younger bulls initiated more affiliative and submissive interactions compared to older bulls. Over time, affiliative interactions decreased as familiarity increased, while aggressive interactions were more frequent during the early stages of group formation before declining as relationships stabilized. Submissive behaviors remained consistently low and were primarily directed from younger toward older bulls. Notably, mixed-age dyads were prevalent across all behavioral categories and proximity networks, suggesting the potential for social learning and highlighting the importance of older bulls in all-male groups. Centrality analyses revealed that Out-Strength and In-Strength were generally higher in affiliative networks compared to aggressive or submissive ones, underscoring the dominance of positive social behaviors among bulls. CV analyses showed that interactions across behavioral categories were generally concentrated among preferred partners rather than evenly distributed within the group, with aggressive and submissive interactions becoming increasingly selective over time. Additionally, specific individuals played more central roles in maintaining group cohesion than others, emphasizing their importance for group stability. Together, these results provide valuable insights into male Asian elephant sociality, emphasizing the need for species-appropriate social housing in captive settings. Enhancing social opportunities for captive male elephants could improve their welfare and better replicate natural social structures. Additional long-term studies in various contexts are now needed to fully understand the social dynamics of male Asian elephants and inform conservation efforts for both in-situ and ex-situ populations.

1. Introduction

Sociality in mammalian societies provides adaptive benefits, including protection from predators, improved access to resources, and increased mating opportunities (Silk, 2007). In many species, male social behavior is predominantly shaped by competition for access to

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females, often limiting cooperation among males (Van Hooff and Van Schaik, 1994). Nevertheless, all-male groups are frequently observed in polygynous species that provide opportunities to gain social skills (Ruckstuhl and Neuhaus, 2002; Shannon et al., 2006; MacFarlane and Coulson, 2009).

Our understanding of elephant social behavior is primarily derived from extensive long-term studies of African savannah elephants (*Loxodonta africana*) (Douglas-Hamilton, 1972; Poole, 1994; Lee and Moss, 1999; Archie et al., 2006). Although research on social dynamics in Asian (*Elephas maximus*) and African forest (*Loxodonta cyclotis*) elephants remains comparatively limited, particularly for males, a growing body of work has begun to examine male Asian elephant social behavior and management in captive settings (Readyhough et al., 2022, 2023, 2024; López Pérez et al., 2025; LaDue and Schiffmann, 2026) . Studies have shown that male elephants may benefit from associating with other males by gaining access to sparring partners (Evans and Harris, 2008; Goldenberg et al., 2014), acquiring ecological knowledge (Allen et al., 2020), and adopting adaptive strategies to cope with anthropogenically altered environments (Chiyo et al., 2014; Srinivasaiah et al., 2019; Muthukumarana, 2023; Puri et al., 2025). Despite these insights, the association patterns within all-male groups have not been studied as thoroughly as those among females.

Male elephant society is dynamic, characterized by dominance hierarchies (O'Connell-Rodwell et al., 2011), complex social networks based on long-term relationships (Evans and Harris, 2008; Chiyo et al., 2011; O'Connell-Rodwell et al., 2011; Murphy et al., 2019), kinship ties (Chiyo et al., 2011), age structure (Evans and Harris, 2008; Allen et al., 2020, 2021a, 2021b), and reproductive status (Chiyo et al., 2011). Young males stay with the natal herd before dispersing at puberty between 8 and 15 years of age (Short et al., 1967; Sukumar, 1992; Lee and Moss, 1999) and alternate between time spent alone, in mixed-sex groups, or in all-male groups (Vidya and Sukumar, 2005; Goldenberg et al., 2014; Srinivasaiah et al., 2019; Allen et al., 2020; Keerthipriya et al., 2021; LaDue et al., 2022). Adolescence is key for social learning, with older bulls playing a vital role in sharing ecological and social knowledge (Evans and Harris, 2008; Jayantha et al., 2009; Muthukumarana, 2023; Puri et al., 2025). Adult bulls are now recognized as highly sociable, and their interactions are essential for physiological and behavioral development within the herd (Veasey, 2006; Srinivasaiah et al., 2019; LaDue et al., 2022).

Of the estimated 50,000 remaining Asian elephants, around 15,000 live under human care across 13 range countries (Menon and Tiwari, 2019) housed under a variety of conditions, such as in zoos, circuses, temples, forest/logging camps, and tourist camps (Riddle and Stremme, 2011; Bansiddhi et al., 2018). The social needs of male elephants are less well understood than those of females, and they are often separated from female groups at a young age and housed individually, a practice that continues into adulthood (Schulte, 2000; Clubb and Mason, 2002; Doyle et al., 2024). In most captive facilities, bulls are only given access to females for breeding (Hartley et al., 2019) and rarely interact with other males (Hartley et al., 2019; Schulte, 2000). This lack of socialization among captive male elephants is a significant welfare concern, as it can negatively affect physiology and behavior (Kurt and Garai, 2001; Clubb and Mason, 2002; Vanitha et al., 2011; Bansiddhi et al., 2019; Doyle et al., 2024). For example, reduced male-male contact has been linked to decreased libido (Hartley et al., 2019; Toin et al., 2020), elevated fecal glucocorticoid metabolites concentrations (Seltmann et al., 2022), increased stereotypic behaviors such as pacing and head-bobbing (Readyhough et al., 2022), and more frequent agonistic interactions among adolescent males in the absence of mature bulls (Readyhough et al., 2024). As wild elephant numbers continue to decline, captive populations are increasingly important for conservation, education, research, breeding, and reintroduction programs. However, proper management of captive elephants is challenging and requires maintaining conditions that closely resemble the physical and social environments of their wild counterparts (Doyle et al., 2024). A foundational understanding of male elephant social patterns and preferences is crucial for effective captive management, including the development of safety protocols and welfare standards.

This study aimed to address a critical knowledge gap in male elephant social behavior in captivity by analyzing social interactions among eight unrelated bulls at the Elephant Conservation Center (ECC) in Lao PDR (Laos), which were gradually introduced to one another over a 2-year period. Changes in group composition during this time provided opportunities to investigate social preferences and network dynamics among the bulls. The primary objectives of this research were to examine: 1) the role of individuals in affiliative, aggressive, and submissive network behaviors; 2) how age affects these roles; and 3) the stability of social networks over time. This exploratory study was designed to identify potential patterns and generate hypotheses for future research. A deeper understanding of male elephant social patterns, preferences, needs, and interactions ultimately contributes to improved care and conservation outcomes for this endangered species.

2. Materials and methods

2.1. Animal ethical consent

This study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand (FVM-ACUC, permit number (S5/2564).

2.2. Study site and subjects

The study was conducted at the ECC in Sayaboury province, Laos (19°14'0.29"N, 101°39'12.06"E) (Fig. S1) from February 2020 to June 2022. The ECC was founded in 2011 and housed 30 elephants (20 females and 10 males) at the time of the study. The ECC serves dual purposes as an ecotourism destination and a conservation center dedicated to preserving Lao elephants through established research, breeding, and reintroduction programs. Of the elephants at the ECC, 18 were owned by the center, one was rented, and 11 were confiscated by the government and placed under ECC care. All elephants resided within a 530-hectare forest concession, with access to specific areas changing daily. During the day, movements were influenced by factors such as food availability, social interactions, health status, and seasonal conditions. All elephants were restrained at night with 30- to 40-m chains in various locations throughout the forest. While elephants had limited access to certain parts of the forest on any given day, they had access to the entire 530-hectare area throughout the study.

This study focused on eight of the 10 male Asian elephants (aged 9–60 years at the time of introduction; see Table 1). One male was excluded because he was part of a release program, while the other was privately owned, and the owner declined to participate in the study. The selected bulls were separated from their mothers at 3 years of age, as is typical for captive elephants in Laos, and grew up with little to no opportunity to socialize freely with conspecifics. Only the youngest male, Suriya, who arrived at the ECC with his mother when he was less than a year old, was not separated and grew up in a female herd, having daily social interactions with other elephants. At the ECC, males are handled by mahouts (keepers) in a traditional free-contact management system (Doyle and Hoffman, 2013). Before the study, males were maintained individually and spent most of their time foraging alone in the forest. Direct social interactions between males were limited, occurring sporadically and only when allowed by the mahout. As such, most males had very limited direct social contact with other males prior to the study, and were largely socially isolated. The social background of each male is described in Table 1. In 2020, all bulls were effectively introduced to one another to provide regular social interactions with other males (López Pérez et al., 2025). Briefly, introductions were conducted three times a week in two phases based on Burks et al. (2004): limited tactile contact (male interactions were conducted under the close supervision of

Table 1
Summary of male elephants participating in the study (n = 8).

Subject	Year of birth	Previous job	Social background before arriving at the ECC	Arrival at the ECC	Social background at the ECC
Thong Khoun (TK)	1960	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Temporary encounters with female elephants for breeding purposes. • Social interactions with the resident males were predominantly non-physical, with physical contact being sporadic and limited. • Regular social interactions with all bulls included in the study since February 2020. • Died the 24th of April 2022.
Xaiyo (XY)	1984	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Temporary encounters with female elephants for breeding purposes. • Social interactions with the resident males were predominantly non-physical, with physical contact being sporadic and limited. • Regular social interactions with all bulls included in the study since February 2020. • Left the ECC the 8th of May 2021.
Bua Ban (BB)	1970	Logging	Little to no opportunity to freely socialize with conspecifics.	2017	• Temporary encounters with female elephants for breeding purposes. • Visual, auditory, and olfactory contact with all the bulls included in the study. • Regular social interactions with all bulls included in the study since February 2020.
Joun Ban (JB)	1987	Logging/ tourism	Little to no opportunity to freely socialize with conspecifics. Daily contact through a barrier with a female elephant from 2017 to 2019.	2019	• Temporary encounters with female elephants for breeding purposes. • Visual, auditory, and olfactory contact with all the bulls included in the study. • Regular social interactions with all bulls included in the study since February 2020.
Boun Pheng (BP)	1992	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Temporary encounters with female elephants for breeding purposes. • Social interactions with the resident males were predominantly non-physical, with physical contact being sporadic and limited. • Regular social interactions with all bulls included in the study since February 2020.
Pai Kham Sing (PKS)	2001	Logging	Little to no opportunity to freely socialize with conspecifics.	2018	• Temporary encounters with female elephants for breeding purposes. • Social interactions with the resident males were predominantly non-physical, with physical contact being sporadic and limited. • Regular social interactions with all bulls included in the study since February 2020.
Do Kham Son (DKS)	2003	Shows	Little to no opportunity to freely socialize with conspecifics.	2018	• Social interactions with the resident males were predominantly non-physical, with physical contact being sporadic and limited. • Regular social interactions with all bulls included in the study since February 2020.
Suriya (S)	2011	NA	Daily social interactions with females.	2011	• Daily interactions with the female herds. • Social interactions with the resident males were predominantly non-physical, with physical contact being sporadic and limited. • Regular social interactions with all bulls included in the study since February 2020.

mahouts); and physical introductions (males were allowed to interact freely or remain solitary based on preference). Limited tactile contact occurred from February 2020 to July 2020, totaling 34 h of behavioral observations. The physical introduction phase occurred from July 2020 to June 2022, during which 351 h of behavioral observations were recorded. Only behavioral data collected during the physical introduction phase were included in the present study. Social group size varied daily, ranging from two to eight bulls (4.91 ± 0.04). These fluctuations were influenced by management-related factors, including the health status of both elephants and mahouts, the quality of the areas where elephants were housed (e.g., natural food availability, shade, and access to water), adverse weather conditions, and individual musth status. Bulls in musth did not participate in introductions due to safety concerns. They were isolated from the group and tethered in the forest on a 20–25 m chain, giving them access to natural food and water until it was safe to rejoin the others.

2.3. Behavioral data collection

An ethogram was developed using descriptions of social behaviors drawn from previous studies (Table S1) (Olson, 2002; Burks et al., 2004; MacFarlane and Coulson, 2009; Poole and Granli, 2009; Fazio et al., 2020). The selected behaviors were grouped into categories considered functionally similar (Burks et al., 2004; Poole and Granli, 2021; Schreier et al., 2021; Readyhough et al., 2022, 2023) and defined as aggressive, affiliative, submissive, and other. The aggressive category was subdivided into aggressive contact and aggressive non-contact as previously described (Burks et al., 2004; Schreier et al., 2021; Readyhough et al., 2022, 2023).

Data on social behavior was collected on each male between 0900 and 1200 h, three consecutive days per week, across four main locations at the ECC concession (Fig. S1) throughout the study period. Due to changes in group composition during the study – specifically the transfer

of XY in May 2021 and the death of TK in April 2022 – behavior data were grouped into three periods: P1 from 15 July 2020–8 May 2021 (124 h of field observations), P2 from 9 May 2021–26 April 2022 (188 h of field observations), and P3 from 27 April 2022–16 June 2022 (37 h of field observations).

Male behavior was assessed using 20-minute focal-animal sampling sessions (Altmann, 1974), during which the start and stop time of each focal follow, the initiator(s) and the recipient(s) of each interaction, and the individuals present in the group during each observation were recorded. Additionally, instantaneous scan sampling (5-minute intervals over 20-minute periods) (Altmann, 1974; Evans and Harris, 2008) was used to record the nearest neighbor and approximate distance to the focal animal to construct proximity networks (Rands, 2015). The lead author collected all behavioral data and, together with the mahouts, maintained a minimum distance of 30 m to prevent disturbance during observations. At the ECC, intervention by mahouts, including verbal commands, occurred only if the elephants moved out of sight (>150–200 m) or engaged in activities requiring immediate action, such as approaching agricultural areas or engaging in aggressive encounters escalated to a level that could compromise the health or safety of any elephant involved in the study. As none of these situations arose during the study period, elephants were allowed to express aggressive and dominant behaviors without interruption.

2.4. Data analysis

2.4.1. Rates of interaction

To construct social networks, rates of interaction were calculated using the following formula for elephants A and B: Rate = $(IntA + IntB) / (TimeA + TimeB)$, where the terms of the numerator represent the number of interactions between A and B during focal follows of A and B, respectively, and the terms of the denominator represent the length of time that A was followed while B was present in the group and the length of time that B was followed while A was present in the group, respectively (Goldenberg et al., 2022; López Pérez et al., 2025). Because the elephants were managed in a captive setting, opportunities for interaction and proximity were determined by management practices. Therefore, periods when one individual was absent were not considered missed opportunities for association, as individuals could not physically interact during those periods. Restricting calculations to periods of co-presence allowed better capture of true opportunities for social association within this managed context, consistent with longitudinal temporal network approaches that account for variation in co-presence among individuals (Gelardi et al., 2019). Calculations were performed using R Statistical Software v.4.1.2 (R Core Team, 2021) and were conducted separately for each of the three time periods described above (P1, P2, and P3) for each behavioral category. Interaction rates were used to create nine separate networks, analyzing different aspects of group structure: affiliative, aggressive, and submissive networks in P1, P2, and P3. Directed affiliative, aggressive, and submissive matrices were generated from these rates, and networks were visualized using Gephi Graph Visualization and Manipulation Software (Bastian et al., 2009). Top partners across periods were identified based on undirected rates of affiliative, aggressive, and submissive behavior. The rates of interaction between top partners across behavioral categories were compared using the Kruskal-Wallis test, followed by Dunn's test for post hoc pairwise comparisons.

2.4.2. Proximity network

In addition to constructing networks from interaction data, proximity networks were generated from nearest-neighbor observations to further explore social behavior. Proximity rates were calculated as the number of scans during which A and B were nearest neighbors – within less than 20 m and in the same group – divided by the number of scans in which A and B were present in the group. These calculations were conducted separately for each time period (P1, P2, and P3) using R statistical Software v.4.1.2 (R Core Team, 2021). Calculated proximity rates were used to create three separate networks with Gephi Graph Visualization and Manipulation Software (Bastian et al., 2009).

2.4.3. Centrality metrics

Centrality metrics were calculated to determine individual roles within networks. We quantified weighted In-Strength and Out-Strength, defined as the sum of rate of interactions received and initiated by each individual, respectively, to assess individual connectivity across the three study periods and characterize the directionality of interactions. Because interactions were weighted by interaction rates, these metrics represent weighted degree (i.e., strength) rather than binary degree based solely on the number of unique partners (Farine and Whitehead, 2015; Díaz et al., 2020).

Betweenness centrality (the number of times the focal node was in the shortest path connecting two other nodes) was calculated based on weighted edge values to determine how individuals differ in group connectivity for a given interaction type (Rose and Croft, 2015; Díaz et al., 2020), and therefore identifies individuals who serve as bridges between different parts of the network (Koyama and Aureli, 2019).

In addition to centrality measures, whole-network metrics were calculated for affiliative, aggressive, and submissive directed networks. These metrics included weighted density representing the average weighted interaction value across all possible dyads within the network and used as a measure of overall network connectivity; average weighted degree, represented the average total interaction strength each individual has with others, reflecting the average level of social connectivity of group members; and the standard deviation of weighted tie strengths, describing the variability in interaction strengths among dyads within the network. Both centrality and network metrics were calculated using UCINET software (Borgatti et al., 2002).

To quantify the distribution of social interactions across partners, we calculated the coefficient of variation (CV) for each individual within each behavioral context (affiliative, aggressive, and submissive) and phase (P1–P3). Dyadic interaction strengths were first derived as undirected weights. This approach provides a single measure of overall relationship strength per dyad and avoids confounding directionality (e.g., initiator vs. receiver effects).

For each individual, the mean and standard deviation (SD) of these weights were calculated, and the coefficient of variation was computed as: CV=SD/Mean. CV values were calculated, including zero-weight dyads, thereby incorporating both the strength of existing relationships and the absence of interactions (i.e., network sparsity). This allows CV to reflect both partner selectivity and interaction exclusivity.

Statistical analyses were conducted to assess the relationship between age (numeric age) and individual centrality measures (In-Strength, Out-Strength, and Betweenness) across behavioral categories (affiliative, aggressive, and submissive). Initially, linear mixed-effects models (LMMs) were fitted, including individual identity as a random effect to account for repeated measures. However, these models consistently resulted in singular fits, with negligible variance attributed to the random effect, likely due to the limited number of individuals ($n = 8$). Consequently, linear models (LMs) were used for subsequent analyses. However, across behavioral categories, model diagnostics revealed persistent issues related to non-normal residuals, heteroscedasticity, sparse interaction patterns, and zero inflation, especially for submissive behaviors. In addition, the relatively small sample size and repeated network measures limited the robustness and stability of more complex mixed-model approaches.

To ensure methodological consistency across behavioral categories and avoid unreliable inference from poorly fitting parametric models, we ultimately used Spearman rank correlations to assess associations between age and centrality metrics within each behavioral category. All analyses were conducted in R statistical Software v.4.1.2 (R Core Team, 2021).

2.4.4. Network stability

Changes in the group structure across P1, P2, and P3 were analyzed by calculating Quadratic Assignment Procedure (QAP) correlations using UCINET software. This method helps determine if the observed patterns of connections between individuals were significantly different from what could be expected by chance. To assess the significance of the correlations, the procedure generated a set number of random permutations of the rows and columns of the interaction matrices, creating a distribution against which the observed correlation was compared. The strength and direction of the linear relationship between the connections in the two matrices were then assessed by Pearson's correlation coefficient (r). The p-value, calculated by comparing the observed correlation to the distribution of random correlations, indicates the likelihood that the observed correlation occurred by chance. An observed correlation consistently higher than random correlations suggests a significant relationship was unlikely to be due to chance. For our analysis, we used 5000 permutations (Díaz et al., 2020) and selected the "Detailed (missing values OK)" analysis type, as XY was absent in P2 and P3, and TK was absent in P3. QAP correlations were generated for the affiliative, aggressive, and submissive behavioral categories.

3. Results

3.1. Rates of interaction and social networks

Mean rates (± SD) of affiliative, aggressive, and submissive behavior among dyads throughout the study period were 0.626 ± 0.748, 0.039 ± 0.064, and 0.038 ± 0.080, respectively. Dunn's post hoc tests revealed that affiliative behavior rates were higher than both aggressive ($Z = 5.56$, $p < 0.001$) and submissive ($Z = 5.64$, $p < 0.001$) behaviors.

Fig. 1 illustrates the interaction networks among male elephants across the three periods (P1, P2, and P3) and behavioral categories. In each behavioral network, individuals are represented as nodes, while directed edges represent the rates and direction of interactions between them. The number and strength of these connections reflect the frequency and directionality of interactions. In the affiliation networks, most bulls interacted with every other bull in every period. However, the structure of the affiliative networks varied across periods.. Weighted density slightly increased from P1 (0.317; n = 8) to P2 (0.325; n = 7) before decreasing in P3 (0.288; n = 6). Similarly, average weighted degree declined across periods (P1 = 2.219, P2 = 1.708, P3 = 1.079), indicating a progressive reduction in overall affiliative connectivity among males over time. Standard deviation values remained relatively high compared to weight density values across all periods (P1 = 0.490, P2 = 0.352, P3 = 0.384), indicating that affiliative interactions were unevenly distributed among dyads, with some males maintaining substantially stronger affiliative relationships than others.

In the aggression network analysis, fewer connections were observed compared to the affiliative networks. Weighted density decreased progressively across periods (P1 = 0.029, n = 8; P2 = 0.014, n = 7; P3 = 0.009, n = 6), indicating a gradual reduction in aggressive connectivity among males over time. Similarly, the average weighted degree also decreased from 0.205 in P1–0.074 in P2 and 0.033 in P3, indicating lower overall aggressive interaction strength per individual over time. Despite the low overall levels of aggression, standard deviation values remained proportionally high relative to the weighted density values (P1 = 0.053, P2 = 0.028, P3 = 0.031), indicating that aggressive interactions tend to be concentrated among specific dyads rather than evenly distributed across the network.

Submissive network connectivity remained relatively low across all periods. Weighted density showed a slight increase from P1 (0.017; n = 8) to P2 (0.018; n = 7) to P3 (0.025; n = 6). Average weighted degree decreased slightly from P1 to P2 and remained relatively stable between P2 and P3 (P1 = 0.117, P2 = 0.092, P3 = 0.094). In contrast, standard deviation values increased over time (P1 = 0.035, P2 = 0.045, P3 = 0.099), suggesting that submissive interactions became increasingly unevenly distributed among dyads, with submissive behaviors concentrated among fewer individuals during P3.

Across the study period, bulls spent a high proportion of time within 20 m of one another, with mean proximity rates remaining relatively stable but showing some temporal variation. During P1, bulls were within 20 m of at least one other bull for an average of 66.2% (SD = 0.12) of the observation time. The proportion decreased slightly in P2–62.8% (SD = 0.12), then increased slightly again in P3–65.7% (SD = 0.15). However, the proportion of time bulls spent within 20 m proximity of another bull did not differ significantly across study periods (Kruskal–Wallis rank sum test: $\chi^2 = 0.669$, $p = 0.716$).

Proximity network diagrams for each period illustrate the evolving structure of dyadic proximity relationships among bulls (Fig. 1). Weighted density increased from P1 (0.133; $n = 8$) to P2 (0.163; $n = 7$) before decreasing slightly in P3 (0.142; $n = 6$), indicating relatively stable spatial connectivity among bulls. The average weighted degree followed a similar pattern, increasing from 0.928 in P1 to1.141 in P2, then declining to 0.851 in P3, suggesting that bulls maintained frequent proximity associations throughout the study, despite a reduction in overall connectivity during the final period.

In contrast, the standard deviation remained relatively stable between P1 (SD = 0.104) and P2 (SD = 0.096), then increased substantially in P3 (SD = 0.138). This increase suggests that proximity associations became more selective over time, with some bulls spending more time in close proximity to preferred partners. This pattern is reflected in the P3 proximity network indicated by thicker edges between specific pairs, while other dyadic relationships became weaker or less frequent, as indicated by thinner edges.

3.2. Centrality metrics

Bulls differed in their affiliative network centrality metrics across time periods (Fig. 2). Affiliative Out-Strength values initially increased by 36.3% from P1 to P2, then declined sharply by 78.2% from P2 to P3, resulting in an overall decrease of 70.3% between P1 and P3 among bulls present throughout the study. Affiliative In-Strength values followed a similar pattern, increasing by 64.9% from P1 to P2 before decreasing by 78.7% from P2 to P3. DKS consistently exhibited some of the highest affiliative In-Strength values across periods (P1 = 3825, P2 = 4957, P3 = 1496) and also maintained the highest Out-Strength values (P1 = 4791; P2 = 6272; P3 = 1365), suggesting that this individual occupied a central affiliative role within the bachelor group. Overall, Out-Strength and In-Strength values were substantially higher in affiliative networks than in aggressive or submissive networks, indicating that affiliative interactions represented the primary form of social connectivity among bulls.

Aggressive Out-Strength values declined progressively across study periods, decreasing by 32.1% from P1 to P2 and by 85.5% from P2 to P3, resulting in an overall reduction of 90.2% from P1 to P3. Aggressive In-Strength values showed a similar pattern, decreasing by 30.4% from P1 to P2 and by 86.0% from P2 to P3. Submissive Out-Strength values increased by 19.5% from P1 to P2 before declining by 63.9% from P2 to P3, resulting in an overall decrease of 56.9% between P1 and P3.

Submissive In-Strength values followed the same temporal pattern. S consistently exhibited the highest submissive Out-Strength values across periods (P1 = 557, P2 = 719, P3 = 407). In contrast, BP showed the highest submissive In-Strength values during P2 and P3 (P2 = 655, P3 = 398), indicating that other bulls increasingly directed submissive behaviors toward him.

Mean betweenness centrality varied across behavioral categories and study periods, indicating temporal changes in the role of specific individuals in maintaining indirect network connectivity. Affiliative networks generally showed low to moderate betweenness values, particularly during P2 (P1 = 0.500; P2 = 0.143; P3 = 0.667), suggesting a relatively cohesive affiliative structure with limited reliance on specific bridge individuals. In contrast, aggressive (P1 = 1.250;

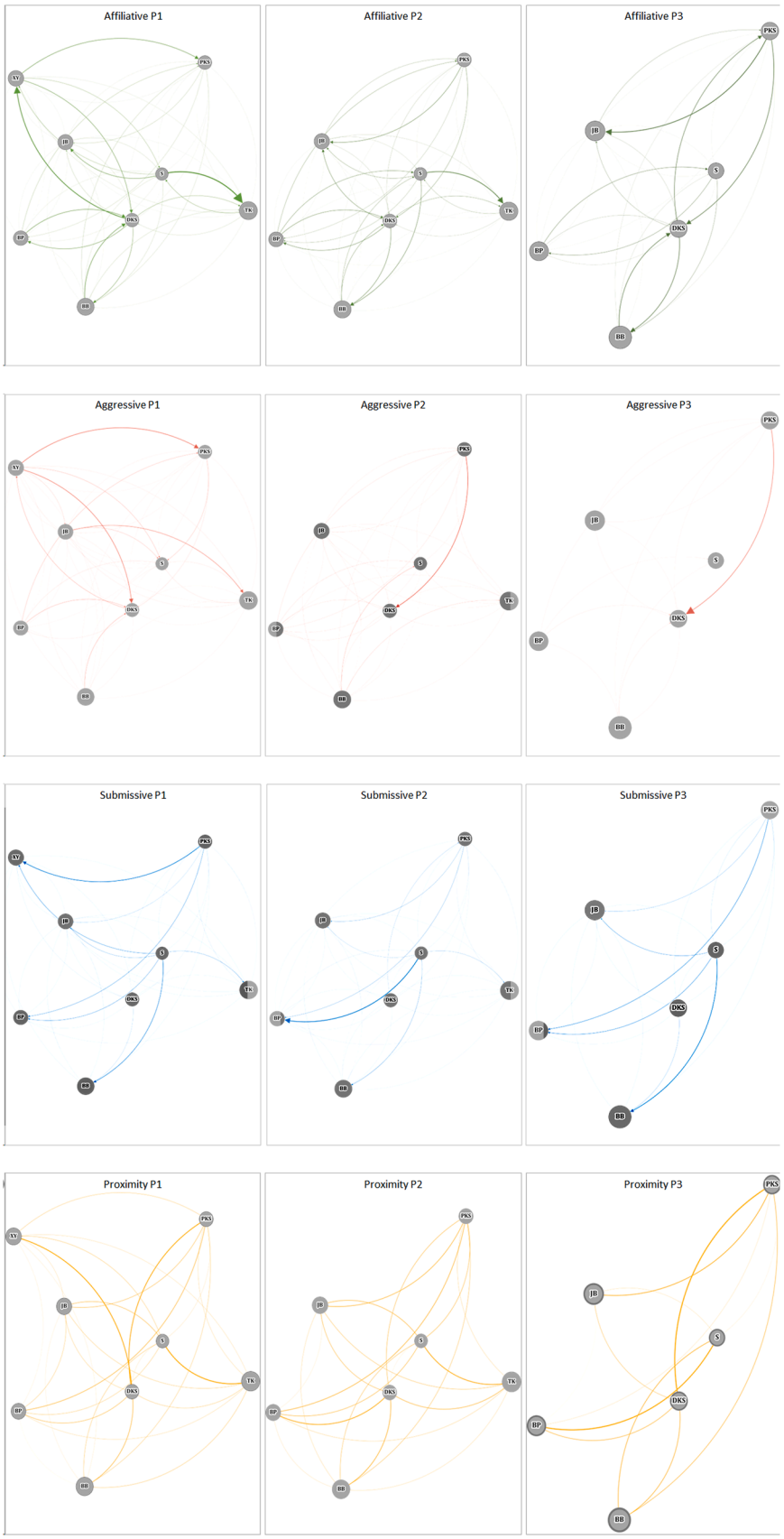

Fig. 1. Directed networks for affiliative, aggressive, and submissive behavior, and undirected proximity networks across three periods (P1, P2, and P3). Nodes are sized by age. The arrows indicate directional interactions. Thicker lines between dyads reflect higher interaction rates. P1: 15 July 2020–8 May 2021. Male group formation. All bulls present (n = 8); P2: 9 May 2021–26 April 2022. XY was relocated to another facility (n = 7); P3: 27 April 2022–16 June 2022. TK passed away (n = 6).

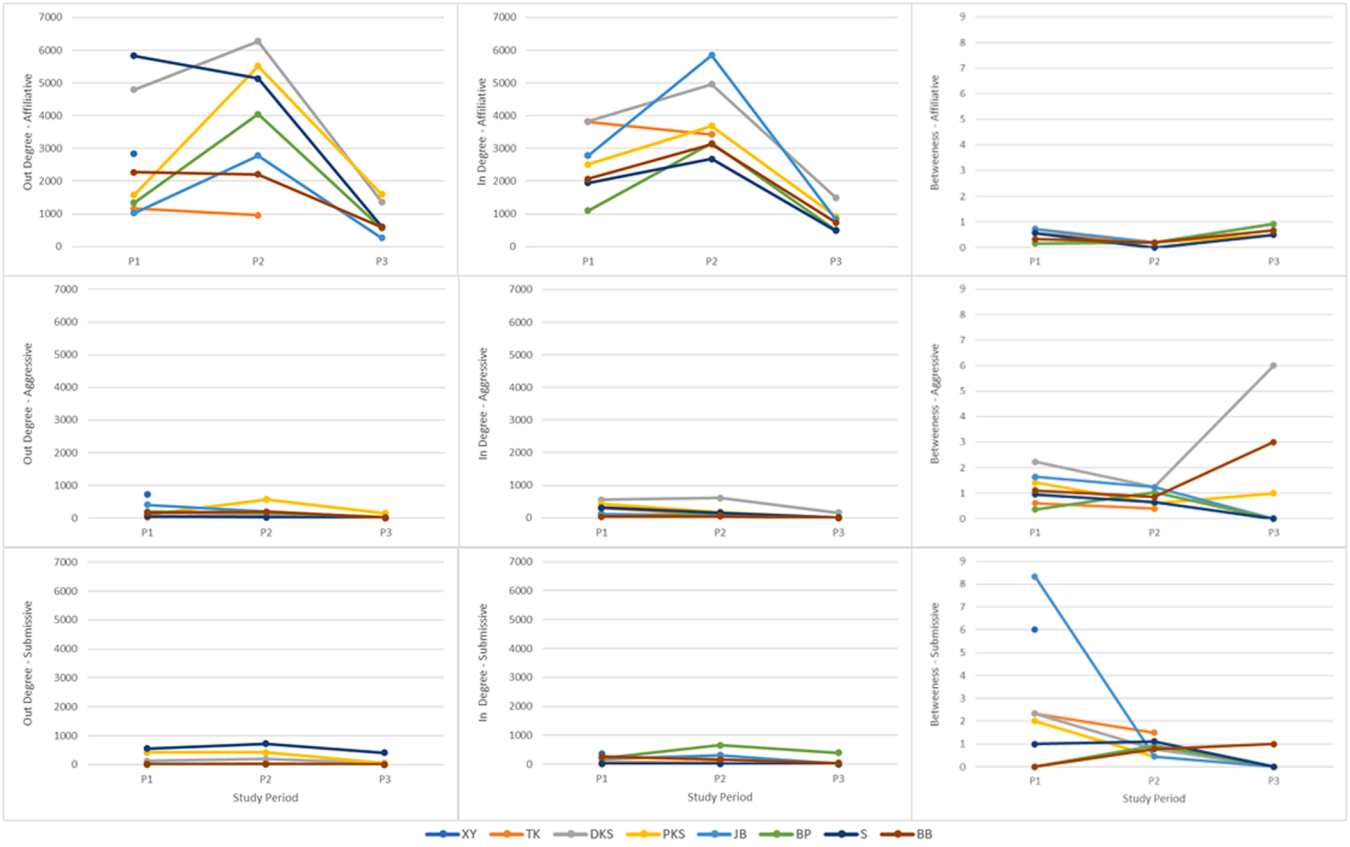

Fig. 2. Individual centrality metric scores across periods. Graphs showed changes in Out-Strength, In-Strength, and Betweenness across different behavioral categories (affiliative, aggressive, submissive) over three periods (P1, P2, P3). Bulls are presented in different colors, and the abbreviation name is included in the legend of each graphic. Out-Strength = number of outgoing connections from a node; In-Strength = number of incoming connections to a node, Betweenness = number of shortest paths passing through a node. P1: 15 July 2020–8 May 2021. Male group formation. All bulls present (n = 8); P2: 9 May 2021–26 April 2022. XY was relocated to another facility (n = 7); P3: 27 April 2022–16 June 2022. TK passed away (n = 6).

P2 = 0.857; P3 = 1.667) and submissive (P1 = 2.708; P2 = 0.857; P3 = 0.167) networks exhibited higher mean betweenness values, indicating that some bulls occupied more central intermediary roles within these interaction networks. DKS consistently showed high aggressive betweenness values, particularly in P3 (betweenness = 6), suggesting that aggressive interactions became increasingly concentrated around this individual. Similarly, JB exhibited high submissive betweenness during P1 (betweenness = 8), indicating an important intermediary role in submissive interactions during the early stages of group formation.

When data from all time periods were combined, Out-Strength was negatively correlated with age in both the affiliative and submissive behavioral categories, while In-Strength was only significantly negatively correlated with Age in the submissive category (Table 2).

3.3. Network stability

A significant positive QAP correlation was found for affiliative and submissive behavior between P1 and P2 and between P2 and P3 (Table 3). No significant correlation was found for aggressive behavior between P1 and P2 or between P1 and P3. However, a significant positive QAP correlation was observed between P2 and P3 for aggressive behavior.

Table 4 indicates that some bulls were more consistent in their partner choices than others. Bulls with higher rates of social interaction for specific individuals across time periods were able to form new partnerships when their original companions were no longer part of the group (e.g., DKS and XY, S and TK). Across all three time periods, mixed-age dyads composed of a mature or older bull (30–45 + years) and an

Table 2
Correlations between age and network centrality measures (Out-Strength, In-Strength, and Betweenness) for three types of social interactions: affiliative, aggressive, and submissive in socially integrated bull elephants at the Elephant Conservation Center in Lao PDR.

	Affiliative		Aggressive		Submissive	
	rho	p-value	rho	p-value	rho	p-value
Age vs Out-Strength	-0.435	0.049*	0.247	0.280	-0.702	0.000*
Age vs In-Strength	0.169	0.464	-0.378	0.091	0.528	0.014*
Age vs Betweenness	0.097	0.675	-0.011	0.962	0.194	0.399

rho = Spearman's rank correlation; Out-Strength = sum of interactions initiated by an individual; In-Strength = sum of interactions received by an individual; Betweenness = measure of an individual's centrality in the social network.
* Indicates significant correlations at $p < 0.05$.

Table 3
Quadratic assignment procedure (QAP) correlation coefficients between time periods (P1, P2, P3) for affiliative, aggressive, and submissive behaviors.

Time period	Affiliative	Aggressive	Submissive
P1-P2	0.800*	0.242	0.650*
P1-P3	0.290	0.261	0.367
P2-P3	0.640*	0.934*	0.933*

P1: 15 July 2020–8 May 2021. Male group formation. All bulls present (n = 8); P2: 9 May 2021–26 April 2022. XY was relocated to another facility (n = 7); P3: 27 April 2022–16 June 2022. TK passed away (n = 6).
* Indicates significant correlations at $p < 0.05$

Table 4
Top social partners for each male elephant across three behavioral categories (affiliative, aggressive, and submissive) during three observation periods (P1, P2, P3). The rate of interaction among dyads is shown in parentheses.

Individual	CV P1	Top partner P1	CV P2	Top partner P2	CV P3	Top partner P3
Affiliative						
XY	1.29	DKS (2.82)	-	-	-	-
BP	1.54	DKS (1.86)	1.01	DKS (1.55)	1.42	S (1.06)
PKS	0.77	XY (1.12)	0.96	JB (1.20)	1.12	DKS (1.91)
JB	1.37	S (1.12)	0.85	PKS (1.20)	1.43	PKS (1.72)
DKS	0.89	XY (2.82)	0.61	BP (1.55)	0.85	BB (1.97)
S	1.28	TK (3.21)	0.86	TK (2.00)	1.33	BP (1.06)
TK	1.60	S (3.21)	1.25	S (2.00)	—	—
BB	1.50	DKS (1.85)	1.18	DKS (1.46)	1.47	DKS (1.97)
Aggressive						
XY	1.08	DKS (0.33)	—	—	—	—
BP	1.02	DKS (0.12)	0.96	PKS (0.05)	1.34	DKS (0.02)
PKS	1.28	XY (0.26)	1.48	DKS (0.19)	1.64	DKS (0.18)
JB	1.23	TK (0.21)	0.72	DKS (0.04)	1.71	PKS (0.02)
DKS	1.11	XY (0.33)	1.20	PKS (0.05)	1.45	PKS (0.18)
S	1.06	XY (0.08)	0.81	BB (0.06)	1.13	BP (0.01)
TK	1.54	JB (0.21)	1.20	BB (0.04)	—	—
BB	1.31	DKS (0.11)	0.86	S (0.06)	1.47	DKS (0.02)
Submissive						
XY	1.40	PKS (0.18)	—	—	—	—
BP	1.25	S (0.07)	1.76	S (0.28)	2.17	S (0.55)
PKS	1.14	XY (0.18)	0.74	BP (0.07)	1.00	DKS (0.06)
JB	1.14	S (0.06)	1.1	PKS (0.06)	1.06	DKS (0.01)
DKS	0.71	BP (0.04)	0.86	PKS (0.05)	1.40	PKS (0.06)
S	0.68	BB (0.14)	1.15	BP (0.28)	1.88	BP (0.55)
TK	1.58	S (0.06)	1.27	S (0.05)	—	—
BB	1.75	S (0.14)	1.37	S (0.08)	1.03	PKS (0.04)

P1: 15 July 2020–8 May 2021. Male group formation. All bulls present (n = 8); P2: 9 May 2021–26 April 2022. XY was relocated to another facility (n = 7); P3: 27 April 2022–16 June 2022. TK passed away (n = 6). CV = Coefficient of variation.

adolescent or young bull (10–29 years) consistently made up the majority of top partner relationships for each behavioral category. In P1, 100% of the affiliative and submissive top partner dyads were composed of mature/older bulls and an adolescent/young bull (10–29 years), while 83% of aggressive dyads were mixed-age. During P2, all affiliative dyads (100%) again involved partners of different ages, with 60% of aggressive and 83% of submissive dyads showing the same pattern. In P3, 75% of affiliative and aggressive dyads, and 81.3% of submissive dyads, were mixed-age. A Kruskal-Wallis rank sum test revealed a difference in the rates across behavioral categories ($\chi^2 = 28.68$, $p < 0.001$). Post-hoc pairwise comparisons using Dunn's test with Bonferroni correction indicated that affiliative dyads had higher interaction rates than both aggressive ($p < 0.001$) and submissive dyads ($p < 0.001$).

In the affiliative behavioral category, 66.7% of CV values exceeded 1 across time periods, indicating that affiliative interactions were generally concentrated among preferred partners rather than evenly distributed among all group members. Affiliative CV values ranged from 0.61 to 1.60. Mean affiliative CV values were lowest during P2 (mean CV P1 = 1.28; Mean CV P2 = 0.96; Mean CV P3 = 1.27), suggesting that affiliative interactions were more broadly distributed across partners during this period, before becoming more differentiated again in P3. The lowest affiliative CV values were observed for PKS in P1 (0.77), DKS in P2 (0.61), and DKS in P3 (0.85), indicating comparatively less selective affiliative relationships. In contrast, the highest CV values were recorded for TK in P1 and P2 (1.60 and 1.25, respectively), S in P2 (1.25), and BB in P3 (1.47), suggesting that affiliative interactions were centered toward specific preferred partners.

In the aggressive behavioral category, 80.95% of CV values exceeded 1 across time periods, indicating that aggressive interactions were largely concentrated among specific partners rather than broadly distributed within the group. Aggressive CV values ranged from 0.72 to 1.71. Mean aggressive CV values increased over time, reaching the highest level in P3 (mean CV P1 = 1.20; mean CV P2 = 1.03; mean CV P3 = 1.46), suggesting that aggressive interactions became increasingly selective and concentrated among fewer dyads during the end of the study period. The lowest aggressive CV values were observed for BP in P1 (1.02), JB in P2 (0.72), and S in P3 (1.13), indicating less selective aggressive interactions. In contrast, the highest CV values were recorded for TK in P1 (1.54), PKS in P2 (1.48), and JB in P3 (1.71), suggesting a stronger concentration of aggression toward specific partners.

Similarly, submissive interactions were also concentrated among specific partners, with 80.95% of submissive CV values exceeding 1 across time periods. Submissive CV values ranged from 0.68 to 2.17 and became increasingly differentiated over time, with mean CV values increasing from 1.21 in P1 to 1.42 in P3. This pattern suggests that submissive behaviors became increasingly focused on particular partners during the study period. The lowest submissive CV values were observed for S in P1 (0.68), PKS in P2 (0.74), and PKS in P3 (1.00), indicating comparatively less selective submissive interactions. In contrast, the highest CV values were recorded for BB in P1 (1.75), BP in P2 (1.76), and BP in P3 (2.17), suggesting increasingly concentrated submissive relationships toward specific individuals.

Table 5 shows that some individuals were more flexible than others in maintaining close proximity to specific individuals. As expected, the top nearest neighbors often aligned with the affiliative top partners across all bulls and time periods. Additionally, during P1 and P2, 100% of the nearest-neighbor dyads within 20 m consisted of mixed-age. Similarly, in P3, 66.7% of nearest-neighbor dyads were also composed of mixed-age pairs.

4. Discussion

The present study revealed important insights into the social behavior of male Asian elephants housed together after a prolonged period of solitary living. Affiliative interactions were more frequent and well-connected compared to aggressive and submissive behaviors. Younger bulls initiated more affiliative and submissive behaviors than older individuals and tended to remain in closer proximity to the older bulls. Overall, elephants interacted frequently with one another, with affiliative behaviors representing the most commonly observed interactions between dyads.

The initial phase (P1) represented the introductory period, during which the bulls were brought together as a social group and allowed to interact freely after an extended period of non-contact socialization (López Pérez et al., 2025). During P1, social relationships appeared to be in an active phase, characterized by frequent interactions as bulls began to familiarize themselves with one another. However, CV values across behavioral categories indicated that interactions were not uniformly distributed, with some dyads already showing stronger or more selective social relationships than others early in the study period. These patterns

Table 5
Nearest neighbor preferences for each male elephant based on proximity within 20 m across three observation periods (P1, P2, P3). Rate of proximity among dyads is shown in parentheses.

Individuals	Nearest neighbor P1	Nearest neighbor P2	Nearest neighbor P3
XY	DKS (0.43)	-	-
BP	PKS (0.24)	DKS (0.37)	S (0.43)
PKS	BP (0.24)	JB (0.25)	DKS (0.45)
JB	S (0.19)	PKS (0.25)	PKS (0.27)
DKS	XY (0.43)	BP (0.37)	PKS (0.45)
S	TK (0.47)	TK (0.37)	BP (0.43)
TK	S (0.47)	S (0.37)	-
BB	DKS (0.24)	S (0.16)	S (0.23)

P1: 15 July 2020–8 May 2021. Male group formation. All bulls present (n = 8); P2: 9 May 2021–26 April 2022. XY was relocated to another facility (n = 7); P3: 27 April 2022–16 June 2022. TK passed away (n = 6).

support the idea that bulls can form bonds not only with relatives but also with unrelated individuals, and that this selective bonding contributes to overall group cohesion (Chiyo et al., 2011).

The aggressive network was more active in P1 and showed a decrease in weighted density across periods indicating a gradual reduction in aggressive connectivity among males over time. This suggests that the bulls were establishing a new hierarchy in P1, which had largely settled by P3. Aggressive centrality measures remained relatively low and stable for most individuals across all periods, suggesting that once dominance hierarchies were established, aggressive interactions played a less central role in the social structure. Because of their large size and powerful physical attributes, conflicts can easily result in serious injuries. A well-defined ranking system can minimize direct confrontations and prevent escalation, as documented in female African elephants (Archie et al., 2011). Reductions in aggressive behaviors were also observed over a 5-month period after introducing a newly integrated bachelor group of Asian elephants to residents at the Denver Zoo (U.S.), as a new dominance hierarchy was established (Schreier et al., 2021). Notably, by P3, aggressive interactions were rare among most bulls but persisted between DKS and PKS. These young males were of similar age and consistently emerged as top partners across affiliative, aggressive, submissive, and proximity networks, suggesting a strong social bond alongside ongoing dominance testing (O'Connell-Rodwell et al., 2011).

Submissive interactions were consistently low across all periods, observed primarily in younger males, likely reflecting stable social hierarchies or minimal need for overt submissive signaling. Notably, elevated aggression in P3 between two young bulls (DKS and PKS), combined with the predominance of submissive behaviors from younger bulls toward adults, supports the hypothesis that younger males actively establish their hierarchical position while older males reinforce dominance to maintain theirs (O'Connell-Rodwell et al., 2011).

Centrality results highlight the variability in individual roles within the social network. Some males exhibited higher centrality values, such as greater Out-Strength, In-Strength, or Betweenness, indicating they held more central positions, while others occupied more peripheral roles, suggesting limited involvement in group interactions. Identifying which individuals are central or peripheral can help managers monitor the social health of the group. Thus, the removal of highly central individuals, whether through transfer, illness, or death, has the potential to destabilize the network, potentially leading to increased aggression or social fragmentation, although this was not observed in the present study.

Out-Strength interactions for affiliative and submissive behavioral categories were significantly negatively correlated with age, indicating that younger individuals initiate affiliative and submissive behaviors more frequently than older ones. The findings align with previous observations in wild African elephants, where adolescent males (10–20 years of age) were the most social age class (Evans and Harris, 2008), engaged in more affiliative and submissive behaviors with mature conspecifics (Readyhough et al., 2023), and greeted at higher rates than adults (Allen et al., 2021b).

In the nearest neighbor preference analysis, most dyads across time periods were mixed-age pairs, consistent with observations that adolescent males often preferred males aged 36 years or older as their nearest neighbors (Evans and Harris, 2008; Muthukumarana, 2023). Associating with older conspecifics and remaining close to more experienced individuals may help young bulls navigate new social and physical environments (Srinivasaiah et al., 2019). Younger elephants learn from more experienced individuals, gaining valuable ecological knowledge and social skills (Rees, 2004; Evans and Harris, 2008; O'Connell-Rodwell et al., 2011; Srinivasaiah et al., 2019; Allen et al., 2020; Murphy et al., 2020; Muthukumarana, 2023; Puri et al., 2025). These findings align with observations in other species. For example, younger adult chimpanzees were more likely to form unequal relationships in which partners did not reciprocate, whereas older chimpanzees had more mutual and equitable friendships (Rosati et al., 2020).

QAP results showed that affiliative and submissive behaviors remained consistent between P1–P2 and P2–P3, suggesting stability in social interactions during these periods. However, behavioral patterns in P1 differed from those in P3, a shift related to the departure of XY and the death of TK. Aggressive behaviors also changed significantly after P1 but stabilized from P2 to P3, reflecting a restructuring of dominance hierarchies following XY's exit. Older males such as these play essential roles in maintaining social cohesion (Chiyo et al., 2011), managing aggression (Allen et al., 2021a), and sharing ecological knowledge (Allen et al., 2020). Their loss may have destabilized the group, a pattern also observed in African elephants, where the absence of mature individuals disrupts social structure (Archie and Chiyo, 2012; Goldenberg and Wittemyer, 2018; Murphy et al., 2019). Although QAP tests are widely used in animal social network analysis to compare interaction matrices, this approach has several limitations that should be considered when interpreting our results. QAP analyses may not fully account for the complex dependency structure of social network data. Additionally, QAP tests primarily provide significance estimates without directly quantifying effect sizes or the directionality of relationships. Therefore, the QAP results presented in this study should be interpreted cautiously, primarily as exploratory assessments of network similarity and temporal social structure.

The top partner-preference results showed that some individuals interacted more often with certain individuals than others (e.g., DKS and XY; S and TK), while other elephants were more flexible in choosing social partners across time periods. In addition, the CV analyses indicated that interactions were generally unevenly distributed across partners, particularly for aggressive and submissive behaviors, with more than 80% of CV values exceeding 1. This suggests that males directed these interactions toward specific individuals rather than interacting uniformly across the group. Affiliative interactions also showed selective partner preferences, with 66.7% of CV values exceeding one. This finding suggests that, in the observed group, male elephants' social relationships were structured and non-random, with individuals concentrating their interactions on preferred social partners across all behavioral categories. Understanding which elephants are more flexible in choosing social partners and which individuals have established strong social bonds can be highly beneficial for management decisions, particularly when planning translocations, work assignments, or daily routines. In addition, elephants with strong affiliative partner preferences can compensate for lost bonds by strengthening other relationships, as demonstrated in wild female African elephants (Wittemyer et al., 2009; Goldenberg et al., 2016; Goldenberg and Wittemyer, 2017). This adaptability in social networks means that management should be flexible when group composition changes, allowing for natural reorganization of social groups.

5. Conclusions

This study demonstrated that male Asian elephants, even after prolonged periods of solitary management, can form complex, stable social relationships when housed together. Across all observation periods during social integration, affiliative interactions were more frequent and well-connected than aggressive or submissive behaviors, challenging the longstanding perception that male elephants are predominantly solitary and aggressive. Age and group composition played significant roles in shaping social dynamics. Mixed-age dyads were common across proximity, affiliative, aggressive, and submissive networks, suggesting that younger bulls benefit from associations with older, more experienced individuals who may facilitate learning and social development. The ability of males to form new relationships following social disruptions, such as the removal or loss of group members, highlights the flexibility and resilience of their social systems. However, identifying individuals who occupy central roles in social networks, and as well as assessing their flexibility in forming new social bonds, is key to maintaining group stability during management interventions.

Several limitations should be acknowledged, including the small sample size, the restriction to a single facility, and the unique management conditions at the study site, where males had access to larger and more naturalistic habitats than those available in many captive elephant facilities across Asia. As such, this study should be considered exploratory, and statistical outcomes should be interpreted with caution, with greater emphasis placed on overall patterns, effect sizes, and trends in social relationships rather than solely on statistical significance.

Nevertheless, the findings provide valuable preliminary insights into the social flexibility and relationship dynamics of male Asian elephants in managed settings and may help guide future confirmatory studies. Further long-term research across a wider range of captive and wild environments is needed to better understand the social behavior of male Asian elephants under different ecological and management conditions. Such knowledge is essential for developing evidence-based management strategies that support species-appropriate social housing, enhance welfare outcomes, and strengthen conservation efforts for this endangered species as both in-situ and ex-situ populations face growing conservation challenges.

CRediT authorship contribution statement

Ana Belén López Pérez: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shifra Z. Goldenberg:** Writing – review & editing, Validation, Resources, Methodology, Formal analysis, Conceptualization. **Janine L. Brown:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Jaruwan Khonmee:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

No conflict.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2026.107067.

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PLOS ONE

Metabolic profiles of captive Asian elephants (*Elephas maximus*) in Lao PDR and Thailand

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RESEARCH ARTICLE

Metabolic profiles of captive Asian elephants (*Elephas maximus*) in Lao PDR and Thailand

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Abstract

This study compared metabolic health biomarkers, lipid profiles, body condition scores (BCS), and adrenocortical function of 78 captive Asian elephants (*Elephas maximus*) managed under contrasting systems in Laos and Thailand. In Laos, elephants are primarily managed in naturalistic forest environments with limited tourist interaction, while many Thai elephants participate in tourism activities like riding and are fed calorie-dense supplemental foods, with restricted access to natural forage. Serum and fecal samples were collected for 1 year to assess serum glucose, insulin, cholesterol, triglycerides, lipoproteins, fecal glucocorticoid metabolites (fGCM), and BCS across seasons, sexes, and management regimens. Thai elephants exhibited significantly higher glucose, insulin, lipid levels, and BCS, reflecting the effects of high-calorie diets and reduced exercise, while Lao elephants displayed healthier metabolic profiles and BCS, attributed to natural foraging and physical activity. In Thai elephants, BCS was positively correlated with insulin, and negatively correlated with the glucose-to-insulin ratio, total cholesterol, HDL, and LDL, suggesting overweight elephants in Thailand are at risk of metabolic derangements. None of those parameters were significantly correlated with BCS in the Laotian population. A surprising finding was higher fGCM in Lao elephants, which could have been related to new social opportunities and pandemic-driven management disruptions at the Elephant Conservation Center. These findings underscore how management practices, particularly diet composition, exercise opportunities, and degree of naturalistic living, can influence the health and welfare of captive elephants and support the adoption of more naturalistic management strategies to improve elephant welfare in captivity.

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the Faculty of Veterinary Medicine at Chiang Mai University Data Access/ Ethics Committee (Professor Dr. Korakot Nganvongpanit, e-mail: korakot.n@cmu.ac.th) for researchers who meet the criteria for access to confidential data.

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Introduction

Elephants are a keystone species with significant impacts on ecosystems and the species therein [1,2]. Despite their ecological importance, wild Asian elephant *(Elephas maximus)* populations continue to decline due to habitat loss and habitat fragmentation, human-elephant conflict, and poaching for body parts such as ivory or skin. The total captive Asian elephant population is approximately 15,000 individuals, corresponding to about one-third of the 45,000 wild Asian elephant population [3]. Maintenance of genetically healthy captive elephant populations can support conservation strategies, such as reintroduction into viable habitats, genetic refreshment of small isolated populations, and research into factors impacting fitness and survival *in situ* and *ex situ* [4–6]. Unfortunately, most captive Asian elephant populations are not self-sustaining due to higher mortality than birth rates [7–9].

Captive elephant numbers are estimated to be ~ 3,800 in Thailand [10] and ~500 in Laos (formally the Lao People's Democratic Republic) [11]. In Thailand, the majority (about 60%) are found in the north and northeast, primarily in Chiang Mai province [12]. In this region, elephants participate in tourist activities, ranging from hands-off opportunities like observation from afar to more interactive experiences such as feeding, bathing, walking alongside, riding with a saddle or bareback, and elephant shows [13]. Most facilities offer only limited access to forests for foraging or free-roaming, so camp owners design much of the captive diet, which includes mostly farm-grown grasses, like napier grass (*Pennisetum purpureum*), bana grass (P. *purpureum x P. americanum*), sweet corn (*Zea mays var. saccharate*) stover, as well as supplements consisting of fruits and sugar cane [14–17]. In Laos, the majority of captive elephants are in Sayaboury province (~80%) [18]. After the 2016 ban on the export of logs and sawn wood [19], many elephants in Sayaboury were left in the forest without employment, or worked in small-scale logging operations, where they had a natural forage diet [20]. Soon thereafter, some of these elephants began participating in tourist activities, ranging from hands-off opportunities to more traditional activities such as feeding, riding, and elephant shows. Today, elephant-based tourism has become a principal component of the national tourism industry in the country, mostly located in Luang Prabang Province [21].

Across western zoos and Asian tourism settings, management practices can strongly affect elephant health and welfare. [8]. Consequently, there has been an increase in studies to assess how management factors impact elephant health and welfare [14,16,22,23], with considerable agreement between countries [8]. Of particular relevance is the relationship between diet and exercise. In Thailand, high-energy foods (bananas and sugar cane) fed by tourists are associated with high body conditions scores (BCS) and alterations in total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides (TG), insulin, glucose, fructosamine, and the ratio of glucose to insulin (G:I) [15]. Both rates of obesity and concentrations of fecal glucocorticoid metabolites (fGCM) are lower in elephants with more physical activity [8,15].

In Thailand, noted changes in management practices based on those results were subsequently observed by veterinarians in the Elephant, Wildlife and Companion

Animals Research Group, Chiang Mai University, who visit camps regularly. But in March-April 2020, the Thai and Laos governments banned all international travel due to the COVID-19 pandemic [24,25], resulting in the closure of all tourist camps through 2021. Studies published by Supanta et al. [12,13,26] showed that the lack of tourists had significant impacts on tourist camp management and elephant health and welfare in Thailand, in the form of reduced exercise opportunities and food provided, resulting in altered stress biomarkers [26], muscle function, and liver function compared with previous years [12]. However, some benefits were also observed, such as healthier BCS and decreases in lipid markers (TC, TG, LDL) from the lack of tourists feeding sweet foods [12].

While considerable physiological data now exist on tourist elephants in Thailand – both pre- and post-COVID-19 – no studies have examined metabolic or stress markers in elephants managed under more naturalistic conditions, like those in Laos. The objective of this study was to compare metabolic and lipid markers, BCS, and fGCM concentrations between captive elephants in Laos and Thailand to evaluate how differing management approaches impact health and welfare across captivity scenarios. These findings are critical for formulating science-based recommendations, identifying areas for improvement in tourist camps to meet elephants' fundamental health and welfare needs, and understanding the physiological effects of contrasting management strategies. Though not intentional, data collection took place during the COVID-19 travel ban in both countries. Thus, this natural experiment provided a unique opportunity to assess the direct and indirect effects of captive management practices, with the enforced pause in tourist activities providing a way to disentangle the influence of visitor interactions from other variables.

Materials and methods

Study sites

Laos. A total of 27 healthy captive Asian elephants, including 10 males (29.0 ± 16.1 years of age, range, 7–60 years) and 17 females (40.0 ± 8.8 years of age, range, 25–57 years) were included in this study (Table 1). From this population, 19 individuals were housed at the Elephant Conservation Center (ECC) in Sayaboury Province. The ECC consists of 530 hectares of mixed deciduous and dry dipterocarp forest. It is open to the public all year, but tourist activities are limited to remote observations only. Elephants spend the morning (0900–1200 hours) and afternoon (1330–1630 hours) socializing and foraging in different natural areas throughout the venue. At night (1800–0800 hours), elephants are restrained with a 40 m chain within different locations of the forest to allow natural foraging and prevent any conflict with surrounding farmers. The remaining eight elephants were located in the Nam Phouy National Protected Area (Nam Phouy NPA), which covers 191,200 hectares of forest [27] and shelters the second largest population of wild elephants in Laos [28]. Of these, four were part of a soft-release reintroduction program into the protected area with complete freedom to roam ('released group'), unless intervention was deemed necessary for safety reasons – either to protect the elephants themselves or nearby communities. Once a month, physical health was evaluated by a veterinarian. Four other elephants were housed at Nam Phouy NPA, where mahouts (elephant keeper) employed traditional management techniques involving periodic release into the forest ('traditional management group'). During the dry season, elephants were alternately tethered on 30–40 m chains or released with a dragging chain in wooded areas near the village. When tethered, they were relocated daily to refresh the feeding grounds and provide access to water. In the rainy season, when human resources were primarily allocated to planting crops, elephants were predominantly released into the forest for extended periods, monitored by mahouts through weekly searches.

Thailand. A total of 51 healthy elephants from five tourist camps in the Chiang Mai area were involved in a welfare study conducted between 2020 and 2022 in Thailand and provided data on husbandry practices [13] and physiology [12,26] for this study (Table 1). The study population included 11 males (34.5 ± 12.5 years of age, range, 19–56 years) and 40 females (38.0 ± 9.2 years of age, range, 20–56 years). Before the travel ban, elephants were involved in riding with a saddle (Camps B, C, and E) or bareback (Camp B), elephant shows (Camps B and C), bathing with tourists (Camp A),

and tourist feeding (all camps). After the lockdown, all tourist activities ceased, leading to significant changes in the care and management of captive elephants. With the absence of visitors, most camps suspended elephant-related activities, resulting in a substantial decline in physical exercise. The average daily walking distance decreased from approximately 4 km to just 0–1 km, and only 47% of camps continued to provide any walking opportunities.

As work activities diminished, chaining time increased. In 77% of camps, elephants were chained for more than 21 hours per day, typically under covered sheds or in woodland areas. Additionally, most camps experienced a notable reduction in the number of employed mahouts, further impacting daily care. Elephant diets were also affected. While the types of roughage provided remained largely the same, the overall quantity of feed was gradually reduced over time [13].

Sample collection

Blood and fecal samples were collected once or twice a month from February 2020 to April 2021 (Table 1). All samples from Lao elephants were collected and analyzed specifically for this study. In Thailand, comparative values were drawn from previously published data using comparable methodology [12,13,26]. Notably, both countries were under international travel bans due to COVID-19 from early 2020 through at least 2021; thus, samples represent data collected during the pandemic, when tourist activities were limited or absent.

Body condition scoring

Body condition was assessed monthly by scoring the back, ribs, and pelvic bone areas using a standardized photographic guide with detailed instructions [29]. Scores ranged from 1 (thinnest) to 5 (fattest), and assessments were made in 0.5-point increments for greater precision [15].

Serum glucose, insulin, G:I

Serum glucose was measured by automated standardized liquid chromatography-mass spectrometry at the International Medical Diagnostic Center in Vientiane, Laos (Roche Cobas C 311; Cobas® Mass Spec) or an automated glucose analyzer (Glucinet T01-149, Bayer, Barcelona, Spain) at Chiang Mai University in Thailand [12]. Serum insulin concentrations were measured by a solid-phase two-site bovine insulin enzyme immunoassay (EIA; Cat. No. 10−1,113−01; Mercodia, Uppsala, Sweden) validated for elephants [12,23]. Colorimetric responses were determined by a spectrophotometer at 450 nm filter with an Opsys MR Microplate Reader (TECAN Sunrise™ microplate reader; Salzburg, Austria) [12]. All samples were analyzed in duplicate; intra- and inter-assay coefficients of variation were <10 and <15%, respectively.

Lipid profiles

Serum TC, LDL, HDL, and TG were quantified by automated, standardized liquid chromatography-mass spectrometry Roche Cobas C311 (Cobas® Mass Spec) in Laos and an Automated Clinical Chemistry Analyzer (Sysmex; BX-3010, Sysmex Corporation of Japan) in Thailand [12].

Hormone processing and extraction

Fecal samples were extracted as described by Norkaew et al. (2018) [15]. Briefly, frozen fresh feces were thawed at room temperature (RT) and dried in a conventional oven at 60°C for ~24−48 hours and stored at −20°C until extraction. Mixed dry powdered feces (~0.1 g ± 0.01) were extracted in 5 mL 90% ethanol by boiling in a water bath (96°C) for 20 minutes and adding 95% ethanol as needed to keep from boiling dry. Samples were centrifuged at 2,500 x g for 20 minutes. Supernatants were transferred into a new glass vial, and the fecal pellets were centrifuged again with 5 mL 90% ethanol. The combined supernatants were dried under air in a 50°C water bath. Dried extracts were reconstituted in 1 mL assay buffer (0.0137 M Trizma base, 0.2 M Tris-HCl, 0.2 M NaCl, 0.2 M EDTA, 0.001% BSA, and 0.001% Tween 20; pH 7.5) and stored at –20°C until enzyme immunoassay (EIA) analysis.

Table 1. Summary of parameters related to the management of elephants at captive venues in Laos and Thailand.

	Laos			Thailand				
Location	Nam Phouy NPA[1] (released)	Nam Phouy NPA[2] (traditional management)	ECC	A	B	C	D	E
Males	1	0	9	5	2	4	0	0
Females	3	4	10	9	8	7	8	8
Age (years)	7-48	32-45	9-60	22-50	23-53	19- 45	28-54	30-56
Blood sample collection	Monthly	Twice a month	Twice a month	Monthly	Monthly	Monthly	Monthly	Monthly
Fecal sample collection	Twice a month	Twice a month	Twice a month	Monthly	Monthly	Monthly	Monthly	Monthly
Diet	A wide variety of natural plants, fruits, barks, and roots are available (up to 114 plant species are commonly consumed by the elephants in the landscape) including bamboo species such as *Bambusa tulda*, *Dendrocalamus strictus*, and *Gigantochloa albociliata*; ginger species including *Zingiber zerumbet* and *Alpinia galanga*; banana plants (*Musa spp.*); and grasses like *Imperata cylindrica* and *Pennisetum purpureum.*		Access to natural food sources, including fruits, bark, roots, and a wide variety of plants, is available throughout the year. However, large seasonal changes affect availability: food is abundant during the wettest months (June, July, and August) but limited during the driest months (March, April, and May).	Napier grass, bana grass, and corn stalk (172.86 ± 2.23 kg/day).	Napier grass, bana grass, and corn stalk (131.90 ± 2.70 kg/day).	Napier grass, bana grass, and corn stalk (162.50 ± 2.97 kg/day).	Napier grass, bana grass, and corn stalk (123.96 ± 1.41 kg/day).	Napier grass, bana grass, and corn stalk, (168.55 ± 2.44 kg/day).
Supplements[4] (kg/day)	0	0	0	9.30 ± 0.68	15.25 ± 1.15	13.88 ± 1.27	14.90 ± 0.99	12.64 ± 0.65
Walk distance (km/day)	0.5-8.0	0.5-5.0	2.0-4.0	0.0-1.5	0.0-6.0	1.0-6.0	0.5-2.0	0.5-3.0
fGCM (ng/g)	49.01 ± 2.71	49.89 ± 2.03	58.17 ± 1.27	50.03 ± 1.97	57.59 ± 1.98	44.94 ± 1.65	53.53 ± 2.17	46.45 ± 1.78

[1]Captive elephants from the Elephant Conservation Center (ECC) released into the Nam Phouy National Protected Area (Nam Phouy NPA) in 2019 as part of a soft-release program.

[2]Captive elephants living within the Nam Pouy NPA under traditional captive management.

[3]Based on interview surveys from April 2020 to April 2021 published by Supanta et al.2023 [13].

[4]Primarily bananas and sugar cane fed by mahouts/camp owners.

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Hormone analyses

Concentrations of fGCM were determined using a double-antibody EIA with a polyclonal rabbit anti-corticosterone antibody (CJM006) validated for Asian elephants [30] and described by Norkaew et al. (2018) [15]. The absorbance was measured at 450 nm by a microplate reader (TECAN Sunrise™ microplate reader; Salzburg, Austria). Assay sensitivity (based on 90% binding) was 0.14 ng/mL. Samples were diluted 1:4 for female elephant samples and 1:6–1:8 for male elephant samples in assay buffer for analysis. The inter-assay CV for high (30% binding) and low (70% binding) control samples was < 15%. Samples were reanalyzed if the duplicate CV was > 10%; thus, intra-assay CVs were <10%. Fecal data are expressed as ng/g dried feces.

Statistical analyses

Statistical analyses were performed using R version 4.1.2 [31]. Physiological measurements were collected monthly from most elephants, although some individuals were sampled twice within the same month. In those cases, the two values were averaged, so that each elephant contributed only one data point per month for each parameter. Repeated measures data were analyzed using Generalized Estimating Equations (GEE) to evaluate the effects of location and country, season, and sex on metabolic and lipid parameters, BCS, and fGCM concentrations. The analysis specifically examined: (1) the country effect; (2) the effect of management practices across the three main study locations (ECC, release/traditional management, and Thai tourist camps); (3) the influence of season (summer, winter, and rainy); and (4) the effect of sex on the outcome measures. To test whether seasonal effects differed by country, an interaction term of season and country was incorporated into the model. To assess sex differences, a separate model was fitted with a country and sex interaction. Individuals were treated as a random effect. GEE was fitted to marginal regression models using the geeglm() function from the geepack package in R, specifying an autoregressive correlation structure of order 1 AR (1) to account for within-subject correlation over time [32]. GEE models were followed by Bonferroni's multiple comparisons of estimated marginal means post-hoc tests with p- value correction. The significance level for all statistical analyses was set at $\alpha = 0.05$. Associations between BCS and metabolic hormones, lipid profiles, and fGCM, were further evaluated using aggregated data. For each physiological category, all parameters were consolidated, resulting in a single representative value per individual per category for the correlation analysis. Given the ordinal nature of BCS data and the presence of tied values, Kendall's tau-b correlation coefficient was calculated using the cor.test() function in R (method = "kendall). To visualize the correlation trends, the geom_smooth() function from the ggplot2 package was also applied.

Results

A total of 1328 serum samples (Laos = 682 samples; Thailand = 646) and 1402 fecal samples (Laos = 806 samples; Thailand = 596) were evaluated in this study. Descriptive metabolic markers, lipid profile, BCS, and fGCM measures for elephants in Laos and Thailand are presented in Table 2. Insulin ($\beta = -0.32$, SE = 0.08, $Z = -4.16$, $p < 0.0001$), glucose ($\beta = -12.80$, SE = 2.62, $Z = -4.88$, $p < 0.0001$), TC ($\beta = -4.42$, SE = 2.02, $Z = -2.19$, $p = 0.029$), HDL ($\beta = -2.04$, SE = 0.56, $Z = -3.66$, $p = 0.0002$), LDL ($\beta = -4.93$ SE = 1.40, $Z = -3.53$, $p = 0.0004$), BCS ($\beta = -0.77$, SE = 0.10, $Z = -7.43$, $p < 0.0001$) were significantly higher in Thai elephants compared to the Lao population. In contrast, fGCM concentrations were higher in the Lao elephants ($\beta = -5.55$, SE = 2.27, $Z = -2.44$, $p = 0.014$).

Table 3 shows differences in metabolic markers between released/traditionally managed elephants living within the Nam Phouy NPA, the ECC in Laos, and several tourist camps in Thailand (Camps A-E). Significant differences were observed across groups, with insulin, glucose, HDL, and LDL being significantly higher for the Thai elephant compared with the release/traditional management and the ECC elephants ($p < 0.05$) (S1 Table). BCS was the lowest, and fGCM concentration the highest at ECC among the three groups. Finally, tourist camp elephants in Thailand had significantly higher TC compared with the release/traditional management elephants ($p < 0.05$).

Table 2. Country effect on physiological parameters in captive Asian elephants. Mean (± SEM), and range (min-max) values in metabolic factors, lipid panel measures, body condition scores, and fecal glucocorticoid metabolite concentrations in captive Asian elephants in Laos (n = 27) and Thailand (n = 51).

Factors	Country	
	Laos	Thailand
Insulin (ng/mL)	0.25 ± 0.02[a]	0.58 ± 0.04[b]
	(0.02-3.26)	(0.02-9.77)
Glucose (mg/dL)	75.16 ± 0.73[a]	88.76 ± 1.00[b]
	(32.00-161.00)	(23.00-203.00)
G:I	793.79 ± 30.48[a]	740.12 ± 35.15[a]
	(48.63-3840.00)	(10.95-3960.00)
TC (mg/dL)	41.69 ± 0.42[a]	46.22 ± 0.57[b]
	(19.33-78.00)	(19.00-124.00)
TG (mg/dL)	23.56 ± 0.59[a]	24.80 ± 0.50[a]
	(4.00-122.00)	(2.00-100.00)
HDL (mg/dL)	10.51 ± 0.15[a]	12.24 ± 0.15[b]
	(4.00-20.50)	(4.80-33.70)
LDL (mg/dL)	25.45 ± 0.29[a]	30.69 ± 0.40[b]
	(9.00-44.50)	(10.30-80.80)
BCS (1–5)	2.93 ± 0.02[a]	3.82 ± 0.03[b]
	(2.00-4.00)	(1.50-5.00)
fGCM (ng/g)	56.07 ± 1.06[b]	50.40 ± 0.88[a]
	(4.94-161.17)	(16.94-175.96)

Abbreviations: G:I = glucose to insulin ratio; TC = total cholesterol; TG = triglycerides; HDL = high density lipoproteins; LDL = low density lipoproteins; BCS = body condition score; fGCM = fecal glucocorticoid metabolites.

[a,b]Different superscript letters within columns indicate significant differences for each variable based on GEE model results ($p < 0.05$). Superscript letters (a, b) were assigned in order of concentration, with a representing the lowest concentration.

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Seasonal effects in Laos and Thailand are summarized in Fig 1 (S2 Table). Overall, none of the parameters appeared to be affected by season within each country, with the exception of fGCM concentrations in Thailand, were the lowest concentrations were found in summer compared to rainy and winter seasons (rainy-summer: β = –8.15, SE = 2.05, Z = 3.98, $p < 0.0001$; summer-winter: β = –10.10, SE = 1.62, Z = –6.23, $p < 0.0001$), and HDL concentration in Laos, were concentrations in winter differed significantly from summer (β = 0.58, SE = 0.24, Z = 2.45, $p = 0.043$).

Between countries, insulin, glucose, HDL, LDL, and BCS were consistently higher across all seasons in Thailand compared to Laos (Fig 2, S2 and S3 Tables). In addition, TC was higher in summer and winter in Thailand compared to Laos ($p < 0.05$), and fGCM was higher in summer in Laos compared to Thailand.

Sex differences were observed within and between countries. In Laos, males had higher G:I (β = 368.00, SE = 105.00, Z = 3.52, $p = 0.0004$) than females (Fig 3, S4 Table). In Thailand, males also had higher G:I (β = 493.00, SE = 142.00, Z = 3.49, $p = 0.0005$) than females, as well as TC (β = 9.85, SE = 4.19, Z = 2.35, $p = 0.02$) and LDL (β = 6.12, SE = 2.81, Z = 2.18, $p = 0.03$), while females had higher BCS (β = –0.94, SE = 0.12, Z = –7.49, $p < 0.0001$).

Among males, Lao elephants had higher fGCM concentrations (β = –4.44, SE = 2.95, Z = –1.50, $p = 0.01$), while Thai males had higher glucose (β = −14.90, SE = 6.61, Z = –2.25, $p = 0.02$), TC (β = –12.10, SE = 5.05, Z = –2.39, $p = 0.02$), and LDL (β = –9.99, SE = 3.40, Z = –2.94, $p = 0.003$) (Fig 4, S4 Table). For females, Thai elephants had higher insulin (β = –0.31, SE = 0.09, Z = –3.93, $p = 0.0001$), glucose (β = –12.00, SE = 2.55, Z = –4.71, $p < 0.0001$), HDL (β = –1.81, SE = 0.57, Z = –3.16,

Table 3. Location effect on physiological parameters in captive Asian elephants. Mean (± SEM) and range (min-max) values on metabolic factors, lipid panel measures, body condition scores, and fecal glucocorticoid metabolites for elephants at the Elephant Conservation Center in Laos (n = 19), those released and traditionally managed in Laos (n = 8), and in tourist camps in Thailand (n = 51).

Parameter	Mean ± SEM	Range
Insulin (ng/mL)		
ECC	0.27 ± 0.02[a]	0.02-3.26
Released/traditional management[1]	0.19 ± 0.01[a]	0.02-0.58
Thailand tourist camps	0.58 ± 0.04[b]	0.02-9.77
Glucose (mg/dL)		
ECC	76.23 ± 0.92[a]	32.00-161.00
Released/traditional management	71.98 ± 0.91[a]	49.00-96.00
Thailand tourist camps	88.76 ± 1.00[b]	23.00-203.00
G:I		
ECC	853.28 ± 36.75[a]	48.63-3840.00
Released/traditional management	616.46 ± 48.48[a]	129.07-2520.00
Thailand tourist camps	740.12 ± 35.10[a]	10.95-3960.00
TC (mg/dL)		
ECC	42.51 ± 0.48[ab]	20.50-78.00
Released/traditional management	39.24 ± 0.84[a]	19.33-56.00
Thailand tourist camps	46.22 ± 0.57[b]	19.00-124.00
TG (mg/dL)		
ECC	21.54 ± 0.47[a]	4.00-57.00
Released/traditional management	29.61 ± 1.75[a]	8.67-122.00
Thailand tourist camps	24.80 ± 0.50[a]	2.00-100.00
HDL (mg/dL)		
ECC	11.09 ± 0.17[a]	5.00-20.50
Released/traditional management	8.77 ± 0.28[a]	4.00-16.50
Thailand tourist camps	12.24 ± 0.15[b]	4.80-33.70
LDL (mg/dL)		
ECC	26.02 ± 0.35[a]	9.00-44.50
Released/traditional management	23.85 ± 0.46[a]	10.67-35.00
Thailand tourist camps	30.69 ± 0.40[b]	10.30-80.80
BCS (1–5)		
ECC	2.79 ± 0.02[a]	2.00-3.50
Released/traditional management	3.35 ± 0.03[b]	3.00-4.00
Thailand tourist camps	3.82 ± 0.03[c]	1.50-5.00
fGCM (ng/g)		
ECC	58.17 ± 1.27[b]	4.94-161.17
Released/traditional management	49.45 ± 1.68[a]	13.11-121.05
Thailand tourist camps	50.40 ± 0.88[a]	16.94-175.96

[1]Elephants living in the Nam Phouy National Protected Area: four from the Elephant Conservation (ECC) that were part of a soft-release program in 2019; four managed under traditional conditions.

Abbreviations: G:I = glucose to insulin ratio; TC = total cholesterol; TG = triglycerides; HDL = high density lipoproteins; LDL = low density lipoproteins; BCS = body condition score; fGCM = fecal glucocorticoid metabolites.

[a,b,c]Different superscript letters within locations indicate statistically significant differences between values, based on GEE model results ($p < 0.05$). Superscript letters (a, b,c) were assigned in order of concentration, with a representing the lowest concentration.

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p = 0.0016), LDL (β = –2.91, SE = 1.26, Z = –2.30, p = 0.0213), and BCS (β = –1.006, SE = 0.12, Z = –8.40, p < 0.0001) than the females in Laos.

Correlations between BCS and physiological parameters had been investigated separately for each country and reported in Fig. 5. In Thailand, BCS showed positive correlations with insulin (τ = 0.39, p = 6.6e-05). Additionally, negative correlations were observed between BCS and G:I (τ = −0.44, p = 6.2e-06), TC (τ = −0.4, p = 3e-05), HDL (τ = −0.21, p = 0.02), and LDL (τ = −0.36, p = 0.00015). In contrast, among elephants in Laos, none of the parameters were significantly correlated with BCS.

Discussion

This study provides the first direct comparison of metabolic, lipid, and stress profiles in captive Asian elephants managed under more naturalistic (Laos) versus intensive tourism (Thailand) systems. The goal was to contrast management practices between tourist camps in Thailand, where diets and activities are highly managed and elephants often participate in feeding and riding activities, and venues in Laos, where elephants spend more time free-roaming, socializing, and foraging naturally, with minimal supplemental feeding and tourist interactions. While the original goal of this research was to compare health and welfare biomarkers between these two disparate scenarios in relation to tourist activities, as it happens, the study began just as the COVID-19 pandemic closed all international travel, curtailing tourism in both

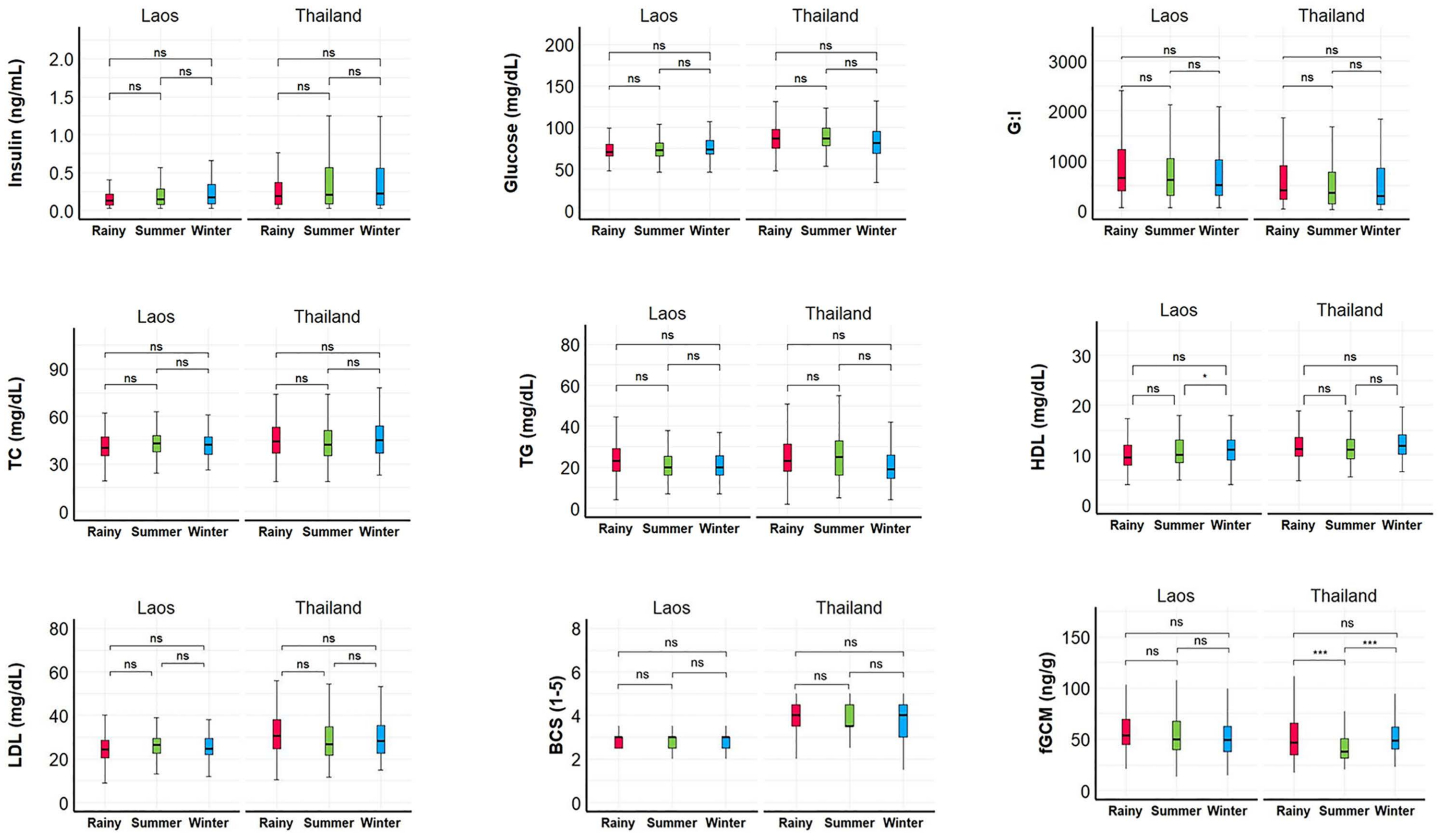

Fig 1. Seasonal effects on physiological parameters in Asian elephants in Laos (n = 27) and tourist camps in Thailand (n = 51). G:I = glucose to insulin ratio; TC = total cholesterol; TG = triglycerides; HDL = high density lipoproteins; LDL = low density lipoproteins; BCS = body condition score; fGCM = fecal glucocorticoid metabolites. Summer = 16 February-15 May; Rainy = 16 May-15 October; Winter = 16 October-15 February. Asterisks above comparisons denote statistical significance as determined by the GEE model: *p < 0.05; **p < 0.01; ***p < 0.001; ns = not significant differences.

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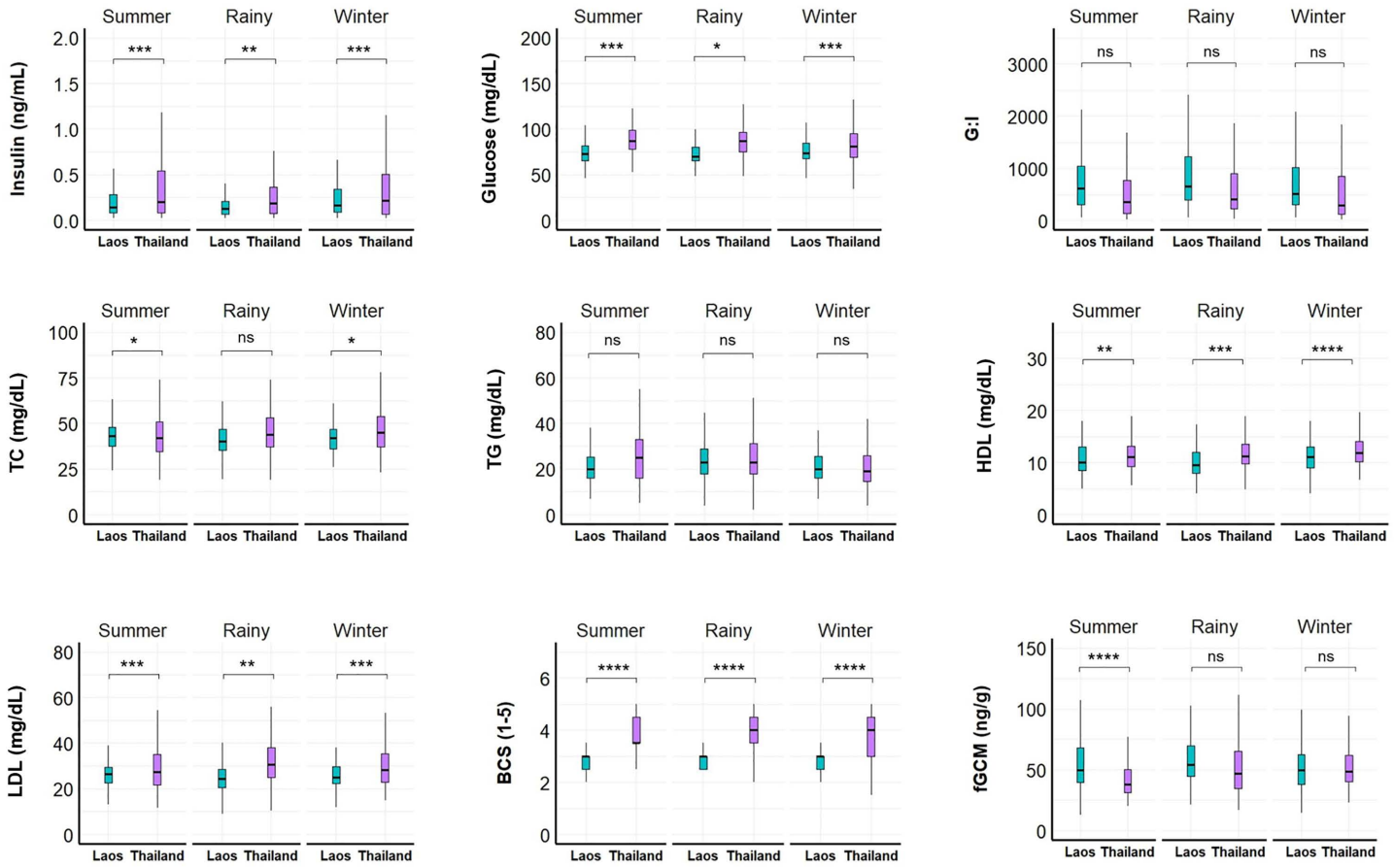

Fig 2. Seasonal differences in physiological parameters between Asian elephants in Laos (n = 27) and tourist camps in Thailand (n = 51). G:I = glucose to insulin ratio; TC = total cholesterol; TG = triglycerides, HDL = high density lipoproteins; LDL = low density lipoproteins; BCS = body condition score; fGCM = fecal glucocorticoid metabolites. Summer = 16 February-15 May; Rainy = 16 May-15 October; Winter = 16 October-15 February. Asterisks denote statistical significance as determined by the GEE model: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns = not significant.

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countries. That provided a unique opportunity to focus specifically on management rather than human contact. Still, even with tourists taken out of the equation, a number of physiological differences were observed between countries. Elephants in Laos displayed more normal metabolic profiles and BCS compared to Thai counterparts, probably related to more natural foraging opportunities. Notably, despite the lack of tourist feeding, Thai elephants maintained higher BCS than their Lao counterparts, which were related to higher insulin, glucose, and lipid concentrations. A surprising finding was that elephants at the ECC had higher fGCM concentrations, which may reflect heightened physiological stimulation from new social opportunities, and disruptions in management practices caused by the pandemic. These results emphasize how management practices, specifically diet composition, exercise opportunities, and social and spatial freedom, can shape elephant health, advocating for more naturalistic approaches to enhance welfare.

Metabolic profiles

Significant location and sex-based differences were observed in circulating glucose and insulin concentrations. Using comparable laboratory techniques, while most values were within normal ranges based on existing references [12,15,16,23,33], concentrations were overall higher in Thai elephants compared to those in Laos. The released elephants

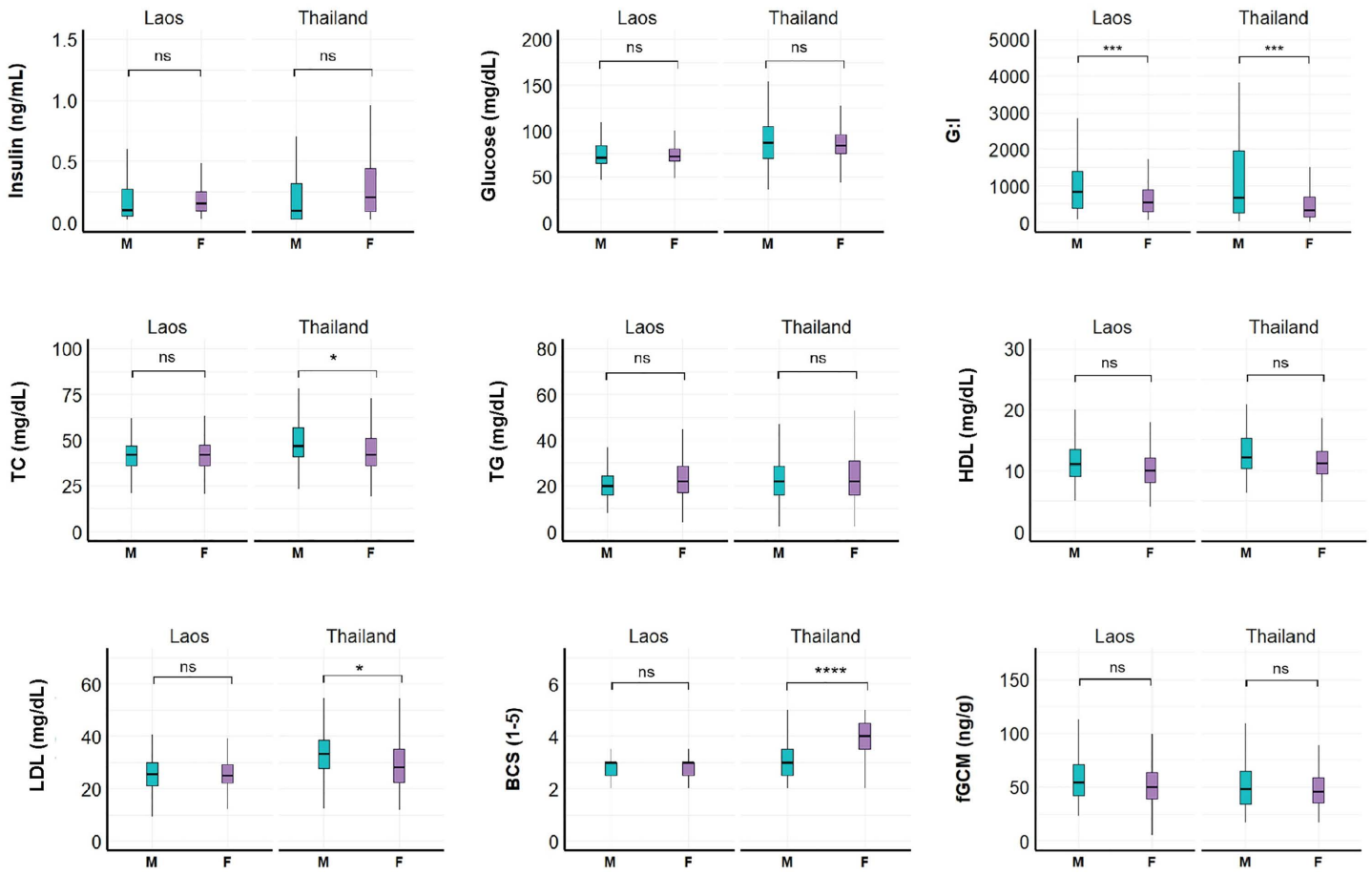

Fig 3. Sex differences in physiological parameters in Asian elephants in Laos (n = 27) and tourist camps in Thailand (n = 51). G:I = glucose to insulin ratio; TC = total cholesterol; TG = triglycerides, HDL = high density lipoproteins; LDL = low density lipoproteins; BCS = body condition score; fGCM = fecal glucocorticoid metabolites. Asterisks denote statistical significance as determined by the GEE model: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns = not significant.

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and those in the traditional management group within the Nam Phouy NPA had the lowest concentrations of metabolic markers (i.e., glucose, TC, HDL, and LDL), except for TG compared to all other groups, followed by elephants at the ECC. These metabolic differences may be related to diet. Elephants in Laos forage on a more natural/diverse diet throughout the year, especially the released/traditional management groups, and food supplements are not part of the diet. By contrast, the traditional diets of Thai elephants are highly managed, consisting of napier grass as the main fodder, with limited natural foraging opportunities, and feeding of high-calorie supplements like bananas and sugar cane by tourists. Even during the tourism ban, camp owners in Thailand continued to provide supplements, albeit in lower quantities [13], but apparently enough to continue having an effect on metabolic status. These results align with previous studies in black rhinoceros (*Diceros bicornis*) [34] and gorillas (*Gorilla gorilla gorilla*) [35], where higher insulin values were observed in captive-fed animals compared to free-roaming counterparts.

Metabolic profile results were associated with BCSs. In Thailand, higher BCS was found to be correlated with elevated insulin concentration, whereas no such associations were observed in the Lao elephants. This discrepancy can be explained by the distribution of BCSs within each group. In Thailand, a substantial proportion of tourist elephants were

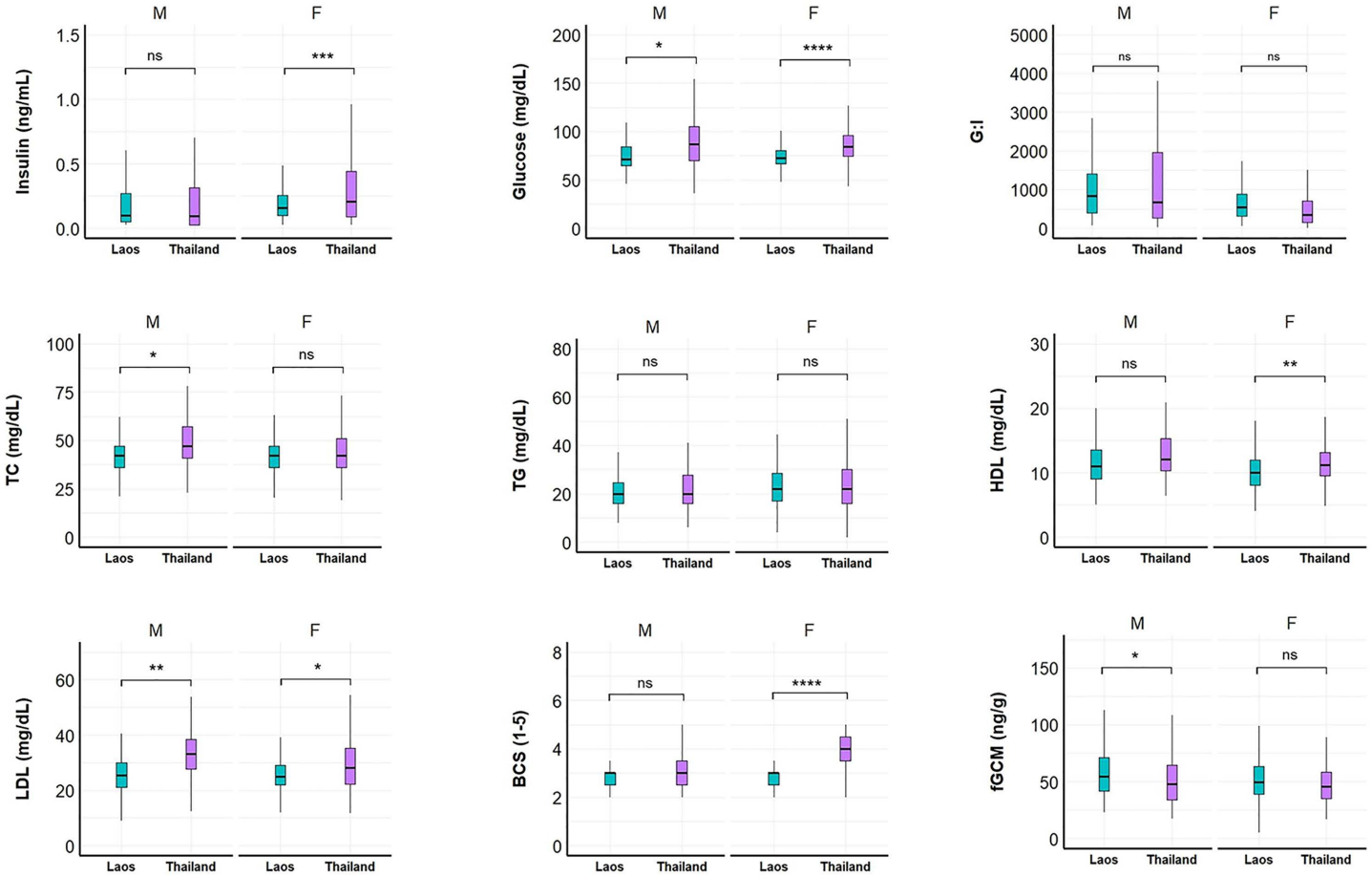

Fig 4. Sex differences in physiological parameters of Asian elephants between Laos (n = 27) and tourist camps in Thailand (n = 51). G:I = glucose to insulin ratio; TC = total cholesterol; TG = triglycerides; LDL = low density lipoproteins; HDL = high density lipoproteins; BCS = body condition score; fGCM = fecal glucocorticoid metabolites. Asterisks denote statistical significance as determined by the GEE model: *p < 0.05; **p < 0.01; ***p < 0.001; ns = not significant.

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classified as overweight or obese: nearly 45% were assigned a BCS of 4, and almost 30% were given the highest score of 5 at the onset of the study [26]. By the end of the study, 23% of Thai elephants still had a BCS of 4. By contrast, only 3.33% of elephants in Laos had a BCS of 4, with none scoring 5 at any time during the study. With so few overweight or obese individuals present in Laos, it was not surprising that metabolic correlations with BCS were not detected.

There were no sex differences in BCS among the Lao elephants, whereas in Thailand, females tended to have higher, though not significant, BCSs corresponding to higher insulin concentrations and lower G:I compared to males. The latter agrees with studies before the COVID-19 pandemic in Thailand [15,16,33] and could be explained by the fact that females tend to be the preferred choice for interactions with tourists, like feeding, as they are more docile and predictable than males [36]. Although there was no tourist feeding in Thailand during the COVID-19 pandemic, female elephants started the study considerably fatter. Within the first 2 months of the study, 91.3% of females in Thailand had BCS = 4 or 5, while only 27.3% of the males did. In both countries, male elephants exhibited lower insulin concentrations than females, which could be due to dietary restrictions as a traditional practice to regulate body weight to better control the time and intensity of musth periods [16]. This phenomenon aligns with findings from a human study, in which insulin levels decreased as

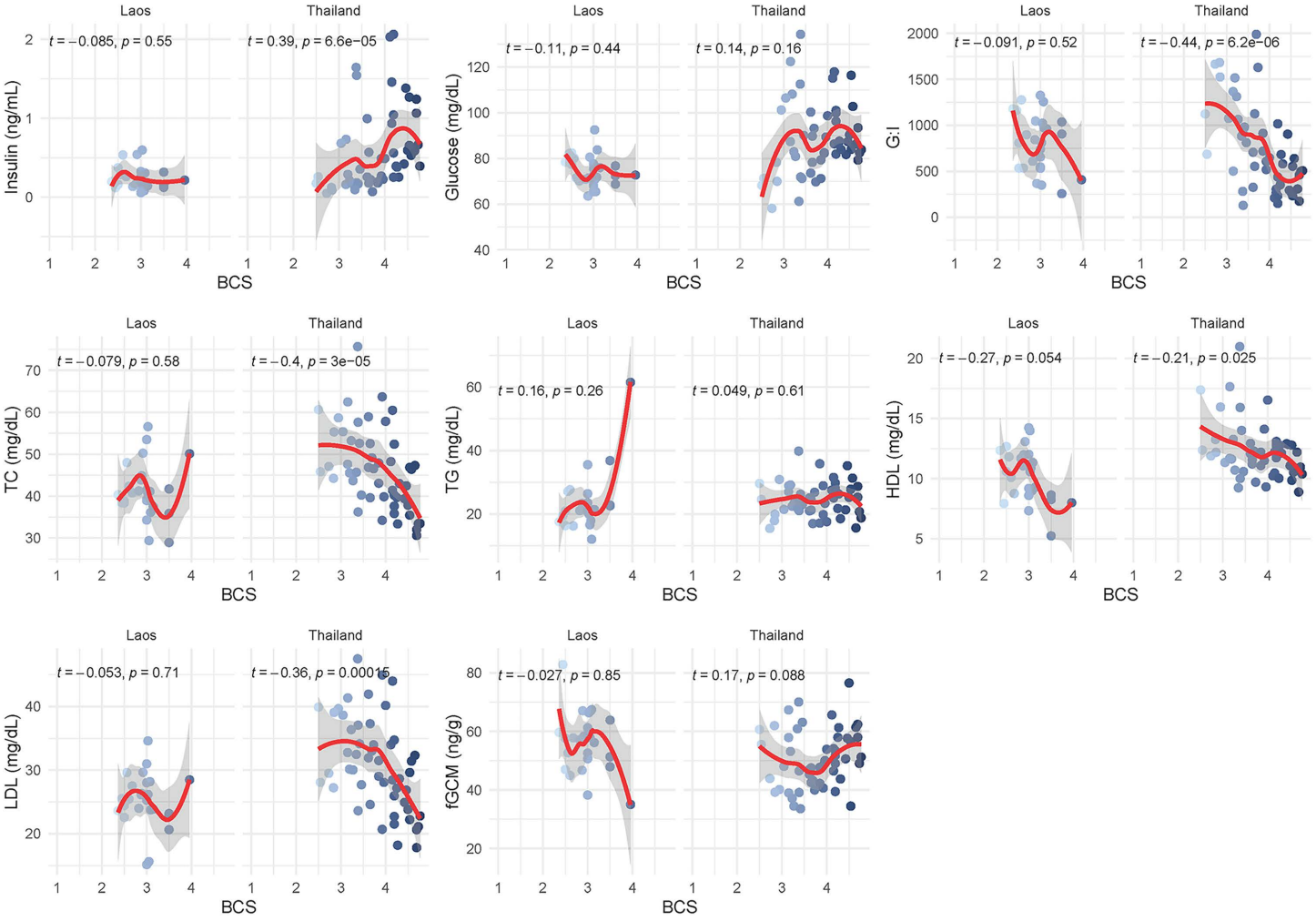

Fig 5. Relationships between body condition and lipid, metabolic, and fGCM factors separated by country. Kendall's tau-b correlation between BCS, metabolic factors, lipid panel, and fGCM concentrations in captive Asian elephants in Laos (n = 27) and Thailand (n = 51). Abbreviations: τ = Kendall's tau-b correlation coefficient; G:I = glucose to insulin ratio; TC = total cholesterol; TG = triglycerides; HDL = high density lipoproteins; LDL = low density lipoproteins; BCS = body condition score; fGCM = fecal glucocorticoid metabolites.

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a result of time-restricted feeding [37]. However, further research is needed to explore and validate this hypothesis, as it could also suggest potential differences in sex-related metabolic or hormonal regulation.

Lipid profiles and BCS

Location differences in relationships between BCS and lipids were also noted. Overall, BCS, TC, LDL, and HDL levels were higher in Thai compared to Lao elephants, which agrees with the observation that BCS remained relatively stable during the study in Laos, likely due to consistent food availability and exercise, even during the COVID-19 tourism ban. Sex differences were also apparent in the lipid panel, where males had higher TC and LDL than females in Thailand. However, these differences were not observed between male and female elephants in Laos. Previous studies in Sri Lankan elephants have reported higher TC in males than females, attributing these differences to physical activity, as exercise can reduce serum cholesterol [38]. However, in the present study, reported walking distances and hours

spent on chains among the Thai population were similar between males (walking distance: 2.24 ± 0.21 km; chain hours: 23.70 ± 0.52h) and females (walking distance: 1.96 ± 0.10 km; chain hours: 22.49 ± 0.58h) [13,26], suggesting that other intrinsic or management-related factors may contribute to the observed metabolic differences.

Among Thai elephants, thinner individuals exhibited higher G:I,TC, HLD, and LDL concentrations. This phenomenon can be explained by the fact that animals with lower BCS have reduced fat reserves and may actively mobilize these stores to meet increased energy demands. During this metabolic process, fat deposits are broken down (lipolysis), releasing free fatty acids and other lipids into the bloodstream, which leads to elevated circulating lipid concentrations. This effect has been reported in pregnant and lactating dairy cows, where periods of negative energy balance, often due to increased metabolic demands, result in intensified fat mobilization and subsequently higher blood lipid levels such as non-esterified fatty acids [39]. In addition diets with low G:I had been shown to have benefits in terms of short-term glycemic control, weight, and adiposity [40]. In Laos, although the correlation was not significant, lower BCS was associated with higher HDL, a finding observed in humans, where an increase in physical activity was associated with an increase in HDL [41]. Lao elephants had regular walking routines that, in addition to naturalistic diets, could contribute to the increase in HDL concentrations and lower BCS. Therefore, these findings underscore the critical value of fostering active lifestyles within captive populations to support better health outcomes.

During the COVID-19 lockdown, Thai elephants experienced a marked reduction in physical activity, with some camps extending chaining periods for up to 48 contiguous hours, and chain lengths restricted to just 3 m during the day and 6 m at night [14,26]. In contrast, walking routines for elephants in Laos were unaffected by the absence of tourists. Elephants at the ECC continued to roam freely in the forests during the day, and at night were restrained with chains averaging 40 meters in length, allowing access to natural feeding grounds within an 80-meter diameter. Released elephants had unrestricted movement both day and night, while those under traditional management in the Nam Phouy NPA were only restrained occasionally.

In other species, moderate exercise is known to improve lipid homeostasis [42,43], while frequent, varied feedings, rather than a few large meals, can help suppress hunger and lower serum insulin [44]. As megaherbivores, Asian elephants are adapted to spend much of the day foraging on a wide variety of plant materials [45–47], and disruptions to this natural pattern, such as those observed in Thai tourist camps, are likely to contribute to the observed metabolic derangements. The Thai elephant's low dietary diversity, reliance on sugar-rich supplements, and limited feeding frequency likely compounded the negative effects of inactivity, explaining the higher BCSs and less favorable lipid and metabolic panels compared to Lao counterparts, underscoring the protective effects of naturalistic management [8,23].

fGCM and BCS

High BCSs have been linked to limited exercise, feeding of high-calorie treats, and higher fGCM concentrations in Thailand [15,16,33] and U.S. zoos [23,48]. By contrast, in the present study, the Lao population presented with lower BCSs and higher fGCM concentrations compared to the Thai population. Although the negative correlation between fGCM and BCS in the Lao elephants only approached significance, it suggests that as body condition decreased, fGCM concentrations tended to increase, a finding that has been reported for free-ranging Asian elephants in India [49]. Low BCSs could produce more physiological stress due to resource scarcity or other environmental challenges [49]. Thus, both low [50–53] and high [54–56] BCSs may be a stressful event for the body. These results highlight that both exercise and diet diversity are critical for maintaining healthy metabolic and lipid profiles in captive elephants, and that management practices mimicking natural conditions can promote better physiological outcomes.

fGCM and seasonality and management factors

Seasonal peaks in fGCM were observed during the rainy (May – October) and winter (October-February) seasons in the Thai population, aligning with another Thai study conducted during the pandemic [26]. Elephants in Myanmar also exhibit higher fGCM concentrations during the monsoon season (May-September) [57]. In addition, these data also align

with pre-pandemic findings in Thailand where fGCM concentrations peaked only during the winter during the high tourist season [15,16], suggesting that natural environmental factors could play an important role the in adrenal activity. Furthermore, since no seasonal variation was observed among the Lao population, it is possible that the elevated adrenal activity observed in Thai elephants is also influenced by management conditions. In Thailand, elephants are often restrained with short chains in non-naturalistic environments, which can increase stress responses during extreme weather events such as heavy rain or strong winds, as they have limited options to seek shelter, find comfort areas, or take refuge near other individuals. Providing elephants with more options to respond to adverse weather could be an important consideration for future management practices. In addition to seasonal variation, numerous other factors can influence GC concentrations. Aversive situations may elicit increases in concentrations, but they can also be increased by events considered to be neutral or pleasant [58]. Behaviorally, the ECC elephants rarely displayed behaviors commonly associated with stress, like stereotypies, whereas those are observed at high rates in Thailand tourist camps (57%) [59]. Rather, ECC elephants are more socially active, as both males and females have daily social interactions, especially compared to Thailand tourist camps. During the COVID-19 pandemic, the ECC initiated a social program for bulls to create all-male groups like those in the wild. Introducing new individuals can elicit stimulatory adrenal responses, especially during the initial months of the introduction [60–62]. During these social encounters, affiliative interactions were performed more frequently than aggressive and submissive behaviors and accounted for over 79.7% of the interactions (López Pérez et al., in prep). Thus, the elevated fGCM concentrations observed in ECC elephants, likely reflect the physiological arousal and adaptive stress response associated with increased social activity and the challenges of group restructuring, rather than distress. However, due to COVID-19 restrictions, several ECC mahouts chose to return to their hometowns, which may have compounded these effects. Studies demonstrate elephants respond more efficiently to familiar handlers, requiring weeks to months to acclimate to new caretakers [63,64]. Thus, higher fGCM at the ECC compared to Thailand and other Lao populations may reflect a multifactorial outcome of proactive social enrichment and pandemic-driven management disruptions, rather than a simple welfare deficit.

Conclusions

This study provides the first comparative analysis of physiological health markers in captive Asian elephants managed under contrasting systems: forest-based naturalistic environments in Laos versus tourism-driven management in Thailand. The findings suggest that differences in diet, management practices, and daily activities play a role in shaping physiological health in captive elephants. Thai elephants exhibited higher glucose, insulin, and lipid levels, likely due to feeding low-diversity diets, supplemental foods rich in sugars, and limited exercise. In contrast, Lao elephants, especially those in the released and traditionally managed groups, had healthier metabolic and lipid profiles and generally maintained normal BCS (3–3.5), likely due to increased physical activity, social opportunities, and access to a diverse, natural diet. These differences persisted even during the COVID-19 tourism shutdown, emphasizing that baseline management practices, rather than tourist interaction alone, are drivers of health outcomes in these populations. Seasonal fluctuations in fGCM were observed among the Thai population, peaking during the rainy and winter seasons, suggesting that natural environmental factors could play an important role in adrenal activity, potentially compounded by the elephants' limited ability to seek comfortable areas during adverse weather due to short restraints. In addition, the ECC elephants presented higher fGCM concentrations than in other locations, possibly due to changes in management (new mahouts and a novel social program for male elephants) that took place during the study period, rather than chronic stress.

These findings highlight the importance of managing elephants in natural environments, including daily exercise and diverse diets to enhance health and welfare. Future management plans should encourage tourist facilities to be located near forests, allowing elephants to forage and walk greater distances, and limit population sizes based on available space to permit free-roaming. When restraint is necessary, longer chains (20–30 m) should be used to allow greater movement, and elephants should be given the opportunity to interact with compatible companions throughout the day rather than

being separated. Implementing these measures could substantially improve the long-term health and well-being of captive Asian elephants.

This study has several limitations that should be considered when interpreting findings. The small sample size of released and traditionally managed elephants in Laos may limit the statistical power and generalizability of comparisons across management types. The cross-sectional, observational design also restricts the ability to draw causal conclusions about the effects of management on specific health outcomes. Differences in laboratory conditions, including water quality, humidity, temperature, and technicians between Laos and Thailand could introduce measurement variability, despite our efforts to standardize assays. Body condition scoring, while using the same guidelines, remains a subjective assessment and may not fully capture differences in fat distribution or muscle mass. Finally, the unique circumstances of the COVID-19 pandemic, including changes in mahout staffing and management routines, may have further influenced the results in ways that are not representative of typical conditions. Now that pandemic restrictions are over, future studies are needed to compare elephants under more typical management conditions, allowing for better separation of the effects of routine practices, especially with respect to tourist activities, from those caused by pandemic-related disruptions.

Supporting information

S1 Table. GEE pairwise comparisons between locations.
(DOCX)

S2 Table. Seasonal effect on physiological parameters in Asian elephants.
(DOCX)

S3 Table. GEE pairwise seasonal comparisons between Lao and Thailand.
(DOCX)

S4. Table. Sex effects on physiological parameters in Asian elephants.
(DOCX)

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Gajah

Guidelines for the Rehabilitation of Captive Elephants as a Possible Restocking Option for Wild Populations

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Context and scope of the guidelines

Asian elephant (*Elephas maximus*) populations have declined across the 13 elephant range countries due to habitat loss and degradation, poaching and human-elephant conflict. Several wildlife management authorities plan to rehabilitate the captive Asian elephants and return them to the wild in order to revive depleted or extirpated populations. This is no simple task. Here we provide an overview of guidelines compiled based on the experience of practitioners and members of the Asian Elephant Specialist Group as a resource for such programs. We first provide a rationale and brief history of elephant reintroduction programs, followed by more specific guidance in sections as summarized below. The full document can be found on the website of the Asian Elephant Specialist Group.

Why (re)introduce Asian elephants?

There are several categorically distinct reasons for wanting to introduce or reintroduce Asian elephants into the wild from captivity, depending on whether one takes the perspective of the elephants, the people, or the landscape. This section considers each of these viewpoints briefly in presenting the rationale for initiating a rehabilitation and reintroduction program.

Which elephants should be considered and who should apply these guidelines?

There are at least two types of source populations that one may consider. One consists of those that have already spent a substantial fraction of their adult lives among people. The other consists of juveniles that may have been injured or orphaned and re-habilitated with the explicit aim of eventual re-introduction. These two types merit separate evaluation. More realistic are animals that are semi-wild, that have experience in foraging, such as those that may have been employed at timber camps. We discuss which types of individuals are appropriate candidates for release. Moreover we highlight the range of expertise that will be required in planning such a program.

Planning stage – primary considerations

The conservation aim of restoring elephant populations needs to be backed by political will and commitment. Important considerations at the outset are broken down as follows, with each discussed in greater detail in the full document:

· Defining the overall objectives of the proposed rehabilitation of captive elephants in the wild - Before the project starts, the overall objectives of this project should be discussed, identified and planned by all stakeholders for the advantages and disadvantages, prospective gains or losses. It requires a long-term commitment with high investment together with understanding that the local villagers will experience long-term impacts from having elephants around.

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· General feasibility and assessment - Many issues need to be considered before starting the project. These include justification of the proposed rehabilitation and translocation, overall objectives, impact of moving the elephants, number of elephants, sex and age groups, government agencies, stakeholders and local communities involved, appropriate rehabilitation and rewilding methods, appropriate relocation approach and timing suitable release sites, logistical coordination and planning mechanisms, socio-economic study of the local communities, mitigation and management of Human-Elephant Conflict (HEC), commitment of long-term financial and political support, and the economic values besides conservation.

· Budgeting - Long term support from both governmental and non-governmental organizations, as reintroduction projects require a lot of funding and resources, especially for a species that is as large, long-lived and behaviourally complex as an elephant.

· Identification of recipient area(s) for Asian elephants - Ecological as well as human dimensions to consider in site selection. The recipient areas should have plenty and variety of elephant food and water across the year, particularly the dry season. Ideally natural areas that once contained wild elephants would be preferable as the required food sources can be expected to be available, and should not ever have human-elephant conflict.

· Identifying source animals - Age, sex, and history of potential candidates to be considered for reintroduction programs. The ideal elephants are from the logging or those raised in natural habitat, who have spent time in the forest.

· Logistical coordination and planning - Programs may have at least four types of key personnel: the elephant team, site preparation team, legal team, and community engagement team. The planning of elephant translocation, quarantine, rehabilitation and reintroduction will require input from each team and execution will require good coordination and communication among them.

· Personnel capacity and experience (including building local capacity) - The expertise of individuals with varied backgrounds will be helpful. This includes ecologists, behaviourists (ethologists), veterinarians, educators, researchers, fundraisers, mahouts, rangers, coordinators from both government and non-governmental organizations. The involvement of government law enforcement, police, or in some cases, military may also be required, as well as representatives of the local community engagement.

· Demographic, genetic and behavioural considerations - Differing needs based on age/sex class and the context for which the reintroduction is being designed. Asian elephants exhibit very dynamic fission-fusion social organization with complication to identify clear dominance hierarchies. Thus, this should be concerned with closed monitoring for social behaviour and demography.

· Veterinary considerations - Release of the captive animals into the wild may result in introduction of disease to conspecific or unrelated species. The planning process is very important in order to prevent disease transmission, and let the animal adapt to the climate and environment.

· Security considerations - Aspects concerning both the safety of the elephants and the people within the landscapes that populations will be situated within or adjacent to.

· Legal considerations - There will likely be country-specific legal frameworks, and possibly loopholes within such frameworks, that must be accounted for.

Rehabilitation period

The actual rehabilitation period can range from months to years, and will likely involve the bulk of the expense. There are three main sections that extensively detail the various needs to prepare for and address during this crucial period:

· Socialization - As elephants are highly social animals, it is absolutely critical to provide opportunities for the development of social

relationships in a careful manner that is age- and sex-appropriate and ensures the safety and well-being of individuals both while in rehabilitation, as well as upon release. This section covers the many diverse aspects of social interaction to be addressed.

· Food and foraging - When releasing the captive elephants back to the wild, their ability to find food and water resources will be crucial for their survival. They should be released in a group with other foraging-experienced elephants, or be trained for foraging, before bringing them to the project, and during the rehabilitation period.

· Health progress - One of the most important indicators that elephants can adapt themselves during the rehabilitation process is their health. Body condition score and dung composition are the most convenient health indicators, and should be regularly monitored.

Post-release period

Post-release monitoring is as crucial as the previous two phases, and no program can be considered complete or deemed a success without it. The main goal is to be able to answer the question: which factors have a positive or negative influence on reintroduction success? The monitoring itself is broken down into phases:

· Intensive monitoring period (1-5 years) - This period should ideally include medical/physical, behavioural, and movement observations. This section details the types of monitoring that programs should strive for in order to be able to evaluate whether animals are properly assimilating to their new surroundings and any potential problems, risks, hazards etc. that might not have been foreseen. If there are wild elephants in the vicinity, it may be as important to include a component studying them as well, and their responses to released individuals, in order to have some basis for determining whether the latter are faring comparatively well or poorly.



Group of released elephants with calves born from natural mating in the forest at Sub Lanka Wildlife Sanctuary, Thailand.

· Long-term basic monitoring (2 or more years) - Depending on the available budget and feasibility, intensive monitoring may need to transition to more basic longer-term monitoring at some point within the first five years. This section covers the types of data that are only obtainable through longer-term observations, such as demographic events.

In both of these components, the need for preparatory and ongoing communication with local communities is emphasized, especially where specialized education programs may be needed.

Lessons learned

Things often do not go as planned. Some of the issues that arise may be wholly unpredictable, but others may benefit from past experience. This final section summarizes experiences collected from various existing initiatives, broken up according to the preceding sections of the guidelines. It is hoped that these aggregated observations will be useful for anticipating what might happen at any stage, to the extent possible.

Community awareness

The absence of good communication and community awareness programs, can foster a lot of misunderstanding, anger and resentment toward reintroduction programs among local people. This is especially true in areas where wild elephants may already be perceived as a risk to life, property, and livelihoods. Released animals may consequently be killed, either intentionally

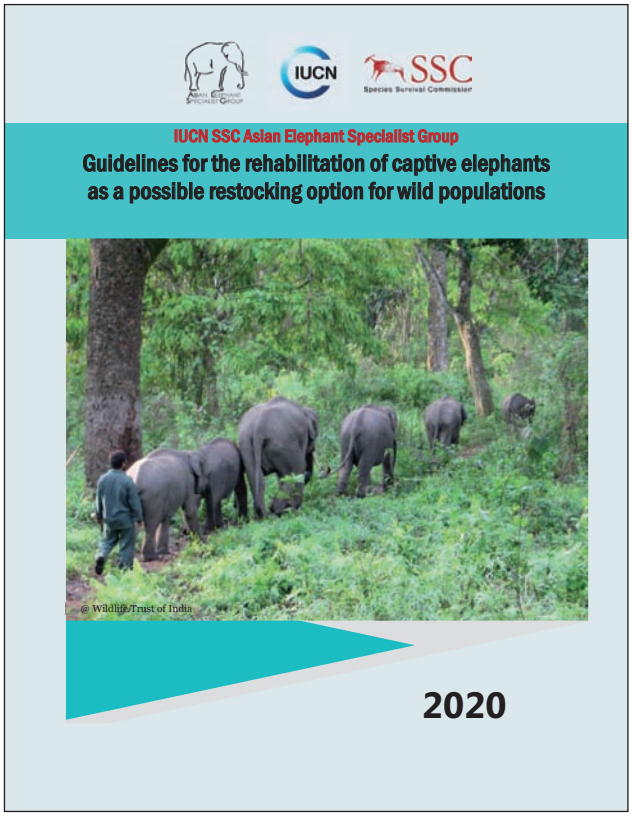
or unintentionally, constituting not only a tragedy but a tremendous waste of resources. The burden is on the re-introduction program to work with people to ensure transparency from start to finish, even if this involves facing opposition or difficult situations.

Over-habituation

Certain individuals from early cohorts of animals that were over-habituated sought out human company to the extent that they tried to find their way back to the care facility or became a nuisance to visitors of protected areas in which they were released. It is therefore crucial to avoid over-habituation.

Social support

If the calves are released in a mixed sex group, male calves will wander large distances and may be more likely to encounter difficulties. It is suggested to release calves at the same sex cohort. Elephant calves need socialization while undergoing rehabilitation to prevent these problems.



Maternal drive

Captive-reared subadult females that are overzealous alloparents can separate offspring from other mothers while being unable to supply milk themselves, and may cause the calves to starve. Conversely, these captive-born mothers might ignore, attack or kill their own wild born calves for possibly due to lack of role models or practiced maternal experience.

Integration to the habitat and wild elephants

Some released calves may be easily accepted by the adults, and integrated to the wild herd. However, elephant-elephant conflict (male-male, female-female, male-female) can occur, and result in deaths of elephants.

Conclusion

When the elephants are released in that particular area, the goal is to ensure persistence for a long-term by integrating with existing wild populations or possibly seeding new populations. The rehabilitation of captive-born or orphaned wild elephants in human care for release back to the wild is a long and complicated process with many issues to consider. This includes not only thinking about the budget and co-operation from various organizations and stakeholders, but about what the elephants themselves truly need to be successful. All three stages - planning, rehabilitation, and post-release monitoring - are equally important. We hope this summary of guidelines helps to understand the various considerations for such program.

Citation

The full document can be found on the website of the Asian Elephant Specialist Group at <https://www.asesg.org/resources.php>.

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Journal of Ethnopharmacology

From plant selection by elephants to human and veterinary pharmacopeia of mahouts in Laos

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From plant selection by elephants to human and veterinary pharmacopeia of mahouts in Laos

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A B S T R A C T

Ethnopharmacological relevance: To what extent has animal observation contributed to the development of human pharmacopeias? We approach this question here through the study of mahouts' knowledge regarding the responses by elephants to their health problems, and the human medicinal uses of plants and the care of domestic animals that result from their observations.

Materials and methods: 32 mahouts were interviewed in Thongmyxay district and 28 at the Elephant Conservation Center in the province of Xayabury. Interviews focused on the elephants' diet, health problems, plant items they consume in particular physiological or pathological contexts and the treatments that mahouts provide them. For each plant mentioned, the part of the plant consumed and mode of preparation and administration if used by mahouts were recorded. Species samples were then collected and later identified by specialists.

Results: 114 species were recorded as being consumed by elephants during interviews with mahouts and forest outings with them to collect samples. Twenty species were identified as used by elephants in particular pathological conditions or physiological states. According to interviewed mahouts, the consumption of certain plants improves the health of the elephant. We observed clear convergences between the observations interpreted by the mahouts as self-medication behaviour from elephants and their own medicinal practices (for human and veterinary purposes).

Conclusion: Beyond a mere reproduction of elephant self-medication behaviours observed, the human or veterinary medicinal cares derived from these observations are the result of complex arrangements integrating all available medicinal and conceptual resources into elaborate preparations.

We recommend that mahouts' knowledge about traditional medicinal care given to elephants be further compiled, as it could have a beneficial impact on veterinary health care provided in elephant resorts and elephants' well-being.

1. Introduction

The study of animal self-medication behaviour and zoopharmacognosy (Rodriguez and Wrangham, 1993) began about 20 years ago with scientific observations made about primates, especially chimpanzees (Wrangham, 1995; Huffman et al., 1997). However, this type of behaviour can be found in many accounts from traditional oral cultures that attribute an animal origin to certain medicinal uses of plants (Huffman, 2003) as well as in ancient literature (Boehm and Luccioni, 2008). Studies have documented convergences of therapeutic uses of some plants between animals and humans (Huffman, 2001; Krief et al., 2005, 2006; Morrogh-Bernard, H.C., et al., 2017) suggesting either independent convergences, or transfers of knowledge from animals to humans. A few cases of transfers have been reported where direct observation of a sick animal's behaviour can be linked to the subsequent and effective use of the same remedy by humans presenting the same condition (Huffman, 2001; Krief and Brunois-Psina, 2017). However, there has been little exploration of how medicinal practices are adapted and shaped by the observation of animal behaviour.

During a survey on traditional plant uses conducted in southern Thailand in 2014, we looked at the knowledge of two mahouts (the English term borrowed from India for a person in charge of a domestic elephant in Asia) that suggested that this particular human-animal relationship between mahouts and their elephants can be a significant source of knowledge to better understand the origin of human medicine (Dubost, 2015). Indeed, in the traditional way of managing elephants within the village context, elephants are not fed but are allowed to feed themselves in the surrounding forests (Maurer et al., 2017; Maurer, 2018). Because of the comparable life span between elephants and humans, mahouts who spend a large part of their lives with the same

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Glossary and abbreviations

ECC	Elephant Conservation Center
EUR	Elephant Use Report
Mahout	A person who takes care of an elephant
NNPA	Namphuy National Protected Area

animal are able to observe its needs and responses according to its state of health, responses that they sometimes interpret as a form of self-medication from these animals. Moreover, the traditional care they provide elephants places mahouts at the interface of these presumed animal self-medication behaviours and local traditional medicine, providing a good opportunity to study the convergence of medicinal plant use between men and elephants. We therefore carried out a study to explore the relationship between traditional human and veterinary medicine and observations on elephant behaviour by mahouts in an area of Laos where mahouts and owners still keep their elephants in the vicinity of villages. Data obtained were supplemented with elephant observations and interviews conducted in an elephant center dedicated to the conservation of this species in Laos.

2. Background

Laos is located in a humid tropical zone. The climate is seasonal, with a rainy season (May–October) a cool-dry season (November–February) and a hot-dry season (March–May). Characterized by extensive forest cover, the country is part of the Indo-Burma biodiversity hotspot (Myers et al., 2000), but this cover has drastically decreased and is becoming more and more fragmented. In the mid-1960s, forested areas accounted for 70% of the territory, compared to 41% in 1999 (Southavilay and Tuukka, 1999) and areas of over 1000 ha decreased from 88% of the total forest area in 1992 to 54% in 2004 (Evrard, 2004), indicating a process of landscape fragmentation. However according to the most recent assessment of forest cover by the Department of Forestry of the Lao PDR, reduction of forest cover decreased significantly from 2002 to 2010, and according to official sources forest cover has increased slightly in the north of the country (Kim and Alounsavath, 2015).

2.1. Elephas maximus in Asia and Laos

The Asian elephant *Elephas maximus* is classified as an endangered species by the IUCN. The loss and fragmentation of its habitats is the main cause of this decline of elephant populations in Asia (Leimgruber et al., 2003), where it is found in 13 countries (IUCN, 2016).

In Laos, the elephant is an emblematic species, and the country was previously called Lan Xang "Land of the million elephants". However, the population estimated at 2000–3000 wild elephants in 1988 (Phantavong and Santiapillai, 1992) had reduced to just 600–800 individuals by 2009 (Khounboline, 2011). Domestic elephant numbers are also declining sharply in Laos and are now comparable to the number of their wild counterparts (Khounboline, 2011; Suter et al., 2014). These elephants living in contact with humans have an intermediate status between wild and captive or domesticated animals. Traditionally sourced from wild populations (Lair, 1997; Sukumar, 2003), they have not undergone a selection process like most domesticated animals. According to traditional management methods, they are not fed and are periodically released in the forest over long periods (Maurer, 2018). It is therefore equally inappropriate to apply the epithet of captives to them, which refers instead to animals whose movements are much more restricted. We will thus use for the elephants of Thongmyxay district the term “village elephant” proposed by Maurer (Maurer, 2018) and used by Lainé (2017) and will use “captive elephant” when talking about elephants kept in camps. Village elephants often have several owners within the same family, but usually just one will be in charge of looking after them and is described as their mahout (Suter et al., 2013).

2.2. Diet and habitat

Elephants are generalist postgastric herbivores that feed on a wide variety of plant items, whether in terms of species consumed, life form (trees, vines, grasses, herbs) or of parts selected such as leaves, fruits, twigs, barks, roots, woody stems (Sukumar, 1992; 2003; Chen et al., 2006; Himmelsbach et al., 2006; Joshi and Singh, 2008; Campos-Arceiz et al., 2008; Santra et al., 2008; Lihong et al., 2007; English et al., 2014). Areas which constitute ecotones between cultivated areas and regrowth or disturbed forests colonized by pioneer species, which display higher levels of understory vegetation than old-growth forest, are optimal for elephants in terms of resources and carrying capacity (Fernando and Pastorini, 2011; Fernando et al., 2005; Evans et al., 2017). These characteristics regularly bring elephants to frequent the outskirts of anthropized areas.

3. Material and method

3.1. Study area

This study was conducted in Xayabury province (Fig. 1), which is home to 80% of the village and captive elephants in Laos (Lassausaie et al., 2015), in two sites:

1. In the district of Thongmyxay, whose inhabited area is encompassed by the Namphuy National Protected Area (NNPA) which covers 1912 km^2 (WWF, 2019) and shelters the second largest population of wild elephants in Laos, estimated at 60–80 individuals in 2009 (Khounboline, 2011). The inhabited area of Thongmyxay is mainly surrounded by forested areas consisting of degraded secondary forests, *Dypterocarpaceae* forests, riverine forests and hill bamboo forests.
2. At the Elephant Conservation Center (ECC) in the north of the province. This center, dedicated to wild and captive elephant conservation and research in Laos, develops ecotourism activities focused on elephant observation. It is delegated by the Ministry of Forests to be responsible for the NNPA's wild elephant protection activities, and has a concession of 512 ha where elephants feed themselves. Vegetation consists mainly of young, dry dipterocarp forest dominated by *Shorea siamensis* Miq. within a broad topographic range covering the area and an abundance of bamboo in patches running along the bank of a stream. Signs of logging are present and the area is surrounded by rice farms.

3.2. Survey

We carried out an inventory of plants which the mahouts indicated were consumed by elephants, specifying the parts consumed. Then we organized forest outings with mahouts (in Thongmyxay district and at ECC) to explore different areas where they let their elephants feed, in order to collect samples of the listed plants, which were dried to prepare herbarium specimens. The taxonomic identification was carried out under the responsibility of Vichith Lamxay in the Department of Botany at the National University of Laos. Plants names were checked and updated according to the standard database “The Plant List” (http://www.theplantlist.org). Voucher specimens were deposited at the Herbarium of the National University of Laos in Vientiane.

We conducted semi-structured interviews with 60 mahouts - 32 in Thongmyxay district (aged 38 to 78) and 28 in ECC (aged 35 to 63) - all of whom were Tai-Lao, the dominant ethnic group in Laos.

The interviews focused on:

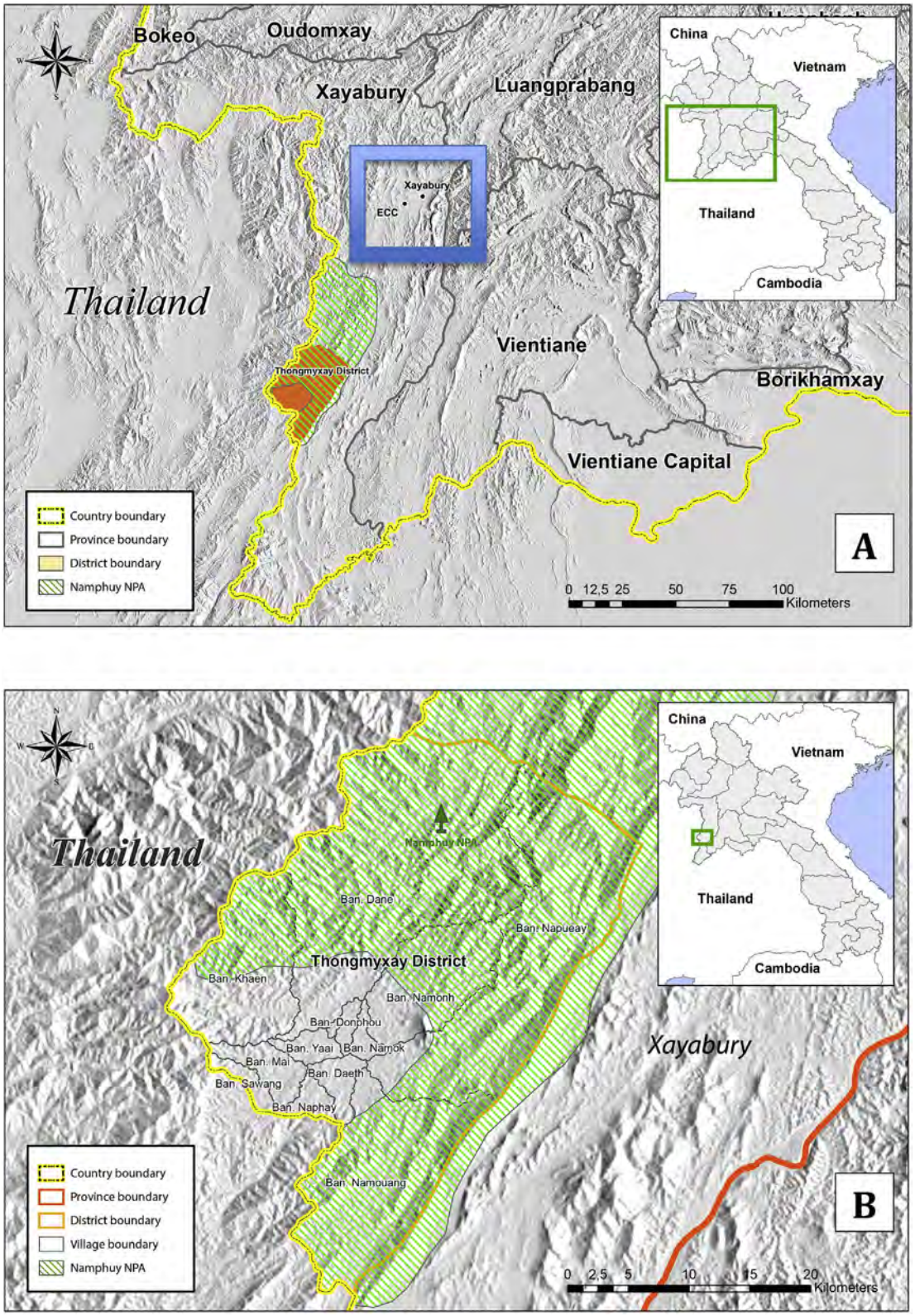

Fig. 1. Contextual maps of Thongmyxay district and location of the ECC centre (Juan A. Torres) wide view (A) close-up view (B).

- Elephant management practices and changes that have occurred since the beginning of their mahout activity, especially issues of access to food resources, in order to assess the context of the observations reported by mahouts.
- The feeding behaviour of their elephants, by requesting qualitative information on the context of consumption of the plant items they consume.
- Elephant health problems, their needs, the possible care provided (including preparation and administration methods) and observations regarding how elephants respond to their own problems. When plants were cited as being consumed by elephants in a particular physiological context, mahouts were also asked if they used them



Fig. 2. Female uprooting a tree (A) to eat the root part (B) (Photo JM Dubost).

themselves in the family environment.

We also followed a female elephant that had been released into the forest (on average 6 h a day for five days) at the ECC site to observe its feeding behaviour. This enabled us to get a better understanding of the observations reported by the mahouts and thus inform our discussions with them on the behaviour of their elephants.

3.3. Ethics and authorizations

Ethical approval was obtained from the National Ethics Committee for Health Research (NECHR) under the supervision of the Ministry of Health of the Lao PDR (0894NIOPH/NECHR). Authorization to collect the plants was obtained from the relevant authorities. The study was approved by the local Nagoya protocol office (N°008/SABS).

Voucher specimens were deposited at the herbarium of the Department of Biology, Faculty of Sciences, National University of Laos (NUoL), in Vientiane.

4. Results and discussion

4.1. Elephant management practices in Thongmyxay district

In recent decades, there have been three successive phases in the evolution of elephant management and use. Of the 32 mahouts interviewed in Thongmyxay district, 21 are no longer active, either because they have sold or rented out their elephant (*cf. infra*) or because they have handed over their responsibility to relatives. They described the traditional elephant management system that prevailed before the construction of the road linking Thongmyxay district, which was built 20 years ago. Elephants were then used mainly for the transportation of goods - mostly rice after the November harvests - and on demand for occasional village-scale logging to provide locally-used timber. These activities took place in the dry season from November to April when the land is passable. During this period, elephants were tethered (with a 30–40 m chain) or simply hobbled in the wooded areas near the village, and were moved once or twice a day to renew their feeding areas and to allow them to drink. During the rainy season, when all available human resources were used to plant crops, elephants were released in small groups in the forest that is now part of the NNPA. They were visited from time to time and sometimes came back on their own at the beginning of the dry season, or the mahouts would go looking for them and bring them back.

About thirty years ago, with the development of the road network and the arrival of vehicles, elephants were no longer used for transport. They were used instead for logging, meeting the demand of forestry companies who were then engaged in a massive exploitation of timber wood. (Suter et al., 2013). As a result, since elephants no longer have any use in terms of work except for occasional logging for the villagers, the owners have tended to free themselves from the burden that their animal now represents by selling it (11 respondents) or renting it out to elephant camps (8 resp.). However, other owners in Thongmyxay chose to keep their elephants (11 resp.). This is due firstly to the fact that the district enclosed in the NNPA offers crop-free areas where they can still release their elephants. Secondly, this protected area, which houses the second largest population of wild elephants in Laos, provides opportunities for the new income activity of elephant breeding for sale. The female elephants are thus left in the forest to mate with wild males (reported by 6 mahouts out of the 11 still working) and an elephant calf can be advantageously sold or rented to elephant camps for which it is a first-choice attraction (Maurer et al., 2017).

4.2. Inventory of plants indicated by mahouts as being consumed by elephants

When they keep their elephant tethered, mahouts can see which species have been consumed by observing changes in the vegetation cover of the area where their elephant has been feeding and the marks left on specimens. The daily movements to bring the elephant to a new feeding area or the regular visits to them when released also provide an opportunity for many direct observations of plant consumption. A total of 114 species were recorded as being consumed by elephants during mahout interviews and forest outings with them, with the parts taken for each amounting to 197 plant items. However, according to all mahouts, bamboo twigs and leaves make up the staple part of the elephants' diet. This inventory will be the subject of a future publication. Parts of plants taken may include roots, stems, twigs, bark, wood, leaves, fruits, and young shoots. Plant forms are also very diverse, ranging from mature trees, saplings and vines to grasses. Other than the consumption of plants, 19 mahouts report the occasional geophagous behaviour of elephants, this issue will be discussed in section 4.4.

Observing elephant feeding behaviour has demonstrated the high selectivity that an individual can show in the choice of items consumed, uprooting a tree to consume only the root (Fig. 2), or stripping twigs to keep either the stem or the leaves. The elephant often first brushes the

plant item with the tip of its trunk before choosing to consume it or not, and uses a whole range of techniques involving the trunk, front legs, tusks and jaws, and the animal's weight and strength. Some parts such as tree roots and bark require a sustained effort to be collected and are consumed in significant quantities (for example, a trunk 15 cm in diameter can be debarked by 50 cm in about 20 min).

4.3. Knowledge about elephant health

The elephant is perceived by mahouts as an animal with generally few health problems, and they explain that in most cases the elephants know how to heal themselves using the resources around them. When asked what they need to do to keep an elephant healthy, mahouts say that the most important thing is to make sure that it can find what it needs on its own. Elephants should therefore be released as often as possible (5 resp.). When tethered, suitable places with abundant and diversified vegetation should be carefully chosen (15 resp.) and the elephant moved once or twice a day to allow it to drink and renew its feeding area. In these conditions, mahouts say that elephants rarely experience health problems. They evaluate the condition of their elephant using various signs. When it is tethered and they go to fetch it, the faeces that are present inform them of its digestive state: diarrhoea, constipation (hard or absent), or presence of macroscopic parasites. A look at the browsed area tells them if the elephant has a normal appetite and what has mainly been eaten. A pathological condition is often reflected in the fact that the elephant sleeps a lot during the day, seems apathetic and eats little (7 resp.), sometimes by an abnormal dryness of the interdigital space which is normally wet because here elephants have glands similar to human eccrine sweat glands (Fowler and Mikota, 2006).

Among the health problems mentioned that could affect elephants are firstly digestive disorders (35 resp.) characterized by diarrhoea (31 resp.), constipation (4 resp.) or bloating (3 resp.). Also mentioned are accidental injuries or abrasions due to the chain used to tether elephants (11 resp.), purulent abscesses (9 resp.), skin rashes (8 resp.), eye pain with discharge (5 resp.), and *amidal* (3 resp.) a local term that refers according to veterinarians to an infection by EEHV (Elephant Endotheliotropic HerpesVirus), and can be fatal to the calves that contract it (Long et al., 2016; Bouchard et al., 2014). The term *amidal* is borrowed from the French word "amygdale" meaning "tonsil" because one of the visible symptoms of this condition in elephant calves, according to mahouts, is swelling of the throat area.

In a study conducted in South India (Jayakumar et al., 2017) investigating ethnoveterinary care given by mahouts to their elephants, digestive disorders are also cited as the most frequent problems among the captive elephants considered. However, constipation is reported to be much more frequent than diarrhoea, unlike in our study where diarrhoea is the main elephant digestive disorder reported. The South Indian study also notes that intestinal disorders, among other problems observed, are related in particular to inadequate husbandry practices such as poor hygiene in the elephant housing area, lack of exercise and monotonous food. These conditions differ in many respects from those experienced by elephants in Thongmyxay District or the ECC, where:

- they probably access a much more diverse diet as they feed themselves in a forested area
- they might spend much more time on feeding than fed animals
- they stay permanently in natural areas and probably have more opportunities for exercise (even when tethered they are moved daily from one area to another and still have to obtain their own food).

Such differences in elephant management practices may explain the different prevalence of constipation versus diarrhoea in the elephants in the South Indian study and ours.

Table 1
EUR presented by species.

Family	Botanical name	Voucher	Vegetal Type	Parts eaten	EUR (nb mahouts)	Mahout medicinal use
Fabaceae	*Acacia caesia* (L.) Willd.	JMD 1127	C	r	diarrhoea: r and s (1)	yes (1)
Fabaceae	*Acacia concinna* Willd. A.DC.	JMD 1149	C	r, l	motherhood: r (1)	no
Zingiberaceae	*Amomum schmidtii* K.Schum. Gagnep.	JMD 1113	H	r, wp	diarrhoea: wp (1)	yes (1) (flatulence*)
Zingiberaceae	*Amomum schmidtii* K.Schum. Gagnep.	JMD 1113	H	r, wp	tonic: r (1)	no
Fagaceae	*Castanopsis indica* Roxb. ex Lindl. A.DC.	JMD 1151	T	r, b	motherhood: r: (2)	no (2)
Fagaceae	*Castanopsis indica* Roxb. ex Lindl. A.DC.	JMD 1151	T	r, b	diarrhoea: r (1) r and b (1)	yes (1) no (1)
Guttiferae	*Cratoxylum formosum* Jack Dyer	JMD 1096	T	l, s, t, b, r, sh	motherhood: r (1)	no (1)
Guttiferae	*Cratoxylum formosum* Jack Dyer	JMD 1096	T	l, s, t, b, r, sh	diarrhoea: r (2)	yes (1)
Moraceae	*Ficus hispida* L.f.	JMD 1134	T	b, t, sh, r	diarrhoea: r (1)	yes (1)
Moraceae	*Ficus racemosa* L.	JMD 1002	T	r, b, l, sh, f	diarrhoea: r (1) r + b (1)	yes (1)
Rubiaceae	*Gardenia sootepensis* Hutch.	JMD 963	T	l, t, r, b	diarrhoea: b (1)	yes (1)
Simaroubaceae	*Harrisonia perforata* Blanco Merr.	JMD 1144	Sh	r, sh	motherhood: r (5)	no (4)
Simaroubaceae	*Harrisonia perforata* Blanco Merr.	JMD 1144	Sh	r, sh	diarrhoea: r (13)	yes (7) no (6)
Araliaceae	*Heteropanax fragrans* Roxb. Seem.	JMD 957	T	b, r, t, sh	motherhood: r (3), b (1)	no (4)
Araliaceae	*Heteropanax fragrans* Roxb. Seem.	JMD 957	T	b, r, t, sh	diarrhoea: r (1), s (1)	yes (2)
Fagaceae	*Lithocarpus auriculatus* (Hickel & A.Camus) Barnett	JMD 1150	T	r	motherhood: r (4)	no (3)
Fagaceae	*Lithocarpus auriculatus (Hickel & A.Camus) Barnett*	JMD 1150	T	r	diarrhoea: r (3)	no
Moraceae	*Maclura cochinchinensis* Lour. Corner	JMD 972	Sh	l, s, b, sh	diarrhoea: r (3)	yes (1) no (2)
Fabaceae	*Mucuna pruriens* (L.) DC.	JMD 1112	C	s, r	diarrhoea: r (1)	no
Euphorbiaceae	*Phyllanthus emblica* L.	JMD 1142	T	f, b	diarrhea: b (1)	yes (1) (cough*)
Euphorbiaceae	*Phyllanthus reticulatus* Poir.	JMD 995	T	l, r, sh	diarrhea: r (3)	no (3)
Theaceae	*Pyrenaria sp.*	JMD 958	T	r, b, s, l, sh	motherhood: r and b (1)	no
Dipterocarpaceae	*Shorea siamensis* Miq.	JMD 1148	T	r, b, s	diarrhoea: r (1)	yes
Anacardiaceae	*Spondias pinnata* L.f. Kurz	JMD 1115	T	b, sh, b, r	diarrhoea: r (2)	yes (1) no (1)
Combretaceae	*Terminalia mucronata* Craib & Hutch.	JMD 1108	T	s, b, r	diarrhoea: r and b (2), b (1)	yes (2) no (1)
Menispermaceae	*Tinospora crispa* L. Hook.f. & Thomson	JMD 1126	C	s, r, l	diarrhoea: r (1)	yes (1)
Menispermaceae	*Tinospora crispa* L. Hook.f. & Thomson	JMD 1126	C	s, r, l	tremor: s (1)	no
Menispermaceae	*Tinospora crispa* L. Hook.f. & Thomson	JMD 1126	C	s, r, l	fever, (apathy + appetite loss): r and s (1)	no

4th column indicates the context of elephant use and items selected, and in brackets the number of mahouts reporting the EUR; 5th column gives the number of mahouts using the item for the same ailment in their household (* indication have been specified when different from EUR). b = bark, f = fruit, r = root, l = leaves, s = stem, sh = shoots, t = twigs, wp = whole plant.

In a report published about veterinary care provided to elephants by Elephant Asia (an NGO which had set up a mobile elephant care unit in Laos), abscesses, intestinal disorders, nails problems, eye problems and injuries were by decreasing order the main problems found in the 315 elephants examined (Labatut et Suter, 2010). Abscesses are most common among the ailments reported, which may be due to the fact that 78% of the elephants examined were working in the logging industry, where, according to the authors, abscesses were caused by the prolonged chaffing of chains.

When asked if there are periods when elephants are more vulnerable, out of the 13 mahouts who had an opinion, 9 mentioned the end periods and transitions of seasons (from dry to rainy season and vice versa) that they believe correspond to a change in food, indicating for example that tender grasses enjoyed by elephants grow back at the beginning of the rainy season. Sukumar (2003) also notes the appearance of intestinal disorders at the changes of seasons and hypothesizes that they can be induced by a change of diet (pre-eminence of browsing in the dry season and grazing in the rainy season) modifying the fiber/protein ratio of their food, to which their digestive system must readapt. Conversely, according to some mahouts, at the end of the dry season the decline in the quality of the available water, which is more stagnant, may also be the cause of these disorders.

4.4. Elephant behaviour interpreted as self-medication

- *Soil use:* When an elephant has localized pain or discomfort (bite, sting, or abscess), it tends to blow on it (4 resp.), drawing attention to a possible external problem on a less visible area of its body, or may also apply mud on the affected area (7 resp.). While mahouts observe regular geophagic behaviour, three of them report that they have observed this type of behaviour particularly when their animal has intestinal problems, with a subsequent improvement noted. These geophagous behaviours are documented in the literature and the composition of soil licks frequented by African (*Loxodonta cyclotis* and *africana*) and Asian (*Elephas maximus*) elephants has been analyzed in the Central African Republic (Klaus et al., 1998), Zimbabwe (Holdø et al., 2002) and Sri Lanka (Chandrajith et al., 2009). Klaus and Holdø note a higher mineral richness in the soils that could compensate for deficiencies in elephant feeding, but Chandrajith does not note significant differences in mineral elements between soil licks and surrounding soils. Klaus and Chandrajit suggest that the clay elements of these soils could help detoxify some of the elements ingested by elephants by adsorbing secondary toxic compounds.
- *Plant use*: In addition to blowing or applying mud to an affected area and geophagy in the event of intestinal problems, of the 49 mahouts interviewed with at least 10 years' experience, 26 reported observations of search behaviour by their elephants and increased consumption of specific items when they have health problems or in relation to their physiological state. We will call this search behaviour and increased consumption of specific plant items described by mahouts “privileged consumption”. For the same condition or physiological state of the elephant, mahouts report a privileged consumption of a single plant item (6 resp.), or of several species, with overlaps that vary from one to the other (19 resp.). We will name this here Elephant Use Report (EUR) referring to an observation described as recurrent by one mahout, where a particular pathological condition or physiological state identified in the elephant they care for is associated with the search behaviour and consumption of one or more items from the same plant species.

We have thus identified 64 EUR in which 20 species are mentioned, representing 30 plant items. These EURs presented in Table 1 fall into two main categories, one relating to health disorders and the other related to female reproduction including gestation, ante and postpartum, and lactation periods. For convenience, we have grouped this last category of EURs, under the generic term of “motherhood”. This group of EURs is detailed in Table 2.

Diarrhoea is the main context of these plant item intakes: 43 EUR, indicated by 19 mahouts with 19 species collected representing 25 items; this is also the elephant health problem most often mentioned by mahouts. Then comes motherhood: 18 EUR indicated by 8 mahouts with 7 species mentioned and 8 items consumed. Other contexts were mentioned by only one mahout for each: dizziness (the elephant shakes), weakness, and ‘fever’, which is described as an apathetic state with loss of appetite.

The 30 items mentioned in these EURs are included among the 197 items recorded in our inventory of items consumed by elephants. When the mahouts specified the reasons for the self-medication interpretation they give to the recorded EURs, two criteria emerged:

- Firstly, the mode of consumption is in relation to the reported condition. Privileged consumption is the general case, in which the item is part of the elephant's usual diet but is particularly sought after and consumed in much greater quantities in certain contexts. Alternatively, exclusive consumption is where the item is only consumed in a particular context (19 EUR - 11 resp.). It should be noted that according to each mahout's own observations, for the same EUR some mahouts mention an exclusive mode of consumption of the plant item for a specific ailment, while others may report

Table 2
Details of EUR related to female elephants motherhood period.

Botanical name	Family	EUR
Acacia concinna Willd. A.DC.	Fabaceae	post-partum and lactation during first months: root
Castanopsis indica Roxb. ex Lindl. A.DC.	Fagaceae	ante and postpartum during 2 months: root
Castanopsis indica Roxb. ex Lindl. A.DC.	Fagaceae	postpartum during 2 months: root
Cratoxylum formosum Jack Dyer	Guttiferae	post-partum and lactation during first months: root
Harrisonia perforata Blanco Merr.	Simaroubaceae	lactation during first months: root
Harrisonia perforata Blanco Merr.	Simaroubaceae	post-partum and lactation during first months root
Harrisonia perforata Blanco Merr.	Simaroubaceae	postpartum during 2 months: root
Harrisonia perforata Blanco Merr.	Simaroubaceae	postpartum for 1 week: root
Harrisonia perforata Blanco Merr.	Simaroubaceae	pregnancy: root
Heteropanax fragrans Roxb. Seem.	Araliaceae	ante and postpartum: root
Heteropanax fragrans Roxb. Seem.	Araliaceae	ante and post-partum: bark
Heteropanax fragrans Roxb. Seem.	Araliaceae	post-partum and lactation during first months: root
Heteropanax fragrans Roxb. Seem.	Araliaceae	pregnancy: root
Lithocarpus auriculatus (Hickel & A.Camus) Barnett	Fagaceae	ante and postpartum during 2 months: root
Lithocarpus auriculatus (Hickel & A.Camus) Barnett	Fagaceae	post-partum and lactation during first months root
Lithocarpus auriculatus (Hickel & A.Camus) Barnett	Fagaceae	pregnancy: root
Lithocarpus auriculatus (Hickel & A.Camus) Barnett	Fagaceae	pregnancy: root
Pyrenaria sp.	Theaceae	pregnancy, ante and post-partum: root and bark

the consumption of the same item as part of the elephant's usual diet.
- Secondly, the consumption of the item is followed by a rapid improvement in the condition of the elephant.

Beyond the observation of the consumption of specific items in a particular physiological context, additional information and observations were provided by mahouts around the question of the interpretation of self-medication by elephants. Five of them have observed that elephants consume certain roots when they have diarrhoea (*Harrisonia perforata* Blanco Merr. for two of them, *Castanopsis indica* Roxb. ex Lindl. A.DC. for another and *Terminalia mucronata* Craib & Hutch. for the last) and have noted that it improves their condition. Knowing this, if their animal suffers from diarrhoea, they will bring it to a place where these plant resources are available. They say that when they return to move the elephant, these plants have largely or entirely been uprooted, their roots consumed, and that the elephant's condition is improving rapidly. The efficacy of these practices to improve the condition of their elephants has not been quantified or demonstrated, but this approach has in a way acted as a test and has confirmed for the mahouts their interpretations of elephant self-medication based on the plant selection behaviour of the sick elephants they observe.

4.5. Interactions between elephant observations and local medicinal practices

Going further than just facilitating access to plant resources, some mahouts also provide them to their animal. Four mahouts report that when their elephant suffers from diarrhoea, they collect and bring it the roots that they know it seeks when it experiences this condition. These are *H. perforata* (2 resp.), *T. mucronata* (1 resp.), *Spondias pinnata* L.f. Kurz (1 resp.). They sometime add salt to make the items more appetizing but the items are given in their raw form. In another similar case though it leads to a more elaborate practice, where the mahout had noted that when the elephant has what he calls a fever (which to him means his elephant is sad and listless) it consumes much more of the *Tinospora crispa* L. Hook.f. & Thomson vine and recovers quickly. If he observes this condition in his animal, he crushes the root and stem of this vine with salt and gives it to be eaten. When he passes through a place where this vine is abundant, the mahout collects some of it and dries it to have some at hand when needed.

These are examples of local veterinary care practices resulting from the observation of elephant behaviour. In the last case described this leads to the creation of a reserve of a therapeutic resource, in a more elaborate form suitable for its conservation that can quickly be used in an assimilable galenic preparation (the root is dried and then reduced to powder with salt added before administration). We see here a veterinary preparation involving the observation of specific elephant behaviour (for the choice of ingredients) and an element from the mahout's medicinal background (in the galenic form). But this integration of elements derived from elephant observation and from the mahout's medicinal background into preparations given to elephants does not only concern the ingredients used and their processing. The medicinal background of mahouts may also be involved in the explanations that sometimes come with the interpretation of self-medication given to some of the elephants' behaviour. These explanations can in turn lead to the addition of other ingredients in preparations given to cure elephants. Thus two mahouts explain the consumption of certain plants by elephants by referring to their warm or cold qualities according to a classification of diseases and remedies in use in Laos for human medicine (Souk-Aloun, 2001; Pottier, 1971, 2007). One mahout states that diarrhoea is a disorder of the category *ok hon* (literally 'feel hot') that the elephant treats by eating *ya yen* plants (literally 'remedy cool') such as *H. perforata* or *Lithocarpus auriculatus* (Hickel & A.Camus) Barnett. Two other mahouts report that when elephants suffer from tremors (1 resp.) or diarrhoea (1 resp.), they consume bitter roots. In the same vein, another mahout told us about this use that in his conception, diarrhoea results from excessive consumption of sour substances such as tamarind fruit, a disorder that he believes can be treated by consuming bitter substances. He noticed that when his elephant has diarrhoea it consumes more *T. mucronata* bark than usual. He himself uses this bark with that of *H. perforata* against diarrhoea, as it is a family remedy. Therefore, when his elephant is affected by diarrhoea and is tethered, he brings it bark of *H. perforata* and *T. mucronata* and adds bitter edible leaves - steamed and then salted - to strengthen the effectiveness of the remedy. This notion of sour/bitter balance is one of the components of a correspondence system found in traditional Lao medicine. In this system the properties of remedies are ordered by associating flavours with the hot/cold polarity mentioned above: sour and salty flavours being "spicy" are hot, while bitter and sweet flavours being "bland" are cold. Hence the possibility of correcting a "hot" imbalance due to an excess of sourness through the consumption of bitter substances of a "cold" nature (Pottier, 2007). Through this last example, we have thus a veterinary preparation combining elements resulting from the observation of an animal's self-medication behaviour - or interpreted as such - (increased consumption of *T. mucronata* in case of diarrhoea), with elements borrowed from partially converging family uses (use of *H. Perforata* and *T. mucronata* in case of diarrhoea) and elements corresponding to concepts stemming from Lao traditional medicine (adding edible leaves for their bitterness).

But this type of complex medicinal construction is not limited to veterinary uses: in Laos, it is common practice to macerate various vegetable or animal substances known for their therapeutic properties in alcohol, to be used for various ailments (Vidal, 1958), but often taken as a fortifier (Strigler, 2011). One of the mahouts told us that when he sees his elephant eating roots, he takes some of them and puts them in alcohol to macerate, a preparation that he then consumes as a tonic. We have here an example of a human health practice that integrates data and knowledge built on the observation of the behaviour of animals frequented on a daily basis (elephants know how to treat themselves, they eat roots for that purpose - *cf infra*) into an already-existing traditional human practice (alcohol maceration of medicinal items).

4.6. Ethnoveterinary preparations

Table 3 shows ethnoveterinary treatments for elephants collected from 11 mahouts. Besides plant items, the ingredients used include clay, elephants' faeces and urine, and human urine. Five out of the twelve species used in these treatments are listed in the EUR: *Tinopora crispa, Entada rheedii* Spreng., *Spondias pinnata, Lithocarpus auriculatus and Harrisonia perforata.* Six species are used by several mahouts, but in different preparations given for different ailments: *Alpinia galanga* (L.). (2 resp.), *Lasia spinosa* (L.) Thwaites, (3 resp.), *Pterocarpus macrocarpus* Kurz (2 resp.), *Spondias pinnata* (L. f.) Kurz (2 resp.), *Tinospora crispa*) (2 resp), *Harrisonia perforata* (2 rep.). One of the mahouts uses the stem of the sappy *Entada rheedii* Spreng. vine on wounds and skin outbreaks. For the latter use, he soaks and twists it in water with detergent added. At first sight that may seem surprising, but the alkaline medium thus formed might facilitate the extraction of secondary active compounds from this vine.

None of the plants used in these preparations are found in the above-mentioned study on ethnoveterinary care for elephants in South India (Jayakumar et al., 2017) in which the authors trace the origin of ethnoveterinary care in India in scholarly traditions, particularly Ayurveda, recorded in treatises dating back to the earliest antiquity. This background may have diffused over time in the practices of mahouts in the region studied, but the possible continuities between these scholarly treatises and local mahouts' care for elephants using "indigenous plant-based medicines" are not considered as it is not the focus of this study. To our knowledge, there are some manuscripts in Laos dealing with elephant disease and care. In his study, Lainé (2017) mentions also manuscripts written in Tai or Pali which are used by

Table 3
Ethnoveterinary preparations given to elephants.

Diarrhoea	Root of *Lasia spinosa* (L.) Thwaites with *Tinosprosa crispa* stem in equal quantity are sliced, dried and then pulverized in a mortar, a little honey is added for palatability. The mixture is dried out and given in a rice ball one time a day for 2–3 days.	Unhusked rice is given with salt and elephant is brought daily to a swampy place where it can eat mud.	Steamed leaves of *Bischofia javanica* Blume with salt added are given to eat.	Chopped barks of *H. perforata* and *Terminalia mucronata* mixed with steamed bitter leaves and salt are given to eat.
Other digestive disorders	*Constipation:* Root of *Lasia spinosa* is sliced, dried and then crushed with unhusked rice in equal parts. A ball of the size of a fist is given 2 times a day.	*Constipation:* Root of *Harrisonia perforata* is boiled and then mashed in the decoction, salt is added and the mixture is given to the elephant.	*Bloating:* Root of *Lasia spinosa* is mixed in equal parts with the fruit of *toona sp.*, husked rice powder and rice bran. Water is added to form a paste. A ball the size of a fist is given 2 times a day until healing.	
Abscess	Abscess is lotionned with a decoction of barks of *Pterocarpus macrocarpus* Kurz and of another tree not identified (mai kao).	Bark of *Dillenia parviflora* Griff. with salt is given to eat.	Grilled unhusked rice is applied on abscess.	
Skin outbreak	A piece of the juicy stem of *Entada rheedii* Spren. is crushed with a mallet and twisted in water with detergent added. Spots are lotioned with the stem dipped into the preparation.	Chopped *Alpinia galanga* (L.) Willd. stems are mixed with elephant urine and applied on the affected area 2–3 times a day until healed.	Crushed fruit of *Spondias pinnata* combined with human urine is then applied to the skin.	
Wounds	A decoction of barks of *Cratoxylum formosum* Jack Dyer, *Pterocarpus macrocarpus*, *Bauhinia malabarica* Roxb. (10 kg each) and fruits of *Spondias pinnata* (4–5 kg), is prepared with 10 L of water, until it reduces to 2 L. A stem of *Entada rheedii*, crushed with a mallet is twisted in preparation and used to lotion the wounds twice a day until cured.	Urine and dry elephant faeces are mixed with crushed *Alpinia galanga* (in equal amounts) and applied by hand on the wound.	A decoction of sliced stem of *Acacia caesia* (500g for 2l of water) is used to lotion the wound everyday.	A mixture of clay and elephant faeces is placed in a squame of banana stem and applied as a poultice on wounds.
Apathetic state	Root and stem of *Tinospora crispa,* dried or fresh is crushed with salt and given to eat.			

traditional healers, the *mo ya,* but none of the mahouts we have interviewed mentioned or referred to such documents. The present study shows that the medicine provided to elephants rather reflects the individual practices and observations of each of the mahouts interviewed as illustrated by the examples given in Section 4.5. As well as by the fact that there is little overlap between the various preparations presented in Table 3.

Fourteen mahouts use conventional veterinary medicine (three among these also use traditional treatments) to treat their elephants, mainly using antibiotic-based treatments for diarrhoea, abscesses and injuries. Four of them give or used to give their elephants energizers or vitamin supplements purchased from the veterinary pharmacy when their elephant is tired or to keep it in good shape. They use products sold for buffaloes and adjust the dose to be injected to their elephant.

4.7. Convergence between EUR and human medicinal uses

Six mahouts told us that sick elephants generally consume more roots than usual. Indeed, roots are present in 90% (58/64) of EUR. Interestingly, the preponderance of roots in Lao pharmacopeia was also highlighted in two studies conducted in Laos (Delang, 2007; Libman et al., 2006). Out of the 20 species mentioned in the EUR, 15 are also used by mahouts for therapeutic purposes in their households, and for 13 of these, the same part is used for the same type of indication (gastrointestinal disorder in all the cases mentioned).

These convergences of medicinal uses found in the mahouts' own practices for treating human diseases and in the EURs could reflect interspecific convergences of medicinal uses of plants between humans and elephants. But it is also possible that the interpretation of self-medication given to the consumption of medicinal items by elephants is induced by the mahouts' own medicinal uses of these plants. However, if we consider the uses of the *H. perforata* root, which is the subject of a significant number of EUR concerning diarrhoea (13 reports), seven of the mahouts told us that they use this item for the same indication in the household context while six did not know about any human use (Table 1). This rules out for the latter the possibility of induction of their own practices and knowledge in their self-medication interpretation by elephants when they search for this item. In addition, many EUR do not have a counterpart in terms of medicinal uses known by mahouts for humans. This is the case in particular for the 18 EUR linked to the motherhood period in elephant females where the seven species mentioned are not known to them to be used by women for similar indications. This in itself is not surprising as among Tai-Lao people the medicinal knowledge about motherhood is mostly transmitted between women (Pottier, 2007) while the management of elephants is a male prerogative. The interpretation of self-medication linked to these EURs in this cultural context of sexual segregation of certain knowledge and activities appears also to be independent of the mahouts' medicinal knowledge. These aspects suggest that even if the medicinal background of mahouts may contribute *a posteriori* to account for certain behaviour observed in elephants (as for the hot or cold plants uses - *cf. supra*), it does not play a decisive role in the interpretation of self-medication that is given to them. It is more likely that the multiple convergences between the observations of privileged consumption of specific plant items by sick elephants and the use made by mahouts of the same items for the same type of pathology reflect convergences of medicinal plant uses between elephants and mahouts.

These interspecific convergences could result either from independent acquisitions of uses in both species or from transfers of knowledge from elephants to humans. The closeness of life that is established in a daily relationship between mahouts and their elephants, and the few examples we have collected of the integration of behaviour observed in elephants into local veterinary and medicinal practices, such as the mahout collecting the roots consumed by elephants to prepare a medicinal maceration, rather pleads in favour of the second hypothesis. A more in-depth investigation is under way into the

medicinal practices of local healers to shed light on this issue.

4.8. Another way to approach interspecific convergences of plants uses

Rather than considering the question of use convergence from the alternative perspective of knowledge transfers versus convergence of independently acquired uses, a more fruitful approach may be the one that Lestel et al. (2006) propose under the term etho-ethnology. According to the authors this approach involves the study of "the hybrid communities comprised of humans and animals sharing meaning, interests and affect" (Lestel et al. (2006)) in which both animals and humans play an active role in the production of knowledge about their environment. Krief and Brunois-Psina (2017) in their study in Kibale National Park in Uganda gives an illustration of such an attempt regarding the particular relationship linking the Batoroo ethnic group and the chimpanzees who share the medicinal use of plant species. The Batoroo's awareness of this knowledge sharing between humans and animals is reflected in the use of a specific term to describe this perceived capacity for "reciprocal imitation between humans and animals" (Krief and Brunois-Psina (2017), our translation). This approach in which mahouts and village elephants would be viewed as a hybrid community seems more appropriate in the context of Thongmyxay district to account for the intertwining that characterizes elephant behaviours, the EURs indicated by mahouts, mahouts own medicinal uses, and the traditional veterinary care they provide to elephants. We have seen for instance in Section 4.5 that some mahouts give their elephants who suffer from diarrhoea the roots that they know it seeks when it experiences this condition.

4.9. Convergences identified in other studies

Fig. 3F shows the number of mahouts who quoted each species mentioned in the EUR.

Table 4 shows ethno-medicinal uses identified and pharmacological properties studied in the literature for species cited in the EUR by at least three mahouts.

The anti-diarrheic property of *H. perforata* reported (Perry and Metzger, 1980) is in accordance with EUR and mahouts own uses. However, one mahout uses this item in case of elephant constipation (Table 2). An observation consistent with this last use is found in the "elephant care manual for mahouts and camp manager" published by the FAO (Phuangkum et al., 2005). In this manual it is reported that elephants purge themselves once or twice a year to remove intestinal parasites using for this purpose different barks and roots, including that of *H. perforata*. Thus this vegetal item, in addition to its anti-infective properties (Table 2), could have a regulatory effect on intestinal transit, as it is the case for instance with *Malva sylvestris* L. which is reported to be used by humans for both diarrhoea and constipation cases (Jabri et al., 2017; Barros et al., 2010; DellaGreca et al., 2009).

Surprisingly, we did not find any mention of medicinal uses or properties in the literature for *Lithocarpus auriculatus*, cited in EUR by seven mahouts, four times in the context of motherhood and three times for diarrhoea.

5. Conclusion

We have recorded numerous convergences between the observations interpreted by the mahouts as self-medication behaviour on the part of elephants and their own medicinal practices. The validity of these interpretations would require a specific ethological study to be established, but what seems remarkable is the dual interface that is at work around a forest environment common to elephants and humans. Village elephants standing at the first interface between the plants they select and the mahouts who observe them become a source of knowledge for humans. The mahouts stand at the second interface between elephants and the human culture they belong to. It is the place where behavioural elements observed on elephants are integrated into the care provided to them and into mahouts' own medicinal practices.

Our initial concern was to better understand how the observation of animal self-medication behaviour has contributed to human medicinal practices. Mahouts' practices in Thongmyxay district show that beyond a mere reproduction of these animal behaviour observations, the human or veterinary medicinal practices from which they may have been derived are the result of complex arrangements, in which all

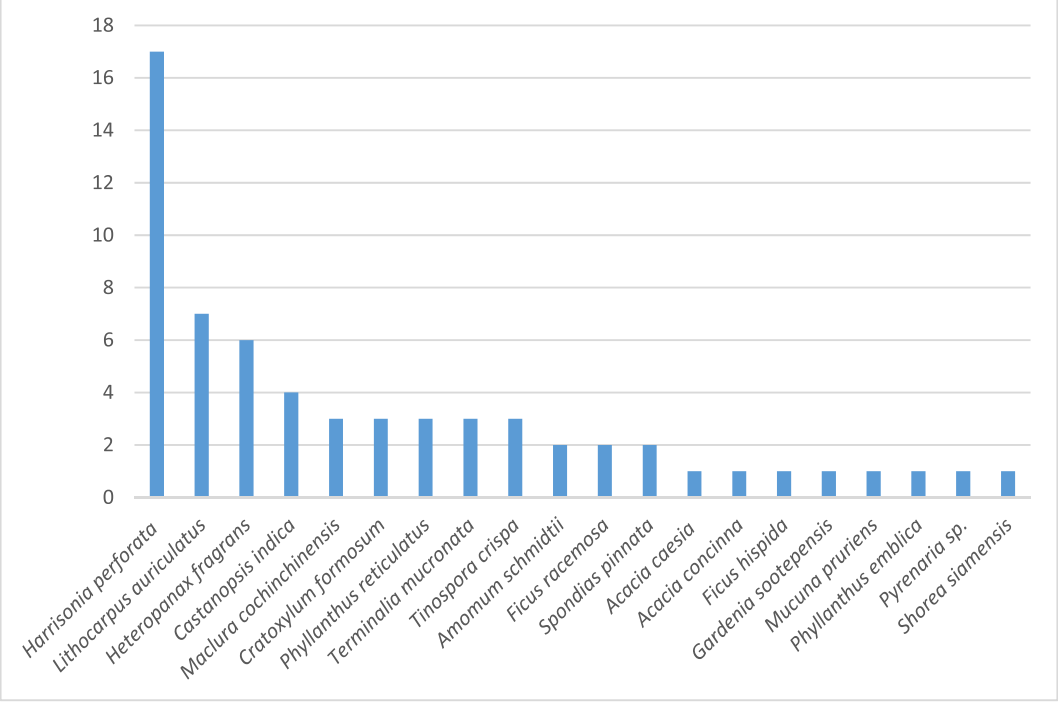

Fig. 3. Number of mahouts citing each species listed in the EURs.

Table 4
Some ethno-medicinal uses and properties found in the literature for the species cited by at least three mahouts. Convergences with EUR on plant parts or indications are highlighted in bold font.

Plant species	Traditional uses	Pharmacological activity
Harrisonia perforata	**Root** is used as a remedy for treatment of wound healing, **diarrhoea**, dysentery and cholera (Perry and Metzger, 1980), and as antitoxic (Libman et al., 2006) and fever (Maneenoon et al., 2015)	Anti-pyretic, anti-diarrhoea properties (Laopaksa, 1988 cited in Batm et al., 2011). Root inhibits *Staphylococcus aureus*, Streptococcus pyrogenes (Batm et al., 2011), Mycobacterium smegmatis (Chea et al., 2007). Leaves have anti Plasmodium falciparum activity (Nguyen-Pouplin et al., 2007). Wood showed activity against HIV-1 IN (Bunluepuech and Tewtrakul, 2009).
Heteropanax fragrans	**Bark is used in a preparation for diarrhoea** **root** and **bark** are included in another preparation given in case of jaundice (Borah et al., 2006)	
Tinospora crispa	**Stem** is used to promotes blood flow, as appetizer, to reduce **fever**, body heat and thirst, against diabetes, (Maneenoon et al., 2015)	Antimalarial activity (Najib et al., 1999), antinociceptive and anti-inflammatory activities (Sulaiman et al., 2008), decreased blood glucose and increased insulin levels in diabetic rats (Noor and Ashcroft, 1989) Stem extract has shown cytotoxicity against HepG2 cells (Weerapreeyakul et al., 2012)
Terminalia mucronata	**Bark** is used in case of sore teeth (Khuankaew et al., 2014). Wood is used in a preparation for leucorrhea and infertility due to menstrual disorders (Chotchoungchatchai et al., 2012)	
Castanopsis indica	Leaves are used to treat stomach disorder and skin diseases. Plant resin is given to treat **diarrhoea**. A paste of leaves is applied for headache (Malla and Chhetri, 2009). **Bark** paste is applied on chest to control chest pain (Joshi et al., 2011)	Free radical scavenging activity of leave extract could have potential hepatoprotective properties (Dolai et al., 2012)
Maclura cochinchinensis	**Root** is used to cure malaria and colds (Kobayashi et al., 1997). Heartwood used to treat chronic fever, skin infection, abnormality of the lymph node, **diarrhoea**, fainting, skin infection; Leakaya et al. (2018)	Extract from heartwood showed antibacterial activity against Staphilococcus aureus, Staphylococcus epidermidis, Bacillus subtilis; anti-fungal activity (dermatophytes) and antiherpes simplex virus (HSV) activity (Kongkiatpaiboon et al., 2017).
Phyllanthus reticulatus	Leaves and **bark** are used as astringent and diuretic. Juice of leaves is used for the treatment of **diarrhoea** in children (Ghani, 2003)	The bark showed antiviral (Saha et al., 2007) and antiplasmodial activity (Omulokoli et al., 1997). Aerial parts Aerial parts extract shows analgesic and anti-inflammatory properties (Saha et al., 2007).
Cratoxylum formosum	The bark, **root** and leaves are used in folk medicines to treat fevers, coughs, diarrhoea, itches, ulcers and abdominal complaints (Nguyen and Harrison, 1999)	Xanthones extracted from root has shown antibacterial and antifungal activity and cytotoxic activity (Boonsri et al., 2006; Boonnak et al., 2010)

available medicinal and conceptual resource are integrated.

The current trend in Laos to transfer village elephants to elephant resorts leads to a disruption in the transmission of mahouts' family knowledge (Suter et al., 2014). It is therefore urgent to continue to collect this knowledge and in particular traditional veterinary care in order to transmit it to the new generation of mahouts employed in these resorts and to the people responsible for the health of captive elephants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2019.112157.

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Maladies émergentes à l'interface Homme-Éléphant au Lao PDR

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Travail Encadré de Recherche :

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Résumé

La tuberculose ainsi que d'autres maladies infectieuses chez les éléphants ont le potentiel d'infecter l'homme et inversement, ce qui représente une préoccupation grandissante tant en santé publique que dans le domaine de la conservation des espèces protégées. Le but de ce projet est d'investiguer par des techniques de biologie moléculaire, la prévalence et la diversité génétique de quelques pathogènes qui affectent les éléphants. Ces pathogènes sont souvent retrouvés aussi chez les populations humaines locales, notamment chez les cornacs, qui sont les maîtres et soigneurs de ces pachydermes, employés dans les travaux de collecte de bois. Un procédé non-invasif va être adopté ainsi que des analyses à partir d'ADN extrait de fèces d'éléphants captifs, collectés dans différentes régions du Lao PDR. Plus précisément la recherche de présence de parasites gastro-intestinaux et de Mycobactéries par amplification PCR de fragments nucléotidiques connus (ITS1-ITS2, CoxI, rpBo, etc.) sera effectuée. Des analyses phylogénétiques suite au séquençage des fragments amplifiés par PCR seront conduites. Ceci a pour but de pouvoir comparer les séquences obtenues avec celles de pathogènes infectant l'Homme et ainsi déterminer le potentiel de transmission inter-espèces de maladies zoonotiques.

1. Introduction

1.1 Les éléphants au Laos

La population d'éléphants d'Asie, *Elephas maximus*, est en déclin et classée en danger d'extinction par l'Union Internationale pour la Conservation de la Nature (IUCN)[1]. L'abondance totale de la population de cette espèce varie de 30 000 à 50 000 individus, bien que ces nombres soient incertains et certainement surestimés[2,3]. Un quart à un tiers de la totalité de cette population est constituée d'éléphants en captivité[4,5]. Les éléphants en captivité sont utilisés pour l'exploitation du bois ou plus récemment pour le tourisme et proviennent traditionnellement de populations sauvages [6,7]. Ces éléphants dits « domestiques » ne constituent cependant pas une espèce domestiquée car leurs caractéristiques génétiques et comportementales sont similaires à celles de leurs homologues sauvages[8]. Au Laos les éléphants captifs étaient traditionnellement utilisés comme animaux de trait dans l'industrie forestière et partageaient le même habitat que leurs congénères sauvages. Au cours des vingt dernières années, l'augmentation de la charge de travail des éléphants a affecté la fécondité de la population et entraîné une forte diminution de la population d'éléphants captifs, passant de 1 000 à 540 individus[9]. Cependant, la demande touristique augmentant le prix des éléphants pourrait-être un facteur d'augmentation de la fécondité, poussant les propriétaires d'éléphants à les faire se reproduire[9]. Elever des éléphants d'Asie au Laos est une tradition séculaire [10]et la plupart vivent dans la province de Sayaboury, qui abrite le parc national de Nam Pouy, foyer de la deuxième plus grande population sauvage du pays [10–13].

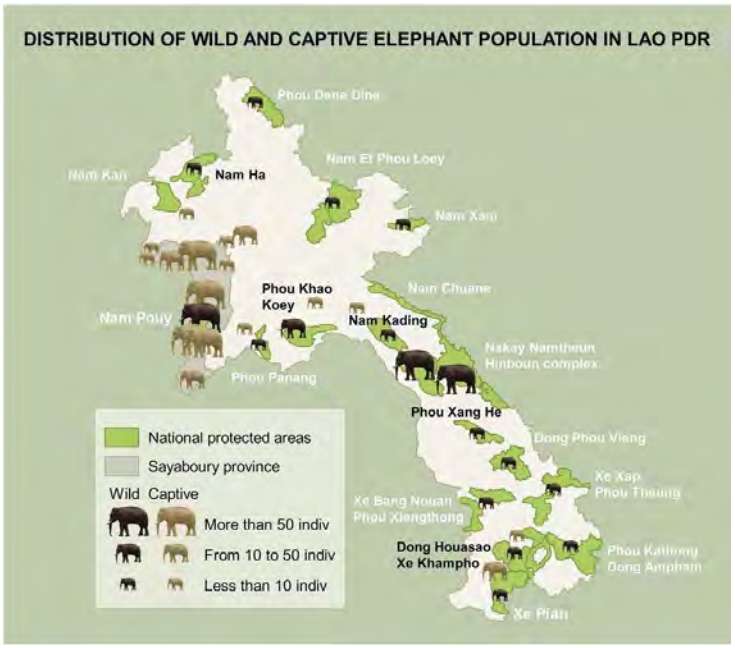

Figure 1. Carte des populations d'éléphants sauvages et en captivité dans la RDP lao

Les interactions entre populations sauvages et captives persistent dans la plupart des pays de l'aire de répartition de l'espèce, principalement par le biais de la reproduction entre femelles captives et mâles sauvages. Au Laos, en Inde et au Myanmar, des femelles captives sont relâchées dans la forêt et s'accouplent avec des mâles sauvages[8,14,15]. Au Laos, près de 80% des éléphanteaux nés en captivité au cours de la dernière décennie sont issus de géniteurs sauvages provenant de l'aire protégée de Nam Pouy[11].

Le Lao PDR est l'un des derniers pays d'Asie qui utilise encore les éléphants pour l'extraction et le transport du bois. Ces éléphants vivent en contact permanent avec leur propriétaire (le cornac ou mahout). Similairement, les éléphants utilisés à des fins touristiques sont en contact constant avec l'Homme. En tout, environ 500 animaux travaillent au Laos dont certains sont en contact avec la population d'éléphants sauvages, tous ces paramètres font que les éléphants du Laos sont une population particulièrement vulnérable aux infections véhiculées par l'Homme, ce qui pose des problèmes d'un point de vue de la conservation de l'espèce.

1.2. La tuberculose et les éléphants

Selon l'Organisation Mondiale de la Santé, la tuberculose (TB) représente la première cause de mortalité par un agent infectieux, et la dixième cause de mortalité mondiale. En 2016, 1.6 millions de personnes en ont péri et 10 millions de nouveaux cas ont été confirmés[16], plus particulièrement l'incidence de la tuberculose chez l'Homme au Laos est de 168/100 000 personnes, l'une des plus élevée en Asie [17]. Cependant l'Homme n'est pas le seul à risque de contracter la TB et l'on retrouve celle-ci dans diverses espèces sauvages ou domestiques telle que chez les bovins [18]. Les éléphants, et majoritairement l'espèce asiatique *Elephas maximus*, est connue pour être particulièrement susceptible à l'infection par *Mycobacterium tuberculosis* et *Mycobacterium bovis*[13]. Cette situation n'est pas nouvelle, en effet la TB chez les éléphants est décrite depuis les temps anciens, mais ce n'est qu'en 1996, lors de la détection de *Mycobacterium tuberculosis* chez des éléphants de cirque, que les

recherches sur ce sujet ont vraiment commencées[19]. La tuberculose chez les éléphants est transmise à partir de l'Homme et peut-être retransmise à celui-ci ainsi qu'à d'autres espèces, et ceci représente une préoccupation importante tant en santé publique qu'en en conservation des espèces protégées. Entre les années 1994 et 2011, la prévalence d'éléphants infectés en Asie a été estimée à 16.4% (45/247), un taux 6 fois plus important que chez les éléphants africains[20]. Cela est d'autant plus inquiétant que l'Asie est le foyer de TB multirésistante aux antibiotiques, ce qui pose un problème de traitement et de contrôle de la maladie, notamment chez les éléphants pour lesquels le traitement est déjà compliqué[16,21].

1.3. Les parasites intestinaux chez les éléphants

Cependant la TB n'est pas la seule maladie à pouvoir être transmise des éléphants à l'Homme et vice versa. D'autres maladies telles que les helminthiases par exemple, peuvent provoquer des problèmes de santé à la fois chez l'homme et les animaux domestiques. Ces maladies peuvent être causées par différents pathogènes et notamment, des nématodes parasitaires. Ces derniers ont été fréquemment observés lors d'études sur des éléphants thaï et africains et plus particulièrement : *Murshidia falcifera, M. neveulemairei, Murshidia sp., Leiperenia leiperi, Khalilia buta, K. sameera, Quilonia spp., Parabronema africanum, P. rhodesiense, Parabronema spp., Oesophagostomum mocambiquei* et *O. mwanzae.* Une étude en particulier, conduite à partir d'échantillons fécaux d'éléphants vivant dans des sanctuaires en Thaïlande indique une forte prévalence de strongyles (93,0%) contre de faibles prévalences de trichuridés (2,3%) et d'ascaridés (2,3%) d'après une observation microscopique. Une analyse moléculaire, a, quant à elle, révélé la présence d'*Oesophagostomum*, pathogène pouvant causer un risque sanitaire chez les animaux domestiques ainsi que chez les habitants locaux. *Oesophagostomum* ou ver nodulaire est un nématode parasitaire appartenant à la famille des Chabertidés, une infection par ces derniers peut provoquer des granulomes dans les parois des petits et gros intestins. *Oesophagostomum bifurcum* est une espèce commune, détectée chez le bétail ainsi que chez les primates non-humains et les humains, de même qu'*O.aculeatum* qui est détecté chez le macaque japonais et peut être transmis à l'Homme[22]. Ce dernier est aussi retrouvé chez les éléphants et peut-être transmis, en plus de l'homme, à d'autres animaux domestiques partageant leurs zones d'habitation avec des éléphants sauvages ou captifs.

Ces zoonoses représentent donc un risque à la fois pour l'Homme en contact avec les éléphants, mais aussi pour la population d'éléphants elle-même, tant sauvage que captive, et elles pourraient être un obstacle dans l'effort de conservation de l'espèce.

2. Projet de recherche pour la période du stage

Le but général de ce stage est de déterminer, par une approche moléculaire, la présence de pathogènes d'origine zoonotique et donc potentiellement transmissibles entre l'Homme et les éléphants captifs et par extension aux éléphants sauvages. La présence de différents organismes pathogènes va être investiguée à partir d'ADN extrait de fèces, incluant celle de vers gastro-intestinaux, de *Mycobacterium tuberculosis* et *Mycobacterium bovis*, bactéries responsables de la tuberculose chez l'Homme et les bovins.

Ces analyses seront effectuées sur des échantillons de fèces d'éléphant préalablement collectés dans les provinces de Xayaboury, Luang Prabang et Champasak (figure 1) ainsi que sur des éléphants hébergés au Elephant Conservation Center (ECC) se trouvant à Nam Tien Lake Sayaboury, dans le district de Sayaboury.

Au cours d'un séjour d'un mois au Laos je m'occuperai de la collecte d'échantillons supplémentaires au ECC. Les éléphants hébergés à l'ECC bénéficient d'un suivi vétérinaire régulier et certaines informations sur leur état de santé pourrons être enregistrées (signes cliniques/ symptômes) ceci, pour ajuster, améliorer et valider la technique de détection de la tuberculose dans les échantillons fécaux d'éléphants. A cet effet des analyses seront tout d'abord à effectuer sur des échantillons provenant d'animaux ayant des antécédents de maladie connus.

Selon les conditions météorologiques et le déroulement des collectes, le champ d'investigations sera élargi aux éléphants sauvages habitant les forêts du Parc national de Nam Pouy. Toujours au Laos, l'extraction d'ADN sera faite à partir du matériel collecté au Centre d'Infectiologie Christophe Mérieux (CICM) de Vientiane avec la collaboration du Dr. Phimpha Paboriboune et sous la supervision de Eric Deharo.

Sur Montpellier, l'efficacité de test non-invasifs à partir d'ADN extrait de fèces d'éléphants sera évaluée. En effet, la présence de métabolites secondaires ainsi que d'autres composants (tannins et restes de nourriture digérée) pourraient inhiber les réactions de polymérase en chaîne effectuées sur de l'ADN

partiellement dégradé car issu d'extractions à partir de matériel de faible qualité. Si cette hypothèse est écartée, la détermination de la prévalence et la diversité génétique d'agents infectieux (Mycobactéries et parasites intestinaux) seront mesurées. Ceci afin de pouvoir comparer les résultats obtenus avec une future étude visant la population humaine et en particulier les personnes en contact étroit avec ces animaux. Cette deuxième partie de stage sera effectué, sous la supervision de Sabrina Locatelli (CRCN) à l'Unité Mixte Internationale 233 « Recherches translationnelles appliquées au VIH et aux Maladies Infectieuses (TransVIHMI) » de l'IRD à Montpellier.

3. Signification, importance et originalité pour le domaine de recherche

Le rapport de l'OMS de 2017 estime que 10 M de personnes sont infectées par la tuberculose (TB) dans le monde, soit une légère diminution par rapport aux années précédentes (2%). La mortalité annuelle s'élèverait à environ 1,6 M de personnes et près de 558 000 personnes seraient atteintes de tuberculose multirésistante (MDR)[16,23], ce qui laisse supposer que la maladie continuera de représenter une menace importante pour la santé publique, en particulier dans les pays pauvres où l'accès aux soins est limité. En RDP Lao, la dernière enquête sur la prévalence de la TB a rapporté 168 cas pour 100 000 personnes[17]. La maladie menace également les éléphants qui jouent encore un rôle économique important dans ce pays. La proximité constante entre l'éléphant et son propriétaire (le mahout) augmente les risques de transmission de maladies dans les deux directions, notamment de la TB. Les efforts pour caractériser la prévalence sont contrecarrés par la difficulté d'effectuer des tests diagnostiques fiables. Les méthodes actuelles de diagnostic de la TB chez les éléphants, fondées sur la culture et la sérologie sont laborieuses et nécessitent des vétérinaires qualifiés. En outre, les méthodes sérologiques sont incapables de distinguer l'exposition passée des formes latentes ou actives de la maladie[24]. Sur la base des méthodes préliminaires d'échantillonnage non invasif mises au point sur les Hommes, nous chercherons de développer une approche fiable, rapide, facile à utiliser et non invasive pour la détection de l'infection par TB. Ce test sera utilisé pour l'analyse des échantillons fécaux prélevés sur des éléphants en captivité, et dans le moyen terme aussi sur des échantillons de crachat et/ou des selles de leurs cornac afin de déterminer comment la prévalence TB, et les caractéristiques moléculaires sont épidémiologiquement liées. Cette méthode de diagnostic à partir d'échantillons fécaux représenterait aussi une méthode de choix pour le diagnostic de populations animales sauvages, en augmentant l'accessibilité à ceux-ci, tout en diminuant leurs contacts avec l'Homme.

4. Description du projet et procédures expérimentales

a. Site d'étude et échantillons collectés

Des échantillons ont préalablement été collectés dans le district de Tongmixai, province de Xayaboury, Lao PDR, provenant d'éléphants captifs, relâchés une bonne partie de l'année en forêt ou attachés en forêt (n=5) ; dans la province de Luang Prabang, au centre Mandalao (n=3), et dans le district de Pathounphone, province de Champasak (n=1) au Elephant Conservation Center (ECC) province de Sayaboury (n=3). L'ECC est responsable du suivi de 29 éléphants. Nous prévoyons de collecter des échantillons fécaux supplémentaires de tous ces individus.

Les autorisations de recherche ont été obtenues auprès du comité d'éthique de l'Institut national de santé publique lao et des autorités laotiennes responsables du protocole de Nagoya.

4.2 Méthode de collecte non-invasive de fèces chez les éléphants au Laos

Les éléphants étant des espèces protégées, difficiles à appréhender et potentiellement agressifs, il est difficile de les étudier. Les prélèvements sanguins ne sont effectués que sur des animaux entrainés et avec la participation de vétérinaires formés à effectuer ces procédures. Il faut donc obtenir des informations sur leur santé en effectuant des prélèvements moins contraignants. Dans le passé, au sein du laboratoire de l'UMI233 des méthodes non-invasives ont été mises au point, afin de détecter la présence d'anticorps, de virus et d'effectuer des génotypages partiels de l'hôte à partir d'ADN et d'ARN extrait de fèces de primates non-humains sauvages[25,26]. Concrètement, environs 20 g de fèces sont mis dans un tube de 50 mL contenant 20 mL de RNA*later* (Ambion, Austin, TX, USA). Ce produit préserve les acides nucléiques de la dégradation pour la durée de la mission et pour un maximum d'un mois. Le mélange sera conservé à température ambiante sur le terrain avant d'être envoyé au laboratoire de Montpellier et conservé dans des congélateurs à - 80°C. La date de prélèvement, le lieu de collecte et les coordonnées GPS ainsi que la fraîcheur estimée, sont notés pour

chaque échantillon. D'autres données peuvent être prises en compte selon l'objectif de l'étude : la présence de graines ou de restes végétaux permettant de déduire le régime de ces animaux, la taille des échantillons fécaux afin d'estimer l'âge des individus, etc.

4.3 Extraction d'ADN et génotypage de l'hôte

4.3.1 Extraction d'ADN

Un protocole modifié pour les extractions d'ADN va être adopté, car certains œufs/cystes de parasites intestinaux ainsi que la paroi des mycobactéries, sont très résistantes aux procédures standards d'extraction. Afin d'extraire donc l'ADN plus efficacement, une étape supplémentaire est ajoutée, qui consiste en l'homogénéisation dans des tubes contenant des billes de silice de différentes taille (lysis matrix E) dans un broyeur FastPrep-24 (MP Biomedicals, Eschwege, Allemagne) et une incubation pendant une nuit à 56°C. Ensuite Le kit QIAampStool DNA Mini (Qiagen, Valencia, CA, USA) sera utilisé. En bref, 2 ml d'échantillon fécal seront re-suspendus dans un tampon de lyse, clarifié par centrifugation, traité avec un comprimé InhibitEx et une protéinase K et passé à travers une colonne de liaison à l'ADN. L'ADN lié sera élué dans 100 µL de tampon d'élution. Cet éluât pourra aussi servir pour le génotypage de l'hôte si nécessaire.

4.3.2 Génotypage de l'hôte

Le gène Cytb de l'éléphant d'Asie sera d'abord amplifié dans tous les échantillons par PCR classique afin de déterminer la spécificité et la qualité de l'ADN extrait. Des échantillons de bonne qualité devraient donner des produits de PCR d'une longueur d'environ 630 pb, taille attendue pour le genre *Elephas.*

Le génotypage de l'hôte sera effectué dans la cas où il y aurait incapacité de discriminer l'appartenance des échantillons fécaux. Ceci sera surement accompli lors de collectes d'échantillons en forêt chez les éléphants sauvages. Pour le moment nous envisageons de nous concentrer sur des animaux captifs et domestiqués. Ce génotypage est effectué par analyse de microsatellites. Les microsatellites sont détectés en effectuant une PCR du locus contenant la séquence répétée suivie d'une migration sur un séquenceur avec un marqueur de poids moléculaire qui permet de situer les allèles en fonction de leur taille.

À partir de l'ADN extrait des fèces, les échantillons seront génotypés sur plusieurs loci (au minimum huit) dont les marqueurs ont été déjà publiés dans la littérature [27–29]Chaque locus a été localisé grâce à un couple d'amorces dont l'amorce sens est marquée par un fluorochrome. Au total, trois fluorochromes seront employés : Fam (signal bleu), Hex (signal vert), Dragon Fly Orange (signal noir). Les PCR seront effectuées en multiplex ; le mélange réactionnel sera le même pour chaque PCR, il comprend à une concentration finale : 1X de tampon de PCR avec MgCl2, 200 µM de dNTPs, 0,2 µg/µL de BSA, 0,4 pmol/µL d'amorces et 0,05 U/µL d'enzyme Qbiogen (Qbiogen, Carslbard, CA, USA) pour un volume total de 50 µL. La quantité d'ADN utilisée varie de 4 à 10 µL. L'amplification est effectuée sur un thermocycleur (PTC-200, Bio-Rad, Hercules, CA, USA). Le programme comprend une première dénaturation de 3 min à 95°C, suivi de 40 cycles de 20 s à 95°C, de 45 s entre 53°C et 60°C (suivant les températures d'hybridation des amorces), et de 45 s à 72°C, puis une extension finale de 5 min à 72°C.

Les PCR seront répétées au moins trois fois lors de l'obtention de motifs hétérozygotes et au moins quatre fois lors de motifs homozygotes afin de s'assurer que le profil obtenu n'est pas le résultat d'un «allelic drop out ». Ceci est un phénomène d'amplification d'un seul allèle, fréquent lors d'analyse de matériel de mauvaise qualité comme dans le cas d'ADN extrait de fèces [30,31]. Le résultat de l'amplification sera analysé avec un marqueur de taille fluorescent (GS400HD, Applied Biosystems, Foster City, CA, USA) sur un séquenceur automatique 3130xl Genetic Analyser (Applied Biosystems). Le logiciel Genemapper version 3.7 (Applied Biosystems) sera utilisé pour la lecture des profils obtenus.

4.4 Analyses moléculaires

4.4.1.1 TB

Une amplification par PCR de l'élément d'insertion ITS (IS6110) et d'un fragment du gène rpoB de *Mycobacterium tuberculosis* selon les procédés décrits dans des études précédentes seront effectuées [32,33].

4.4.1.2 Parasites gastro-intestinaux

La présence d'Oesophagostomum sera être détectée dans les échantillons d'éléphants en ciblant la petite sous-unité de l'ADN ribosomal (SSU rDNA) comme marqueur génétique, celui-ci étant largement utilisé afin de

préciser la taxonomie et les parentés moléculaires chez différents groupes de parasites [34,35,36].

4.4.2 Séquençage des produits amplifiés

Les produits d'amplification seront purifiés, si nécessaire, en utilisant le kit Geneclean Turbo (Qbiogen) et séquencés directement par la méthode de Sanger avec le kit ABI PRISM BigDye Terminators (Applied Biosystems) avec les amorces de la seconde étape de PCR. Les séquences seront lues sur un séquenceur automatique 3130xl Genetic Analyser (Applied Biosystems). La vérification et l'assemblage des séquences seront effectués sur le logiciel Seqman (DNAstar, Madison, WI, USA).

4.4.3 Analyses phylogénétiques des pathogènes amplifiés

Les séquences vont être alignées à l'aide du programme informatique ClustalX.[37]contre des séquences de références provenant de la Genbank. Des méthodes de phylogénie seront utilisées pour cette étude comme la méthode classique de distance (les plus proches voisins) et la méthode plus puissante basée sur les probabilités (maximum de vraisemblance).

5. Résultats préliminaires

Ceci est un projet nouveau au sein de l'équipe, en revanche méthodes de diagnostic à partir d'échantillons fécaux obtenus auprès de la faune sauvage ont déjà été appliquées avec succès lors d'études permettant notamment de détecter la présence de différents pathogènes[38,39]. Comparées à l'homme, les options de diagnostic chez l'éléphant sont limitées, notamment pour la tuberculose, les signes cliniques n'apparaissant que lorsque la maladie est à un stade avancé, une radiographie des poumons n'est pas faisable chez l'éléphant adulte et les résultats des tests intra-dermaux sans corrélation avec les résultats des cultures. Un test sur lavage de trompe, est peu sensible à cause de l'inefficacité de la collecte d'échantillons et doit être répété plusieurs fois dû à l'intermittence de la décharge bactérienne[24,39]. Les éléphants développent en revanche une importante réponse aux anticorps à *M. tuberculosis*, particulièrement au stade avancé de la maladie et la sensibilité du test sérologique ElephantTB STAT-PAK est significativement plus élevée que chez d'autres mammifères[40]. Une prise de sang demande la présence de vétérinaires même si l'éléphant est entrainé, et reste une pratique invasive et stressante pour l'animal. Une méthode de diagnostic alternative est donc nécessaire pour l'étude, notamment de la TB, chez ces animaux.

6. Les outils mis à disposition et justification du budget à demander pour la réalisation du projet

Outils mis à disposition	Matériel nécessaire	Coût pour un échantillon (EUR)
Matériel L3	Kit extraction ADN fèces	3
Broyeur	Microbilles broyage	1
Thermocycleur	Produits PCR (enzymes, amorces, tampons)	3
Appareil pour migration sur gel	Migration et purification de produit PCR	1
Thermocycleur	Réactions de séquence (enzymes, amorces, tampons)	8
Centrifugeuse	Précipitation des séquences	1
Séquenceur	Analyses des séquences	6
	Consommables	14
Séquenceur	Analyses microsatellites (multiplex PCR mastermix, amorces analyse sur Applied Pop7, consommables)	23
Total estimé pour un échantillon		**60**
Total estimé pour 60 échantillon		**3600**

A ces coûts imputés à l'utilisation de matériel nécessaire aux analyses, s'ajoutent les frais de transport et de séjour aux Laos d'une valeur de 3000 EUR environ.

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Gajah 57 (2024) 26–29 · Short communication

Using Satellite Tracking for Health Monitoring and Treatment of Released Work Elephants

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Using Satellite Tracking for Health Monitoring and Treatment of Released Work Elephants

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Introduction

The Elephant Conservation Center (ECC) has been working with Asian elephants (*Elephas maximus*) in Laos since 2001. The ECC initially started as a captive elephant welfare organisation, assisting work elephants with veterinary care. In 2011 it transitioned into an eco-tourism venture, allowing people to observe captive elephants in a natural forest, providing time for captive elephants to form social bonds while giving tourists the opportunity to see elephants in a similar setting as they may find wild elephants.

In 2018 the ECC begun working with the government of Laos to assist in wild elephant conservation issues, working mainly in Nam Poui National Protected Area to help monitor and protect the second largest population in the country. The ECC also runs Laos' only reintroduction project in the south-west of Nam Poui, monitoring four released captive elephants as they roam freely for just over four years.

The ECC's reintroduction project hopes to lay the foundations for a successful captive elephant release program in Laos. The ECC believes there is a lot of potential for reintroduction across the country, with limited options currently for work elephants and several small wild populations that could benefit from augmentation (Ministry of Agriculture and Forestry 2022). The project focuses not only on captive elephants but also on mahouts, how their traditional knowledge can be used to facilitate soft releases and how they could transition into a monitoring and tracking role in the future.

The current release is centred around four non-related captive elephants, three adult females, 35–40 years old and one juvenile male approximately 11 years old (Table 1). All individuals in the soft-release are captive born, born to captive females that have bred with wild males inside Nam Poui National Protected Area. All three of the adult females are former logging elephants that worked in and around the area they are now released pulling logs. The juvenile male was trained specifically for circus and was destined to be the star of a show in a middle eastern zoo. All four elephants were part of a 13-elephant sale to a zoo that was stopped at the last moment due to significant external pressure. All these elephants were then given to the ECC by the government.

The reintroduction program started in March 2019 when these four individuals were released into an area of the NPA well-known by the mahouts of these elephants. This was initially a 3-month project to give the ECC team a large continuous forest where we could release elephants and know they are far away from farms with ample food sources. The first goal was simply to understand if these four individuals, who seemed to stay together consistently at the ECC (a much smaller and fragmented forest) would stay together when they have a large forest and real opportunity to disperse.

The first three-months saw the group stay together at all times with no instances of human-

Table 1. List of released elephants.

Name	Age	Collar ID
Mae Mah	35	5331
Mae Noy	35	5332
Mae Boun Me Yai	38	5330
Dor Khoun Mueang	11	5329

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elephant conflict. Because of the success of the first period, we asked for an additional six-months extension to continue the program. After this permission was granted for an additional year and currently, we can continue at least until December 2024, however talks are ongoing with the government to legally change the status of these four elephants, granting them permeant freedom as wild elephants.

After the first three months, once it was clear we would pursue a long-term project keeping these individuals free-roaming for extended periods of time, we start to collect faecal samples every two-weeks and blood samples once a month to monitor different parameters. Bodyweight and general health checks were also carried out monthly. All individuals were tested for infectious diseases such as tuberculosis pre-release and were vaccinated against tetanus.

The team in charge of tracking the elephants and monitoring their behaviour, consisting of 5 mahouts, 1 local assistant, 1 biologist and project manager stayed in the forest and went to check and monitor the group every day for the first three months. The release and monitoring were made possible thanks to the extensive knowledge of the landscape by the mahouts and their ability to track the elephants' using spoors, dung and a small 3 m drag chain on one leg.

With no real threats identified to the elephants or the local community the team took a less hands-on approach, seeing the mahouts go to check the group three times a week for the next 3 months. In 2022, after two years of using the traditional mahout skills to track the elephants, GPS collars were added to each individual thanks to financial support from The Smithsonian Institute. The tracking team now checks approximately once a week on the group, which spends the majority of its time together as a group of four. There have only been two instances of human-elephant conflict, both raiding rice and cassava plantations that reside inside NPA boundaries happening within three days of each other in late 2022 amounting to a total of 250 USD but the group does not appear motivated to crop-raid.

Treatment of Dor Khoun Mueang

The general movement of our released elephants seems to follow a pattern of spending 1–3 days in a small space of a few hectares before moving on to new feeding grounds (Fig. 1). However, on the rare occasions Dor Khoun Mueang leaves his surrogate group, he tends to be more active. This behaviour is normal for a male of his age in the wild, as they explore new areas independently, as they gradually leave the maternal herd and associate with adult bulls

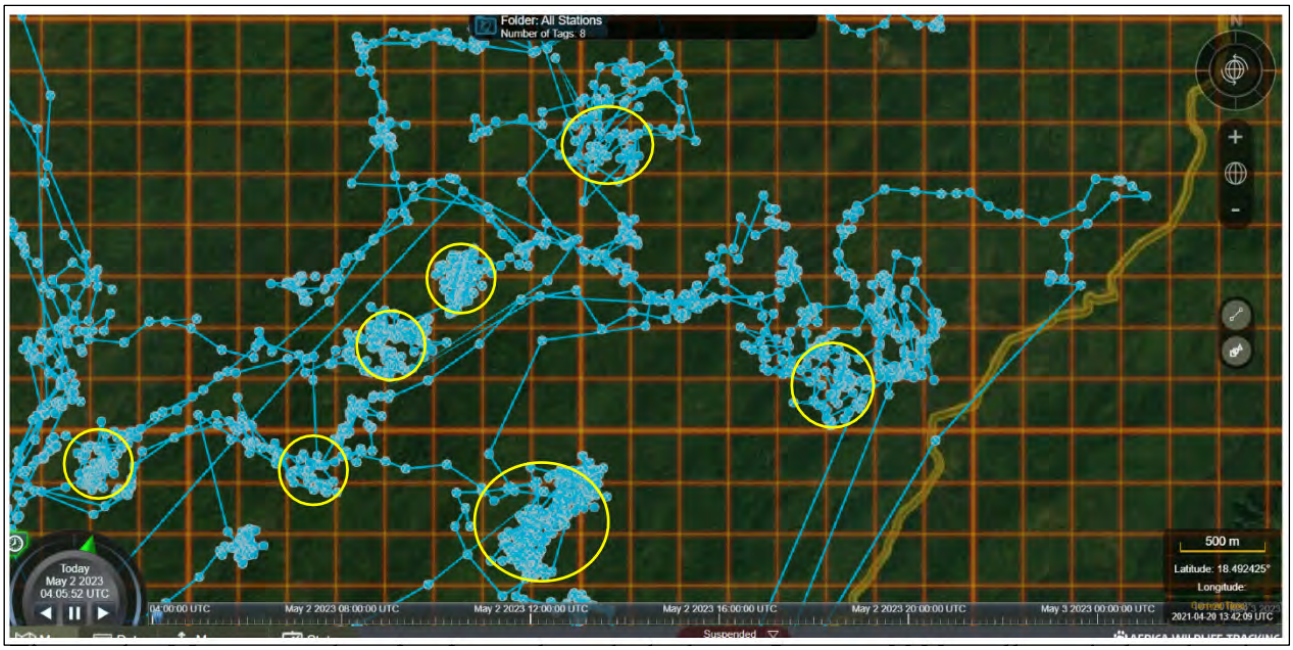

Figure 1. Movement data for four released elephants January 2023, yellow circles showing clusters where elephants' graze.

and/or looking for females in oestrous (Keerthipriya *et al*. 2021).

After a few days solitary roaming we noticed that Dor Khoun Mueang moved very little and remained in an area no larger than 300 m^2 for 3 days, prompting us to send the tracking team to check on him. After the first day the team called back to say they couldn't find him. They continued to reach for him the following day and luckily found him at 10 am the following morning. The team explained that he seemed very weak, had diarrhoea and was not eating. Due to the remote area DKM was found in it was not possible to perform any sort of blood, urine or parasitic analysis, however, based on the description and physical examination our veterinarian suspected a bacterial infection leading to dehydration. Our vet assistant and team then spent the next 4 days in the forest performing treatments (Figs. 2–4) including:

- Rectal and IV Fluid administration: Dextrose 5% (5 l), Ringer (4 l), Saline solution (4 l), for 4 days
- Enrofloxazine 10% (100 mg/ml): (5 mg/kg), IM, QD, for 7 days
- Flunixine Meglumine: dose 1.2 mg/kg, IM, QD, for 4 days
- Cathosal 10%: 100 ml, IV, QD, for 4 days
- Biosan TP: 100 ml, IM, QD, for 3 days
- Ivermectine: 0.1 mg/kg, IM, 1 time

The body score for each elephant is calculated using Fernando *et al*. (2009) description where 1 is very skinny and 10 is obese. Weight for

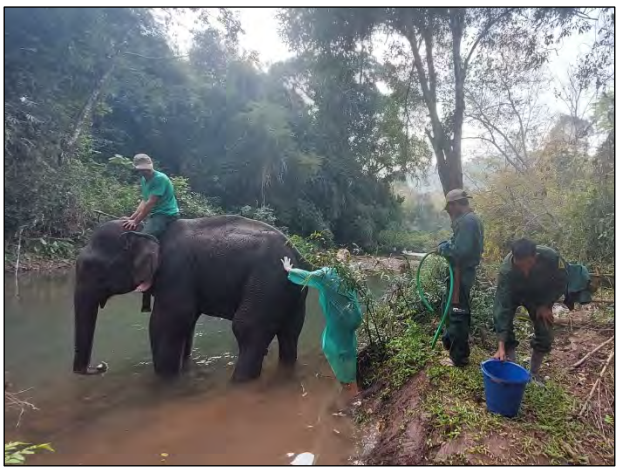

Figure 2. Rectal fluid administration for Dor Khoun Mueng.



Figure 3. Team assisting with the treatment.

dosage was calculated using the following calculation:

Girth (cm) x Girth (cm) x Shoulder Height (cm) x 0.93 ÷ 10,000 = Weight (kg)

After two days of treatment Dor Khoun Mueang showed a drastic improvement in his energy levels and appetite. He was eating, drinking and defecating regularly. The mahouts moved DKM to a more accessible area of the forest on day three so we could drive to the area with a 4x4 car in less than 1 hour instead of the 2.5-hour tractor trip at his current location and the mahouts and vet team continued to monitor him.

For the rest of January, Dor Khoun Mueang was kept isolated from the released group and managed in a more traditional way, on a 40 m chain tied up in the forest (Fig. 5). His mahout and the



Figure 4. Tracking team assisting with treatment.



Figure 5. Dor Khoun Mueng eating some watermelon after treatment.

rest of the tracking team camped nearby to continue monitoring until they assessed he was at full health. He was then released back with his surrogate herd and made a full recovery (Fig. 6). All of our mahout tracking team can still handle and manage their elephants without sedation, making these situations much easier.

Conclusion

Although a lot of the success of this soft-release program stems from the mahouts traditional tracking knowledge, it is highly likely that without the satellite data the team would not have been aware of his disease or in time and Dor Khoun Mueng would have not recovered from the illness. However, with satellite data alone, in a large forest such as Nam Poui, there is a likelihood that the veterinary team would not have been able to find him in the forest so efficiently. These situations perfectly highlight the need to merge traditional knowledge and skillsets with modern technological tools. This project aims to lay foundations for reintroduction programs in Laos which emphasises and champions traditional mahout skills.

This small group will continue to be monitored by their mahouts, now dubbed the 'reintroduction program monitoring team' for the foreseeable future. Even if the legal status of these elephants is changed to wild, we still see the team of 5 mahouts as a valuable unit to continue to monitor and provide information on this group. Their continued employment will help us fine-tune exactly what roles mahouts will be able to have long-term in elephant conservation. With potential across the country for elephant reintroduction, we hope expansion into new areas can be beneficial for elephants and mahouts alike, providing a new life for elephants and sustainable employment for mahouts.

Acknowledgements

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Ministry of Agriculture and Forestry (2022) *National Elephant Action Plan of the Lao PDR 2022 – 2032*.



Figure 6. Dor Khoun Mueng (far right) back with the group in late April 2023.

Gajah 57 (2024) 23–25 · Short communication

Camera Trap Study of Wild Elephants in Nam Poui National Protected Area

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Camera Trap Study of Wild Elephants in Nam Poui National Protected Area

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Introduction

Camera trapping is a useful tool to help conservationists understand the diversity of species present in a given area, population dynamics and abundance of species as well as aid understanding in population ranges. Camera traps are especially useful for elusive and dangerous species that are nocturnal and or live in remote areas. Asian elephants (*Elephas maximus*) are hard to observe due to their living predominately in forests, avoidance of humans and potentially aggressive nature. Therefore, deploying camera traps in elephant range can help obtain information about them,

The total elephant population estimate for Laos is 300–400 wild individuals, however, none of these population estimates are backed by rigorous data and could be significantly lower (Ministry of Agriculture and Forestry 2022). There is a need for better understanding wild elephant populations in Laos in order to allocate resources to correct areas. Many smaller populations, for example, may be on the brink of becoming functionally extinct without intervention and it will be important for the survival of elephants in Laos to identify those areas and consider what interventions could take place. Nam Poui National Protected Area (NPNPA) is home to what is likely the second largest population in Lao PDR (Laos). The population estimates range from 40–80 individuals (Ministry of Agriculture and Forestry 2022) However, this, like all other population estimates in Laos, is mostly guess-work from sightings by both locals and forestry officials. In an aim to gather some baseline information about the elephant population in Nam Poui, The Elephant Conservation Center (ECC) deployed ten camera traps around locations known to be frequented by wild elephants to try and identify individuals and lay the foundation for more rigorous, scientific population surveys.

NPNPA is situated in Sayaboury Province, in Northern Lao PDR, west of the Mekong River. It is one of Laos' original 18 National Biodiversity Conservation Areas (now called NPAs). Nam Poui NPA covers an area of 191,200 ha. NPNPA, which borders Thailand's Doi Phou Kha National Park and Phou Fa non-Hunting Area of Nan Province. A camera trapping study conducted jointly by Word Wildlife Fund (WWF) and ECC previously, found a number of large and medium-sized mammals including, Asian elephant, gaur, Asian golden cat, marbled cat, clouded leopard, sambar, Asiatic black bear and dhole.

The main objective of this study was to provide the NPNPA staff with some information on its elephant population, with a particular interest in identifying breeding-age males, evidence of breeding and to identify potential candidates for GPS collaring in the near-future.

Methodology

Ten camera traps were set around salt licks, paths and water sources where wild elephants were frequently spotted in Nam Poui during ranger patrols in 2022 and 2023. Four cameras were located in the west and six in the east (Fig. 1). Camera traps are still in use inside the park, some in the same locations, some in new areas, however the data described in this report is from January – April 2023.

Following Arivazhagan & Sukumar (2008) individuals were assigned to one of four categories:

- Calf: <1 year old
- Juvenile: 1–5 years old

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- Sub-adult: 5–15 years old
- Adult: >15 years old

Individuals were only identified as adult males if there was clear evidence (tusk, penis, sign of musth and/or head size/body composition), otherwise they were assumed to be female. Body condition scoring followed Fernando *et al.* (2009) using a scale of 0–10.

Results and discussion

Two camera traps were stolen (ECC_002 + ECC_011) while all other camera traps captured photos of elephants. Cameras ECC_009 in Paklai District and ECC_007 in Phieng District provided the clearest photographs. Two distinct elephant groups were identified, one in the west of NPNPA and one in the east. Photos of both groups were captured within one day of each other and given the distance and topography it can be confidently assumed that these elephant groups did not consist of the same individuals. The two groups are separated by a large mountain range and have been known by our ranger teams as distinctly different for some years. Therefore, we are quite certain that these are two distinct populations, separate from each other. We plan to GPS collar individuals from both groups to verify this in early 2024.

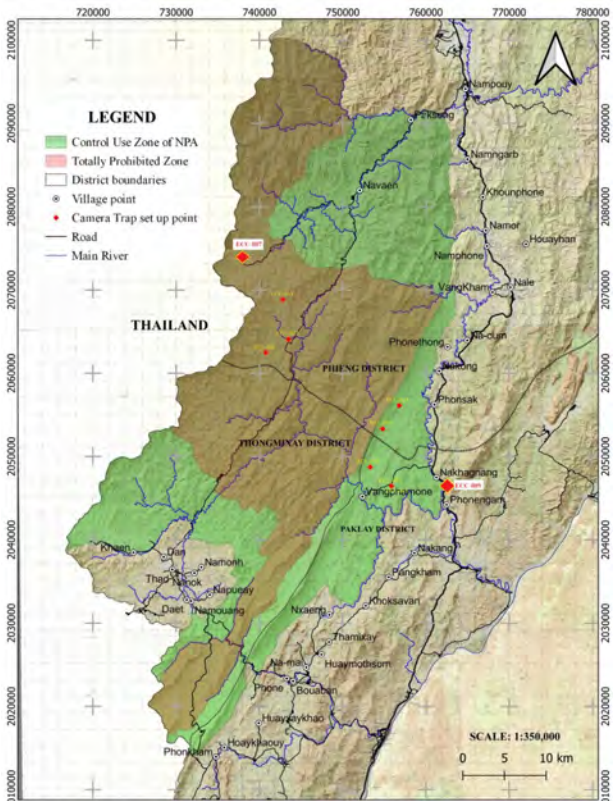

Figure 1. Camera trap locations in NPNPA.

A total of 52 individuals were identified including two tuskless males, with 26 individuals identified in each location (Table 1). In each instance, the groups were captured moving as a unit, which made identifying the number of individuals and age estimation straightforward as they moved almost single file across the camera.

The results of this survey show the presence of on-going reproduction with adult males, adult females, and juveniles being present in both the east and the west of Nam Poui (Table 1). All individuals seemed to be in good health, with no visible injuries and with a body score range of 5–7. In conversations with locals from the surrounding area many had stated that there is only one breeding-age male across the landscape, which was not the case, with three separate adult males identified and at least three sub-adults, ensuring a new generation of breeders.

These results, although far from a complete survey of the wild elephants in NPNPA, is a positive step and demonstrate the applicability of camera trap studies for population surveys in Nam Poui and other areas in Laos. This 'snapshot' into the wild elephant population in Nam Poui provides a baseline to work from, something lacking for elephant populations across Laos. Organizations such as ECC, WWF and Association Anoulack are pushing to better understand and protect the remaining wild elephant populations in Laos. ECC has dedicated elephant monitoring patrol units and will attempt to collar wild elephants in Nam Poui in

Table 1. Elephants identified in Paklai (camera ECC_009) and Phieng (camera ECC_007).

	Paklai	Phieng
Calf	2	2
Juvenile	10	5
Female sub-adult	1	7
Female adult	9	10
Male sub-adult	3	0
Male adult	1	2
Total	26	26

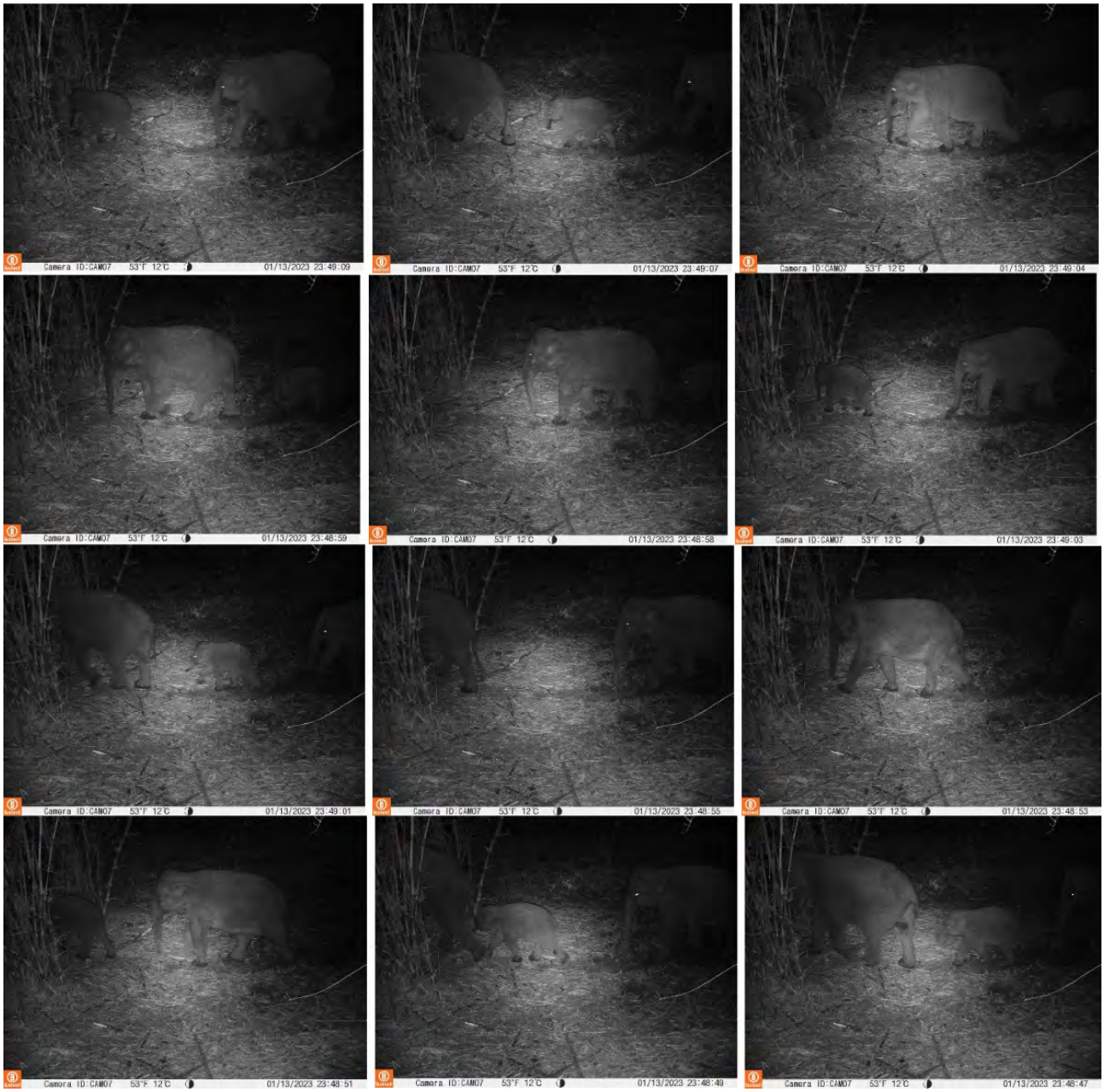

Figure 2. Elephants walking in a straight line past the camera ECC_007.

2024, together with assessing the genetic diversity of the captive elephant population. WWF works on law enforcement and will attempt a comprehensive population survey in Nam Poui in 2024.

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First Reported Case of Elephant Endotheliotropic Herpes Virus Infection in Laos

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FIRST REPORTED CASE OF ELEPHANT ENDOTHELIOTROPIC HERPES VIRUS INFECTION IN LAOS

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Abstract: The elephant endotheliotropic herpesvirus (EEHV) is now recognized as one of the main causes of death of young Asian elephants (*Elephas maximus*) in North American zoos. Its impact in wild and domestic elephant populations in Asia is not clearly understood. This article describes the first case of EEHV infection in Lao People's Democratic Republic of a 2.5-yr-old domestic male Asian elephant. Clinical signs and pathological findings reported here are consistent with previous infections in Asian elephant calves. Phylogenetic analyses showed 100% homology with other EEHV-1A strains identified in Asia, Europe, and North America. Contamination of the molecular assays was ruled out, because the DNA polymerase sequence identified in this study differed from the positive control by two base pairs.

Key words: Asian elephant, elephant endotheliotropic herpesvirus, *Elephas maximus*, Laos, phylogeny.

BRIEF COMMUNICATION

Infections with elephant endotheliotropic herpesvirus (EEHV) can cause severe hemorrhagic disease in juvenile Asian elephants (*Elephas maximus*). Epidemiological data have shown that EEHV is responsible for more than half of the deaths of Asian elephants born in the United States since 1980.[5] Fatal cases have also been reported in wild and domesticated elephants from India, Cambodia, and Thailand, which suggests that the virus is widespread.[7,10,12] Asian elephants are endangered worldwide and the species has been listed on the Appendix I of the Convention on International Trade of Endangered Species in Wild Fauna and Flora (CITES) since 1975. Whether its survival is threatened by EEHV remains unclear. In the Lao People's Democratic Republic (Laos), elephant populations are highly endangered. There are approximately 500 wild and 450 domesticated individuals left (Lao Elephant Care and Management Programme [LECMP], Bouchard, unpubl. data). Here, the authors report this is the first confirmed case of fatal EEHV infection in the country.

On 23 February 2011, veterinarians from the LECMP were asked to investigate the death of a 2.5-yr-old domesticated male Asian elephant in the village of Ban Naxeng, district of Paklay in the province of Sayabouri, northern Laos. Like most of the domesticated elephants in Laos, the calf had been raised with its dam under traditional, semicaptive conditions. On 15 February, the two elephants were taken to the town of Paklay, Paklay district, for the week-long elephant festival where they were examined by the festival veterinarians from the LECMP. They were both found in good health and were dewormed with the use of injectable ivermectin 0.1 mg/kg s.c. (Ivomec 1%, Merial, Lyon 69007, France). According to the owner, the calf showed no abnormal clinical sign during the festival except a mild diarrhea and a progressive weakness that started 2 days before death, on the last day of the festival on 21 February. No similar signs were observed for the other elephants, including the dam. Once the celebrations finished, mother and calf came back to their village, walking for 8 hr.

Apart from a significant swelling in the intermandibular region, the owner noticed no abnormal lesions on the elephant's body or skin until the dead calf was discovered the following morning. The owner also noticed a straw-colored effusion in the abdominal cavity while he retrieved the heart, spleen, parts of the submandibular muscle, liver, and intestines before burying the calf under advice from district livestock officials. The organs were kept refrigerated until gross pathology examination was performed by LECMP veterinarians the next day. Gross examination revealed extensive petechial hemorrhage in the epicardium and in the myocardium, petechiae on the liver, diffuse hemorrhage in the spleen and hemorrhage in the submandibular muscles. The small and large intestines appeared normal and contained only a small amount of

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watery feces. No parasites were found. Collected organs were found to be exempt of suppurative or fibrinous lesions. Microscopic examination of formalin-fixed tissues revealed extensive autolysis that prevented any clear diagnosis. Samples of the heart, liver, spleen, intestines, and submandibular muscle were sent at 4°C to the central veterinary laboratory (National Animal Health Center, Vientiane, Laos) for bacteriological culture while duplicate samples were stored at −80°C until molecular detection of EEHV was performed. Fresh samples were cultured aerobically onto blood and MacConkey agar for 48 hr at 37°C as well as anaerobically onto blood agar in the same conditions to check for *Clostridium* growth. Only one colony was isolated from MacConkey medium. It was identified as a *Serratia* spp. with the API-20 E/NE test (bioMérieux, Marcy l'Etoile 69280, France) and was considered a contaminant. Infection due to *Salmonella* seemed unlikely, because no significant microorganism was grown and because intestines appeared normal. An encephalomyocarditis virus infection also seemed less probable because hemorrhages affected multiple organs and because the myocardium did not show any pale streaks.[3] Further molecular testing was therefore performed, and DNA was extracted from homogenized pooled organ samples (cell-free supernatant) with the use of the FavorPrep viral Nucleic Acid Extraction Kit I (Favorgen Biotech Corporation, Ping-Tung 908, Taiwan). The EEHV terminase (TER) and DNA polymerase (DPOL) genes were targeted by conventional polymerase chain reaction (PCR) and nested PCR, respectively, such as described before.[1,8] Negative controls were included in every run and DNA extracted from liver samples of an infected elephant in Thailand was used as positive control. The PCR and nested PCR products were revealed by electrophoresis on 1.5% agarose gel. Both first and second rounds of the DPOL assay yielded positive results. The final products of the TER and DPOL PCR (337 base pairs [bp] and 316 bp, respectively, including primers) were then purified with the use of the GEL/PCR Purification Mini Kit (Favorgen Biotech Corporation, Ping-Tung 908, Taiwan) and sent to First Base Laboratories (Seri Kembangan 43300, Selangor, Malaysia) for sequencing. The identified nucleotide sequences (Fig. 1a, c) were deposited in GenBank (accession numbers KF471084 and KJ400033) and BLASTed against the GenBank database. They were found to share 100% identity with EEHV-1A detected in other domesticated elephants in Thailand, India, and Europe (TER), and North America (DPOL). Contamination of the molecular assays was ruled out, because the DNA polymerase sequence identified in this study differed from the positive control by 2 bp. Phylogenetic trees were generated with the use of the MEGA 5 software (Center for Evolutionary Medicine and Informatics, Tempe, Arizona 85287-5301, USA) (Fig. 1b, d).

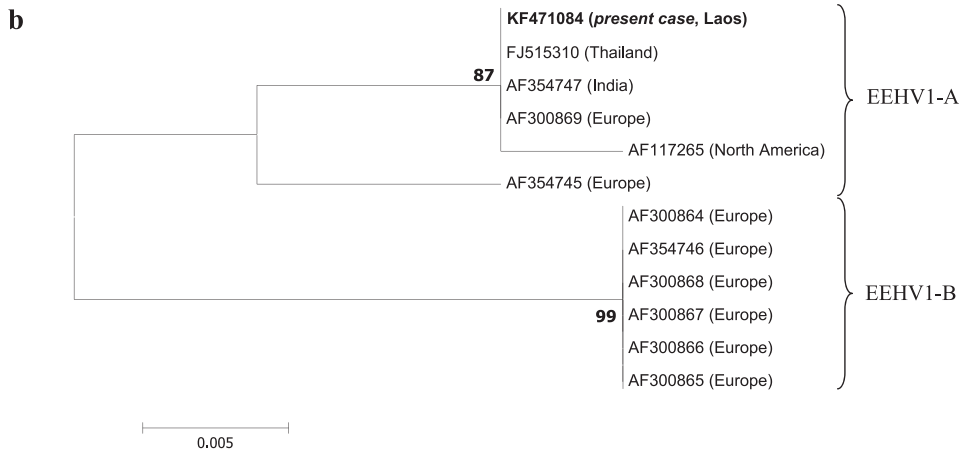
Clinical signs and pathological lesions observed during this investigation, such as general lethargy, cardiovascular failure, and extensive hemorrhages, are congruent with previous reports of EEHV-1 infections in elephants.[4,6–8,10] Among all EEHV subtypes described to date, EEHV-1A appears to be the most widespread in Asian countries with PCR-confirmed cases reported in India, Thailand, Cambodia, and unpublished reports from Nepal and Indonesia.[7,10,12] Although EEHV-4 can cause similar hemorrhagic diseases in young elephants, it has only been reported twice, in India and Thailand.[10] Both EEHV-1A and 1B subtypes have been shown to be carried and shed by healthy animals.[11] Their widespread distribution in range countries and high level of genetic diversity suggest that EEHV-1 is endemic in Asian elephant populations.[12]

Interestingly, the case reported here occurred during the country's largest elephant festival, where the calf started to show clinical signs. While predisposing factors involved in the EEHV pathogenicity have yet to be clearly identified, several hypotheses have been suggested. Stress has been considered by some to be the most important factor to trigger the clinical disease.[7,9] During festivals, elephants that are usually raised in remote areas under semicaptive conditions could well experience high levels of physical and psychological stress. They are being exposed to unfamiliar environments, food and sometimes exhausting activities for several days. Through multiple cellular mechanisms including the hypersecretion of glucocorticoids,[2] such stress may impair the immune system of young elephants and render them more susceptible to develop a clinical disease from a new or latent EEHV infection. Another risk factor present during large elephant gatherings (the festival hosted 50 elephants including calves) is the transmission of EEHV strains between elephants of different locations. Some strains could be pathogenic for naïve young elephants, particularly since no evidence of cross-protective immunity has been reported before.[11]

More studies are needed to confirm these hypotheses as well as to assess the real impact of EEHV as it relates to elephant conservation in

a **TERMINASE**

5'GTGAAGAAGACACCGACTCAAATATTAAATGTGATATCCTACGTATGTGAAGAACA
CTTATATAGCTTCGGAGAGAGGTCTGAAGCAATCACGTGTCCATGTTATCGATTACA
TAAGCCCACTTTCATATCACTTAATTTGAATATCAAAAAAACTGCAAACGCATTCTTA
AAAGATTCGTTCAACGAGGAGATATTAGGCACCACCAACACGAGCTTCTTAGCCAAT
CCCATTCTAACAGATTCCAGTGTCAATGAATTTGACATGATAAGATATAGTACTGTA
AACAAACAGTTA3'



c **DNA POLYMERASE**

5'TCATAACTGGATATAACATAAATGCTTTTGATATACCCTACGTGATAGGGCGATGT
AGGTACTACAATATTCAATGCGGCGCGTTTACCAAGATGAAACGTGGGCGCATGACG
TGTTTTAAGGGCATGGAATCTTTCCTAAACAGATGTCAGTGTAAAGTGACTATCTCG
GGAATAGTTATCATAGACATGTATCGTGTGTGTATGGATAAGGTCTCGGCCCCTAAT
CATAAATTAGACACGGTGGTAGACATGTTACTCGGTGAAAAGA3'

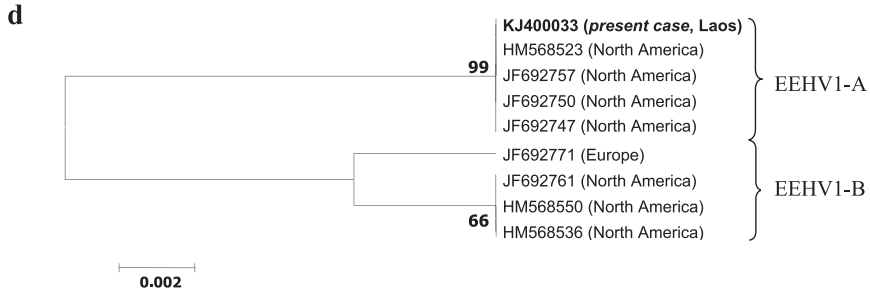


Figure 1. Phylogenetic analyses of the polymerase chain reaction (PCR) products obtained from pooled organ sample from a 2.5-yr-old Asian elephant calf. (a) DNA sequence (excluding primers) of the 297–base-pair (bp) PCR product and (b) neighbor-joining tree of the partial sequence of the elephant endotheliotropic herpesvirus (EEHV) terminase gene. (c) DNA sequence (excluding primers) of the 270-bp PCR product and (d) neighbor-joining tree of the partial sequence of the EEHV DNA polymerase gene. Neighbor-joining trees were generated with our isolate and with isolates obtained from the GenBank database with the use of 1,000 bootstraps.

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Tuberculosis in Laos, who is at risk: the mahouts or their elephants?

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Tuberculosis in Laos, who is at risk: the mahouts or their elephants?

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SUMMARY

Tuberculosis (TB) in elephants has the potential to infect humans and is an increasing public health concern. Lao PDR is one of the last countries where elephants are still used for timber extraction and where they live in close contact with their mahouts. There are 500 animals at work in the country, some interacting with wild herds. Although human TB prevalence is known to be high in Laos, studies on elephant TB had yet to be undertaken. From January to July 2012, screening was performed using the ElephantTB Stat-Pak assay on 80 elephants working around the Nam Pouy National Park in Sayaboury Province. This represents more than 18% of the total registered national working elephant population. Here we report that 36% of the elephants were seroreactive to the test. Of these, 31% had contacts with wild individuals, which suggests potential transmission of mycobacteria to the local wild herds. Clinical examination, chest X-rays, sputum microscopy and culture were performed on their 142 mahouts or owners. Despite high TB seroreactivity in elephants, no participant was smear- or culture-positive for *Mycobacterium tuberculosis* or *M. bovis*, although atypical mycobacteria were isolated from 4% of participants.

Key words: Elephant, Laos, mahout, *Mycobacterium tuberculosis*, tuberculosis (TB).

INTRODUCTION

According to the World Health Organization (WHO), tuberculosis (TB) is the second most common cause of death from infectious diseases after HIV. In 2011, an estimated 1·4 million people died from the disease and 8·7 million new cases were reported [1]. Humans are not the only ones at risk of contracting TB. Elephants, predominantly the Asian species *Elephas maximus*, are known to be particularly susceptible to *Mycobacterium tuberculosis* and to *M. bovis* infections [2–10]. TB has been diagnosed worldwide in captive elephant populations [3–6, 9, 11–18], and a wild African elephant with previous human contact recently died from the disease [10]. Palaeontologists have also hypothesized that TB may have played a role in the extinction of mastodons (*Mammut americanum*) since 52% of skeletons ever found showed typical TB lesions [19]. The transmission of *M. tuberculosis* between

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elephants and humans was documented for the first time in 1998 in an exotic animal farm that housed four infected elephants in Illinois, USA. Half of the 22 animal handlers had positive tuberculin skin tests and one was diagnosed with active TB. Molecular analyses showed that isolates from the elephants and the infected handler were identical [5]. Several reports have since confirmed that caretakers who have close and prolonged contact with elephants shedding tubercle bacilli were at risk of being infected through aerosols [2, 4, 12, 20–22]. A recent study in Malaysia using the QuantiFERON assay (Cellestis Limited, Australia) showed that elephant handlers had much higher TB prevalence than the general population (25% *vs.* 4–6%) [17]. Four cases of culture-positive Asian elephants with strains of human origin were also recently reported in Thailand [14].

Lao PDR (Laos) is one of the last countries where Asian elephants are still used in the timber industry. There are about 500 at work in logging camps and as many in the wild [Lao Elephant Care and Management Programme (LECMP), unpublished data]. The ownership of an elephant can be shared between several people, and some owners may employ more than one mahout to ride their elephants. As a result, there are often two or more persons working every day in close contact with each animal. According to the WHO numbers, Laos was ranked second in terms of human TB prevalence among the 13 Asian elephant countries in 2011 [1]. TB prevalence, incidence and mortality were estimated to be 540, 213 and 11/100 000 inhabitants, respectively [23]. Given the proximity between mahouts and elephants, there is a concern that transmission of *M. tuberculosis* from both parties may be frequent. Because contacts between captive and wild elephants are common in several areas of Laos, the risk of spreading TB to wild populations could also be significant [24].

The prevalence of TB in animals has never been studied in Laos and literature on human TB is scarce. The purpose of this study was thus to estimate the prevalence of the disease in domestic elephants and their mahout in Laos, in order to determine whether exposure to elephants is a major risk factor of being infected.

METHODS

Study design

A cross-sectional study was conducted from January to July 2012 in the four districts surrounding the Nam Pouy National Park in the province of Sayaboury, Laos: Thongmixay, Paklay, Phieng and Sayaboury (Fig. 1). This region hosts more than 80% of the domestic elephants and is home to the second largest population of wild elephants in the country, estimated to be 60–80 animals (LECMP, unpublished data). In each district, the elephant owners were located and contacted by the local District Agriculture and Forestry Office. Based on this information, a field survey was organized to examine the elephants, the mahouts and the owners either in their village, in logging camps, or at the annual elephant festival in the town of Sayaboury. Data was collected by a team of veterinarians and medical doctors coordinated by the LECMP. Written informed consent was obtained from all recruited participants and ethical approval was granted by the Lao National Ethics Committee for Health Research.

Recruitment and TB screening

Elephants

Elephants aged $\geqslant 10$ years were included in the study group. Data on health status and on previous contacts with humans and other elephants (wild or domesticated) were collected from owners and mahouts. The veterinary team performed a clinical examination of the animal and collected 5 ml of whole blood. Fresh serum samples were aliquoted, transported on ice to the National Animal Health Centre in the capital city of Vientiane and stored at −80 °C. Samples were then tested with the ElephantTB Stat-Pak test (Chembio Diagnostic Systems Inc., USA), based on serological detection of IgM and IgG antibodies specific for *M. tuberculosis* and *M. bovis*. The rapid tests were performed and interpreted according to the manufacturer's instructions and read by two operators. Trunk washes could not be performed in the field because of difficulties in implementing the method on untrained elephants.

Mahouts and elephant owners

Every person aged >15 years owning and/or working with an elephant for >1 month was included in the study. Socioeconomic and medical data were recorded as well as frequency and duration of contact with elephants. Comprehensive clinical examinations were then conducted by our medical team. Chest X-rays were performed at the district hospital by the local radiologist and double checked by a

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Fig. 1. Sayaboury province, Lao PDR.

pneumologist at the referral hospital of Mahosot in Vientiane. Two sputum samples were collected from each participant, the first at the time of consultation and the second in the morning upon waking [25]. Salivary samples or sputum specimens of insufficient volume were discarded. Microscopic examination of Ziehl–Neelsen-stained smears [26] was performed by a laboratory technician from the district hospital. At least 200 microscopic fields were read at 1000× magnification before asserting the absence of acid-fast bacilli. Aliquots of sputum samples were also transported on ice by plane or bus within 72 h to the National TB Reference Laboratory at Mahosot Hospital for a second independent smear microscopy and for *Mycobacterium* culture. Briefly, after homogenization and decontamination for 15 min with an equal volume of sterile 4% sodium hydroxide solution, three drops of specimen were inoculated on slopes of solid Ogawa medium [27]. Cultures were incubated at 36 °C for 8 weeks and tubes with contaminants were discarded. Culture-positive tubes were sent for *Mycobacterium* species identification and for susceptibility testing at the Centre d'Infectiologie Christophe Mérieux du Laos, Vientiane, using the GenoType MTBDRplus and GenoType Mycobacterium CM/AS assays (Hain Lifescience GmbH, Germany) according to the manufacturer's instructions.

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Table 1. *ElephantTB Stat-Pak assay results according to different epidemiological variables*

	Total, *n* (%)	Reactive, *n* (%)	Non-reactive, *n* (%)	*P* value
Total	80 (100)	29 (36·3)	51 (63·8)	
District of origin				0·08
Hongsa	15 (18·8)	8 (45)	7 (55)	
Botene	2 (2·5)	1 (50)	1 (50)	
Sayaboury	10 (12·5)	5 (50)	5 (50)	
Paklay	20 (25)	9 (45)	11 (55)	
Thongmixay	19 (23·8)	4 (21·1)	15 (78·9)	
Phieng	14 (17·5)	2 (14·3)	12 (85·7)	
Sex				0·12
Female	49 (61·3)	14 (28·6)	35 (71·4)	
Male	31 (38·8)	15 (48·4)	16 (51·6)	
Age (years)				0·51
10–19	9 (11·3)	2 (22·2)	7 (77·8)	
20–29	25 (31·3)	12 (48)	13 (52)	
30–39	21 (26·3)	7 (33·3)	14 (66·7)	
40–49	23 (28·8)	8 (34·8)	15 (65·2)	
50–59	2 (2·5)	0 (0)	2 (100)	
Clinical signs				0·17
0	50 (62·5)	21 (42)	29 (58)	
1	23 (28·8)	4 (17·4)	19 (82·6)	
$\geqslant 2$	7 (8·8)	4 (57·1)	3 (42·9)	
Contact with wild elephants				0·63
Yes	25 (31·3)	8 (32)	17 (68)	
No	55 (68·8)	21 (38·2)	34 (61·8)	
Contact with work elephants				0·53
Yes	67 (83·8)	23 (34·3)	44 (65·7)	
No	13 (16·3)	6 (46·2)	7 (53·8)	

Statistical analysis

Data entry was performed using EpiData v. 3.1 software (EpiData Association, Denmark) and analysis with Stata v. 8.0 (Stata Corp LP, USA). Qualitative variables were compared with χ^2 or Fisher's tests. Quantitative variables with normal distribution were compared with Student's *t* test and ANOVA tests while Wilcoxon and Kruskal–Wallis tests were used for the comparison of quantitative variables with abnormal distribution. A 0·05 level of significance was used for the analyses.

RESULTS

Elephants

Study population

A total of 82 elephants, representing 23% of the domestic population of the province of Sayaboury and 18% of the national population (LECMP, unpublished data), were examined by the veterinary team. Two elephants were too aggressive to be approached and were excluded from the study. The remaining were aged 13–59 years (mean 33 years) with a F/M sex ratio of 1·58/1 (Table 1). Twenty (25%) were from Paklay district, 19 (24%) from Thongmixay, 14 (18%) from Phieng and 10 (13%) from Sayaboury. The remaining 17 elephants were working in Phieng during the study, but came from nearby districts: 15 (19%) were from Hongsa and two (3%) were from Botene. All the animals were used for timber extraction. The majority (84%) were living with or had regular contacts with other working elephants while 25 (31%) had regular contacts with wild elephants. Of those with regular contacts with wild animals, 22 (92%) were females that had been approached by wild males when kept in the forest near their logging camps or villages, during the breeding season.

Clinical findings

When the veterinary team visited, 19/80 elephants (24%) had abscesses, wounds or injuries, mainly on

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the shoulders, head or back. Six females were lactating and six others were thought to be pregnant. Clinical signs suggestive of TB in elephants [3, 6, 14, 15] were investigated: fatigue, weight loss, loss of appetite and trunk discharge. Of the 80 screened elephants, 50 (62%) showed no clinical signs, 23 (29%) showed one sign and seven (9%) showed two or more (Table 1). Interviews revealed that 13 (16%) elephants had trunk discharge when working hard in hot weather.

ElephantTB Stat-Pak reactivity

Of the 80 elephants tested, 29 (36%) were reactive to the ElephantTB Stat-Pak assay (Table 1). Males were more frequently reactive (48%) than females (29%), although the difference was not significant ($P = 0{\cdot}07$). Reactivity differed with the district of origin and was higher in Hongsa (53%), Sayaboury (50%) and Paklay (45%) while lower in Thongmixay (21%) and Phieng (14%). However, this difference did not appear significant ($P = 0{\cdot}08$). Reactivity of the elephants did not significantly correlate with clinical signs suggestive of TB ($P = 0{\cdot}17$) or with age ($P = 0{\cdot}51$) and sex ($P = 0{\cdot}17$).

Elephant owners and mahouts

Study population

In total, 142 participants (after exclusion of the two mahouts of the two aggressive elephants) were enrolled in the study, with 1–4 individuals (mean 1·8) per elephant. All were men, aged between 16 and 68 years (mean 35·7 years), mostly married (77%) and belonging to the Lao Loum ethnic group (92%), the predominant ethnic group in Laos. Only 40% had received the bacille Calmette-Guérin (BCG) vaccine. Most participants were mahouts (48%), 30% were only owners (some owned several elephants) and 23% were both mahouts and owners. They had spent an average of 17 years with work elephants (range 1–50 years) and an average of 10 years with the elephant included in this study (range 1–50 years). Of the participants, 35% owned or worked with an elephant reactive by the ElephantTB Stat-Pak assay.

Clinical findings

Upon examination, 90 (63%) participants had at least one clinical sign suggestive of TB [28]: chest pain (23%), dyspnoea (22%), night sweats (21%), cough for >15 days (19%), weight loss (20%), crackling or wheezing during pulmonary auscultation (13%), dysorexia (7%) and fever (6%) (Table 2). Thirty-one (22%) showed only one suggestive sign, 28 (20%) two signs and 30 (21%) $\geqslant 3$ signs. Although 55% of the participants declared smoking cigarettes, this was not significantly associated with any clinical signs suggestive of TB or with abnormal chest X-rays ($P = 0{\cdot}77$).

Table 2. *Clinical and radiological signs suggestive of tuberculosis among mahouts and elephant owners*

Clinical signs	Total ($N = 142$) *n* (%)	X-rays suggestive of TB ($N = 8$) *n* (%)	*P* value
Chest pain	33 (23)	4 (50)	0·09
Dyspnoea	31 (22)	3 (38)	0·43
Night sweats	30 (21)	4 (50)	0·06
Cough >15 days	27 (19)	0 (0)	0·51
Weight loss	29 (20)	3 (38)	0·36
Dysorexia	10 (7)	1 (13)	1·00
Fever	9 (6)	0 (0)	0·67
Abnormal pulmonary auscultation	19 (13)	1 (13)	1·00

Chest radiography

Eight (6%) participants had chest X-rays suggestive of pulmonary TB (Table 3). Three of these (nos. 86, 99, 104) worked with a Stat-Pak-reactive elephant and seven had familial or personal history of TB. Six of these eight participants also had clinical signs suggestive of TB. They were all seen 3 months later; clinical examinations were then normal and Ziehl–Neelsen stain and culture gave negative results.

Culture and microscopy

Two sputum samples per participant were collected for a total of 286 samples, of which 164 were suitable for analysis. All samples were negative for acid-fast bacilli. Upon culturing, 19 (8%) were discarded because of contamination. Seven samples from six different participants were positive for *Mycobacterium* species. All these mycobacteria were identified as Mycobacteria other than tuberculosis (MOTT): *M. gordonae* ($n = 2$), *M. fortuitum* ($n = 1$), *M. abscessus* ($n = 1$) and unidentified species ($n = 2$). Some of these MOTTs were isolated from participants with clinical or X-ray signs of TB (nos. 136 and 143),

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Table 3. *Demographic and clinical features of participants with medical history of TB, abnormal X-rays or with positive mycobacterial culture*

Participant no.	Age (yr)	Personal or familial history or TB	BCG*	Clinical signs	Chest X-ray pulmonary signs	Mycobacterial culture	ElephantTB Stat-Pak test	Contact with current elephant (yr)
13	27	Grandfather treated	+	Weight loss	–	–	–	5
21	16	Under treatment; brother also under treatment	–	Dyspnoea, cough	–	–	–	7
26	35	–	–	Dyspnoea, weight loss, weakness	Micronodular opacities	–	–	2
65	32	Grandfather under treatment	–	–	–	*M. gordonae*	–	11
67	32	–	+	–	–	*Mycobacterium* spp.	–	15
84	59	–	+	Dyspnoea, chest pain, weight loss	Pleural effusion	–	–	11
86	31	–	–	Chest pain, weight loss, weakness and night sweats	Atelectasis with large pulmonary nodule	–	+	12
91	35	–	–	Chest pain, night sweats	Hilar adenopathy	–	–	24
97	37	–	+	–	–	*M. fortuitum*	+	5
99	52	Treated	–	–	Opacity of the two summits	–	+	15
101	31	–	+	Night sweats	Bilateral infiltrate	–	–	3
104	40	Grandmother died of TB	+	Dyspnoea, chest pain, dysorexia, weakness, night sweats	Hilar adenopathy	–	+	21
113	20	Father treated	+	Dyspnoea, chest pain, weight loss, weakness	–	–	–	1
114	21	Father treated	+	–	–	–	–	1
115	20	–	+	–	–	*M. gordonae*	–	1
136	50	–	–	–	Right axillary opacity	*M. abcessus*	–	4
143	64	–	–	Cough, purulent & bloody sputum, dysorexia, weight loss, weakness	–	*Mycobacterium* spp.	–	15

* Previous bacille Calmette-Guérin (BCG) vaccination.

with TB-infected relatives (no. 65), or with a Stat-Pak-reactive elephant (no. 97) (Table 3).

Data analysis revealed no significant relationship between culture, clinical or radiographic signs suggestive of TB and elephant reactivity with the ElephantTB Stat-Pak test.

DISCUSSION

The aim of this survey was to report the seroreactivity of TB in working elephants in Laos and the TB-related health status of their mahouts and owners. A total of 80 elephants and 142 mahouts/owners were examined either in their village, in logging camps, or at the annual elephant festival in the town of Sayaboury. This represented more than 18% of the total national population of registered working elephants (LECMP, unpublished data). The seroreactivity in the elephant population found in this study appears surprisingly higher (36%) than in the other Asian countries where the ElephantTB Stat-Pak assay has been used before. In Nepal and Malaysia, TB seroreactivity was found to be 15% ($n = 106$) and 20% ($n = 63$), respectively [15, 17]. In India, a recent study on 179 serum samples randomly selected from 600 working elephants showed a seroreactivity of 18% [18]. Higher rates were found in temple elephants (25%) compared to other groups of working elephants (12–15%) [16]. These findings suggest that close contacts between humans and elephants in countries with high TB may increase the risk of TB infection in elephants. The higher elephant seroreactivity observed in our study could reflect the high prevalence of human TB in the country. In Laos, the TB burden has been found to be 540/100 000 whereas in Malaysia it is 101/100 000, in Nepal 243/100 000 and in India 249/100 000 [1, 23]. The recent increase in elephant resale in Laos could also contribute to the high seroreactivity. National data show that about 50% of working elephants have changed owners in the last decade (LECMP, unpublished data). This implies increased contacts between elephants and sequential owners, mahouts and their community.

Our results show that elephant seroreactivity was significantly influenced by the district of residence (from 14% to 53%). This relationship has already been reported in other studies, notably in Malaysia where seroreactivity ranged from 0% to 26% in herds from different locations. However, groups there were too small to highlight the factors explaining these differences [17]. In our study, the lowest rates were found in the districts of Thongmixay (21%) and Phieng (14%). In those districts mahouts traditionally work around the Nam Phouy National Park where logging is intensive; therefore, they are thus less likely to travel long distances to reach new logging camps. Isolation from other elephants and mahouts could be a potential protective factor against TB exposure in working elephants.

Our results also suggest a greater susceptibility of male elephants to TB infections (48% *vs.* 29% for females, $P = 0{\cdot}07$). To our knowledge, such a difference has never been reported before. In Nepal, no association was found between sex or age and reactivity to the ElephantTB Stat-Pak assay [15]. Similarly, US data from 1994 to 2011 showed that the incidence of TB culture-positive elephants was 11% in both males and females [8]. In the logging camps of Laos, male elephants are usually given higher workloads than females in order to avoid aggressive behaviours, particularly during the musth period. A chronic state of fatigue in males could result in an impairment of their immune system and explain their greater susceptibility to TB infection, as suggested for humans [29]. Further studies would be needed to confirm such claim.

Around a third (31%) of the elephants included in this study had contacts with wild elephants (Table 1), exclusively inside or around the Nam Phouy National Park. Most of them (88%) were domesticated females temporarily released into the forest during the breeding season to be approached by wild tuskers. This traditional method of elephant breeding is by far the most successful in Laos and remains crucial for the survival of the population. In 2011, when at least 17 deaths were recorded, 5/6 newly born elephants in the whole country had a wild father (LECMP, unpublished data). The high TB seroreactivity in working elephants suggests that the wild breeders could be infected. It is likely that these males also breed with the wild females that are known to live in the park in three or four matriarchal groups comprising a total of 60–80 individuals (K. Khounboline, personal communication), and hence transmit mycobacteria to the wild population. Little is known about the consequences of the introduction of TB in a wild Asian elephant population, especially its effects on reproduction. Later stages of the disease are, however, known to markedly affect the general health condition of the animals [5]. This could hasten the extinction of small and isolated populations of elephants like those living in the Nam Phouy National Park.

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Despite high TB seroreactivity in elephants, neither mahouts nor owners were smear- or culture-positive for *M. tuberculosis* or *M. bovis* although MOTTs were isolated from six (4%) participants and although eight (6%) participants had chest X-rays suggestive of TB. These atypical mycobacteria have been isolated from 15% of smear-positive participants in a recent TB prevalence survey in Laos (National Tuberculosis Programme, unpublished data). Infections by MOTTs are not considered contagious for humans or other animals [30] but can be pathogenic and are probably underdiagnosed in regions where TB is endemic, e.g. in Southeast Asia [31, 32]. *M. gordonae*, which was cultured from the sputum samples of two participants is generally found in contaminated water networks but is considered non-pathogenic [33].

The discrepancy between the high seroreactivity in elephants and the absence of active pulmonary TB in their mahouts or owners could partly be explained by the tests used in this study. The ElephantTB Stat-Pak assay is a screening test that detects both IgG and IgM. Anti-TB IgG may be detected long before the appearance of clinical signs and may persist for years, even after treatment of the elephants [34]. False-positive results have been known to occur with the ElephantTB Stat-Pak; some have been attributed to MOTT infections [7] or to chronic inflammatory diseases such as arthritis [34–36]. In our study, 7/29 reactive elephants (24%) presented with inflammation (cutaneous abscesses, wounds or corneal perforations). This could have impacted their reactivity status. It has also been suggested that an anergic immune response in an advanced TB case could interfere with the ElephantTB Stat-Pak assay [14]. Clinical manifestations of TB in elephants are usually observed in later stages of the disease. This may explain why no correlation was found between seroreactivity and clinical signs in this study, or in others [14, 37]. Further tests should indeed be performed in order to confirm the Stat-Pak-reactive results. It is generally assumed that the response to TB by the immune system is cell-mediated. However, the tuberculin skin test is not applicable in elephants and the development of an interferon gamma release assay (IGRA) is currently underway in Thailand [38]. Several studies have found that serodiagnosis in elephants is more accurate than in other species. Many African as well as Asian elephants did produce strong antibody responses years before mycobacteria were isoled by trunk wash [34]. Serological tests have therefore been used as well as trunk-wash cultures. Although culture remains the most reliable technique to confirm active TB in elephants, it has inherent limitations as screening diagnostic technique. Several weeks of incubation are needed before results can be obtained, it requires prior training of the elephants and has limited sensitivity [39]. As well as the Elephant Stat-Pak test, other assays have been developed. However, multi-antigen print immunoassay (MAPIA), which allows the detection of a broad range of antibodies, must be performed in a laboratory-based setting and is more useful for post-treatment follow-up [37, 40]. The Dual Path Platform assay (DPP Vet TB test; Chembio Diagnostic Systems Inc.), that allows the discrimination between MOTTs and *M. tuberculosis* complex [35, 37], was unfortunately not available when this study was conducted.

In this study mahouts and owners were considered TB-positive if sputum samples turned culture-positive. Only active pulmonary TB would therefore have been diagnosed here, but this could have been underestimated; although the combination of sputum microscopy and culture is extremely specific and is considered the gold standard, the sensitivity has been reported to be only 78% [41]. Sensitivity of the Ziehl–Neelsen stain on its own has been shown to vary greatly [42] and chest X-rays might not reveal suggestive signs of TB in the early stages of the disease, when damage to the lungs have not yet become apparent. Numbers of TB-infected participants might thus have been underestimated in our study.

The absence of detection of active pulmonary TB cases in the 142 participants suggests that mahouts and owners do not show a higher risk of disease than the general population of Laos. The country has the second highest TB burden of the 13 Asian elephant countries after Cambodia [23]. In this context, it is likely that the transmission of TB occurs from humans to elephants. A medical monitoring of people working or living with elephants should thus be implemented.

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DECLARATION OF INTEREST

None.

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ElefantAsia in the Lao PDR – An Overview

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ElefantAsia in the Lao PDR – An Overview

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The Lao PDR is an often forgotten range country of the Asian elephant (*Elephas maximus*). Despite having the largest remaining Asian elephant population in the Indochina region, little robust research on either wild or domesticated elephant populations has been undertaken in Laos. Any research that has occurred is either aging beyond relevance, or recent and ongoing. Population estimates range from 500-1000 wild, and 500 domesticated elephants remaining in the Lao PDR (Lair 1997; Blake & Hedges 2004; Sofranco 2006; ElefantAsia 2007).

One International Non-Government Organisation working in Laos since 2001 is ElefantAsia. A French-based INGO, ElefantAsia focuses efforts primarily on domesticated elephant registration, reproduction and veterinary care. Other projects include the creation of alternative income-generating activities for elephant owners, and environmental education activities such as the annual Elephant Festival. All programs endeavour to conserve and protect the domesticated Asian elephant from regional extinction in Laos.

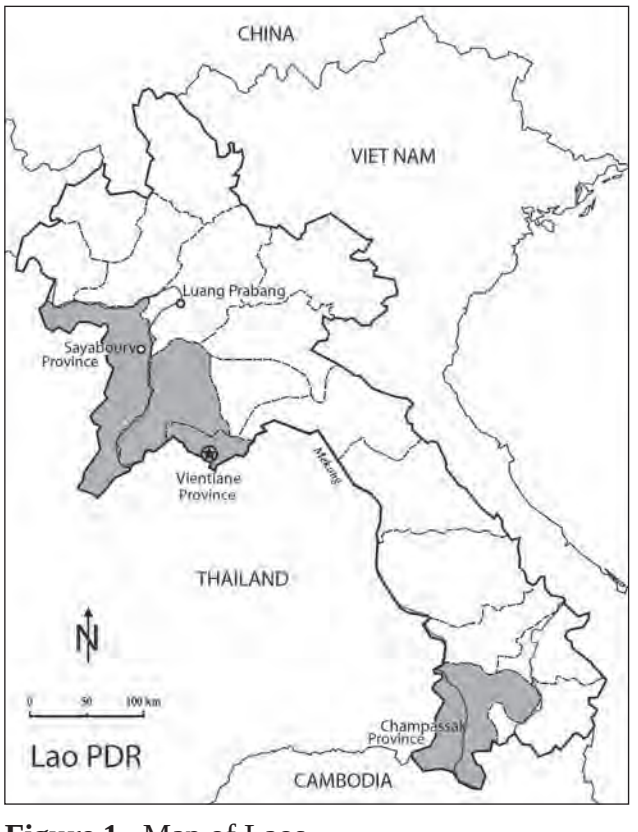

Figure 1. Map of Laos.

Of the estimated 500 remaining domesticated elephants in Laos, 80% reside in the northwest province of Sayaboury (ElefantAsia 2007, Fig. 1). Sayaboury is a mountainous region still lacking many access routes or the infrastructure to make broad-scale logging viable. While selective logging for village use with domesticated elephants (Fig. 2) has occurred in the Sayaboury province for centuries, it has only been during the past 20 years that organised logging companies have exploited domesticated elephants for professional timber extraction. Acknowledged as one of the poorest districts in Lao PDR by the United Nations Development Program (2009), elephant owners in this area have embraced this much-need income-generating activity offered by the logging industry, despite the adverse and often disastrous results it can have on domesticated elephant populations.

With calves economically worthless in the logging industry until age 14, consultations with mahouts indicate that many are opting to work



Figure 2. Logging camp in Laos.

their cows intensively rather than allowing them time for reproduction (ElefantAsia 2008). Over a 20-year period this had resulted in an inverted age pyramid, with the mortality rate of domesticated elephants greatly outweighing the birth rate. Sadly, many deaths are either fully or partially caused by work in the logging industry. Severe exhaustion, lacerations, fractures, and other disorders are often left untreated as mahouts work for months in remote forested regions isolated from veterinary assistance (ElefantAsia 2008). These issues are the focus of ElefantAsia's primary projects: veterinary care, and alternative income-generating abilities.

ElefantAsia has two Mobile Veterinary Units (MVUs) that travel throughout the provinces of Laos where domesticated elephants reside. The MVUs are all-terrain vehicles equipped with veterinary supplies suited to the Lao climate, context, and local availability (Fig. 3). International volunteer veterinarians participate in all MVU trips, along with Department of Livestock and Fisheries government officials, and other specialised ElefantAsia staff. With most domesticated elephants located in Sayaboury Province, the majority of MVU time is spent travelling throughout this large province. Scheduling meetings with elephant owners and their elephants takes approximately two weeks in advance to prepare from ElefantAsia's head office in Vientiane. This includes consultations with district and provincial Department of Livestock and Fisheries staff; attempts to contact mahouts working in areas with poor mobile phone reception, and purchasing veterinary products from the National Animal Health



Figure 3. Mobile Veterinary Unit.



Figure 4. ElefantAsia vet at work.

Centre supply unit. Once in the field, veterinary care is undertaken at either pre-arranged meeting areas, or simply by the roadside where elephant owners come out of the forest to meet the MVU (Fig. 4). The most commonly treated disorders observed by MVU veterinarians are abscesses; parasitic abscesses; eye disorders; cracked nails and superficial wounds (Labatut & Suter 2010).

ElefantAsia does more than just attend to injured elephants, then leave. The MVU supplies all elephant owners, who bring their elephant to the MVU for treatment and registration, with an Elephant First Aid Kit (Fig. 5). The kit is a waterproof plastic container full of basic yet effective veterinary items that mahouts can administer to their elephants as required. Such products include eye cleansers, broad-spectrum antibiotics, vitamins, de-worming medications, iodine, gauze pads, and prophylactic materials. A new addition to the Elephant First Aid Kit is a translated version of the *Elephant Care Manual for Mahouts and Camps Managers,* co-published by the Food and Agriculture Organization of the United Nations and ElefantAsia. This is the first and only book written in the Lao language specifically relevant for elephant ownership, and includes chapters about how to assess the weight of an elephant for medical dosage requirements; how to treat common diseases; dietary requirements; life cycles; and control techniques.

ElefantAsia also spends a significant amount of time and resources providing product training and education to elephant owners and mahouts (Fig. 6). As the daily care-givers to this endangered species, ElefantAsia believe it



Figure 5. Elephant First Aid Kits.

is imperative that owners and mahouts have the skills and knowledge required to independently and effectively administer treatment to their elephants when immediately required. The application of all products in the Elephant First Aid Kit is comprehensively explained to mahouts by ElefantAsia staff members, with all supplies available for purchase at district pharmacies or veterinary centres. Feedback regarding the Elephant First Aid Kits has been extremely positive, with all elephant owners and mahouts enthusiastic to learn about product usage and how to provide better care in remote situations. Funding for Elephant First Aid Kits is provided by private sponsors, and ElefantAsia intends to continue ongoing first aid kit distribution, as it is an incentive to owners and mahouts to seek assistance from ElefantAsia's MVUs.

Another project undertaken by ElefantAsia is the ongoing "Baby Bonus" incentive. With ElefantAsia's registration records showing domesticated elephant births occurring at an exceedingly low rate, ElefantAsia has implemented a motivational program aimed at encouraging elephant owners to breed their cows. Through a binding agreement between ElefantAsia and an elephant owner, ElefantAsia agrees to provide a tak-tak (a motorised rotary hoe used for agricultural use); pre and post pregnancy veterinary care; and elephant life insurance to owners willing to stop working their cows in the logging industry and actively attempt to breed them. To date, ElefantAsia has four cows signed to the Baby Bonus program with more owners interested. Once the calf is born, ElefantAsia will attempt to place mother and calf in a quality tourist camp where they can undertake light trekking work. Laos does not currently have an elephant ultrasound machine, so it will be some time before veterinarians and the owners' experience tells whether or not their cow is pregnant.

There are various approaches through which domesticated elephant cows in Laos can become impregnated. In the north-west district of Thongmixay, cows are often impregnated by wild bulls from the neighbouring Nam Phui Protected Area. Nam Phui is believed to have a wild elephant population of approximately 130 individuals, although only rudimentary population counts have been undertaken (National Elephant Management Action Plan 2009). Nevertheless the Nam Phui Protected Area is believed to contain the second largest wild population of elephants in Laos (National Elephant Management Action Plan 2009).

While working in remote forested regions, mahouts may see a wild elephant and tether their cow at night close to where the bull was observed. On the 26th of January 2010, two calves were born on the same day to domesticated cows, with the male believed to be a bull from the Nam Phui Protected Area. ElefantAsia staff are aware of at least another six cows that are pregnant by wild bulls in Thongmixay. In southern Laos, the breeding of domesticated elephants is currently poor. With approximately 30 domesticated elephants residing in the Patoumphone District



Figure 6. ElefantAsia staff train mahouts in product usage.

of the Champassak Province, cows and bulls are generally not kept together, making reproduction an unlikley occurence. Unlike northern Laos where domesticated elephant owners can earn a good income through logging, this does not occur in Patoumphone, a flat, previously logged region. Consultations with elephant owners in southern Laos have indicated that breeding is viewed as an unnecessary, expensive, and unsafe practice to undertake. Poor reproductive knowledge and a lack of financial incentives were other major explanations as to why owners are not breeding their elephants in southern Laos (Maurer 2009).

Despite elephant domestication occurring for centuries, many domesticated elephant owners in Laos do not necessarily have reproductive knowledge and techniques. Until the 1980's it was legal to capture calves from wild elephant populations as required, hence reproductive knowledge has never been required. Since the Government of Laos banned the taking of elephants from the wild, domesticated elephant population numbers have declined significantly (Lair 1997; Norachuck 2002). ElefantAsia's mahout training includes how to visually observe and assess a cow's oestrus cycle, and how to chart oestrus cycles on a calendar.

Poaching of domesticated elephants is another problem ElefantAsia is trying to tackle. The latest poaching occurred in the Vientiane Province of Laos in March 2010. ElefantAsia is aware of 10 domesticated elephants poached since 2008, with no arrests for any of the crimes. Laos has been recognised as a nation that lacks both capacity and transparency to enforce laws relating to the illegal trafficking of ivory (Norachack 2002; Dublin *et al.* 2006). Within Southeast Asia, Laos has been singled out as a major source of ivory, and as a well-used channel to smuggle African and Asian elephant ivory products to neighbouring nations (Nooren & Claridge 2001). With the risk of arrest for killing or smuggling elephant parts throughout Laos extremely low, the motivation by poachers to kill Asian elephants for ivory remains high.

Laws in the Lao PDR are clear regarding the killing of elephants. In 1989 the Government of Laos declared a ban on the hunting and killing of protected, endangered species; and the country became signatories to the *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES) in 2004 (CITES 2009). However, poaching continues to be a major cause of death for both domesticated and wild elephants.

ElefantAsia has several environmental outreach programs aimed at teaching local Lao communities about the need to protect and conserve their national icon. These include posters and children's books distributed to schools and libraries in areas of Laos where domesticated and wild elephants reside. Mobile Book Parties to remote villages via elephant is another novel way ElefantAsia teaches young generations about the need to protect, not poach elephants. ElefantAsia's annual Elephant Festival is also a major drawing card in raising awareness about elephant conservation.

Held annually in the Sayaboury Province, the Elephant Festival has attracted over 80,000 attendees, mainly Lao nationals (Fig. 7). With up to 60 domesticated elephants attending each year, visitors to the two-day event learn about ancient domesticated elephant culture, logging techniques, and the life of an elephant owner. Consultations with elephant owners indicate that they enjoy attending the festivals as their profession is shown a high status and is respected by Lao nationals and foreigners alike. Now one of Laos' major festivals, many national and international television crews and journalists travel to Laos purely to attend ElefantAsia's Elephant Festival.



Figure 7. Elephant Festival in Laos.

While ElefantAsia has many ongoing projects, the main focus is to increase domesticated elephant populations for future generations to use. Tourism in Laos is a growing industry (Lao National Tourism Administration 2009), with approximately six elephant tourist camps in the Luang Prabang municipality. Private hotels and guesthouses throughout Laos also own elephants and offer elephant treks to visitors. Tourism in the form of elephant trekking may be the domesticated elephant's saving grace in Laos. Once logging quotas are filled, elephants will become unemployed and ownership will be financially unsustainable. ElefantAsia is endeavouring to create Laos' first breeding sanctuary for domesticated elephants, enabling pregnant cows to do light treks while pregnant or with a calf. As a change from working in the intensive logging industry, quality elephant trekking can be a viable way to continue Laos' ancient, traditional, domesticated elephant ownership.

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Changes in Elephant Ownership and Employment in the Lao PDR: Implications for the Elephant-Based Logging and Tourism Industries

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*Communities in the Lao People's Democratic Republic (PDR) have been utilizing captive Asian elephants (*Elephas maximus*) for centuries. The elephant handler (mahout) profession has remained fundamentally unchanged. Captive elephants, however, are no longer necessary in roles where they were once considered vital, particularly the logging industry. To gauge mahout demographics and assess problems associated with elephant industries, we surveyed 133 mahouts in the Lao PDR. We found that mahoutship is an aging industry with a vanishing family association. Contrasts between logging and tourism mahouts were apparent when observing family tradition, finances, and industry-related experiences. Logging mahouts rely on tourism to provide them with future employment; however, tourism cannot currently employ elephants on a scale similar to that of logging operations. The need for the traditional mahout and a large population of captive elephants may have reached its finality in Laos.*

Keywords Asian elephant, *Elephas maximus*, Lao PDR, mahouts, tourism

Introduction

The Asian elephant (*Elephas maximus*) has been captured and tamed by humans for at least 4,000 years (Baker & Kashio, 2002; Lair, 2004; Norachack, 2002; Sukumar, 2003). Captive elephants are credited with facilitating the expansion of human settlements and for playing important roles as instruments in warfare and symbols of spirituality and national identity (Csuti, 2006; Singh, 2012; Sukumar, 2003). Working and owning an elephant is a conventional practice in many Southeast Asian nations, with mahouts (someone who owns or works with elephants) only recently facing significant industry changes (Fowler, 2006; Sukumar, 2003). Mahoutship is the term used to portray the handling skills required

All surveys were undertaken with The University of Queensland human ethics approval (School of Integrative Systems, 28/06/2010). We thank staff at the Lao Department of Livestock and Fisheries and National Animal Health Center for their organizational assistance, in particular Dr. Douangchanh Sirivongsa, Dr. Bounmee Saymounty, and Phouang Singngam. Special thanks goes to Sébastien Duffillot, Gilles Maurer, Vatsana Chanthavong, Nittaphone Sivanthong, Thonsavath Doungdy, and all the veterinarians at ElefantAsia for providing essential support and assistance for which we are graciously indebted. In fond memory of Dr. Hanh Choundara.

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of mahouts to successfully commander an elephant (Lair, 2004). Throughout all Asian elephant ranges, men would inherit the mahout profession from their father or male relative, continuing to work with the family elephant(s) throughout their life (Hart, 1994; Lair 2004; Stanley-Millson, 1991; Vanitha, Thiyagesan, & Baskaran, 2009). Modern-day technology, deforestation, anti-logging legislation, and globally declining Asian elephant populations are shifting the manner in which captive elephants work, the practices of the mahout, and calling into question the modern-day relevancy of this tradition.

Captive elephants were traditionally sourced from wild populations, with bans on wild capture only implemented since the 1970s (Csuti, 2006; Pimmanrojnagool & Wanghongsa, 2002; Sukumar, 2003). There are considerable variations in population estimates of remaining wild Asian elephants, with consensus placing total populations between 30,000–50,000 (Dublin et al., 2006). The captive elephant population is estimated to be one quarter of the total remaining Asian elephant population (Leimgruber et al., 2008), making the captive elephant population significant for the species conservation. Captive elephants are not a genuinely domesticated species, being genetically and behaviorally identical to their wild counterparts (Lair, 2004). Interrelationships between wild and captive populations persist in most range nations, typically reproduction between captive cows and wild bulls (Lair, 2004). Captive elephants maintain a high degree of "releaseability" back into the wild, acting as a genetic reservoir while contributing to scientific research and species education (Lair, 2004; Smith & Hutchins, 2000). Despite the International Union for Conservation of Nature (IUCN) listing Asian elephants as endangered, it is recognized that most captive elephant populations are ill-managed and lack cohesive conservation management plans (Choudhury et al., 2008; Dublin et al., 2006).

While still holding strong historical and cultural significance, the importance of captive elephants has fundamentally declined. Elephants are no longer necessary in roles where they were once considered indispensable (Csuti, 2006; Lair, 2004; Vanitha et al., 2009). Their once frequent use in warfare became redundant and so too is their usefulness for transportation and logging (Csuti, 2006; Mar, 2007; Sukumar, 2003). Technology is challenging the relevance of this cultural tradition, the viability of captive elephant populations and affecting the employment of the once widespread mahout profession.

Over the past two human generations, logging restrictions have rendered large numbers of captive elephants and their mahouts jobless. Changes in national logging legislation have altered mahout employment in countries such as Thailand, India, and Sri Lanka (Bist, Cheeran, Choudhury, Barua, & Misra, 2002; Csuti, 2006; Jayewardene, 2002; Martelli, 2006; Sarma, Deka, Chakraborty, & Hazarika, 2003; Tipprasert, 2002; Vanitha et al., 2009). Additionally, sons from traditional mahout families now wish to avoid outdoor, agricultural-style labor (Lair, 2004). Mahoutship is no longer viewed as a financially viable or desired profession; men now prefer employment in more modern environments (Lair, 2004; Vanitha et al., 2009). Nevertheless, most range nations still employ captive elephants for village, tourism or temple/festival purposes (Dublin et al., 2006; Jayewardene, 2002; Vanitha et al., 2009). Elephant tourism now employs nearly all of Thailand's captive elephants, a complete industry shift in just one generation, although hundreds may still work in illegal logging (Duffy & Moore, 2010; Kontogeorgopoulos, 2009; Mecir, 1998). While tourism provides some employment, it does not demand the same number of elephants once essential for logging. A century ago 1,000 elephants were used in Indian logging operations, compared with 200 elephants needed for that nation's current ecotourism industry (Bist et al., 2002; Dublin et al., 2006). The decline in logging has left many mahouts and elephants unemployed with similar industry downturns likely to affect mahouts in the Lao People's Democratic Republic (PDR).

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The Lao PDR was formerly named Lang Xang, the "Land of a Million Elephants" (Stuart-Fox, 1997), yet little research has been undertaken regarding Lao mahouts or elephants. Obtaining accurate data for Lao captive elephant populations is difficult due to a lack of funding, capacity and a restrictive political environment (see Duckworth, Salter, & Khounboline, 1999; Lair, 2004; Norachack, 2002; Phanthavong & Santiapillai, 1992; Sukumar, 2003). Estimates of the national captive elephant population range from 500 to 1,350 (Dublin et al., 2006; Duckworth et al., 1999; Lair, 2004; Sukumar, 2003). The most recent population surveys have been performed by the international nongovernmental organization (INGO) ElefantAsia. ElefantAsia has been actively registering elephants in Laos for 7 years and places the Lao captive elephant population between 420 and 460 (S. Duffillot, ElefantAsia, personal communication).

Through surveys, we assessed whether Lao mahouts face professional difficulties similar to those experienced in other range nations. We approached this task using a quantitative results and empirical research. Specifically, we address the following: (1) Mahout demographics, income generation, and elephant reproduction and (2) the problems and challenges Lao mahouts experience with elephant work.

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Study Context and Area

This study was undertaken in the Lao PDR. Laos remains a least-developed, single-party, authoritarian Communist nation (Stuart-Fox, 2006; United Nations Development Programme, 2011). In general, limited study has been undertaken within Laos, with the government making social-science research difficult and remaining distrustful of foreigners undertaking projects in remote areas (Linter, 2008; Stuart-Fox, 2011).

We interviewed mahouts within the Champassak, Luang Prabang, Sayaboury, and Vientiane provinces (Figure 1) as these four provinces contain the largest remaining captive elephant populations (G. Maurer, ElefantAsia, personal communication). Sayaboury remains a mountainous, remote province lacking the infrastructure needed for logging machinery. Bordering the Sayaboury Province is the Luang Prabang Province, containing Luang Prabang municipality, a UNESCO World Heritage city considered one of Laos' leading tourist destinations.

When designing the survey we were mindful that the official number of mahouts in Laos is unknown, as mahoutship is typically dependent on family timeshare arrangements. However, we know that there are approximately 3.6 owners per elephant, with work assigned between family members on seasonal agreements (G. Maurer, ElefantAsia, personal communication). Some elephant owners work with their own elephants while other owners may employ one to two mahouts. There are also unemployed mahouts and those currently without elephants (G. Maurer, ElefantAsia, personal communication). However, if there are currently 450 elephants working in Laos, there may be around 1,600 mahouts in the country. It should be recognized that not all these mahouts work full time with their elephant and some do not even work with their elephant every year (G. Maurer, ElefantAsia, personal communication).

Methods

Surveys were conducted during or after ElefantAsia mobile veterinary unit field missions. Surveys in Luang Prabang were conducted in elephant tourist camps. Sayaboury and Vientiane surveys were conducted in logging camps, on the side of the road or in small villages. Champassak interviews were conducted at a tourist center or local villages.

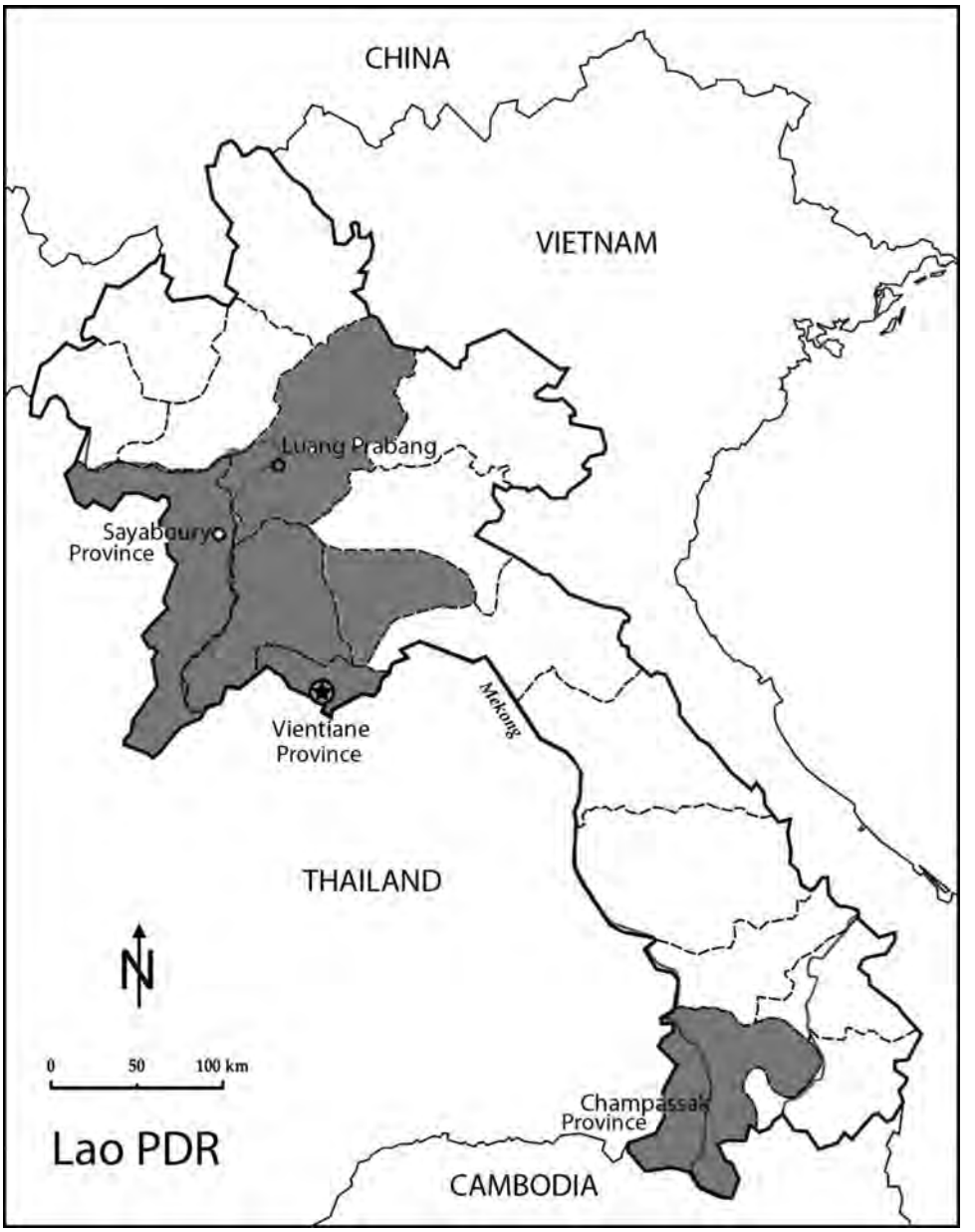

Figure 1. Lao PDR.

A total of 133 mahouts were surveyed between June 2010 and March 2011 and 51 villages in 15 districts were visited. The selection of respondents was based upon willingness to participate, typically dependent on the mahout's work schedule and availability. Given that mahoutship is a male-orientated occupation, all survey respondents were male. Translators with previous experience working with mahouts or livestock laborers were used to conduct and scribe surveys. Forty of the mahouts we encountered were unable

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to participate in the interviews due to work or time commitments. However no mahout who had sufficient time directly refused to participate in the interview.

Our survey focused on mahout demographics and captive elephant information. We chose semi-structured and multiple choice questions for the survey. This allowed mahouts to respond in their own words and choose the most appropriate response to the question raised. Open ended questions were followed with a "Why?" or "Please specify" question. Questions with multiple choice answers were given a selection of responses previously identified by ElefantAsia as issues frequently affecting mahouts. Statistical analyses of data were performed using analysis of variance (ANOVA) and chi-square tests of association with Yates Correction. A significance level of .05 was adopted. Before performing the ANOVA, the data were tested for homogeneity of variances using Hartley's test and for normality by examining a plot of residuals. The Vientiane Province was not independently analyzed due to the small sample size.

Respondents were categorized into three age groups; under 30, 31–39, 40+ years old. These age brackets were selected as over half the Lao population is economically active from age 10 with average life expectancy for Lao males being 59 years (Messerli et al., 2008). Elephant work was classified into four industries: professional logging, elephant tourism, small-scale family or village work, and split industries (a combination of several industries).

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Results

Most mahouts (51%) were at least 40 years old, with the mean age being 41 years. Of the 133 respondents, the majority (53%) worked in the professional logging industry ($\chi^2 = 59.63$, $df = 3$, $p < .01$) (Table 1). Mahouts were more likely to work in the logging industry in the Sayaboury Province and tourism in the Luang Prabang Province ($\chi^2 = 94.45$, $df = 9$, $p < .01$). Nearly half of all logging mahouts (49%) were aged 40 years or older. In contrast, 52% of mahouts working in tourism were under 30 years of age. Mahouts in the Champassak and Sayaboury Provinces were significantly older than mahouts in the Luang Prabang and Vientiane provinces ($F = 3.45$, $df = 3$, $p < .05$).

Elephant Inheritance and Elephant Income

Nationally, there was no relationship between mahouts working with elephants and having a family link to elephant ownership (Yates $= 1.69$, $df = 1$, $p > .01$). However, a family inheritance of elephants and/or mahoutship was still observed in logging mahouts, with 45% of respondents citing family tradition or no other choice for job/money as the reason they worked with elephants. From all professions, mahouts working in tourism were more likely to work with elephants because they loved elephants ($\chi^2 = 32.49$, $df = 9$, $p < .01$).

A lack of assistance or interest in mahoutship by adolescents was revealed when mahouts were asked whether or not they wanted their son or male relative to become a mahout. Significantly more mahouts wanted their son or relative to inherit the mahout tradition than not ($\chi^2 = 14.63$, $df = 2$, $p < .01$). However when asked if they believed their son/relative wished to become a mahout, only 19% of respondents replied in the affirmative.

Mahouts employed in tourism earned significantly less (between \$1,000-\$2,000/year) than mahouts in the logging industry (over \$6,000/year) ($\chi^2 = 66.92$, $df = 24$, $p < .01$) (Table 2). Besides income earned from elephant work, 70% of mahouts indicated they had an additional source of income (Yates 21.12, $df = 1$, $p < .01$). Over three quarters of

Table 1
The elephant-related profession of mahout survey respondents in the Lao PDR, categorized by province

	Champassak Province	Sayaboury Province	Vientiane Province	Luang Prabang Province	Total
Number of surveys	17	68	2	14	100
Sole or main industry of mahout					
Tourism	8	1	0	14	23
Professional logging	2	50	1	0	53
Small-scale village work	6	2	0	0	8
Split industries	2	14	1	0	17
Total	17	68	2	14	100

Note. Numbers are percentages of replies. $N = 133$.

Table 2
Annual income mahouts earn from elephant-related employment categorized by industry

Amount	Tourism	Professional logging	Small-scale village work	Split industries	Total
Under $100	0	0	2	0	2
$100 to $500	0	0	0	1	1
$500 to $1,000	6	2	2	1	10
$1,000 to $2,000	9	11	2	5	26
$2,000 to $3,000	4	11	1	2	17
$3,000 to $4,000	2	7	1	3	13
$4,000 to $5,000	0	3	0	2	5
$5,000 to $6,000	1	3	0	2	6
Over $6,000	1	12	0	2	15
Not stated	0	5	2	0	6
Total	23	53	8	17	100

Note. Numbers are percentages of replies. $N = 133$.

mahouts (77%) earned additional income through agriculture, livestock or fishing, typically tending the family plot of land. The tourism industry differed from all other industries in that these workers were not likely to have another source of income ($\chi^2 = 25.40$, $df = 3$, $p < .01$).

Elephant Demographics

In total, the respondents owned or managed 136 elephants; 56 bulls and 80 cows. The average age of bulls was 32 years ($SD = 10$) and cows 35 years ($SD = 13$). Only three elephants were 15 years or younger and none were under age 10. Bulls were more likely to

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work in the logging industry, with 79% of all bulls employed in logging ($\chi^2 = 24.89$, $df = 3$, $p < .01$). In contrast 90% of elephants working in tourism were cows.

A low captive elephant reproduction rate was recorded with only 21 cows (26%) reported to have experienced pregnancy during their working lives. Of these pregnancies, 76% were considered unintentional by respondents. Cows were likely to fall pregnant by wild bulls accidently, with 61% of cows believed to have been impregnated in this manner ($\chi^2 = 15.38$, $df = 3$, $p < .01$). Only 14% of mahouts surveyed intentionally arranged breeding with the owner of a bull.

Problems Mahouts Experience With Elephant Work

Mahouts were asked to state the biggest problems they experienced when working with elephants (Table 3). The most common problem (22%) was finding elephant work too tiring, primarily affecting logging mahouts. There was also a positive association between logging work, having to live away from home and the stress induced by potential elephant poaching ($\chi^2 = 79.12$, $df = 24$, $p < .01$). None of these issues were considered problematic by tourism mahouts. The leading response by tourism mahouts (35%) was having no problem or difficulty working with elephants.

Mahouts employed in the logging industry stated that they did not want another elephant because they did not have enough help or labor to assist with elephant husbandry ($\chi^2 = 27.92$, $df = 5$, $p < .01$). In contrast, there was a significant relationship between mahouts working in tourism not wanting an elephant because they were too expensive to

Table 3
Major problems identified by Lao mahouts when working with elephants (Respondents were able to select more than one answer)

	Industry				
Major problems (%)	Tourism	Professional logging	Small-scale village work	Split industries	Total
Not enough money to live on	4	1	0	4	9
Too time consuming—Taking elephants to forests, needing food & water	3	12	1	2	17
Too many elephant illnesses & diseases. Too hard work for elephants	2	1	1	0	3
Elephant poaching/Scared of having elephant poached	0	5	0	1	6
Too expensive to upkeep	0	2	1	1	12
Having to live away from home	0	9	1	2	12
Unsafe/dangerous for people	0	2	0	1	3
Tiring job	0	19	2	2	22
No problem/Not difficult	5	9	3	3	22
Other/Not stated	2	0	0	0	2
Total	15	61	9	15	100

Note. Numbers are percentage of all responses. $N = 203$.

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buy ($\chi^2 = 54.0$, $df = 5$, $p < .01$). Too expensive to purchase was the only reason cited by tourism mahouts as to why they did not want an elephant.

Mahouts were asked if there was anything they would like assistance with relating to their domesticated elephant or elephant work. The most frequent request was mahouts seeking elephant medicine or health care advice (29%).

Mahouts in the logging industry were significantly more likely to make comments specifically relating to their industry and their future ($\chi^2 = 70.34$, $df = 21$, $p < .01$). Logging mahouts expressed concern for the future of their industry with indicators of logging cessation already apparent in some Sayaboury districts. Logging mahouts were significantly more likely to show concern or fear for the future termination of their industry than tourist mahouts. Concern for future employment was reflected with a significant number of logging mahouts (7.5%) directly requesting help to find work in the elephant-based tourism industry.

Tourism mahouts were the most likely to request further information about elephants, commenting they wanted to learn more about mahout training and elephant reproduction. Small-scale village work and split industries were not significantly associated with any comment or request.

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Discussion

Elephant Inheritance and Elephant Income

Similar to findings in other Asian elephant range nations, Lao mahoutship is an aging profession (Lair, 2004). Our study shows that males from traditional mahout families did not wish to follow the footsteps of their elders and confront the unemployment dilemmas facing their older relatives. In contrast, young tourism mahouts from non-traditional mahout backgrounds are willing to work in their chosen field.

Our results suggest that domesticated elephant work is shifting away from traditional logging toward the emerging tourism industry. This transfer is similar to observations in Thai, Indian, and Sri Lankan elephant industries (Duffy & Moore, 2010; Godfrey & Kongmuang, 2009). While the logging industry in Laos still employs the majority of mahouts, these mahouts are likely to soon experience the same unemployment dilemmas felt by their international counterparts (Duffy & Moore, 2010). Even in the remote Sayaboury Province, men from traditional mahout families are likely to be the last generation working in the logging industry on such a large scale.

Hiring unrelated men to work as mahouts has seen mahoutship shift from a family business to a profession no longer associated with elephant inheritance. A vocation once transferred via male lineage, family association with elephant ownership remains for logging mahouts only (Hart, 1994; Hart & Sundar, 2000; Vanitha et al., 2009).

In contrast to the traditional logging industry, there is a growing trend among elephant owners to hire young men to work in Luang Prabang tourist camps. The hiring of non-traditional mahouts has shown to have a detrimental effect on elephant husbandry, public and mahout safety (Lair, 2004). Hiring non-traditional mahouts for tourism risks diluting traditional mahout skills and knowledge, a concern previously raised for Indian and Thai domesticated elephant culture (Bist et al., 2002; Lair, 2004). With tourism mahouts requesting more information about elephant care, there is a concern that these mahouts lack fundamental elephant knowledge. This is supported by similar observations indicating much elephant knowledge is inadequately shared with younger generations from non-traditional mahout families (Lair, 2004).

Mahouts working in the Sayaboury Province believed they had no other choice for a job or income other than working with the family elephant. Conversely, mahouts in tourism chose to work with elephants rather than having family tradition forced upon them. With Luang Prabang one of the busiest and most accessible regions of Laos, these mahouts had greater access to a variety of employment options, particularly in tourism. Whereas the remoteness of Sayaboury means a selection of employment opportunities is rare.

The mahout tradition is losing its appeal to younger generations from traditional mahout families. A lack of assistance or interest in mahoutship by adolescents was confirmed. While elder mahouts wanted their tradition continued, realistically they knew mahoutship was not their son/relative's aspiration. This leaves the mahout tradition and culture with an uncertain future

Mahouts working in tourism are the lowest earners compared with all other elephant-related industries (Table 2). Tourism mahouts are the youngest of all elephant-based professions, have no mahout background or alternative source of income. Hired by tourist camp managers or elephant owners, these young men are vulnerable to job and financial insecurity if the tourism industry experiences a downturn. Conversely, logging mahouts can earn over eight times the national per capita income of $500–$700 USD/year (Lao Insight Books, 2009; Linter, 2008). Despite this, the wealthiest mahouts experience the highest level of job dissatisfaction, mainly due to the recent decline in logging quotas and hard living conditions associated with logging.

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Elephant Demographics

Our study shows that industry suitability for elephants is strongly related to gender. Tourism favors docile, smaller cows suitable for carrying passengers whereas logging requires larger, stronger bulls capable of pulling heavy loads. Gender preferences are likely to limit logging mahouts wishing to participate in tourism. The nature of elephant-based tourism leaves limited opportunities for bull-owning mahouts.

The decrease in Sayaboury logging opportunities combined with an extremely low calf birth rate will see both industry and elephant numbers become markedly reduced. The accidental nature of captive elephant breeding suggests mahouts place little value on breeding or utilizing captive bulls for reproduction. This concern has been raised previously by ElefantAsia staff, confirming an inability for elephant owners to agree on costs associated with elephant reproduction. Often mahouts want calves but disputes can arise when attempting to calculate compensation for lost time, covering costs of birthing and raising the calf, and eventual calf ownership (G. Maurer, ElefantAsia, personal communication). Most calves that are born are sired by wild bulls, illustrating the beneficial role wild elephants play in captive elephant reproduction.

Problems Mahouts Experience With Elephant Work

Satisfaction with working conditions varied significantly between logging and tourism mahouts as tourism mahouts seemed to have a much higher rate of job satisfaction. Variation in satisfaction rates can be attributed to industry-specific lifestyles. Similar to the Thai experience, the labor-intensive nature of logging is affecting desire to follow family tradition (Lair, 2004). Logging mahouts must live in remote, isolated logging camps for months at a time, where many of them experience stress at the anticipation of their elephant being poached. None of these issues were considered problematic by tourism mahouts. Tourism mahouts have fixed working hours; live close to regional centers; have safer, less

strenuous working conditions and work in socially active environments. They are represented by a younger demographic and are less likely to have the same family commitments as middle-aged logging mahouts.

There were disparities between industries in the desire of mahouts to own more elephants. Aging logging mahouts lack the family assistance required for the upkeep of a domesticated elephant. Younger family members have observed the demanding workloads of elephant ownership and have little desire to work under such tiring conditions. Conversely, young tourism mahouts would like an elephant but without family inheritance they are unable to afford the large investment required for elephant ownership. With an adult male bull fetching over $20,000, family inheritance of an elephant is economically fortuitous. Similar issues have been reflected in the supply and demand of Sri Lankan elephants. With a lack of calves born into captive populations, elephants are increasingly expensive to purchase and considered out of reach for young contemporary mahouts (Jayewardene, 2002).

Logging mahouts face greater challenges and employment uncertainties than those working other elephant-related industries. Mahouts in the logging industry expressed a multitude of negative attitudes and challenges regarding their profession. This group of men perceive themselves as having a much higher rate of job insecurity than any other industry, with deep concerns about their future once forests "close" indefinitely. Men are actively seeking advice on how to transition from the logging industry into what they view as more secure future in tourism. Given the significant shift away from logging in Thailand, India, and Sri Lanka, Lao mahouts have a justified basis for their unemployment concerns (Duffy & Moore, 2010; Godfrey & Kongmuang, 2009; Tipprasert, 2002). However with only an approximate 50–60 elephants working in tourism nationally, the ability for tourism to employ logging elephants on a large scale is unfeasible and should not be seen as a solution to mahout unemployment.

Unlike logging mahouts, who are worried about the future, tourist mahouts expressed a strong desire to learn more about the elephants they work with. This is an indication of their inexperience and insufficient on-the-job training. Their concerns are justified as mahout deaths have sporadically occurred in Lao tourist camps. Verbal control skills, husbandry and musth detection are all skills tourism mahouts should be competent with. Studies undertaken by Vanitha et al. (2009) demonstrate a higher incidence of mahout casualty in non-traditional mahouts. This danger is avoidable as training received by non-traditional mahouts in Indonesia has proved very successful in enhancing the quality of their elephant skills (Nazaruddin, Stremme, & Riddle, 2010).

Conclusion

The cessation of logging suggests there are some serious implications facing this captive elephant population. The results of our survey suggest there will be some future demand for captive elephants but populations will be smaller than in previous years and will be susceptible to local extinction. The current disinclination for elephant reproduction will see a dearth of domesticated elephants within a single generation, risking unemployment for all elephant-related professions. Reproductive and conservation management plans should be implemented to ensure a higher calf birth rate without relying on wild elephant populations for breeding.

A needs-based assessment of the elephant-based tourism industry is recommended to examine Laos' potential to expand elephant tourist camps or elephant trekking operations.

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The geographical locations of elephant-based tourism operations need to be assessed and complemented with visitor demographics, and local and national infrastructure.

Our findings suggest that logging mahouts are very willing to work in the emerging tourism industry. However, a transition into tourism may be unachievable for most mahouts if tourist camps prefer to employ young, inexperienced mahouts willing to work for significantly lower wages and if the industry maintains its preference for cows over bulls. Older mahouts can be an added service to the tourism industry by mentoring and training young, non-traditional mahouts; however, elephant tourist camps may be unwilling to financially invest in this arrangement. The nation of Laos appears to be following the same decline in mahoutship and elephant-based employment as other Asian elephant range nations. Given that the captive elephant population of the Lao PDR is estimated to be 33% of the total population of the country (Khounboline, 2011), any commercially induced decline in the captive population will have important implications for elephant conservation in this country.

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Report · International Elephant Foundation / ElefantAsia

Domesticated Elephant Advanced Registration (DEAR)

International Elephant Foundation · ElefantAsia

1st January 2010 – 1st January 2011

INTERNATIONAL ELEPHANT FOUNDATION

ElefantAsia

Domesticated Elephant Advanced Registration (DEAR)

1st January 2010 – 1st January 2011



2010: Phieng District, Sayaboury. A logging elephant is microchipped as part of the DEAR project. Assisted by his mahout; ElefantAsia's Registration Officer Mr Thongsavath Doungdy and Department of Livestock & Fisheries Officer Mr Phouang Singngam (left).

TITLE OF THE PROJECT: *Domesticated Elephant Advanced Registration (DEAR)*

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Budget

Total Project Budget: **USD $27,629**
Total Received from the International Elephant Foundation: **USD $8,000**

Timeframes

Project Start Date: 1 January 2010
Project End Date: 1January 2011

Project summary

The Lao PDR is one of the Asian elephant's range countries afforded the least amount of conservation management, population assessment, veterinary care or governance. ElefantAsia remains the only organisation dedicated solely to the protection and conservation of Asian elephants in Laos. An estimated 1,000 - 1,200 elephants remain in Laos. Wild population surveys are rudimentary with population counts recognised as baseline assessments only. More is known of the domesticated elephant population, of which there are an approximate 460 elephants remaining in Laos. ElefantAsia works in collaboration with the Department of Livestock and Fisheries (DLF) to provide the best healthcare, registration and employment options for elephants and elephant owners.

ElefantAsia recognised a clear need for enhanced Asian elephant registration in Laos. Paper registration was proving to be duplicative and inefficient, with some elephants having multiple registration papers while other registration papers were lost in provincial offices. These meant proper elephant census was unattainable and elephants with ongoing health issues not followed though with appropriate attention. Given the Lao context, microchipping the entire domesticated elephant population of Laos was seen as the best management option. Many Asian elephant range countries have already seen the benefit of microchipping domesticated elephants, with five out of 13 nations microchipping captive populations (Dublin *et al* 2006).

The Domesticated Elephant Advanced Registration (DEAR) project timeframe was 1st January 2010 – 1st January 2011. During this period ElefantAsia has made significant improvements for the domesticated elephant population of Laos.

DEAR project highlights:

- The microchipping of 77% of Lao's domesticated elephant population.
- The creation of Lao's first comprehensive elephant database containing all field missions, consultations, images and stakeholders.
- The straightforward ability to follow-up elephant vet care and health via the database 'flagging' system.
- Capacity building and training of Lao nationals in registration, database usage and microchipping.
- The ongoing use of microchip and database information for the international community.

All activities have been completed by the anticipated dates with some activities ongoing. The DEAR project has occurred in five provinces of Laos, with a focus on elephants used in remote logging areas and tourism. ElefantAsia and DLF staff members have been trained in microchipping and database use and continue to create Lao's first accurate and functional domesticated elephant catalogue.

Methodology & work plan

(From project application)

Activity	Methodology	Expected results	Status (Pending, Ongoing, Completed)
Activity 1. Acquire microchip equipment	• Telephone & email micro-chip the company in the USA for best prices available for equipment.	• Receive a written quote for equipment. • Microchips and scanners obtained. • Database software obtained.	Completed
Activity 2. Establish microchip database.	• Communications with Asian elephant experts regarding the most effective requirements for the database. • Employ a local database consultant.	• Software is relevant and compatible with microchip software used regionally. • Software must be in both Lao and English.	Completed
Activity 3. Train staff in microchip use and data input.	• Conduct training during a field visit by the Mobile Vet Unit. • Training to be held at district DLF offices • Provide interactive instructions and training methods in Lao language. • Provide handouts in Lao language.	• Training in microchip and database usage. • Stationary. • Photocopying of documents • Human resources and travel costs	Completed
Activity 4. Registration of all domesticated elephants in Lao PDR.	• Microchip elephants during field visits by the Mobile Vet Unit. • Microchip elephants during the annual Elephant Festival. • Visit provinces where the Mobile Vet Clinic does not currently operate to microchip elephants.	• 480 domesticated elephants are micro-chipped. • Database compiled.	Ongoing

Activity	Methodology	Expected results	Status
Activity 5. Monitor DEAR success, microchip & database usage.	• Compare field notes with database information. • Feedback from district and provincial staff, mahouts.	• Database is effective • Ongoing data collection.	Completed, Ongoing
Activity 6. Identify any gaps in knowledge or technical skills (ongoing).	• Compare field notes with database information • Communications with staff and mahouts during field visits	• Quality review of database. • Feedback from district and provincial staff	Completed Ongoing
Activity 7. Provide database information/access to national and international stakeholders.	• Publication of findings in academic literature. • Via database software sharing. • Presentation of findings at Regional Asian elephant meetings • Broadcast on relevant internet websites	• National and international stakeholders will have accurate figure of domesticated elephant populations in Lao PDR	Completed Ongoing

Activity outcomes, monitoring & evaluation

Activity 1. **Acquire microchip equipment.**

Outcome: All microchips and scanners have been received.

Monitoring & evaluation: The type of microchipped used has proved to be very effective. The decision was made to purchase pre-loaded, individually wrapped microchip implanters. This leaves fewer margins for registration error and affords the safest form of hygiene as microchips remain sealed and untouched until moments before injection. Only a handful of elephants have been considered too aggressive to be microchipped. The decision to not microchip these elephants for personal safety reasons was noted and recorded in the database.

Activity 2. **Establish microchip database**.

Outcome: The database is a fully functioning and is now an integral part of ElefantAsia's elephant management system. All elephant details are entered into the database, including each individual elephant microchip number, sex, location, age, body mass index, health issues, medications given, advice given to owners, required follow-up reports, pregnancies, births, deaths, owner contact details and other unique features. All Mobile Veterinary Unit field missions and consultations are entered into the database and can be cross-referenced using the elephant's name, owner's name or microchip number.

Monitoring & evaluation. The ability to cross-reference data greatly reduces the room for error or elephant duplication. The database is routinely assessed for quality control (see Activity 5).

Activity 3. **Train staff in microchip use and data input.**

Outcome: Relevant ElefantAsia and DLF staff have been trained and are competent in database use. ElefantAsia's registration officer and vet technicians enter all data into the software after each field mission. Relevant information is entered in the Lao language, making the database comprehensible for Lao nationals. "How to" database instructions have been created, allowing new staff members or DLF staff to interact with the software. Staff names are in the database so it is possible to see which person has added, changed or deleted information.

ElefantAsia and the DLF's veterinarians and vet technicians have been trained by international veterinarians in microchip implantation. Implantation is not difficult however some specific measures must be undertook to ensure implantation is successful and people are not placed in danger. To date there has been no problems with microchipping elephants. Dangerous elephants are identified early and microchipping is not attempted.

Monitoring & evaluation: Database training is an ongoing process as new ElefantAsia and DLF staff require the database. ElefantAsia staff capacity has been built so most staff members can train others in database usage. After each microchip has been implanted vet technicians scan the area with the reader to ensure the chip is read and the microchip number is unique. This also applies before each microchip implantation to ensure the elephant has not been microchipped previously. Elephants are always double-checked for microchips as several microchips have been found in single elephants in other range countries such as Thailand.

Activity 4. **Registration of all domesticated elephants in Lao PDR.**

Outcome: It was always understood that not all domesticated elephants in Laos would be microchipped within the 12-month project period. To date 355 elephants have been microchipped, **totalling 77% of the entire domesticated population**. 36 elephants were microchipped at the 2010 Elephant Festival and 45 microchipped at the 2011 Elephant Festival. Elephants from five provinces have been microchipped: Sayaboury, Champassak, Vientiane, Luang Prabang & Bolikhamxay. Two p provinces have been added to ElefantAsia's Memorandum of Understanding with the Lao Government; Luang Prabang and Bolikhamxay. This is a significant achievement as more elephants are being sold to Luang Prabang tourist camps and veterinary care to these elephants is vital. Most elephants microchipped work in either the tourism or logging industries.

Monitoring & evaluation: Microchipping the entire domesticated elephant population of Laos is occurring as efficiently as possible. ElefantAsia has two Mobile Veterinary Units, allowing microchipping to occur at a faster rate than previously. Given the poor state of roads and six-month wet season, filed missions (and subsequent microchipping) can only occur under ideal conditions. Often roads are unexpectedly inaccessible by 4WDs because of flooding or erosion. It is anticipated that 90% of the population will be microchipped with a year, with the remaining 10% taking another year to complete.

Activity 5. **Monitor DEAR success, microchip & database usage.**

Outcome: While the designated Registration Officer enters all information into the database, this can be checked and verified by all ElefantAsia staff. The database is designed so that local and international database users can access the most recent and updated version without having to be in the ElefantAsia office. This makes the database extremely effective for instantaneous review and querying. Feedback from all stakeholders including elephant owners and DLF staff has been positive. One of the best design features of the database is the ability to 'flag' an elephant. This may be a sick or injured elephant that need follow-up attention, or a suspected pregnant elephant. Flagging an elephant means an automatic alert will occur, ensuring vets or DLF staff remember to ring an elephant's owner for follow-up observations. Given the remoteness of domesticated elephants in Laos, this system is the most effective way to ensure all elephants are treated post veterinary unit visitation.

Monitoring & evaluation: It is still too early to gauge the success of the DEAR program. Once at least 85% of the population has been microchipped, population viability analysis will be undertaken, with results published in academic journals and presented at conferences. Database usage is a big success with ElefantAsia and the Lao DLF. Elephant owners can be identified and contacted promptly if necessary. This is required for follow-up health checks for an elephant and can be done from anywhere in the world.

Activity 6. **Identify any gaps in knowledge or technical skills (ongoing).**

Outcome: The database went through a review process during the design phase. Negative feedback about its use and features were reviewed with changes made as necessary. Database design took longer than expected but has since proven to be extremely effective in containing all data required.

Monitoring & evaluation: Quality review of ElefantAsia's Registration Officer is regularly undertaken to ensure all data is entered into the database accurately. This is achieved by veterinarians or other staff members reviewing field mission reports, microchip numbers and elephant records against the information entered in the database. Any oversights are shown to the Registration Officer with changes made accordingly.

Activity 7. **Provide database information/access to national and international stakeholders.**

Outcome: ElefantAsia's microchipping has been documented in media including environmental magazines and websites. Academic publications are currently been written for publication in scientific journals. Findings will be presented at conferences, including the *Environmental Research Event*, Stradbroke Island, Australia June 2011.

Monitoring & evaluation: Academic peer review takes time to occur and is an ongoing process. ElefantAsia staff members notify all interested parties at all opportune moments. Without international assistance, elephants in Laos will continue to be overlooked.

Conclusion

The DEAR project will continue until all domesticated Asian elephants in Laos have been microchipped. This will be an ongoing project, given births will occur and calves will need to be microchipped at appropriate ages. ElefantAsia is pleased to update Laos' registration system to that of internationally recognised criteria. This includes the database, now a comprehensive tool for all domesticated elephant information in Laos. Already staff members of IUCN Laos have requested domesticated elephant database from the DEAR database. Ongoing funding will be necessary for the purchase of additional microchips and field missions.

It is anticipated that the flow-on benefits of the DEAR program will be felt for many years. Potential breeding partners have been identified, and are participating in ElefantAsia's "Baby Bonus" programme. Cows of breeding age are paired with bulls in the region. A comprehensive database showing each veterinary visit is vital to follow an elephant's development, including flagging sick or pregnant elephants for further observation. None of this would have been possible without the DEAR program and the International Elephant Foundation's assistance.