WILDLIFE SOS

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WILDLIFE SOS

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NOTE FROM THE CO-FOUNDERS

It has been more than two decades since Wildlife SOS was started and in the time that has passed, we have only moved from strength to strength. After eradicating the 'Dancing Bear' practice from the streets of India, we turned our eyes to other issues – rescuing abused elephants from the cruel practice of performing in circuses, used for begging or riding, rehabilitating leopards caught in man-animal conflict situations and spreading awareness about conservation efforts, with extremely successful results. In the midst of these activities, we have continued conducting research on issues concerning the country's fauna and working on tribal rehabilitation programs.

As India's wildlife loses its habitats and faces mounting pressure on food sources and shelters, there are a host of factors threatening their peaceful existence. We have spent the last twenty years trying to provide a safe haven to injured and abused animals across the country. Because of this extended association, we have had the good fortune of gathering immense knowledge about the physical, emotional and behavioral tendencies that wild animals exhibit; this has enabled us to find and develop better ways to care for those under our aegis. Our work involves countless issues like geriatric care, dental treatment and intensive medical care, cub-rearing, conservation research, anti-poaching, habitat protection and behavioural research.

It has been an exciting journey of enlightenment, and we are proud to showcase this compendium of research covering multiple species that we have worked with. We hope it will light the path for young wildlife veterinarians and researchers when they face similar circumstances.

Geeta Seshamani

Geeta Seshamani
Co-founder & Secretary
Wildlife SOS

Kartick Satyanarayan
Co-founder & CEO
Wildlife SOS

53 Parvis Road

West Byfleet

Surrey KT14 7AA

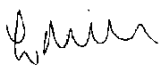
United Kingdom

FOREWORD

It is an honour to be asked to write the foreword for this book, having worked with its authors, my esteemed colleagues at Wildlife SOS, for the past fifteen years. I have been privileged to visit the bear rescue facilities run by Wildlife SOS in Agra and Bannerghatta on many occasions to perform dental surgery on the rescued dancing bears alongside Dr Arun and his team. Over the years I have come to admire and respect their tremendous passion for India's wildlife and the natural world.

Dr Arun and his colleagues have gained a wealth of experience in treating all kinds of wildlife with a wide range of illnesses and injuries. In particular they have become experts in the physical and psychological rehabilitation of rescued dancing bears and in the provision of treatment to relieve painful chronic conditions caused by years performing on the streets of India.

This second collection of articles is testament to the dedication of these extraordinary vets. Their commitment to wildlife treatment, rehabilitation, protection and research is exceptional and their approach is always compassionate, conscientious and highly professional, as is illustrated in this excellent book.



Lisa Milella BVSc Dip EVDC MRCVS

Dr. JACOB ALEXANDER

Assistant Director
Zoological Gardens
Thiruvananthapuram
Dept. of Museums & Zoos
Govt. of Kerala-695033

FOREWORD

I am privileged and happy to introduce the third volume of the compendium of the research publication of Wildlife SOS, which proudly owns a successful model in place for rescue and rehabilitation of displaced wildlife in India.

It is heart-warming to say that I have personally observed that the veterinarians and professionals of the organisation are keen on presenting papers in most of the scientific seminars and science congresses in India. I believe that the support is given by the managers at the helm of affairs, and the urge they give to document and present should have been the nidus that created this brilliance of research publications. Only few passionate ones choose wildlife as their profession, and not all of them continue in the profession and are thriving. Several veterinarians who opted for wildlife are given chances of employment and a place to grow up in this career with the excellent mentorship of senior Wildlife veterinarians, including Dr. Arun A Sha and Dr. Ilayaraja Selvaraj at the Wildlife SOS. These veterinarians are continuously being consulted by zoo veterinarians and managers inside and outside the country for the diagnosis and treatment of captive-wild animals, especially sloth bears and elephants.

Most often, the overburdened wildlife vet elsewhere seldom publishes their rich experiences. This is a substantial intellectual loss. It is here the importance of publication of these papers in the form of essence comes in place. I am sure that this compendium of publications of Wildlife SOS will be one of the sought-after reference books among zoo vets, researchers, aspiring students as well as the managers of wildlife collections.

I pray that these pieces of information be used to soothe the sufferings of the speechless and help their managers in preventing its occurrence wherever possible by better management and understanding. I am anxiously waiting for the fourth volume of research studies, which I am sure with continuous striving for improvement will get better each time.

Dr. Jacob Alexander

M: +919446464418, E: zoovettvm@gmail.com, W:www.museumandzoo.kerala.gov.in

Dr. A Sha Arun
B.V.Sc., M.V.Sc (Wildlife Medicine), M.S. (Psychotherapy).

Wildlife SOS
Director - Research & Veterinary Operations
Bannerghatta Bear Rescue Centre
Bannerghatta Biological Park, Bangalore - 560 083

ACKNOWLEDGEMENTS

We are pleased to launch the third volume of " A compendium of scientific publications of Wildlife SOS". This stands testament to the commitment of the staff of Wildlife SOS to research and scientific inquiry that we have tried to imbibe in our culture. We owe a debt of gratitude to our co-founders who have supported our work all these years, and we hope that they continue to embrace and encourage us in these endeavors.

Furthermore, this volume is a result of the relentless dedication of our staff, and I'd like to specifically acknowledge the contributions of Dr.Nithin, Ms.Prajakta, Mr.Sandeep and Mr.Lenu, in this regard. I would also like to thank all our animal keepers for their steadfast cooperation that did the research that constitutes these volumes possible. I'm also grateful for all the positive encouragement and feedback from my peers and colleagues that helped shape our work.

Lastly, I would be amiss if I did not acknowledge the role of the various forest departments across the country that have supported our research and look forward to continued cooperation.

Happy Reading!!

(Attur Shanmugam Arun)

M +91 9980145785 | E arun@wildlifesos.org| E arun4wildlife@gmail.com| W www.wildlifesos.org

A COMPENDIUM OF SCIENTIFIC PUBLICATIONS OF WILDLIFE SOS

– TWO DECADE REVIEW

Volume - III

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Art- 152. REPRODUCTION IN CAPTIVE SLOTH BEARS *(Melursus ursinus)*: BEHAVIOR, DENNING AND MATERNAL CARE

Vibha Raghuram K., Arun Attur Shanmugam, Harikrishnan C., Simone Ayoob

Abstract

The Sloth Bear (*Melursus ursinus*) is an omnivorous, seasonally monoestrous, crepuscular bear found within the Indian Subcontinent. Mating has been observed during the peak summer (April to July) (n=6). Cubbing occurs about 6 to 7 months later (November to January) (n=9) owing to the availability of insects during winter and monsoon and seasonal fruit resources during summer. The behavioural changes mark the early estrus period. These include decreased food consumption, increased scent marking, restlessness with frequent position changes, rolling, urination and olfactory investigation in addition to swelling and reddening of external genitalia. Presenting the anogenital region to the male, constant looking at males, aggressive vocalization (moaning and barking) by females are characteristic observations of Receptivity peak period.

Parturition dens used by the sloth bears vary. While few

bears use the crevices between big rocky boulders and spaces between terrestrial roots of many tree species for shelter, some were found to make earthen dens 5, 6.

Litter count is usually one or two, rarely three in number. The cubs are born blind. They grow rapidly; opening their eyes by 4 weeks of age and begins to walk out by about 2 months of age. The mother (n=4) seldom feeds self for the first 6-10 weeks of cubbing. Cubs leave the den at about six months of age on the females back, thus defending predator attacks 2, 4. Cubs stay with the mother for about 2.5years, which lets females breed at either two- or three-year interval in the wild. However, for the need of better management in captivity, weaning of the cub at one year of age is practiced. Further research in this area is being undertaken by us.

Keywords: estrus, vocalization, behavioural changes, denning, cubing.

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Art – 153. THE SLOTH BEAR OF MYSORE: A REALLY ROGUE BEAR, A TYPICAL SLOTH BEAR, OR SEVERAL DIFFERENT BEARS?

Thomas Sharp

Introduction

Sixty years ago, Kenneth Anderson (1957) published the story "Alam Bux and the Big Black Bear" in his book Man-Eaters and Jungle Killers. The bear at the story's center has become infamous as the Sloth bear of Mysore and appears on Internet top 10 lists of the "greatest animal serial killers." This bear supposedly killed at least 12 people and mauled more than 24 others near Mysore in the state of Karnataka, southern India. Re- reading the story more than a half century later, what can we now ascertain? What insights does the story offer about sloth bear *(Melursus ursinus)* attacks? And do the last 60 years of sloth bear research shed any more light on these events?

Anderson wrote that sloth bears *(Melursus ursinus)* "are excitable, unreliable and bad-tempered animals," a view still widely held today by both local people as well as sloth bear biologists. Yet he also appeared to have a true affection for this species: "As I have mentioned somewhere else, Bruin is an old friend of mine, against whom I have no antipathy. I was

therefore most disinclined to go after him." Anderson's writing makes clear that he was both fond of and knowledgeable about sloth bears and their ecology. His writing is filled with the details of a sloth bear's life, including their diet, daily lifecycle, activities like crop raiding (though he does not use this term) ground nuts (peanuts), and, of course, attacks.

Anderson set the stage for the story by stating that sloth bears "have a reputation for attacking people without apparent reason, provided that person happens to pass too close, either while the bear is asleep or feeding, or just ambling along." He later described how a sloth bear attacks a victim. Recounting one specific event (but saying it was emblematic of all sloth bear attacks), he wrote that the bear "invariably attacked the face of the victim, which he commenced to tear apart with his tremendously long and powerful claws, in addition to biting viciously." However, Anderson also made it clear that for the most part, sloth bears are generally "vegetarian", meaning not carnivorous, eating almost exclusively fruits, vegetables, and insects or insect products such as honey. Anderson also mentioned that these bears eat carrion, and he thought that reports of the sloth Bear of Mysore partially eating its victims were at least plausible — in fact, sloth bears partially eating their victims has been documented as recently as 2005 (Bargali et. al. 2005). The bear encounters that Anderson described

were apparently brought about by a food source that lured the bear into the human domain. Anderson wrote specifically about figs, which lined the road to the shrine where his friend's son was killed. He also mentioned ground-nut that was planted behind the same shrine. However, Anderson also commented that this particular bear "would go out of his way to attack people even when he saw them a long distance away," though he offered no evidence of this claim. The habitat in which this bear lived - rocky hillocks surrounded by farmlands, which the bears raided on a nightly basis, as Anderson described it— is very similar to the habitat of our Wildlife SOS (WSOS) sloth bear study area in eastern Karnataka. Additionally, Anderson's description of the bears' nightly habits seems to describe the vast majority of bears that we observe: "Hungry by sunset he could be seen coming forth from his cave, and, as twilight deepened into nightfall, he would amble down the knoll and come out on the ground-nut fields. Here he would spend a busy night, eating, uprooting and generally shuffling about over a wide area throughout the hours of darkness. Leisurely he would climb back to his abode, there to spend the hot hours of the day in deep and barely slumber."

Given the similarities of the famous "Sloth bear of Mysore" and the many bears that we have seen in eastern Karnataka, it seems fair to ask if all the attacks and deaths were likely from

this single bear. And what's more, could they be ascribed to the bear that Anderson eventually shot and killed? Sloth bear attacks in this hillocky habitat were not uncommon then, as Anderson clearly indicated, and they are not uncommon now, as indicated by our study of sloth bear attacks during the last 5 years.

Typical open farmland with dwellings surrounded by rocky hillocks occupied by sloth bears in Karnataka, India.

In Karnataka, India, sloth bears typically emerge from rock crevices in the evening and come down from the hillocks to feed at night. They are widely regarded as being prone to attack people Anderson first hunted the bear where his friend Alam Bux's son was killed. Having no luck, he returned to Bangalore for a month. Upon hearing of 2 more bear attacks roughly 30 km from the first location, Anderson "concluded that it was the same bear." However, several things suggest otherwise. First, the 30 km between these 2 attacks is a large distance for a sloth bear: this species may have the smallest home range of any bear species, with study area-specific averages for males spanning just 4–14 km2 (Joshi et. al. 1995, Ratnayeke et. al. 2007; although Yoganand et al. 2012 suggested ranges could be much larger). Therefore, the distance between attacks would be unlikely to fall within the home range of a single male bear. Also, since sloth bear home ranges can overlap extensively (Joshi et al. 1999), the area in which these 2 attacks occurred was likely inhabited by multiple bears. Supposedly both attacks were by a large male, not a young dispersing animal that could be traveling extensively. Anderson describes the area where the bear was killed, as "wanting for nothing." If true, then the bear would likely not be searching widely for food or water. It is impossible to know with certainty whether this was indeed 1

marauding bear. Perhaps there were reasons not explained in the literature that led to the single-bear theory. However, it seems more plausible that several different bears were responsible for the many attacks. It is clear that Mr. Anderson understood sloth bears, but perhaps his determination to rid the area of a problem bear clouded his judgment in this case. Or perhaps he realized that his narrative would not be quite as compelling if the tale was not about a single aberrant bear that was dispatched at the end of the story. Whatever the case, this would not have been the first time, nor obviously the last, that a bear would have been killed to make the public feel safer after an attack or a series of attacks. This happens not just in India, but anywhere where bears or other potentially dangerous wildlife still occur.

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Art – 154. KAZIRANGA NATIONAL PARK — PLENTY OF SLOTH BEARS, BUT ARE THERE OTHER BEAR SPECIES?

Nishith Dharaiya, Dave Garshelis, **Thomas Sharp**, Arzoo Malik, Nandita Patel, Nilmani Rabha

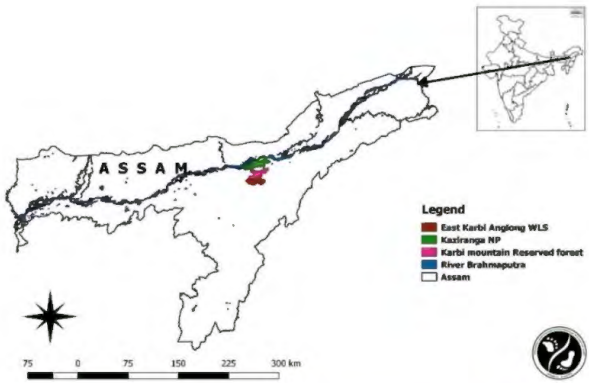
Introduction

India's Kaziranga National Park, located in the state of Assam in northeastern (NE) India is most known for its one-horned inhabitant, the Indian rhinoceros *(Rhinoceros unicornis)*. But it contains a host of other fascinating wildlife as well, including tigers, leopards, elephants, water buffalo, gaur, sambar, and swamp deer. Kaziranga comprises a lush mixture of grasslands, marshlands, riverine forests, and mixed-deciduous and semi-evergreen tropical forests, crosscut by 4 rivers and interspersed with lakes. During the peak of the monsoon in July – August, much of the park is flooded. We were interested in what bear species occupy this park and surrounding areas.

Three bear species occupy NE India (Asiatic black, *Ursus thibetanus*, sloth, *Melursus ursinus*, and sun, *Helarctos malayanus*), but due to confusion over identification of these species, great uncertainty exists about where each of them lives within this region. We specifically sought to find whether

there might be places where all 3 overlap, and if so, what conditions permitted this sympatry. Kaziranga was a possibility of such a place. Before spending time at Kaziranga, we were aware that the park was inhabited by a robust population of sloth bears: Choudhury (2011:15) described the park as the most important site for the species in NE India." We were aware of no evidence for the presence of Asiatic black bears or sun bears within or adjacent to the park. However, there are old records of sun bear cubs formerly being found in tea farms bordering the park's grassland and Karbi Mountains areas (Choudhury 2011). Kaziranga National Park, bordered by the Brahmaputra River to the north and Karbi Mountain range to the south, offers a possible intersection of habitats favorable to 3 bear species. Diggings for termites, claw marks and half-eaten honeycomb indicated that a sloth bear feasted at this site within a patch of riverine forest.

Sloth bear scat was found on the second floor of this watchtower overlooking the Kaziranga grasslands. The grasslands of Kaziranga National Park, Assam, India, harbor most of the world's Indian rhinoceroses, and apparently a sizeable population of sloth bears. The Karbi Mountains possibly inhabited by Asiatic black bears and/or sun bears are in the background.


ASSAM
Legend
East Karbi Anglong WLS
Kaziranga NP
Karbi mountain Reserved forest
River Brahmaputra
Assam
75 0 75 150 225 300 km


KATHPORA

With the help of a research and conservation grant from the IBA we were able to visit 4 sites in NE India, intending to lay the groundwork for future studies of the overlap of these 3 bear species in NE India. The field visit to Kaziranga involved taking jeeps along safari roads through the park's grass- lands for 8 – 10 km. With forest officers and staff, whom we had just trained about identification of bear sign, we searched mainly for bear diggings into termite mounds — a definitive sign of sloth bears. We found that many mounds had at some time been broken into. We also found 3 different sloth bear signs at a single site: diggings into a termite mound that surrounded a tree; sloth bear claw marks on the tree; and the remains of honeycomb at the base, which explains the bear's motivation for climbing it. We also found sloth bear scat (recognizable by the termite remains) on a footbridge over the Difolu River, and at a watchtower that we checked based on information from a local mahout (elephant caretaker), who had seen a sloth bear climbing down one morning. The large amount of sign that we observed in such a short time and small area, and along well-travelled safari routes, suggests that sloth bears are indeed very common in the park.

Sloth bears are known to relish grasslands with abundant termite mounds, which are a hallmark of this park. Asiatic black

bears and sun bears are forest-dependent species. Although the grasslands contain patches of alluvial woodlands, these are too small to support a forest-dependent bear species. However, the Karbi Mountains (not part of the national park but protected as reserved forest), south of grasslands, are well forested and contain potential habitat for all 3 bear species. The grasslands and mountain areas are only separated by a single-lane highway and, in certain places, tea farms or other small developments. We were not able to conduct any sign surveys in the Karbi Mountains on this trip. Additionally, whereas by-catch of camera-trapping for tigers has provided many photos of bears, we were informed that no camera traps have been set in the reserved forest of the Karbi Mountains. Therefore, the species of bears in this area remains unknown. Thus, this area will be a prime target for our search for that rare remnant patch of habitat possibly shared by 3 species of bears.

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Art – 155. USE OF ADVANCED DIAGNOSTIC AND THERAPEUTIC EQUIPMENT IN ROUTINE ELEPHANT HEALTH CARE IN INDIA

Ilayaraja Selvaraj, Yaduraj Khadpekar, Arun. A. Sha, Gochalan. E, Kamalanathan. M.

Abstract

In this modern world, science and technology have taken an upper hand. As a result, we are seeing regular advancement in every field including human and veterinary diagnostic and medicine. Although the latest technology and equipment in these fields are available and well established in developed countries; in developing countries, adopting them still has lot of challenges such as cost, availability of expert technicians and lack of infrastructure. In India many captive elephants suffer various health issues caused by improper captive management by the owners and mahouts. Wildlife SOS established Elephant Conservation and Care Centre (ECCC) in India to help and rehabilitate the captive elephants that need urgent medical attention; and realized that bringing in the modern technology in the veterinary care of these elephants is necessary to provide better results and improve their welfare. Wildlife SOS took great efforts to make

the equipment such as thermal imaging camera, therapeutic ultrasound, therapeutic laser machine, ultrasonography machine and portable x-ray machine with Computerized Radiography, available for their veterinarians. Through this presentation, we explain the effective utilization of each of this equipment in our routine elephant health care at ECCC.

Keywords: Elephant health care, Thermal imaging camera, Therapeutic ultrasound, Therapeutic laser, ultrasonography, portable x-ray machine, Computerized Radiography.

Art – 156. POINT OF CARE TUBERCULOSIS SERO-DIAGNOSIS KIT FOR WILD ANIMALS: COMBINATION OF PROTEINS FOR IMPROVING THE DIAGNOSTIC SENSITIVITY AND SPECIFICITY

Maroudam Veerasami, K. Venkataraman, Chitra Karuppannan, **Arun Attur Shanmugam,** Mallepaddi Chand Prudhvi, Thomas Holder, Polavarapu Rathnagiri, K. Arunmozhivarman, Gopal Dhinakar Raj, Martin Vordermeier, B. Mohana Subramanian

Abstract

Tuberculosis is a significant problem globally for domestic animals as well as captive and free ranging wildlife. Rapid point of care (POC) serology kits are well suited for the diagnosis of TB in wild animals. However, wild animals are invariably exposed to environmental nonpathogenic mycobacterium species with the development of cross reacting antibodies. In the present study, POC TB diagnosis kit was developed using a combination of pathogenic Mycobacteria specific recombinant antigens and purified protein derivatives of pathogenic and nonpathogenic Mycobacteria. To benchmark the TB antibody detection kit, particularly in respect to specificity which could not be

determined in wildlife due to the lack of samples from confirmed uninfected animals, we first tested well-characterized sera from 100 M. bovis infected and 100 uninfected cattle. Then we investigated the kit's performance using sera samples from wildlife, namely Sloth Bears (n = 74), Elephants (n = 9), Cervidae (n = 14), Felidae (n = 21), Cape buffalo (n = 2), Wild bear (n = 1) and Wild dog (n = 1).In cattle, a sensitivity of 81% and a specificity of 90% were obtained. The diagnostic sensitivity of the kit was 94% when the kit was tested using known TB positive sloth bear sera samples. 47.4% of the in-contact sloth bears turned seropositive using the rapid POC TB diagnostic kit. Seropositivity in other wild animals was 25% when the sera samples were tested using the kit. A point of care TB sero-diagnostic kit with the combination of proteins was developed and the kit was validated using the sera samples of wild animals.

Keywords: Mycobacteria. POC. Rapid test. Tuberculosis. Wild TB diagnostic kit

Introduction

Tuberculosis (TB) is an emerging zoonotic disease of captive and free-ranging wildlife species with severe consequences in biodiversity and species conservation. Tuberculosis bacteria

has a wide wild life host range which includes Elephants [1–3], sloth bears [4, 5], Arabian oryx [6], White tailed deer [7], Reindeer [8], European badgers [7, 9, 10], cervids [8], rhinoceros [11], lion [12], badgers [13] and non-humane primates [2, 14]. So far 60 different wild mammal species were proven to be infected with TB [15, 16]. TB in wild animals is an emerging global concern as some of the wild species were proven to be maintenance and reservoir host for TB [17, 18]. Interspecies transmission of TB at wildlife livestock- human interface poses public health, conservation and economic threats. Ante-mortern diagnosis of TB in wildlife is difficult due to the subclinical nature of the infection and limited choice of diagnostic tests for wildlife [19]. Gold standard TB diagnostic test in humans is Mycobacterial culture. However, difficulties in sample collection, long incubation period, cumbersome sample processing and sample transport methods render the test impractical for implementation in wildlife. Cell mediated immune (CMI) response based diagnostic methods such as skin testing or interferon-gamma release assays are the commonly employed TB surveillance assays in domestic animals. However, CMI based assays require species specific reagents and skin testing is not practical in wild animals. In this context, sero-diagnosis of TB is an alternative in wildlife

with a scope for improvement in assay sensitivity and specificity [20]. Rapid pen-side sero- diagnostic kits are preferred for TB diagnosis in wild animals as they are simple, inexpensive, rapid and relatively non-invasive diagnostic methods. Moreover, the sero-diagnostic method does not require bio-containment facility, unlike the Mycobacteria detection by culture methods [21]. Multi-antigen prints immunoassay (MAPIA) is being used as an appropriate tool for the identification of sero-dominant antigens of TB organism. The MAPIA methodology was adopted in various animal species such as Cattle [22, 23], Elephants [7], Reindeer [8] and European Badgers [24] for determining antigen recognition patterns of serum samples. Early Secretory Protein-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) are the immuno-dominant antigenic candidates in Elephants [25]. However, the immuno-biology of TB may vary with different species and is poorly understood in wildlife. Therefore, sensitivity of the assay can be compromised if only a limited number of antigens are used in the development of sero-diagnostic assays. At the same time wild animals are invariably exposed to environmental nonpathogenic Mycobacterial species [26] and various atypical mycobacterium species outside the Mycobacterium tuberculosis complex. Wild animals can mount an antibody

response to environmental Mycobacterium resulting in false positivity in TB diagnosis [27].

The present study was aimed at developing a Rapid TB antibody detection kit using recombinant fusion protein of ESAT-6:CFP-10 along with purified protein derivatives (PPDs) of M. bovis for the improved diagnostic sensitivity and also with PPDs of M. avium for assessing the diagnostic specificity. To the best of our knowledge, this is the first report on the development and evaluation of a point of care kit using the above combination of antigens for the diagnosis of TB.

Materials and Methods

Immunochemicals and Reagents Purified protein derivatives of Mycobacterium bovis (BoPPD) and Mycobacterium avium sub species avium (AvPPD) were procured from Prionics AG (Wagistrasse, Schlieren-Zurich, Switzerland). Wild animal sera samples were sourced from various zoos, rescue centers and temple elephants around India. Bovine positive and negative reference sera were from Animal and Plant Health Agency (APHA), UK. Cloning and Expression of Recombinant ESAT- 6: CFP-10 Fusion Protein Coding sequence of ESAT-6 and CFP-10 of M. tuberculosis was synthesized as fusion gene construct (GenScript, USA). The gene construct was cloned into prokaryotic expression vector pET28a (Novagen) and the

plasmid clone was used to transform chemically competent E. coli BL21 DE3 cells (Invitrogen). The protein expression was induced in the E. coli clone using 1 mM IPTG for overnight at 25 C. The HIS6 tagged ESAT-6: CFP10 fusion protein was purified from the soluble fraction of the bacterial lysate using Ni–NTA agarose (immobilized metal affinity chromatography). Briefly, a 5 ml Ni–NTA agarose column was equilibrated with 10 column volumes of tris buffered saline (TBS) and the soluble fraction of the bacterial lysate was passed through the column and the column was washed with 20 column volumes of TBS containing 50 mM imidazole and the recombinant protein was eluted using 500 mM imidazole. The pooled protein fractions were dialyzed against PBS (pH 7.4) and purity of the protein was assessed using SDS-PAGE. The protein was identified in a western blot using anti-His antibody.

LPS removal from the purified TB antigens

LPS from recombinant fusion protein ESAT-6: CFP-10 was removed using Triton X100 as per the procedure [22]. Briefly, Triton X -100 was added to the protein sample to a final concentration of 1% (v/v) and incubated at 40C for 1 h with continuous mixing. The sample was centrifuged at 7500rpm for 10 min at 300C and the upper micellar phase was collected

without disturbing the LPS rich middle and lower phase. Triton X-100 was added again to the upper phase to a final concentration of 0.5% (v/v) and the remaining steps were repeated as mentioned above. Then, the recombinant protein was analyzed in SDS-PAGE and western blot.

Characterization of recombinant protein

The ESAT-6:CFP-10 fusion protein was characterized using reference culture positive and culture negative sera samples in immuno-blot. Identity of the protein was further established by LC–MS/MS analysis of the trypsin-treated protein sample.

Lateral Flow Assay (LFA) design and manufacture

LFA Design and Principle Immuno-chromatographic strip test was designed as a two-module system using the combination of recombinant fusion protein, BoPPD and AvPPD. BoPPD was used in the test line 1 and the recombinant fusion protein was used in the test line 2of test module 1. Similarly, AvPPD and recombinant fusion protein was used in the test lines 1 and 2, respectively, of module 2. The conjugate pad of the assay kits contained gold conjugated protein G and streptavidin. The protein G could bind to antibodies from wide range of animals. The immune

complex migrated along the membrane due to the capillary activity and the TB specific antibodies were trapped by the proteins in the test lines 1 and 2. Streptavidin gold was trapped by the biotin in the control line. Colour development in the test line 1 of the module 1 or test line 2 (either of the modules) indicated the presence of antibodies against pathogenic Mycobacteria whereas the development of colour in test line 1 of the module 2 indicated the presence of antibodies against environmental non-pathogenic Mycobacteria (Fig. 1).

Gold conjugation of protein G antibodies and streptavidin

Gold conjugation of the protein G and streptavidin was performed using gold chloride (Sigma Aldrich, USA) as per the method described [28]. Briefly, the proteins were mixed with 50- nm gold solution and pH of the solution was adjusted to 7.2 with 50 mM potassium carbonate (pH 9.6) to achieve a final concentration of 15 lg/ml. The unbound protein G from the gold conjugate solution was removed by washing with 15% bovine serum albumin solution. The gold coupled proteins were resuspended in 2% BSA in 10 mM sodium carbonate (pH 9.6) and stored in a refrigerator until further use. The Protein G-coupled gold particle was diluted in dye

dilution buffer containing 1% casein and 100 mM sodium phosphate (pH 7). The diluted gold solution was spread onto conjugation pad presoaked in pretreatment buffer containing 1% NP-40, 0mMEDTA, 0.25% L- 7600, 1% polyvinylpyrrolidone, 10 mM sodium phosphate and 0.1% sodium azide (pH 7.0); dried in a lyophilizer; stored in a low-humidity room until further use.

LFA Lamination assembly and manufacture

The assay membrane and pads were cut as 4.2 mm wide and 60 mm long composite strips using MDI strip cutter Model-M70 to fit in a plastic assay device that provides the metrics for even flow of analytes and reagent buffer. The strips were laminated on a 300 mm 960 mm plastic backing consists of a nitrocellulose detection strip in the middle, flanked at one end by a sample pad followed by conjugation pad and at the other end by an absorption pad. The completely laminated strips were cut into 4.2 mm-wide strips and were housed manually into plastic casing and packed in dehumidified room in an aluminum-plastic pouch with a desiccant to ensure the longevity of the product.

Validation of the assay kits

Diagnostic Sensitivity and Specificity of the Assay 17

samples from Sloth Bears with confirmed TB based on the postmortem

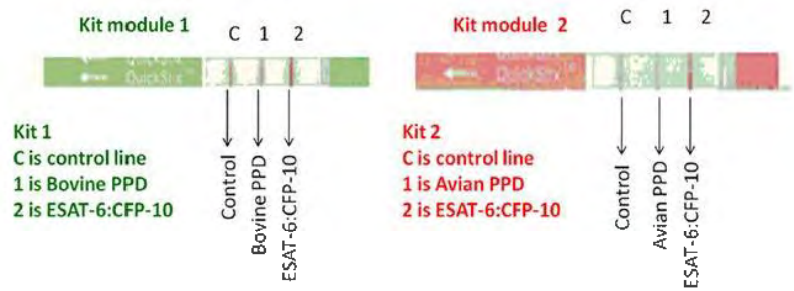

Fig. 1 LFA kit modules. In kit module 1, the test line 1 was printed with BoPPD and the test line 2 was printed with recombinant ESAT6:CFP10. Whereas in kit module 2, the test line 1 was printed with AvPPD and the test line 2 was printed with recombinant ESAT6:CFP10

lesions and acid-fast staining which were tuberculosis specific were used for the study. Diagnostic sensitivity of the assay was determined using these known positive sera samples of Sloth Bears (N = 17). Considering the difficulty in determining the TB infection status in wild animals using other tests such as skin test or IGRA, TB positive and negative bovine sera samples were used to estimate the diagnostic sensitivity and specificity of the assay. For this purpose, 200 cattle sera were obtained from APHA, UK (100 from skin-test positive cattle with culture confirmed *M. bovis* infection and 100 from TB-

free animals). The cattle sera samples were designated negative on the basis of IFN c release assay (IGRA) and skin test results. Additional Sera Samples from Sloth bears and other Wild Animals. Sloth Bear sera samples (n = 74, including the 17 from animals with confirmed TB, referred to in the previous paragraph) were from Wildlife SOS, India and 48 sera samples from other wild animals such as Elephants (n = 9), Felidae (n = 21), Cervidae (n = 14), Cape Buffaloes(n = 2), Wild dog (n = 1) an d Wild Bear (n = 1) were collected from captive animals housed in various zoological parks and temples in India. 17 of those sloth bears died later due to TB as described above. The remaining 57 sloth bear sera samples were from in-contact animals with unknown disease status. The other wild animals were with unknown status for TB infection.

Screening of the study sera samples and interpretation

Sera samples and the LFA kits were brought to room temperature and a drop of sera sample (approximately 5 ll) was drawn using a disposable dropper. The sample was placed onto the sample pad (middle of sample well 'S') by holding the dropper vertically and avoiding air bubbles. Additionally, two drops of sample buffer (PBS) was added to the sample well to enable sample flow, through the

membrane. The results were read in the results window after 10 min. Sera samples with positive line in either of the test lines (T1 and T2) of test kit 1 or both the test lines in test kit 1 was considered as seropositive against tuberculosis. Positive line for the recombinant fusion protein was the indication of seropositivity against pathogenic Mycobacteria. Development of positive line only for the AvPPD (Kit2—T1) was interpreted as sero-reactivity against non-tuberculous mycobacteria. However, for the routine testing positive line only for T1 of kit 1 (BoPPD) warrants the use of kit 2.

Batch quality control for assembled LFA

Every batch of the kit was checked for its performance using known positive and negative sera.

Batch signal strength

The signal strength of the device was considered sufficient when apparently visible lines developed with 5 ll of known positive sera.

Batch reactivity

Aliquots of positive and negative reference sera samples of Sloth Bear (n = 4), Elephant sera samples (n = 4) and bovine sera samples (n = 4) were stored in-20 Cand each

batch of the LFA kits were tested with these reference sera samples.

Batch specificity

Cross reactivity of every batch of LFA kits were tested with reference sera samples of other animal diseases such as Infectious Bovine Rhinotrachitis, Foot and Mouth Disease, Leptospirosis and PPR.

Ethics statement

The study did not involve any experimental animal usage. The study was conducted using the sera samples available in the repository of Wildlife SOS, India. Testing of bovine sera samples were performed at APHA, UK using the samples available in their repository.

Results

Cloning and expression of recombinant protein

The genetically fused coding sequences of ESAT6 and CFP 10 were cloned into prokaryotic expression vector and the ESAT6::CFP10 fusion protein was expressed as double His6 tagged protein and the positive clones were selected based on the identification of 28 kDa protein band in the immuno-blot using anti-His antibody. The recombinant protein was

purified using Ni–NTA agarose column and the purified protein was verified by SDS-PAGE followed by staining with Coomassie brilliant blue. Protein estimation was done using BCA kit and the protein was aliquoted and stored at -80 C. The protein yield was approximately 20 mg/lt of shake flask culture.

Characterization of recombinant protein

Each batch of the ESAT6:CFP10 recombinant protein was verified in SDS-PAGE for purity and the protein was also subjected to immune-blot using anti-His antibody, TB positive cattle sera and TB negative cattle sera. The immunoblots using anti-His antibody and TB positive reference sera produced a protein band of *28 kDa as shown in Supplementary Fig. I and II whereas the TB negative reference sera did not react with the recombinant protein. In the MALDI TOF analysis, the fusion protein had 100% amino acid identity with the available ESAT-6 and CFP-10 amino acid sequences of M. tuberculosis and M. bovis.

Kit assembly and batch release criteria

Line drawing and LFA kit assembly was carried out as mentioned in the methods section. Batch quality of the kits was assessed using reference positive and negative sera

samples. The following parameters were assessed for batch clearance: 1. Reference positive sera developed lines with the recombinant fusion protein and BoPPD (Fig. 2 Panel A); 2. Reference culture negative sera developed only the control lines (Fig. 2 Panel B); 3. Rabbit hyper-immune sera against PPD of M. avium developed specific line with AvPPD (Fig. 2 Panel C). Additionally, a visible control line should appear in all the tests. All the batches of the LFA kits were cleared based on the above-mentioned batch release criteria.

Interpretation of result using the LFA Kits

Reactivity of the kit was verified against sera samples from various wildlife species as mentioned in the materials and methods sections. Antibody responses against the recombinant fusion protein, BoPPD and AvPPD were assessed using the kit. Reactivity of the sera samples against the recombinant fusion protein or BoPPD or both was considered positive test result in the LFA. Reactivity against AvPPD alone in the absence of any positive lines against ESAT6:CFP10 and BoPPD was classified as antibody response against non-pathogenic Mycobacteria.

Application of the kit for cattle samples

To benchmark test performance, particularly in respect to

specificity, which is very challenging to determine in wildlife species without samples from confirmed uninfected animals, we tested well characterized samples from GB cattle (100 skin test positive cattle with culture-confirmed bovine TB and 100 from TB-free cattle). The results of these experiments are presented in Tables 1 and 2. Applying the interpretation criteria as stipulated above, we estimated test sensitivity and specificity to be 81% (71.93–88.16% at 95% CI) and 90% (82.38–95.10% at 95% CI), respectively in cattle (Tables 1 and 2).

Diagnostic sensitivity in wildlife species

Given the constraints of working with wildlife and the low number of samples typically available for any study, the present kit was evaluated using a total of 74 Sloth Bear sera samples and 48 sera samples from other wildlife species. 17 out of the 74 Sloth Bears were confirmed for tuberculosis based on postmortem granuloma lesions and acid fast bacillias shown in Fig. 3. The postmortem granuloma samples were also confirmed in PCR for Mycobacteria. These 17 Sloth Bear sera were considered as known positive samples to estimate diagnostic sensitivity of the rapid wild TB kit. 16/17 of these sera samples gave positive responses using the LFA kit which indicated 94% diagnostic sensitivity (71.31–99.85%

at 95% CI; Fig. 4 - I). We next assessed the complete set of 122 wildlife samples including the 17 samples described in the previous paragraph. The reactivity of these wild animal sera samples (n = 122) against recombinant fusion protein, BoPPD and AvPPD is provided in Fig. 5. Twenty seven out of the 57 live-in- contact Sloth bear sera samples were positive using the LFA kit indicating 47.4% (Fig. 4 - II) positivity for TB. This experiment was conducted in a Bear rescue center where TB is prevalent. None of these in-contact sloth bears showed any obvious symptoms of TB. However, 27 of these animals were diagnosed positive by the kit. These animals were followed subsequently and seven of these positive animals (7 out of 27) died so far. All these seven animals showed typical TB granulomas subsequently confirmed in the lab test also) during postmortem examination indicating that the kit detected these asymptomatic TB positive animals. Sera samples from Elephants (n = 9), Felidae (n = 21), Cervidae (n = 14), Cape buffaloes (n = 2), Wild bear (n= 1) and Wild dog (n = 1) were also tested and the seropositivity in these animals was 25%. Reactivity of individual antigens in the LFA kit for the sera samples from Sloth bears (n = 74), Elephants (n = 9), Felidae (n = 21), Cervidae (n = 14), Cape buffaloes (n = 2), Wild bear (n = 1) and Wild dog (n = 1) were presented in Table 3.

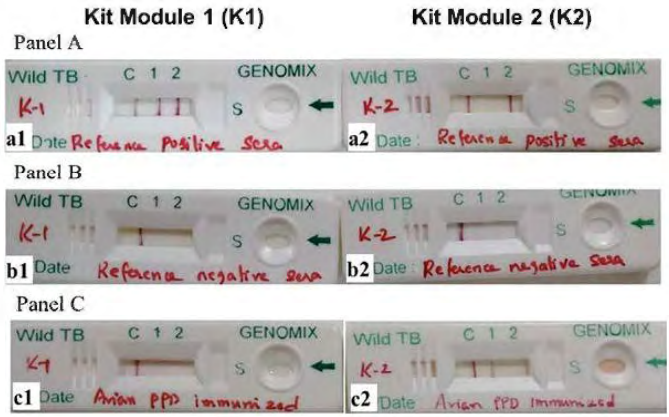

Fig. 2 Panel A: a1: Reference positive sera in Kit1 developed positive lines for Fusion protein and BoPPD. a2.: Reference positive sera in Kit2 developed positive line only in Fusion protein. b1 and b2: Reference negative sera developed only control lines. c1 and c2: Avian PPD immunized sera developed only control lines in Kit1 and developed line for Avian PPD in Kit2 Indian J Microbiol 123

Table 1 Interpretation of results and result summary of known positive cattle sera using the LFA kit

S. no.	Fusion protein	BoPPD	AvPPD	Interpretation	Number of reactive sera/total TB positive cattle sera	Conclusion based on the LFA kit
1	Positive line	Positive line	Positive line	TB sero-positive	15 out of 100	TB seropositive (81 out of 100 samples)
2	Positive line	Positive line	Negative line		15 out of 100	
3	Negative line	Positive line	Negative line		41 out of 100	
4	Positive line	Negative line	Negative line		0 out of 100	
5	Positive line	Negative line	Positive line		2 out of 100	
6	Negative line	Positive line	Positive line	TB sero-positive/inconclusive	8 out of 100	
7	Negative line	Negative line	Positive line	TB sero-negative (positive for environmental mycobacteria)	3 out of 100	TB seronegative (19 out of 100 samples)
8	Negative line	Negative line	Negative line	TB sero-negative	16 out of 100	

The sera samples (n = 100) were from cattle which turned positive in skin test and IGRA. The estimated sensitivity of the LFA kit was 81% (71.93–88.16% at 95% CI) in cattle

Table 2 Interpretation of results and result summary of known negative cattle sera using the LFA kit

S. no	Fusion protein	BoPPD	AvPPD	Interpretation	Number of reactive sera/total TB negative cattle sera	Conclusion based on the LFA kit
	Positive line	Positive line	Positive line	TB sero-positive		TB sero-positive (10 out of 100 samples)
2	Positive line	Positive line	Negative line		1 out 100	
3	Negative line	Positive line	Negative line		3 out 100	
4	Positive line	Negative line	Negative line		3 out 100	
5	Positive line	Negative line	Positive line		2 out 100	
6	Negative line	Positive line	Positive line	TB sero-positive/Bacillus issue	1 out 100	
7	Negative line	Negative line	Positive line	TB sero-negative positive for environmental mycobacteria	6 out of 100	TB sero-negative (90 out 100 samples)
8	Negative line	Negative line	Negative line	TB sero-negative	84 out of 100	

The sera samples (n = 100) were from cattle which turned negative on skin test and IGRA. The estimated specificity of the LFA kit was 90% (82.38–95.10% at 95% CI) in cattle

19.67% of all samples tested (n = 122) produced positive lines for the recombinant ESAT6:CFP10 fusion protein as well as BoPPD.

1. 18.85% were reactive against one of these two proteins in the LFA.
2. 4.91% of samples were reactive against all the three proteins in the test (ESAT6:CFP10, BoPPD and AvPPD).
3. One of the negative wild animal samples (negative by the kit of the present study) produced visible line for AvPPD alone (one out of 66 negative samples).

Therefore, these animals were seropositive against pathogenic mycobacteria (Sr. No. 1 and 2 of the above combination), environmental mycobacteria (Sr. No. 4) and a combination of pathogenic and non-pathogenic mycobacteria (Sr. No. 3). Apart from the above-mentioned combination of results, other interesting combinations of sero-reactivity were also observed. Two of the positive samples were reactive

against the BoPPD and AvPPD with no visible line for the fusion protein. Similarly, one of the positive samples produced visible lines for the fusion protein and AvPPD.

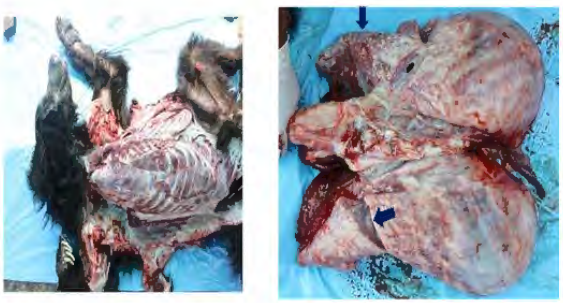

Fig. 3 Postmortem confirmation of TB specific granuloma lesions in Sloth Bear lungs. Arrows indicate the pustular lesions

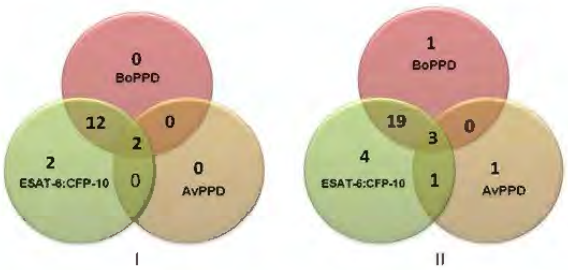

Fig. 4 Venn diagram depicting the reactivity of Sloth Bear sera samples against BoPPD, ESAT- 6::CFP-10 and AvPPD using the LFA kit. I: Sera from TB positive sloth bear (n = 17). The LFA kit had 94% diagnostic sensitivity (71.31–99.85% at 95% CI); II: sera from in-contact Sloth Bear (n = 57)

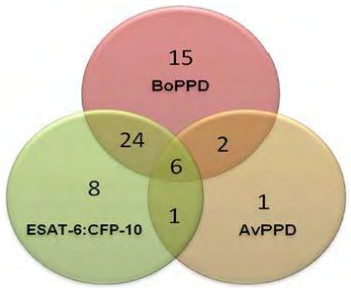

Fig. 5 Venn diagram depicting the reactivity towards BoPPD, ESAT-6::CFP-10, AvPPD using the LFA kit for all the wild animals sera samples (n = 122) of the present study.

Based on the above results, it was decided that the Kit 1 (with BoPPD and recombinant protein) was to be used routinely for the sero-diagnosis of TB. The Kit 2 would be used whenever the kit 1 produced visible line against BoPPD alone which was 12.29% of our study samples.

Advantage of using combination of TB Specific Proteins over single proteins

Using both the fusion protein and BoPPD, sensitivity of the kit had increased considerably. 41.07% of the positive sera (or 18.85% of the total wild animal sera samples tested) produced line for only one of the two antigens.

1. 26.78% of the positive sera had produced line for PPD of M. bovis alone.
2. 14.28% of the positive sera had produced line for

recombinant ESAT6:CFP10 fusion protein alone.

Specificity of the diagnostic method was assessed using AvPPD. Only two out of 124 wild animal sera generated positive lines both in BoPPD and AvPPD without any line development for the fusion protein. Cross reactivity with the environmental Mycobacteria is not ruled out in these animals. In such cases, the test may be repeated after some time using fresh sera and the diagnostic decision can be arrived based on the reactivity against ESAT6:CFP10. Additionally, the diagnostic decision can be based on the disease prevalence and local rules of the country wherein the requirement of 'ruling out' or 'ruling in' of TB in wild animals can determine the diagnostic decision.

Table 3 Interpretation of results and result summary of sera samples from wild animals (n = 122) using the LFA kit

S. no	Fusion protein	BoPPD	AvPPD	Interpretation	Number of reactive serum/total positive sera[a] (percentage within positive sera[a])	Number of non-reactive serum/total negative sera[a] (percentage within negative sera[a])	Overall percentage out of 122 samples (%)
1	Positive line	Positive line	Positive line	TB sero-positive	6/56 (10.71%)		4.91
2	Positive line	Positive line	Negative line		24/56 (42.85%)		19.67
3	Negative line	Positive line	Negative line		15/56 (26.78%)		12.29
4	Positive line	Negative line	Negative line		8/56 (14.28%)		6.55
5	Positive line	Negative line	Positive line		1/56 (1.78%)		0.81
6	Negative line	Positive line	Positive line	TB sero-positive/ Inconclusive	2/56 (3.57%)		1.63
7	Negative line	Negative line	Positive line	TB sero-negative (positive for environmental mycobacteria)		1/66 (1.51%)	0.81
8	Negative line	Negative line	Negative line	TB sero-negative		65/66 (98.48)	53.27

[a]The sera samples were declared positive and negative based on the present LFA kit

Discussion

India is endemic for tuberculosis and the disease is prevalent in humans, domestic and wild animals. The populations of TB susceptible wild animals such as elephants, bears and boar are high in Asian and African countries. However, the data on TB epidemiology is limited in these countries with a severe effect on the management and control of TB [18, 29]. In developing countries both zoonosis and reverse zoonosis of TB are common; Mycobacterium species isolates from animals include M. tuberculosis and M. bovis in India and other developing countries [30–32]. M. tuberculosis also infects animals with similar virulence like M. bovis. The antigen complements of both organisms are almost completely identical. Thus, a test detecting M. bovis will also detect M. tuberculosis, i.e. bovine PPD response will also detect M. tuberculosis and human PPD will detect M. bovis infection. Indeed, in early bovine TB control programs for cattle, human PPD was used successfully. As both M. tuberculosis and M. bovis can infect animals including wildlife and cause severe pathogenesis a differential diagnosis between M. bovis and M. tuberculosis is not required nor is possible given the degree of antigen identity between these two pathogens. Wild- animals infected with either of the species are culled or segregated depending on the country's

law. Aim of the current kit is to identify animals which are sero-positive for pathogenic TB (which includes M. tuberculosis and M. bovis) and help in the zoo or wild-life authorities to make appropriate decision or to study the TB sero-prevalence of tuberculosis in wildlife. Additionally, differentiating the pathogenic Mycobateria from environmental Mycobateria (such as M. avium sub sp. avium) is more important in the context of animal infections. Genome wide sequence search of M. avium subsp. paratuberculosis and M. avium subsp. avium revealed absence of any sequences with similarity to ESAT 6 and CFP 10 and the proteins were useful for the specific diagnosis of tuberculous Mycobacteria [33]. These proteins or their peptides had been used in IGRAs for the specific diagnosis of Tuberculosis [23]. The degree of specificity in general of these two antigens is high and they are also the mainstay of TB diagnosis both in cattle and humans [34–36]. Thus, many of the FDA and OIE approved kits such as QuantiFERON and BOVIGAM 2G uses ESAT6 and CFP10 peptides due to their proven ability to differentiate pathogenic TB (M. tuberculosis and M. bovis) from environmental Mycobacteria and BCG [37]. Mycobacterial species like M. marinum or M. klansasii express almost identical homologues of ESAT6 and CFP 10 [38, 39]. However, infection with these Mycobacteria is rare (both in humans and

animals) and therefore, not considered to be major confounders. These two bacilli can also cause pathology and have zoonotic potential and removal of animals infected with these species will be advantageous. However, and most importantly, the homology (and therefore cross-reactivity) of M. bovis/M. tuberculosis ESAT6 and CFP10 compared to other more prevalent environmental Mycobacteria like M. avium sub sp. Avium or M. avium sub sp. paratuberculosis is low.

Tuberculin skin testing and IGRA are the common TB diagnostic methods in domestic animals and humans. However, point of care serology assays are more practical for TB diagnosis in wild animals. To improve the sensitivity of detecting intracellular bacteria such as TB using sero-diagnostic kits, cocktails of antigens are required [40, 41]. At the same time, cross reactivity in antibody response due to the exposure to environmental Mycobacteria should also be taken care of while selecting the antigens for serology [42].

In the present study, BoPPD was used for increasing the sensitivity of the assay and AvPPD was used to mitigate against the cross reactivity from environmental bacteria. Tuberculosis antigen recognition pattern varies from species to species in serological assays. ESAT 6 or ESAT 6:CFP 10 fusion proteins were the earliest and most frequently recognized antigens in elephants infected with tuberculosis.

The antibody response against the proteins was much earlier than the diagnosis by detecting the organism in trunk washes [2]. Though, MPB 83 and MPB 70 were the sero- dominant proteins in cattle and deer, ESAT 6 and CFP10 were also detected in majority of the tuberculosis infected animals [23, 34]. Moreover, the cross-reacting antibodies from other Mycobacteria can also detect MPB 83 and MPB 70 resulting in a reduction in specificity of the assay. Therefore, a ESAT6:CFP10 fusion protein was used in the current diagnostic kit to enhance the specificity of the assay.

Performance of the point of care immune-chromatographic strip test was assessed using sloth bear sera samples. 16 out of the 17 known positive sera samples turned positive in the LFA kit also. All the 16 samples were detected by the recombinant fusion protein and 27 of the in-contact sloth bear samples were detected by the protein. 6 out of 44 positive sloth bear sera samples reacted with AvPPD and all those six samples were positive for the fusion protein indicating the infection with pathogenic Mycobacteria. Therefore, the use of fusion protein aids in making the diagnostic decisions. To benchmark test performance against a larger number of animals with confirmed TB, the sensitivity and specificity of the kit was estimated using reference cattle sera (n = 100), which was lower (81%) than the estimate in sloth bears

although the small sample size of our sloth bear assessment resulted in large confidence intervals overlapping those estimated with the cattle sera. The sero- dominant TB proteins in cattle are MPB 83 and MPB 70, and current kit did not contain these proteins as an individually expressed protein. These proteins were not included in the current kit to avoid detecting the cross-reacting antibodies from environmental Mycobacteria.

A commonly encountered limitation of testing wildlife samples is the lack of samples from animals that are confirmed TB-free. Specificity estimates are therefore difficult to ascertain. To partially overcome this limitation, we have used cattle sera from TB-free animals. Thus, the specificity of the assay was determined using known negative cattle sera from TB-free herds in TB-non-endemic areas of GB. Further, freedom of infection of individual cattle was confirmed by negative tuberculin skin and interferon gamma test results. Using these TB-free cattle sera (n =100) the LFA kit showed 90% specificity. This is comparable to the data by Da Silva et al. [43] reported a sensitivity and specificity of 82 and 91% for their indirect ELISA using BCG as coating antigen. Similar kind of tuberculosis antibody detection kits were tested in wild animals earlier. DPP vet TB kit was tested in white tailed deer (sensitivity 65.1 and specificity 97.8%) [44] and South

American Camelids (sensitivity 74 and specificity 98%) [45]. Elephant TB STAT-PAK Kit was tested in Asian and African elephants and the reported sensitivity and specificity was 100% and 95–100%, respectively [3]. Similarly, Prima TB STAT- PAK kit was tested in Non-Human primates and the reported sensitivity and specificity was 90 and 99% respectively[46].

The major difference between the other LFA kits and the current kit is the combination of proteins present in the kit. The other kits uses MPB83 protein and ESAT6/CFP10 fusion protein. The kit which is described in the current manuscript uses ESAT6:CFP10 fusion protein and native Mycobacterial proteins (BoPPD and AvPPD). Inclusion of BoPPD in the kit had increased the sensitivity by 41% in cattle (Table 1). However, BoPPD can compromise the specificity of the kit and therefore, AvPPD was included in the kit to detect the cross reactivity (if any) from the environmental Mycobacteria. One out of 100 negative cattle (Table 2; Sr No. 6) showed BoPPD positive line which may be due to the cross reactivity of environmental mycobacteria. Considerable percentage of positive samples (41.07%) were reactive against either BoPPD or fusion protein alone. Sero-reactivity against tuberculosis varies between the species and use of multiple proteins as in MAPIA increased the sensitivity of detection.

Instead, BoPPD which is a mix of Mycobacterial proteins was used in the present kit for enhancing the sensitivity. 26.78% of positive samples were reactive against BoPPD alone. Use of kit II which contained AvPPD was recommended in those circumstances to rule out any cross reactivity with antibodies against environmental Mycobacteria. Two of the total 122 samples were reactive against BoPPD and AvPPD. These animals may be tested again to confirm the TB sero-positivity.

The kit was used in wide range of wild animals including Elephants (n = 9), Felidae (n = 21), Cervidae (n = 14), Cape buffalo (n = 2), Wild bear (n = 1) and Wild dogs (n = 1). Use of protein A and Protein G conjugate (instead of anti-species antibody) enabled the kit to be used universally for any of the wild species.

The results indicated that 47.4% of in-contact sloth bear samples which were tested using the kit turned sero-positive for tuberculosis. 25% of samples from other wild species were also positive by the test. This indicates the high prevalence of TB in Indian wild animals. The situation is further complicated by the extensive wild animal and human (animal handlers of captive wild animals and tribes in the periphery of the forest areas) as well as wild and domestic animal interactions. In India the disease is highly prevalent in

domestic animals and humans. These results emphases the need for systematic TB surveillance and control programs in India for wild animals. Sero-diagnosis of tuberculosis in wild animals was permitted in countries like USA and UK (OIE 2014), since the conventional TB diagnosis by culture/IGRA/skin testing have serious practical difficulties in implementing them in wild animals [47, 48]. For an efficient surveillance and management of Wild TB, rapid, accurate, affordable and reliable TB diagnostic kits are needed. The available commercial sero-diagnostic kit was licensed for use in few countries and the kit is for fewer species. Moreover, the cost of the kit is too high for developing countries like India. The current kit addresses some of these problems.

In the present study, a rapid TB sero-diagnostic kit which can detect antibodies due to pathogenic as well as environmental Mycobacteria infection in wild animals was developed and validated using the sera samples of wild animals. Using this kit, sero-diagnosis of TB in wild animals is enabled.

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Art – 157. ELECTROCARDIOGRAPHIC OUTLINE AND SERUM CONCENTRATION OF CLINICALLY IMPORTANT CARDIAC BIOMARKERS IN ANESTHETIZED INDIAN SLOTH BEARS *(Melursus ursinus)*

Swagat Mohapatra, **A. Sha. Arun,** Tiushar Jyotiranjan, Suchitra Sahoo and Akshaya Kumar Kundu

Abstract

The Indian Sloth bear (*Melursus ursinus ursinus*) can easily be recognized by its shaggy black coat, long muzzle, protruding lip and by a white V-shaped patch on the chest. There are no reference values available in the literature to interpret electrocardiogram and to detect abnormal levels of serum cardiac biomarkers while dealing with suspected case of cardiac abnormality in Indian sloth bear. Our study reports the electrocardiographic reference values, configuration of ECG waves and serum levels of clinically important cardiac biomarkers in the Indian sloth bears.

Electrocardiographic variables were measured in six healthy semi-captive Indian sloth bears (*Melursus ursinus ursinus*) tranquilized with a combination of Xylazine and Ketamine. The electrocardiograph was set with a paper peed

of 50 mm per sec and sensitivity of 20mm = 1mV and the electrocardiograms were recorded in bipolar limb lead II with the electrodes clamped into the skin over the olecranons and patellas. The electrocardiographic parameters along with morphologies of different waves of ECG were analyzed.

All sloth bears had sinus rhythm and three of them had respiratory sinus arrhythmia. The P waves in all electrocardiograms recorded above the baseline. ECG of some bears revealed wider P wave (n=2) and some had strikingly peaked P wave. The amplitude of P wave ranged between 0.05 mV and 0.15 mV while the P wave duration ranged from 0.03 to 0.06 sec. Q waves were absent in electrocardiograms of three bears while its amplitude in remaining electrocardiograms ranged from 0.1 to 0.2 mV. The QRS complex featured a positive configuration in all electrocardiograms with the amplitude of QRS complex ranged from 0.5 to 1 mV. Pronounced notching of QRS complex was seen in the electrocardiograms of two bears. The amplitude of S wave reported wide variation. All T waves recorded positive configuration with amplitudes ranging from 0.25 to 0.6 mV and duration ranging from 0.1 to 0.14 sec. The PR interval ranged from 0.12 sec to 0.28 sec, QT interval

ranged between 0.26 sec and 0.36 sec and RR interval ranged from 0.88 sec to 1.44 sec. The heart rate ranged between 41.66 beats per minute (bpm) and 68.18 bpm. The mean QRS electrical axis varied from 66 to 77 degrees. Clinically important serum cardiac biomarkers levels were estimated in our study. Serum concentrations of AST (range 103 - 130 U /L), LDH (range 693 - 785 U /L), Troponin - I (negative) and CRP (range 0.20 - 0.30 mg/L) were reported. The authors highly recommend the use of ECG during routine clinical examination in captive Indian sloth bears and infer that the wildlife clinicians will find this beneficial for detecting cardiac abnormalities in this rare wildlife species.

Art – 158. INFLUENCE OF AGE AND SEX ON THE ELECTROLYTE BALANCE OF SLOTH BEAR

(Melursus ursinus)

Arun A. Sha, S. Ilayaraja, K. Nithin, Sidharth Prasad Mishra and V. Sejian

Abstract

A study was conducted to assess the influence of age and sex on the electrolyte balance of sloth bears. Twenty-eight Sloth bears (*Melursus ursinus ursinus*) were used in the study from Bannerghatta Bear Rescue Centre of Wildlife SOS, Karnataka, India (12°48'9N; 77°34'9E) from December,2016 to August 2017. Arterial samples were collected from femoral artery of anaesthetized sloth bears by using lithium analyzer (IDEXX VetStat, USA). Among different electrolyte balance, the influence of age and sex was determined on the Na, K, and Cl. The pH was found to be almost same in the entire population with no significant ($p<0.05$) difference neither between different age groups nor with sex. Moreover, K concentration of the population was determined to be within the reference range of bears with no significance differences ($p<0.05$). Nevertheless, Na

and Cl were established to be influenced by age with lower concentration ($p<0.01$) between cubs, sub-adults and adults. However, sex factor did not influence these parameters which was evident from the non-significant difference between the males and females. The mean (_+S.D) of Na and Cl of sloth bear cubs was discovered to be 149.6 +_ 1.73 and 112.0 +_1.41 but with the advancement in age the level significantly reduced to 136.55+_5.27 and 105.00+_4.33 in adults respectively. This could be attributed to the fact that the needs of water and electrolyte requirements per unit body mass were very high after birth and its requirement slowly decreases with advancement in age. These findings may serve as baseline information and may be useful to develop and evaluate health profiles of sloth bears under different age groups for better diagnosis and management.

Art – 159. NON-INVASIVE MONITORING OF REPRODUCTIVE HORMONES IN CAPTIVE FEMALE SLOTH BEARS

Yaduraj Khadpekar, John Whiteman, Barbara Durrant, Megan Owen, Sant Prakash

Abstract

Studies on reproductive physiology of sloth bears *(Melursus ursinus)* including the timing and duration of oestrous cycles in females are limited. Normal levels and profiles of oestrogen and progesterone in cycling sloth bear females remain largely unknown. A study has been initiated by Wildlife SOS, San Diego Zoo Global and Dayalbagh Educational Institute to develop a non- invasive method to monitor reproductive hormones in rescued captive female sloth bears under rehabilitation at the Agra Bear Rescue Facility, Uttar Pradesh, India. Urine samples of unmated study bears (n=31) are non-invasively collected throughout the year from specially designed collection trenches in den floors and assayed for oestradiol and progesterone metabolites (standardized to creatinine concentration) by quantitative enzyme immunoassays (EIAs). Urinary hormone profiles are generated for each individual study bear. The physical changes in vulva appearance and visibility are also noted and

tracked throughout the year. Vulva appearance is then correlated with the hormone levels and profiles. Preliminary results show that the levels of urinary oestradiol metabolites in female sloth bears increase during mid-May to mid-July, and this increase occurs during a time period of high vulva visibility. Urinary progesterone metabolite analysis so far has indicated a visible rise in the levels of urinary progesterone metabolites in one female bear beginning 70 days after the end of oestrus. Sloth bears appear to have a single, seasonal oestrous period similar to most other bear species. Urinary hormone assays and data on visible changes in the vulva are currently under study. Profiling the oestrous cycle of the sloth bear and understanding the reproductive biology of this iconic species is our goal.

Art – 160. APPROACHES TO STUDYING BEHAVIOR IN CAPTIVE SLOTH BEARS THROUGH ANIMAL KEEPER FEEDBACK

Yaduraj Khadpekar, John Whiteman, Barbara Durrant, Megan Owen, Sant Prakash

Abstract

Animal keepers at zoos and wildlife rescue centres often possess in-depth knowledge of the health and behavior of the individuals under their care. While it is often not feasible for keepers to regularly collect behavior data through formal scientific methods, efforts should be made to find alternative means to capture this knowledge. We investigated the use of keeper feedback to study the behavior of sloth bears at the Agra Bear Rescue Facility (ABRF; Agra, India). We prepared a survey with 5 questions focused on behaviors indicative of playfulness, boldness, aggressiveness, and the tendency to express self- directed behaviors (SDB).We asked keepers to rate bears on a Likert scale from 1(least likely to exhibit a behavior) to 5 (most likely) for 44 adult female bears (5–21 years of age).We validated this method by comparing keeper ratings of SDB with formal behavior observations, finding that

time of day had an influence on the accuracy of keeper assessments. We found a significant negative correlation between housing bears in larger groups (>15) and SDB. In addition, we correlated ratings given by keepers for all study behaviors. Social play had significant negative correlation with aggression toward people. There was no correlation between social play and aggression toward other bears, possibly due to the existence of cohesive social groups in group housing or high dimensionality of the data. We found that keeper feedback is an efficient tool to gather behavior data on captive sloth bears and recommend its use in future studies.
Keywords: enrichment, self-directed behavior (SDB), social interactions, stereotypy, questionnaire

Introduction

Conservation management of species threatened with extinction is often hindered by a lack of basic biological knowledge, particularly for large carnivores (Karanth & Chellam, 2009). For species that have been rescued from inadequate or inhumane human care, this biological knowledge can also be used to ensure that rehabilitation strategies are optimized, welfare is maintained, and that

reproductive behavior is appropriately managed, especially when conservation breeding is relevant to the species' overall conservation strategy.

Zoos and wildlife facilities housing captive wild animals have a great opportunity to study the species under their care (Hutchins & Thompson, 2008; Kleiman, 1992). Some facilities, like wildlife rescue centers, may also hold large populations of rare and understudied species. This scenario presents a unique opportunity to robustly study various aspects of a species' biology, filling in critical knowledge gaps that may aid conservation management. However, most rescue centers do not have the scientific staff to manage and implement the formal collection of biological data. Thus, alternative methods for data collection must be developed, and animal care staff can play a valuable role in this process.

Animal keepers often possess in-depth knowledge of the health and behavior of individuals under their care. Indeed, there are examples where zoo keepers have been provided with a systematic framework with which to summarize their experiences and generate useful assessments and basic knowledge of species' behavior over time (Swaisgood et al., 2006), or to engage in research questions related to conservation breeding (Carlstead, Mellen, & Kleiman, 1999)

and welfare (Szokalski, Litchfield, & Foster, 2013; Whitham & Wielebnowski, 2013). It has been reported that data or behaviour ratings by observers who are familiar with the individual animals, such as their caretakers, tend to be reliable and valid (Gosling, 1998, 2001; Meagher, 2009). Keeper- centric data collection frameworks have taken a variety of forms, often tailored to the resources and the most pressing questions related to species management, and have been applied to a wide range of species including black rhinoceros (Diceros bicornis; Carlstead et al., 1999); cheetah (*Acinonyx jubatus*; Wielebnowski, 1999); gorilla (Gorilla gorilla gorilla; Less, Kuhar, Dennis, & Lukas, 2012); and Asian elephant (*Elephas maximus*; Duer, Tomasi, & Abramson, 2016). Robinson et al. (2016, 2017) demonstrated that keeper questionnaires can be a useful tool in collecting data on animal welfare and personality. These examples provide a strong theoretical framework for the value of keeper feedback in assessing animal behavior (King & Landau, 2003; Whitham & Wielebnowski, 2009). However, to our knowledge, there are no reports on the use of keeper data to study captive sloth bears.

The study ofanimal personality—i.e., between individual differences in behavior that persist through time (Carter,

Feeney, Marshall, Cowlishaw, & Heinsohn, 2013)—is gaining prominence in a range of conservation applications including breeding, maintaining optimal welfare and husbandry management, translocations, and for understanding the range of personalities present in and between populations (Razal, Pisacane, & Miller, 2016). For species with ranges that overlap extensively with human-habitation or activities, the role that personality plays in human-wildlife coexistence is especially important as it may influence post-release survival. For example, Bremner- Harrison, Prodohl, and Elwood (2004) found that for swift foxes *(Volpes volex)*, early death after translocation was positively correlated with bold personality types. Similarly, the study of behavioural syndrome, i.e., the correlation of particular personality traits is also relevant to conservation management. Both are particularly relevant to rewilding programs especially when human wildlife conflict can result in the loss of life or property (Merrick & Koprowski, 2017). Thus, developing the means by which to assess personality and related traits should be prioritized.

The Agra Bear Rescue Facility (ABRF) is located inside the Soor Sarovar Bird Sanctuary near Agra, India. As of April 2015, the ABRF housed 224 sloth bears rescued from

members of the Kalandar community who used them in traditional “bear dancing” (Seshamani & Satyanarayan, 1997) and from human- wildlife conflicts. Sloth bears are currently found in India, Nepal, and Sri Lanka (Yoganand, Rice, & Johnsingh, 2012), and were recently declared extirpated from Bangladesh (Islam et al., 2013). Although there is no reliable global estimate, their population is thought to be declining due to degradation and fragmentation of their habitat (Akhtar & Chauhan, 2008) and the species is vulnerable to extinction (IUCN, 2016). Captive populations of sloth bears are thus an important resource for developing knowledge of their biology and behavior, as conservation breeding for reintroduction (or other forms of translocation) may become an important component of their conservation.

While the ABRF provides access to a large number of bears for study, it should be noted that there are aspects of bear behavior and management typical of captive setting that are not consistent with those of free-ranging populations. For example, bears in captivity tend to exhibit repetitious self-directed behaviors (SDB) such as pacing, head swaying, paw-sucking, and masturbation (Anderson, Arun, & Jensen, 2010; Forthman et al., 1992) that are not typically observed in their wild counterparts. While sloth bears, like other bears species,

are considered to be solitary under free-ranging conditions (Laurie & Seidensticker, 1977), bears at the ABRF are housed socially. As social housing is common practice for a range of bear species at rescue centers and zoos around the world, the influence of captive social housing on well-being is of interest to the managers. For example, in captive polar bears, larger group sizes have been shown to be associated with low SDB (Shepherdson, Lewis, Carlstead, Bauman, & Perrin, 2013).

In India, keepers in zoos and other captive wildlife facilities play a pivotal role in routine animal care and management (Rajeev, Rajkamal, & Saseendran, 2008). At the ABRF, no keepers have scientific training; in fact, many of them are former bear handlers from the Kalandar community. Capitalizing on this relationship, we developed a systematic rating system for the keepers to evaluate the behavior of sloth bears in their care. Our primary goal in this study was to test the efficacy of this approach and we discuss potential implications for future behavioral research, including the assessment of personality or behavioral syndromes.

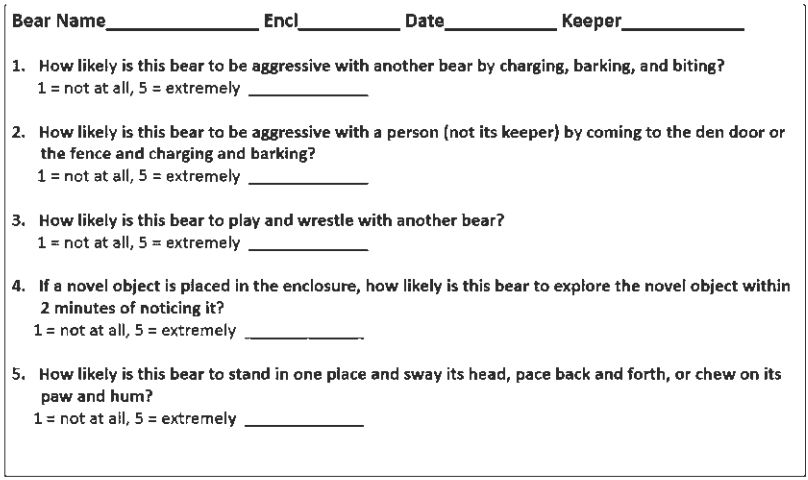
Materials and methods

The study was carried out from 5th April 2015 to 8th May 2015 at the ABRF, located inside the Soor Sarovar Bird

Sanctuary in the state of Uttar Pradesh, in India. It was conducted as a pilot study for a larger research project, for which adult, prime age female bears in good health were of primary interest. Thus our study subjects were 44 female bears ranging in age from 5 to 21 years (Mean = 14.69, SD = 3.34). The ABRF has 14 enclosures, each of which housed sloth bears in social groups of 2–30 depending on the size of enclosure. All but one group was mixed sex. Keepers selected for providing feedback had worked with the same group of bears in a single enclosure for 3–9 years (Mean = years) but they did not have any prior training in the systematic collection of Sloth bears behavior data.

At the start of the study, the primary keeper for each bear rated Multiple behavioural characteristics for each bear on a Likert scale, from 1 (least likely to exhibit the behavior) to 5 (most likely) (Figure 1). The rating system was designed to assess four aspects of individual bear behavior: a) aggressiveness toward other bears and people (Questions 1 and 2), b) playfulness (Questions 3), c) boldness (Question 4), and d) SDB (Question 5). The ethogram used for the questionnaire is shown in Table 1. The ratings recorded by the keepers for all behaviors were cross- correlated using a total of 10 ordinal logistic regression analyses (program R–package

MASS) to assess the relationships among those behaviors and thus to provide an initial metric of the utility of this approach for future study of personality or behavioral syndromes. To validate the accuracy of the keeper assessments, authors YK and JW conducted formal 30 min behavior observations on each bear.



Bear Name_____ **Encl**_____ **Date**_____ **Keeper**_____

1. **How likely is this bear to be aggressive with another bear by charging, barking, and biting?**
   1 = not at all, 5 = extremely _____
2. **How likely is this bear to be aggressive with a person (not its keeper) by coming to the den door or the fence and charging and barking?**
   1 = not at all, 5 = extremely _____
3. **How likely is this bear to play and wrestle with another bear?**
   1 = not at all, 5 = extremely _____
4. **If a novel object is placed in the enclosure, how likely is this bear to explore the novel object within 2 minutes of noticing it?**
   1 = not at all, 5 = extremely _____.
5. **How likely is this bear to stand in one place and sway its head, pace back and forth, or chew on its paw and hum?**
   1 = not at all, 5 = extremely _____

FIGURE 1: Questionnaire given to bear keepers at the Agra Bear Rescue Facility (ABRF), India (translated from Hindi to English).

Observations were conducted in the morning (10:30–12:00) and in the evening (16:45–18:30) relative to the daily feeding schedule, and to account for potential diurnal behavioral

variations. In their dens, bears were fed porridge (08:30–09:30), fruits (12:00– 13:00), porridge (16:00–17:00), and scattered small food items (e.g., nuts, dates, coconut pieces) in their yards as enrichment (17:30). Morning observations were timed to avoid food-oriented behaviors, but evening observations occasionally coincided with scattered food enrichment. YK and JW observed the bears with or without binoculars from outside the enclosure, the den hallway, or the roof of the dens. To compare author-and keeper-collected data, we used SDB because it is easily distinguished from other activities.

Behavior characteristic	Definition
Aggressive with another bear	Charging towards other bear, baring teeth, high pitched vocalization, biting, clawing. Injury is likely.
Aggressive with a person	Charging towards a person standing outside enclosure boundary or outside the den in the den hallway, with vocalization.
Play and wrestle with another bear	Physically interacting with another bear while mounting, rolling, wrestling with the bear. Injury is unlikely.
Exploring the novel object	Sniffing and physically manipulating the novel object.
Self-directed behavior	Standing in one place and swaying head, pacing back and forth, chewing on paw and humming.

TABLE: 1 Ethogram used for keeper questionnaire

Point-sampling (Altmann, 1974; Martin & Bateson, 1993) was done at 1-minute intervals to classify the bear into behavioral coding as: a) inactive, b) active and not engaging in SDB, or c) engaging in SDB. Of 44 bears for which keeper ratings were obtained, authors were not able to observe one particular bear either in the morning or in evening, while for another bear morning observations were not possible. For each of the remaining 42 bears, both morning and evening observations were conducted once each. The observations were conducted in compliance with the "Guidelines for the Use of Animals in Research" (Anonymous, 1992).

All statistical analyses were performed using program R (version 3.5.0; R Core Team, 2013). The proportion of all visible activity that was coded SDB was calculated for each bear for both the morning and evening observations. Binomial logistic regression analysis was carried out with the response variable of the observed proportion of SDB codings and the predictors of keeper SDB ratings, time of day, interaction between time of day and keeper SDB ratings, and group size. For this analysis, the time of day was categorized as morning and evening, and the group sizes were the ordered categories of small (0–5), medium (6–15), and large (>15).We also examined the relationship between the formal morning and

evening SDB codings across all bears, using linear regression. We evaluated the model fits by plotting residuals versus fitted values.

Results

The keeper SDB ratings were related with formal SDB codings. Pooling all data in the binomial logistic regression analysis, the keeper SDB ratings as predictor showed a significant correlation with formal SDB codings (odds ratio = 1.30, $z = 3.34$, $p < 0.001$). The regression coefficients and 95% confidence intervals for all regression analyses are presented in Table 2. The interaction between the keeper SDB ratings and observation timing showed a significant effect on the formal SDB

TABLE 2 Confidence Intervals (95%) for regression coefficients

Model type	Independent covariates	Dependent variable	Regression coefficient	Upper CI	Lower CI
Binomial Logistic regression	• Keeper SDB ratings	SDB codings	0.266	0.423	0.110
	• Time of observations[a]		−3.060	−2.213	−3.908
	Morning				
	• Keeper SDB rating * time of day		0.782	1.028	0.536
	• Group size[b]				
	Medium		0.109	0.415	−0.196
	Large		−0.394	−0.055	−0.734
Linear regression	• SDB codings in the evening	SDB codings in the morning	0.347	0.607	0.087
Ordinal logistic regression	• Playfulness with other bears	Aggression towards people	−0.785	-0.252	−1.317

[a]Categorical variable: Evening = reference category.
[b]Categorical variable: Small = reference category.

codings (odds ratio = 2.19, z=6.24, p < 0.001) confirming that the time of day played an important role in identifying the relationship between the formal SDB codings and the keeper ratings for SDB. Figure 2 depicts the results of morning and evening observation sessions. Authors' codings for SDBin the morning correlated positively with keeper ratings.

However, there was no correlation between the evening SDB codings and the keeper ratings. Despite this difference, the regression analysis between the morning and evening SDB codings yielded a significant correlation (odds ratio = 1.41, t=2.7, p = 0.01). The large group size (>15) had a significant negative relationship with the formal SDBcodings (odds ratio = 0.67, z = −2.27, p=0.02).

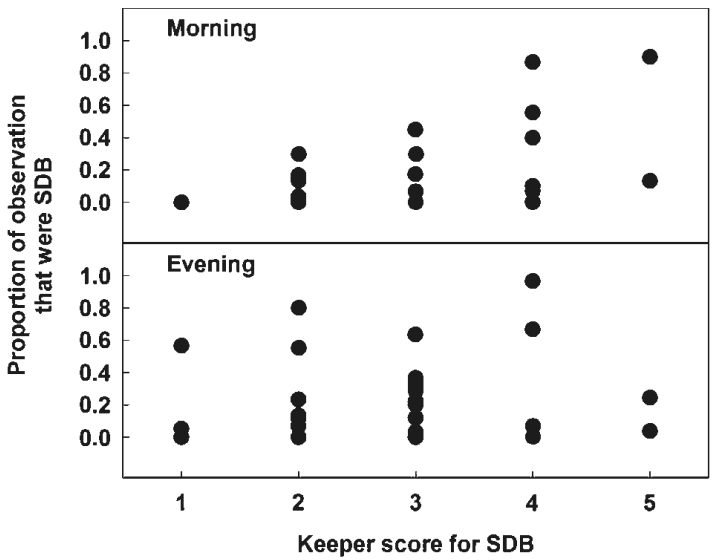


FIGURE 2: Comparison between the keeper's ratings and codings from formal behavioral observations for self-directed behaviours (SDB) in the morning and evening.

Authors' observations for SDB in the morning correlated positively with keeper ratings. However, there was no correlation for the evening SDB observations. Binomial logistic regression analysis indicated a significant correlation of keeper SDB ratings as a predictor, with the formal SDB codings. However, the interaction between the keeper SDB ratings and observation timing showed a significant effect on the formal SDB codings confirming that the time of day played an important role in identifying the relationship between the formal SDB codings and the keeper ratings for SDB We also used cross-correlation among keeper ratings for all behaviors as a preliminary assessment of the utility of this approach for future studies of personality or behavioral syndromes with rescued sloth bears. In Figure 3, a negative correlation indicates that bears that were more playful with other bears in their enclosure were less aggressive toward people. Ordinal logistic regression model applied to these two behavior variables supported the finding that social play had significant effect on aggression toward people (odds ratio = 0.46, t = −2.89, p= 0.004). We found no significant

correlations among the rest of the behavior categories rated by keepers (Figure 4A through 4I). This significant finding may be useful for future assessment of release candidates in translocation or rewilding programs.

Discussion

The divergent results from the comparison between keeper ratings for SDB and formal morning and evening codings partially validate

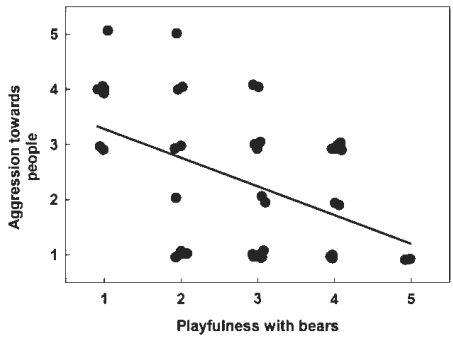


Figure 3 A negative correlation was found between playfulness toward other bears and aggression toward people. Ordinal logistic regression model indicated a significant effect of social play on aggression toward people. Data points are slightly offset for visual clarity. Linear regression line is shown only to illustrate the trend, not for statistical purposes

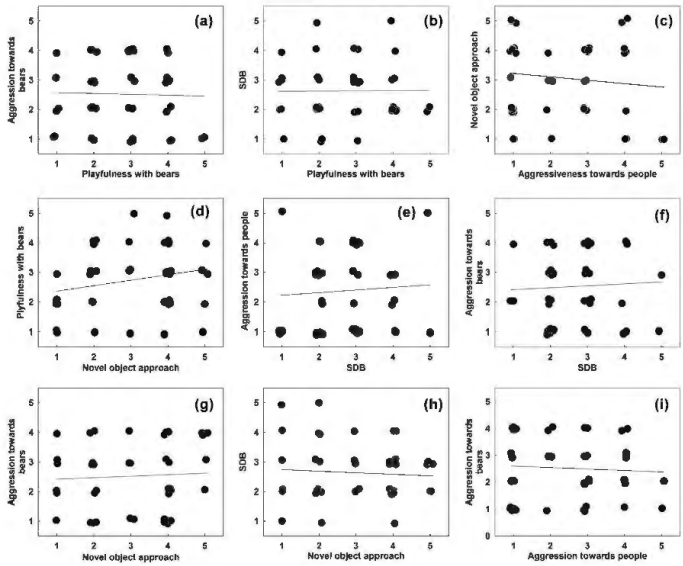


Figure 4 Comparison of keeper ratings for captive sloth bear behaviors. No clear relationship was observed among these behaviors. Data points are slightly offset for visual clarity. Linear regression lines are shown only to illustrate trends, not for statistical purposes.

the use of keeper ratings but also provide insight into environmental factors that may influence results. In the evening, bears receive a scatter feed that is timed with some variability. Such feeding is known to decrease the expression of SDB in bears (Anderson et al., 2010; Bauer, Babitz, Boedeker, & Hellmuth, 2013; Carlstead, Seidensticker, & Baldwin, 1991; Fischbacher & Schmid, 1999; Gupta, Sinha, & Prakash, 2001). Thus, it is likely that food enrichment at least

partially explains the low proportion of SDB observed by researchers in the evening for some bears with high SDB keeper ratings. It should be noted, however, that not all bears observed by the authors showed a lower level of SDB than was recorded by keepers. In fact, some bears demonstrated higher levels of SDB in author codings. Our current analysis cannot account for this discrepancy. This may be due to the fact that behavior data for each bear was collected only from its single primary keeper, and we would suggest garnering ratings from multiple keepers when feasible to make for more robust ratings. For example, in the personality research, it has been recommended to collect the behavior data from multiple observers and combining those data, to give more reliable results on personality assessment (Block, 1961; Gosling, 2001). Although for each bear we selected the keeper that we believed had the best knowledge of that bear, collecting data from multiple keepers may have possibly explained this discrepancy. It is also important to note that although the comparison between morning and evening SDB codings showed a significant correlation, the predictive power of the variables was considered weak due to the high variation in the data points possibly resulting from the effect of scattered feeding in the evening. This analysis thus merits further

investigation.

SDB in captive wild animals is generally considered awelfare concern rather than a part of personality (Mason, 1991; Mason & Latham, 2004). However, in their review, Ljichi, Collins, and Elwood (2013) found broad evidence that suggests that some personality types (i.e., reactive individuals) may be more inclined to cope with stress through SDB. Because the "bear dancing" trade, from which these bears are rescued and brought to ABRF, involves conditions of cruelty (both physical and psychological), restriction of movement and natural behavior (Seshamani & Satyanarayan, 1997) we know that all bears rescued and residing in the ABRF experienced a significant amount of stress prior to their arrival, and we have documented variable, but often severe SDB in this population. However, there are inter- and intra-individual variations in the rate of change in exhibition of SDB during the rehabilitation process at ABRF, in spite of following uniform management practices for all bears (personal observation). Thus, while the expression of SDB may reflect a reactive coping style, we emphasize that this current work does not represent a formal exploration of personality in this species. The keepers from whom behavior ratings were collected had known the individual bears for a number of

years. Comparing keeper ratings for different behaviors thus gave us an insight into the general behavior and personality trends in this captive sloth bears population, through the keepers' assessment of individual bears under their charge. The expression of negative relationship between playfulness with other bears and aggression towards people is a particularly interesting finding given the sloth bear's reputation for being prone to aggression when confronted with humans in the wild (Bargali, Akhtar, & Chauhan, 2005; Dharaiya, 2009; Sharp et al., 2017), a characteristic that challenges the successful translocation or rewilding of sloth bears. Additionally, that social playfulness is a common characteristic in this population is somewhat surprising given the generally solitary nature of the species (Laurie & Seidensticker, 1977). However, such behavior may be attributed to long-term captivity, group housing, and the social flexibility that has been documented in a range of bear species. Previous studies of other naturally solitary bear species have indicated that group housing and social interaction in captivity provide opportunities for physical activity which may substitute for foraging and may reduce the prevalence of SDB (Ottewell, 2016; Shepherdson et al., 2013). Our finding that the group size of >15 had a negative

relationship with the SDB, is consistent with these observations. Although little is known about socialization among sloth bears in captivity, they may experience benefits from group housing.

The lack of significant relationships among other behaviors is may be due to the fact that we designed our keeper survey to ensure that keepers could reliably interpret the behavioral categories, resulting in broad, mutually exclusive behavior questions. In essence, each trait that was rated was representative of different personality trait (e.g., boldness, playfulness, etc.) and thus unlikely to be correlated unless part of a behavioral syndrome. Another possible explanation may be related to the existence of cohesive social groups within the entire group housed together. For example, there was no relationship between the playfulness and aggression of the bears towards other bears in the group. During discussions with keepers, they mentioned that some bears were very playful with a few particular bears while being aggressive toward the other bears in the group. The existence of such individual relationships may have obscured any correlation between playfulness or aggression and other behaviors. Another possibility is that, the nature of data gathered through these

broad behavior trait questions was highly dimensional (i.e., representing potentially unrelated traits) thus resulting in the lack of such correlations, as demonstrated with aggression and fear in dogs (Duffy, Hsu, & Serpell, 2008).

Conclusions

Long term monitoring of behavior is essential for scientific studies as well as for husbandry management of captive wildlife (Watters, Margulis, & Atsalis, 2009). Our data suggest that such behaviour monitoring can be integrated into routine husbandry practices by utilizing keeper knowledge and observations. This is particularly relevant for sloth bears, as a very little research has been done on their captive behavior despite being a commonly exhibited bear in zoos worldwide.

The aim of this study was to reliably gather generalized behaviour data on group-housed sloth bears using keeper ratings and to assess the potential utility of keeper knowledge in behavior studies. In spite of the lack of scientific training, keeper ratings and comments correlated with researcher observations. In addition, keeper familiarity with individual bear interactions helped explain the unexpected lack of correlation between the aggression and playfulness with

other bears in the group. We conclude that keeper feedback is an efficient tool to gather behavior data on captive sloth bears.

Acknowledgments

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Art – 161. PROTECTED CONTACT AND FREE CONTACT – ARE THEY MUTUALLY EXCLUSIVE?

Yaduraj Khadpekar, Gochalan E., M. Kamalanathan, Ilayaraja S., Arun A. Sha, Baijuraj M.V., Kartick Satyanarayan, Geeta Seshamani

Abstract

Elephant Conservation and Care Centre (ECCC) at Mathura, Uttar Pradesh, India is run and managed by Wildlife SOS, a charity wildlife conservation and welfare organization in India. Captive elephants, both males and females, from different backgrounds such as begging, circuses, temples that are in bad shape and need retirement and intensive care, are rescued, transported and rehabilitated at ECCC.

The efficient management practice for captive elephants - Free Contact or Protected Contact – has been a topic of much discussion and debate around the world. In a country like India, where the captive elephants have been an important part of culture and history for centuries, it has always been, and still majorly is, a Free Contact handling and management. At ECCC, we introduced Protected Contact management for the first time in the country! Over the years, we gradually limited the Free Contact handling to specific situations and requirements. Currently we work with both management systems. All the elephants get trained for Protected Contact,

but also have free contact with the staff as and when it's necessary or more efficient. With this presentation, we explain and discuss the simultaneous use of both management practices at ECCC.

Art – 162. AN ATTEMPT TO ESTABLISH BASELINE DATA OF FECAL GLUCOCORTICOID METABOLITES OF CAPTIVE SLOTH BEAR (*Melursus ursinus)* FOR PRACTICAL APPLICATIONS AND INTERPRETATION

Tista Joseph, Sneha Mishra, **Ilayaraja Selvaraj**, **Attur Shanmugam Arun**, Nagalingam R. Sundaresan

Abstract

Fecal cortisol levels are considered as a reliable non- invasive biomarker to assess the physiological (endocrine) stressors in wildlife. Sloth bear *(Melursus ursinus ursinus)* fecal samples at the Wildlife SOS, Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park, Karnataka, India (12 degrees 48'N; 77 degrees 34'E) were analyzed using the ELISA technique to obtain a reference range for the species. The geographical location of the Bear Rescue Centre is within the natural habitat of free- ranging wild sloth bears. All the captive sloth bears sampled were maintained under the same husbandry and nutrition practices. Sampling was done between 1100 hrs to 13:00 hrs., post-feeding, considering the diurnal patterns of secretion of hormones. A methanol-based extraction was carried out followed by sample preservation at -20ºC until ELISA. This study has kept in mind the difficulties

faced in sampling and sample storage in field areas and has used field-friendly methods for both. The results of fecal cortisol levels were analyzed based on sex [10 (M):11 (F)] and estrus (n=5). The mean fecal cortisol level was 144.73 ng/g of feces in males, 156.34 ng/g feces in females not in estrus and ng/g of feces in estrous females. We conclude that the fecal cortisol level was significantly higher in non-estrus females than in male sloth bears ($p<0.05$), whereas fecal cortisol levels in estrous females were 1.5 to 3 times higher than non-estrous female sloth bears($p<0.05$), indicating increased stress during estrus. We further intend to study the effects of estrus, nutrition, age, disease, and environmental stress on fecal cortisol along with a comparison of captive to free-ranging wild sloth bear stress levels to conclude the sensitivity, specificity, precision, and accuracy of the protocol. With an increased focus on captive animal welfare and man-induced stress on free ranging wildlife populations, this baseline set for non-invasive monitoring of adrenocortical activity in sloth bears will be useful for a more efficient management of captive and rescued sloth bears.

Art – 163. INCIDENCE OF CLAW DISORDERS IN GERIATRIC CAPTIVE SLOTH BEARS *(Melursus ursinus)* AND ITS MANAGEMENT

Rashmi S. Gokhale, **Arun A. Sha, S. Ilayaraja**, **Pushpendra Kumar Singh, and M. V. Sharma.**

Abstract

The present study describes the occurrence and management of claw disorders in geriatric captive sloth bears of more than 16 years age at Agra Bear Rescue Facility, Wildlife SOS. Sloth bears *(Melursus ursinus)*, family Ursidae are insectivores found mostly in southern part of India. Their diet in the wild consists of insects, fruits, honey, carrion and certain vegetations. Bears have 5 digits on each limb and each digit has a claw. Sloth bears have long, curved front claws that are typically about 7cm long which help them break open terminate mounds and climb trees. Hind claws are 3cm in length approximately. Average lifespan of sloth bears in captivity is 25 years. Claw disorders are a collection of structural abnormalities of claw such as onychia, onychodystrophy, onchychogryposis, oycholysis etc.

Objective of the study was to identify claw abnormalities in ages sloth bears implement management practices to prevent

secondary traumatic wounds and stress caused due to the same. The study was conducted at Agra Bear Rescue Facility, Wildlife SOS, Agra, UP. Total 08 bears having same environmental and nutritional provisions of more than 16 years age with claw deformities and recurrent secondary traumatic wounds were selected for the present study. History of the selected bears were collected, and field observations were performed for the duration of one year. 04 out of 08 bears had secondary traumatic wounds specifically in foreclaws, claw wound healing took 20-30 days for complete granulation and epithelization, but new nail growth was rarely seen. Sterile swab culture of claw wound was used to rule out infectious cause and colonies of Salmonella sp., Shigella sp., and E. coli were found to be contaminating colonies. Common symptoms such as recurrent inflammation, irregular keratinization, excessive claw curvature, and ventral cavitations were observed in rescued captive sloth bears of Agra Bear Rescue Facility. These old bears had shown limited field accessibility, reduced instinctive behavior of nail scratching, rarely shown behavior of digging holes as of young and adult bears and four out of eight bears have abnormal gait with arthritis.

Preventive management practices such as periodic claw trimming by positive reinforcement, typical fly repellents in

cavitated claws, providing suitable muddy substrate to avoid paw abrasions and giving special enrichments to promote were and tear of claws has been carried out.

Claw deformities were seen in bears more than 16 years of age and proved to be an age-related phenomenon. Severity of the disorder was specific to health of the individual bear. Success of preventing secondary traumatic claw wounds was dependent on management practices followed. Onchetomy (declawing) was mandatory to prevent pain and retrograde infection. Deformed claw was concluded as a good indicator of age in captive sloth bears and periodic claw trimming was the key management for the same.

Keywords: bear, claw disorders, geriatric, *Melursus ursinus*, onychetomy

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Art – 164. BODY CONDITION SCORING IN CAPTIVE SLOTH BEARS *(Melursus ursinus)*

Laura Maillard, **Arun A. Sha**, Michelle Mousel, **Simone Ayoob**

Abstract

The exaggerated size of the sloth bears owing to their shaggy coat acts as a challenging factor in assessing body condition of captive sloth bears. A five-point scoring system from one to five of both visual and palpable body conditions was developed at the Bannerghatta Bear Rescue Center of Wildlife SOS. The score system ranged from one to thin, two for underweight, three for ideal, four for overweight and five for obese. The primary parameters taken into consideration for visual scoring was the appearance of the neck, abdomen, limbs and rump, while the palpable score was based on the prominence of the scapular spine, pelvis and ribs. Visual body condition scores assigned to all 85 sloth bears housed at the center in autumn 2015 and 2016, and spring 2015. The population was divided into age groups: cubs 0-2, juveniles 3-4, adults 5-20 and geriatrics 21-25. The effect of season, age and gender on body condition score was evaluated.

Significant relationships were found between season and

BCS, and between age and BCS. Gender had no effect on BCS. With the increased incidence of diseased, overweight and obese sloth bears in captivity, a scoring system will allow veterinarians to keep a constant tab on their physical condition and welfare. Further studies to be carried out on the relationship of the score to diet, gender, season, disease conditions and various age groups.

Art – 165. STRESS MANAGEMENT IN CAPTIVE WILD ANIMALS

A.S. Arun, V. Sejian, M. Bagath, G. Krishnan, C. Devaraj, V.P. Rashamol and R. Bhatta.

Introduction

Animals housed in artificial habitats are confronted by a wide range of potentially provocative environmental challenges. These include abiotic, environmental sources of stress such as artificial lighting, exposure to loud or aversive sound, arousing odors, and uncomfortable temperatures or substrates. In addition, confinement-specific stressors such as res tricked movement, reduced retreat space, forced proximity to humans, reduced feeding opportunities, maintenance in abnormal social groups, and other restrictions of behavioral opportunity. All captive wild species experience a release from most of the dangers with which their wild conspecific struggle. Their lives in captivity ensure them year-round stable supplies of quality food and water, veterinary care, protection against predators and hostile conspecifics, shelter against non-optimal climate conditions. Some are even ensured stable artificial species-specific environments and a number of other more or less obviously needs. In spite of all the improvements, most of the

health risks in captivity nowadays seem to be related to chronic, husbandry-related stress (Clubb and Mason, 2007). These include both abiotic, environmental sources of stress such as artificial lighting, exposure to loud or aversive sounds, arousing odors, uncalm fortable temperatures or substrates, and confinement specific stressors such as restricted movement, reduced retreat space, forced proximity to humans and similar restrictions of behavioural opportunities (Morgan and Tromborg, 2007). The captive environment will inevitably have both positive and negative effects on captive animals. This chapter will identify and describe some of the types of stress and stressors animals are affected by in the captive environment, and their consequences for the affected animals. Further, this chapter will address animal's coping mechanisms and a few management plans to decrease or avoid stress in captive animals.

Stress and Stress Response

According to Moberg (2000) stress is a response towards a stressor. This response is an evolutionary survival- mechanism the individual establishes in order to survive threats or direct danger with the goal to re-establish its homeostasis. Stress response includes three elements: (1) A stressor: An event, internal or external, which holds a reel or potential

threat against maintenance of the individual's homeostasis; (2) A stress-response: All behavioural or physiological responses, caused by the interaction between the individual and the stressor, in order to re-establish homeostasis and (3) The consequence: A change in the individual's biological function. There are four types of stress the captive animals are subjected to: This include acute stress (few seconds), intermittent stress (no constant intensity), long term stress (less than a week) and chronic stress (constant).

Sources of stress in captivity

Some stressors in captivity are highly visible: unstable hierarchical groups, forced proximity to humans and deprivation of species needs. But a lot of stressors are less obvious since stress-responses can be a sign of weakness. The following are some of the common stressors that captive animals are subjected to: (1) Abiotic Environmental Stressors - Sound, light, odours, temperature and substrate; (2) Confinement specific stressors - restricted movement, absence of retreat space, forced proximity to humans, restricted feeding and foraging opportunities and abnormal species-relevant social grouping.

Measurement of stress in captivity

By measuring stress in captivity, it is important to pay attention to both behavioural and physiological reactions. Since many physiological stress-responses are rather much alike, no matter if they are a result of positive or negative stress, it is important to compare the results of for instance corticosterone measurements with the behaviour prior to the measurement. Unfortunately, the measure of stress is potentially stressful for all animals. This paradox is caused by the fact that stress traditionally is measured by taking blood samples. Inevitably, the chase, restrain and handling associated with the collection of such a sample can as such result in elevated glucocorticoid levels in the blood stream of the animal (Bonier, et al., 2004).

In order to avoid such deleterious effects of data on stress in captivity, some techniques have been developed to detect stress-responses in a non-invasive way. Some indicators on behavioural disorders, have proven to be useful to measure stress just as, well as or even better than, the traditional physiological ones (Manning and Dawkins, 1998). The predominant advantage of using behavioural indicators to measure stress is, that it can be done without physiological damage, stressful handling or even without the animal being aware, of its being surveyed. One of the big challenges by this measurement is of course to find accurate descriptions of

normal behaviour in order to have a reliable comparative baseline. The most obvious way to measure stress in a non-invasive behavioural way, is to survey the animals without human presence. This can for instance be done by monitoring the animal with recordable TY equipment. Such recordings have a wide variety of pros, compared to direct observations. The most normal methods include observations of: undisturbed behaviours, specific periods or behavioural patterns and specific behavioural tests.

One of the most striking characteristics of the physiological response to stress is the release of glucocorticoids (Moberg, 2000). Detection of e.g. elevated glucocorticoids in a blood or urine sample is therefore an extremely valid method to measure stress (Moreira, et al., 2007). However, blood collection procedure itself Alternative non- invasive measuring methods are fortunately under constant development. Such methods can be used to monitor levels of reproductive and stress related hormones both in captivity and in the wild (Bonier, et al., 2004).

The excretion of metabolized blood steroids into feces permits the monitoring of physiological functions without disturbance of the animals. A new non-invasive method of obtaining blood samples has been developed (Stadler, et al. 2007). This revolutionary method relies on the "kissing bug"

(Dipetalogaster maxima), that pierces the skin of its host, releasing pain-reducing substances, and feeds on the host's blood. The host will barely never notice its temporary parasite, which enables the kissing bug to collect as much as 3.8 ml blood, before it discharges itself from the host (Stadler, et al.,2007). The blood obtained by the bug can now be collected by a syringe foranalysis.

Needs of wild animals in captivity

When thinking about the welfare of captive wild animals, we can look to see if their basic needs, also known as the "Five Freedoms", are being met. At the very least, captive establishments have a duty to ensure these basic needs are fulfilled. Also, having some control over their environment and the freedom to make choices is essential to the well- being of animals, and if these elements are lacking this will lead to poor welfare.

Freedom from hunger and thirst

All captive animals should have access to sufficient clean drinking water at all times, and must be given food of a quantity, quality and type that is appropriate to their species and will keep them healthy.

Freedom from discomfort

The substrate that animals are given is a vital component of their overall welfare. All animals should be provided with areas of soft substrate (e.g. grass, sand, mud) and soft bedding (e.g. hay, straw) if appropriate to the species. Enclosures should be well drained and should not become waterlogged. Animals should not be housed on concrete or wire mesh floors. If animals are living on hard or wire mesh floors, their welfare is immediately compromised. Wire mesh floors can cause discomfort, pain, infection and injury. Hard surfaces such as concrete can be uncomfortable or physically damaging to animals and are inherently boring. Concrete also increases the thermal load animals experience, by radiating heat in hot weather. Animals need sufficient shelter from the rain and shade from the sun, and there needs to be enough shelter for every individual in an enclosure. They need to be able to keep warm or cool, depending on the ambient conditions. Animals should be kept in a climate that is similar to that to which they are adapted to. Animals should not be subjected to temperatures that will cause them discomfort, for example Arctic animals should never be kept in tropical climates. Animals should never be chained, as chains can cause serious discomfort.

Freedom from pain, injury or disease

All animals should be handled and treated as gently as possible at all times. Enclosures and their interiors should not pose any dangers to the animals. Enclosures and fixtures should be hygienic and not harbor disease. Visitors should not be able to feed animals, as this may cause them harm. Animals should receive good veterinary care. injured animals should never be on display and should receive appropriate medical care away from visitor view.

Freedom to express normal behavior

Wild animals need the space and opportunity to engage in their natural behaviours. Animals who climb in the will need to be able to climb. Runners need to be able to run. Swimmers need to be able to swim. Diggers and burrow need to be able to dig. Animals who live in trees need trees. The list goes on. These things should not be considered as extras, but as basic needs of the animals. Food should always be fed in a way that encourages the animals' natural foraging behaviours. In the wild, many animals will spend several hours a day travelling, often over vast distances. Sometimes to find food, water and resting places, but they also seem to roam for reasons we do not fully understand. Roaming and moving around not only keeps their minds active, it also helps to exercise their bodies

and keep them fit. Animals in captivity need space to roam. For some animals, such as polar bears and elephants, who roam over vast distances in the wild, it is very difficult to provide them with the space they need in captivity to keep happy and healthy. Animals should also be housed in appropriate social groups. For example, social primates, such as macaques, should never be housed alone. One of the cruelest things we can do to a social animal like a macaque is to keep him or her on their own. Animals should never be chained, as this denies them of even their basic need to move freely. Animals need something to do. As zoo enclosures can only provide fairly static environments, it is essential that environmental enrichment programmers, which stimulate all of the animals' senses, are in place for all animals, to allow them to express their natural behaviours. Structures and objects must be added to enclosures for the animals to explore. The animals can be given challenges to sort out- for example food can be hidden or put in hard-to-reach places or puzzle feeders. Such environmental enrichment needs to be changed regularly, as animals will quickly become bored with the same old toys, furniture and challenges and they will no longer be stimulating. Token feeding sessions can be considered a form of enrichment, but only if they occur at different times each day. Token feeding sessions that take place at the same time each

day simply become a part of the animals' day to day routine and are no longer enriching. It's important to remember that they best enclosures shouldn't actually need environmental enrichment, as they should already provide complex, stimulating environments. However, in most cases in today's zoos the animals will definitely benefit from environmental enrichment.

Freedom from fear and distress

In the wild animals need places to hide- to avoid predators, to look after their young and to rest safely. They need them perhaps even more in captivity, to escape from the constant view of visitors when they want to, and from all the unnatural sights, smells and sounds of a zoo full of visitors. There needs to be enough private areas for each individual to retreat comfortably. When animals can't escape to a private place, they may become stressed. Similarly, animals need to be able to retreat from each other and even from each other's view. Less dominant animals need to be able to retreat from dominant individuals, to avoid stress and conflict, which may result in unnaturally high levels of aggression and injury. Unnatural aggression between animals, which may result in serious injuries, is far more likely to be seen in situations where animals cannot retreat from each other. Animals should not be

housed in noisy areas, for example near to speakers playing music or amusement rides, as this may distress them. Visitors should not be able to tease and harass the animals in any way. Any staff taking care of animals needs to be respectful towards every animal in their care, to avoid causing them fear and distress. Animals should not be subjected to any activities that may cause them fear and distress, such as photography sessions or being made to perform unnatural tricks, which may both best stressful for them.

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Art – 166. INGESTED FOREIGN BODY (THORN): PRIMARY CAUSE OF DEATH IN A WILD PYTHON

(Python moluru smolurus)

Ilayaraja S, Mukul CG, Acharya PR, Pradeep R and **Arun A. Sha**

Abstract

A wild Indian rock python *(Python molurus molurus)* was rescued by Wildlife SOS rescue team at Agra district, Uttar Pradesh, condition of python was debilitated with external parasitic infestation and bleeding from mouth. The oral cavity examination revealed stomatitis with blood clots and broken tooth. The radiographic examination of the head not revealed any fracture on skull. Though all the necessary treatment has been carried out to stabilize the condition the animal succumbed to death after a month. The detail post- mortem examination revealed presence of acacia thorn which pierced the Gastro-intestinal tract and penetrating the liver. The liver was swollen, congested and blood clots were found at the site of penetration.

Keywords: Python *(Python molurus)*, Acacia Thorn, Foreign body, Bleeding, Stomatitis.

Introduction

The light-colored, largest Indian rock pythons found in tropical and sub-tropical areas of Southern Asia (ITIS, 2009). This snake species is native to India, Pakistan, Sri Lanka, Myanmar, Southern Nepal, Southern China, Thailand, Laos, Vietnam, Cambodia, Peninsula, Malaysia and Indonesia (McDiarmid et al., 1999). P. molurus molurus is listed as Lower Risk/Near Threatened by International Union for the Conservation of Nature and Natural Resources (IUCN, 1994, 1996), enlisted in U.S. ESA (United States Endangered Species Act) as endangered throughout its range (Coborn, 1991; De Vosjoli, 1991; Jurgen et al., 1988; Murphy and Henderson, 1997). Pythons are non-venomous snakes. They are general omnivorous (Grzimek, 1975), but could be entirely carnivorous depending on their environment (Bogart, 1974). In forests, they feed indiscriminately on reptiles, birds and small mammals such as Leo pardus, wild pigs *(Susscrofa)* and antelopes *(Hippotragus equines)* (Grzimek, 1975; Bogart, 1974). When around human habitations, pythons never hesitate to prey on cats, dogs, domestic pigs and on rare occasion humans (Bogart, 1974). The ritual hunting for flesh (IUCN, 1996; Jurgen et al., 1988), international skin trading (Mukherjee, 1982; Tikader, 1983; Groomridge and Wright, 1982; Murthy, 1979), habitat loss, road kill mortality, killing

due to conflict in agricultural fields and around water bodies due to its larger size; due to misidentification and confusion with venomous species Russell's Viper which shared almost same kind of habitat (indiansnak.org), these are the main threats that resulted in an alarmingly decline of natural populations. The present case study was based on the post-mortem finding of a foreign body; ingested acacia thorn was the cause of death in a rescued debilitated python. The authors took effort to document this experience to create the awareness among the wildlife veterinary neophytes.

Case History, Diagnosis and Treatment

An adult debilitated python *(Python molurus)* with bleeding mouth (Fig 1) and external parasitic infestation was rescued by wildlife SOS rescue team and brought to wildlife hospital. The python was 5kg and 9feet in length. On clinical examination it was found that the animal was having stomatitis (Fig 2), with broken tooth and blood clots in oral cavity. Rest of the body was normal, and no injury was noticed. Radiography of head was performed as suspected for head injury, but radio graph not revealed any fracture neither in skull bone nor in jawbone (Fig 3).

The oral cavity was gently opened to remove the blood clots and broken tooth. Diluted chlorhexidine mouth wash solution

was used to rinse the oral cavity. The ticks were removed manually by using forceps. The animal was fed with two eggs after fixing feeding tube (Fig 4). Styptic solution (Inj-Revici, Kee Pharma Ltd) was sprayed inside the oral cavity to stop bleeding. To manage the pain and prevent the further infection Inj. meloxicam at 0.1mg/kg and Inj enrofloxacin eat 5mg/kg was given intramuscularly. In order to avoid hypothermia, the snake was exposed to sunlight in day hours and kept in a wooden observation box with bedding material, electric bulb (100 watts) used as heat source in night hours. However, the bleeding from mouth stopped, python was not showing much improvement in activity. Feeding was repeated with 2 eggs and de wormed with Fenbendazole at 50 mg/ kg orally on 15^{th} day. In spite of all the care and treatment the python died on 30th day.

Fig 1: Bleeding from Phthon's mouth.

Fig 2: Inflamed oral cavity of Python.

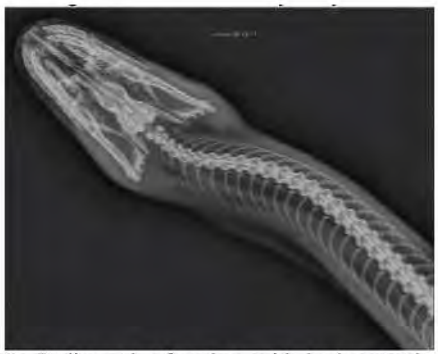

Fig 3: Radiograph of python with broken tooth and normal skull bones.

Fig 4: Feeding the python with eggs after fixing feeding tube.

Discussions

Post-mortem examination of the python revealed congested oral cavity, 6cm long acacia thorn was found in the Gastro-intestinal tract which pierced the esophageal wall and penetrated the liver (Fig 5). The liver was swollen, and blood clots were noticed at the site of penetration (Fig 6). Foreign bodies in gastrointestinal tracts are common findings in reptiles and it documented well in green iguanas (Anderson, 1992). Gastro-intestinal tract pathologies revealed major accidental findings in large sea turtles. Di Bello et al. (2006) reported a case of intestinal obstruction in the Loggerhead sea turtle (*Caretta caretta*). Oros et al. (2005) also mentioned gastrointestinal tract disease such as perforations and obstructions in sea turtle due to ingested foreign bodies at Postmortem. These foreign objects are either often derived from the natural environment or human-related and cause

different clinical signs depending on the shape and size of material (Kik and Nickel, 2001; Büker et al., 2010). Ingestion of bedding material, especially sand, gravel and shavings, may cause gastrointestinal obstruction in pet reptiles (Rahal et al., 1998). The affected animals may suffer with depression, anorexia and vomiting (Anderson, 1992; Wellehan and Gunkel, 2004). Diagnostic imaging like x-ray, ultrasound, computed tomography (CT) and endoscopy should be combined to reliably diagnose the localization of the foreign body (Hernandez-Divers, 2001; Schumacher and Toal, 2001; Banzato, 2013). Gastroscopy is another diagnostic option to locate and even remove foreign bodies resting in the stomach (Lumeij and Happe, 1985). If removal via gastroscopy is not possible, the foreign bodies have to be removed via celiotomy (Mitchell and Diaz- Figueroa, 2005; Büker et al., 2010). It is noteworthy to mention that snake uses sense of smell and taste to choose its prey (McKeown, 1996). Therefore, there could be some kind of smell that contaminates the foreign body and cause the snake to mistakenly swallow. Accidental swallow of a variety of foreign bodies such as stone, golf ball, heating pad, artificial chicken egg, cotton and plastic bottle have been reported in snakes (Smith, 1953; Jacobson et al., 1980; Zwart et al., 1986; Souza et al., 2004; Vasaruchapong and Chanhome, 2013). If the snake cannot pass the foreign

bodies via feces or regurgitation, total obstruction and death are the consequences (Vasaruchapong and Chanhome, 2013).

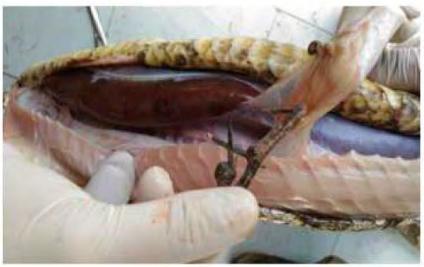

Fig 5: Acacia thorn in python's GI tract.

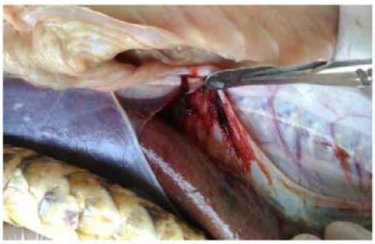

Fig 6: Bleeding from the inflamed liver at site of thorn penetration.

In our study the ingested foreign body was an acacia thorn with multiple sharp edges, thus caused the difficulties and more pain for the python to eliminate the same by normal peristalsis movement of its GI tract.

The sharp edges of the thorn repeatedly caused damage while it's migrated inside the GI tract which resulted in ulceration, bleeding, hematemesis and constant pain, that leads to severe debility with diminished activity as the python become anaemic, anorectic.

Conclusion

The authors concluded that an ingested acacia thorn foreign body is the primary cause for death of the victim as it caused severe trauma to the GI tract and liver.

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Art-167. SLOTH BEAR (*Melursus ursinus*) MATERNITY DENNING AT THE WILDLIFE SOS BANNERGHATTA BEAR RESCUE CENTRE, INDIA

A. S. Arun, T. R. Sharp, H. C. Pillai, S. Swaminathan, K. Satyanarayan & G. Seshamani

Abstract

Little is known about sloth bear *Melursus ursinus* birthing behaviour and denning. The Wildlife SOS Bannerghatta Bear Rescue Centre, India, which houses rescued bears, is visited by wild male sloth bears during the breeding season (April–July). Two female sloth bears have been impregnated by these wild males, giving researchers the opportunity to observe maternal behaviour closely. One female bear made use of an excavated a den to give birth and raise her cubs. A second bear gave birth in an excavated shallow cavity. Neither mother left the cubs for food or water for weeks (22 and 28 days, respectively). In the first case, the cubs eventually joined their mother leaving the den for food and water 20 days after the mother had initially left the den alone, whereas in the second case, the cub still had not joined the mother on outings for provisions after 68 days, when both were moved from the den area.

Keywords: Bannerghatta Bear Rescue Centre; cubs; dens; India; maternal; sloth bear; Wildlife SOS.

Introduction

Sloth bears *Melursus ursinus* occur in the warmer climates of the Indian subcontinent and do not hibernate (Ward & Kynaston, 1995; Akhtar et al., 2007). However, these bears make use of dens or shallow cavities for resting as well as for birthing and raising cubs in a defendable, secure environment. Dens can be either naturally occurring caves hollows or excavated. This document uses two terms to distinguish between den types used for different purposes. The term 'resting den' refers to a shelter where Sloth bears spend time after foraging. Usually, resting dens are used during daylight hours, as sloth bears are generally nocturnal. Likewise, the term 'maternity den' refers to a shelter used by a pregnant sloth bear to give birth and rear cubs.

When sloth bears make use of natural caves or hollows for maternity dens, they appear to choose them with extreme care. The bears appear to have a preference for caves with two or more cavities, especially in areas with predators such as Striped hyenas (*Hyaena hyaena)* and Leopards (*Panthera pardus)* that could potentially prey on cubs. The innermost den cavity is generally deep enough to make access difficult, even for the mother bear (Seshamani & Satyanarayan, 1997).

Little is known or has been published about sloth bear maternity dens and how they might differ from resting dens. Although sloth bear maternal denning behaviour has not been well documented or studied, some valuable work has been conducted. Joshi et al. (1999) used radio telemetry to investigate the maternal denning of wild Sloth bears. Between 1991 and 1993 five pregnant females were observed entering maternity dens. The pregnant bears excavated all the dens in the banks of dry streams facing the stream beds, and all den sites were obscured with dense vegetation. Joshi et al. (1999) were unable to determine precisely when females gave birth. However, the telemetry data indicated that females did not leave the dens for c. 2 months, apparently going without food or water during that period. When the mothers first exited the dens, they only left for short periods to get food and water before returning to the den. The cubs made their first sojourn from the dens with their mothers roughly 2 weeks after their mother had first left the den. Jacobi (1975), who worked with sloth bears at Amsterdam Zoo, the Netherlands, reported that two of three breeding females did not take food from day 3 to day 36 after parturition, and a third did not take food from day 7 to day 70.

This paper documents two cases of female sloth bears at the Bannerghatta Bear Rescue Centre (BBRC) in Bangalore, India, being impregnated by encroaching wild males, giving birth

and rearing cubs. The free-ranging area at BBRC is located on 26.3 ha of natural habitat within the range of the species. This unique situation of two female sloth bears becoming pregnant provided an unprecedented opportunity to document cubing behaviour. We will discuss what was discovered, what this information can tell us about the reproductive biology of the species in the wild and any implications for potential captive-breeding programs.

Bannerghatta Bear Rescue Centre

Wildlife SOS houses 77 sloth bears in a 26.3 ha free- ranging enclosure at the BBRC. The free-ranging enclosure allows for observation and documentation of sloth bear behaviour in a captive though somewhat natural setting. During the breeding season (April–July), Wildlife SOS researchers have observed wild male sloth bears venturing from the surrounding Bannerghatta National Park to the BBRC perimeter, apparently drawn by the scent of female bears in oestrus. The males leave definitive signs around the perimeter, including scat and footprints, and often knock over equipment. Camera traps set specifically to monitor wild bears approaching the Centre have captured several images, one of which shows a male bear on the moat/trench wall at the BBRC perimeter on 29 June 2014. Several wild male bears have managed to breach the perimeter and enter the BBRC

enclosure to mate with females before leaving the Centre and returning to the wild. It would appear that the wild bears are simply more capable of out manoeuvring the electrical fences and moats than the captive bears. Two such visits resulted in the impregnation of two female sloth bears (Bear 1-G and Bear 2-K; hereafter referred to as 1-G and 2-K). Although these pregnancies were unexpected and unanticipated, they provided the opportunity for close scientific observation of a little-known behavior in sloth bears.

All sloth bears at the BBRC were rescued from the 'dancing-bear' trade, poaching or other human–bear conflict situations. The BBRC neuters all male sloth bears that come into the facility. However, females at the BBRC are not spayed because of the invasive nature of that surgery and because all males are neutered. Bear 1-G and Bear 2- K both arrived at the BBRC after being removed from the dancing-bear trade. As such, they had been harvested illegally from the wild as cubs and had spent several years in captivity before being impregnated by the wild males at BBRC. Sloth bear cubs poached from the wild for the dancing bear trade are generally taken from dens and their mothers at c. 1–2 months of age. Because of this, the cubs have usually not spent enough time with their mothers to learn all the behaviours necessary for adulthood, which may impact their denning behaviour in later life.

Study area

The BBRC is part of the safari zoo at the Bannerghatta Biological Park and borders the relatively large Bannerghatta National Park (310 km^2) that is home to wild sloth bears, Leopards and Asian elephants *Elephas maximus* (Fig. 1). The BBRC habitat is characterized by boulders, rocks, trees and hills, which is similar to the landscape and habitat outside the Centre. A deep dry trench lined with smooth granite walls and electric fencing encircles the entire BBRC facility, which encloses the 26.3 has free ranging area.

Enclosure, husbandry and behaviour

The enclosure for Sloth bears at BBRC is a 26. 3 ha free-ranging area surrounded by electrical fencing and a dry moat (Fig. 2). Males (n = 43) and females (n = 34) occupy the free-ranging area together. Cubs that are less than 18 months old are kept in a separate, smaller area. Each individual bear has its own concrete den in which they are fed 7–9 kg of a millet porridge, along with two hard-boiled eggs, honey and vegetables twice a day. The bears are also fed fresh fruit once a day in the free-ranging area. The bears are usually only in their dens at feeding times. When the bears are not in their dens, they are free to roam around the whole area at will. Other than an occasional squabble over food, the bears appear to get along well with each other. Each bear receives a full

health examination once every 12 months (or more often in the case of a sick or injured animal). Forty different health parameters are checked.

Materials and Methods

Two pregnant females (1-G and 2-K), housed in the free-ranging area at the BBRC, provided the opportunity for researchers to observe and document Sloth bear breeding behaviour in a captive environment that is somewhat natural in its setting and where the mothers could select their own denning locations. Wildlife SOS's highest priority in the case of the breeding females at the BBRC is the health and well-being of individual bears, which meant that it was not possible to observe the denning bears without a degree of human interference. The keepers continually offered food and water to the females and called to them in an effort to coax them out of their dens. This was done because the keepers were worried about the health of the bears and this was the first-time cubs had been born in the BBRC.

Because of the sloth bear's dense fur and the fact that they do not exhibit obvious behavioural changes during pregnancy, direct observation is the only means for determining pregnancy at the BBRC, unless a pregnant bear is randomly given a physical. Wildlife SOS keepers monitor the behaviour and diet of all the sloth bears at the facility, and after

parturition both mothers and cubs were monitored closely. After 1-G gave birth, the den area was fenced off by BBRC workers so that the new family unit would not be negatively affected by the other 76 bears in the enclosure. This also allowed for food and water to be left at the den entrance and monitored each morning for consumption rates. Researchers also monitored sounds from within the den to ensure that the cubs were still alive. The den area 2-K selected could not be fenced off because of its location. However, the location and shallowness of the den meant that researchers could directly observe and monitor the female and cubs. After the mothers and cubs were removed from their respective den sites, researchers entered the sites to inspect them and gather information.

Results

Case study 1: Bear 1-G There were no behavioural indications that 1-G was pregnant until the day she gave birth. Although 1-G had a slightly depressed appetite during pregnancy (based on a later comparison of her normal consumption rate to

Fig. 1. Bannerghatta Bear Rescue Centre and Bannerghatta National Park, India.

that during the week before parturition), she continued to eat until 24 hours before giving birth. The only other physical characteristic indicative of pregnancy that she exhibited was the shine of her coat, which appeared sleek and shiny, unlike the dull appearance of the coats of non-pregnant bears. Bear 1-G made use of a den for birthing that had originally been excavated by a different female that had never been pregnant. When 1-G did not appear for her morning meal on 22 November, keepers checked the den site, as she had been observed spending more time in and around it. From the den's entrance, the keepers could hear 1-G and her cubs. After giving birth, 1-G stayed in the den without consuming food or water for 22 days, even though both were available at the entrance.

Fig. 2. Facility map of the free-ranging enclosure for Sloth bears (*Melursus ursinus)* **at Bannerghatta Bear Rescue Centre, India.**

After 22 days, she emerged alone, consumed some porridge (a nutritious gruel made from wheat/barley mixed with dates and eggs), drank roughly 2 litres of water and quickly returned to the cubs inside the den. She repeated this routine, leaving her cubs in the den, for an additional 20 days. Bear 1-G emerged from the den in the morning 42 days after giving birth, for the first time accompanied by two cubs. After all three consumed porridge

and water, they returned to the den. This routine continued twice a day (morning and evening) until day 89, at which point the entire family group was moved to a concrete den area. Once the cubs had been weaned from the mother, she was returned to the general free-ranging area. The cubs were then placed in the separated cub area until they were 18 months of age and could be released into the general free-ranging area along with the adults.

The maternity den used by 1-G has a single, elliptically shaped, south-east-facing entrance (Fig. 3). The two-chambered den opens into a main corridor, which has a low ceiling that widens progressively deeper into the den. The primary chamber is situated at the rear and appears to be where the cubs were delivered and nurtured. The secondary chamber, which is near the den's entrance, appears to have been used solely for waste, as urine and scat were found there (Table 1; Fig. 3). Based on what appeared to be recently moved dirt when the den was first inspected, 1-G likely modified the den after entering it.

Case study 2: Bear 2-K

As with 1-G, 2-K's pregnancy went undetected until the day she gave birth. The situation was identical: she behaved normally while pregnant other than a slightly depressed appetite, although she did eat within 24 hours of giving birth.

Like 1-G, her coat exhibited a noticeable sheen. Bear 2-K gave birth in a shallow cavity that she had dug on the side of the moat that surrounds the BBRC's free-ranging enclosure.

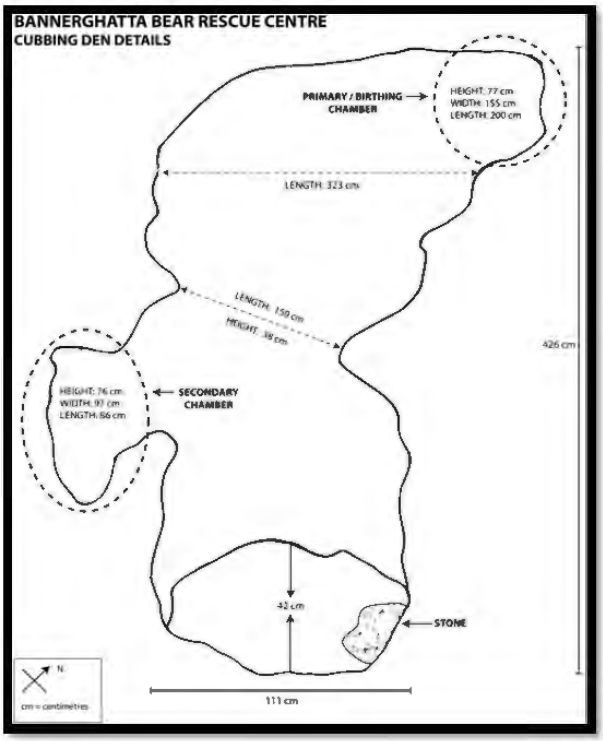


Fig. 3. Layout of maternity den for Sloth bear *(Melursus ursinus)* **(Bear 1-G) at Bannerghatta Bear Rescue Centre, India.**

Bear 2-K was not present for the 6 December morning feeding but was soon observed in the shallow cavity. The cavity's location and structure allowed for close visual observation of 2-K and her cubs but the area could not be fenced off from the

free-ranging enclosure. Bear 2-K remained with the cubs in the cavity for 27 consecutive days after giving birth without leaving and was observed grooming and positioning the cubs close to her (Plate 1). One cub died during a storm on day 16, when rains caused the banks of the cavity to cave in. From day 28 until day 68 (when the mother and surviving cub were moved from the den area by keepers to protect them from other bears in the free-ranging enclosure), 2-K left the den to eat and drink twice a day every day, always leaving her cub in the den cavity. The shallow den excavated and used by 2-K was 91 cm wide, 76 cm high at the entrance and 137 cm deep. The cavity was excavated 1.5 m below the main level of the enclosure on a slope facing the dry trench.

Discussion

Breeding and birthing season Sloth bears typically breed May through July in Nepal (Laurie & Seidensticker, 1977; Joshi, 1996) and India (Gopal, 1991), and in the state of Karnataka (Iswariah, unpubl.), where the BBRC is located. Laurie & Seidensticker (1977) suggested that breeding occurs at other times of the year. It has also been suggested that there is not a specific breeding period further south Sri Lanka (Phillips, 1984). However, Norris (1969) reported that cubs are born in the summer in Sri Lanka, suggesting that mating occurs in the winter. At the BBRC, which is in southern India,

wild male bears begin to arrive at the forest edge overlooking the 26.3 ha free ranging enclosure as early as April, indicating that females may already be in oestrus. Wild bears frequent the area through July, possibly meaning that the breeding season in the Bannerghatta National Park may run from April through July, slightly longer than previously reported.

Most cubs in Nepal and India are born November through January (Jacobi, 1975)

Table 1. Birthing den for Sloth bear Melursus ursinus (Bear 1-G) at Bannerghatta Bear Rescue Centre, India: dimensions given in centimeters (cm); N/A, not available.

AREA OF DEN	HEIGHT (cm)	LENGTH (cm)	WIDTH (cm)	NOTES
Entrance	43	N/A	112	elliptical shape, opening to the south east
Main chamber	38	427	152–320	
Secondary chamber	76	86	97	evidence of urine and faeces
Primary 'birthing' chamber	76	198	158	no nesting material

Plate 1. Sloth bear *(Melursus ursinus)* **(Bear 2-K) with cub in shallow cavity den at Bannerghatta Bear Rescue Centre, India. Wildlife SOS.**

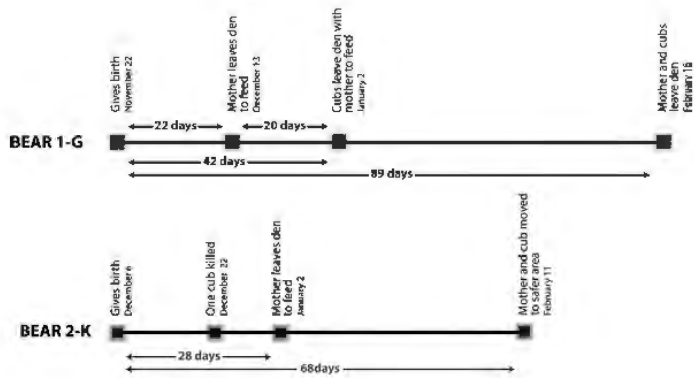
Laurie & Seidensticker, 1977; Joshi, 1996; Iswariah, unpubl.) after a period of delayed implantation (Puschmann et al., 1977). Cubs born in the BBRC were also born during that time period. Although 1-G and 2-K ate less than normal during their pregnancy, they were still eating enough to make a pregnancy determination difficult. The shiny coat that pregnant sloth bears exhibit, coupled with the depressed diet, could be indicators that help researchers identify pregnant females in the future. Birthing behaviour and timing Previous research suggests that female Sloth bears remain in their dens for 1– 2 months before coming out to drink or eat (Jacobi, 1975; Joshi et al., 1999), consistent with observations at the BBRC. Bear 2-K gave birth in a shallow cavity and left the den to eat and drink 28 days postpartum. Bear 1-G gave birth in a larger den and did not eat or drink for 22 days postpartum (Fig. 4).

There are two possible reasons that these two individuals at BBRC left their dens for food and water after slightly less than a month. First, these bears were in a protected environment where detection by conspecifics was not a threat. Second, keepers began calling 1-G by her name ('Gayathri') a few days after parturition to see if she could be drawn from the den and continued to do so until she finally emerged. This was done out of concern for the mother's health, as the keepers were not experienced in sloth bear cubing behavior and wanted to

ensure the well-being of the mother and cubs.

Bear 1-G's cubs did not emerge from the den until 42 days after birth and 20 days after their mother first emerged. Bear 2-K's cub, which was healthy and survived to adulthood, still had not joined her outings for food or drink after 68 days, when the pair were moved from the shallow cavity to a different denning area to protect them from other bears in the enclosure. The number of days that 2-K's cub did not leave the den was longer than usual based on observation of 1-G's cubs as well as the scant information available from other studies. The cubs that Joshi et al. (1999) observed were not thought to have left the den for the first time until 1–2 weeks after the mother initially emerged.

Data collected at the Wildlife SOS sloth bear rescue and rehabilitation facilities show that Sloth bear cubs, like those of other bear species, are born altricial. The cubs are deaf, or at least cannot hear very well, for 14– 16 days because the ear canal is blocked. Additionally, Sloth bear cubs do not open their eyes until 20–25 days after birth, rendering them blind for the first 3 weeks of life.



Maternity dens provide females and their cubs with protection from both the elements and predators (Oli et al., 1997; Linnell et al., 2000). Potential predators, such as Leopards and Tigers *(Panthera tigris)*, are not a concern for denning Sloth bears in the free-ranging enclosure at the BBRC; however, protection from the elements is still important. The fact that 2-K gave birth in a small cavity, rather than a more protected den complex, probably is the reason she lost 50% of her litter (one cub) to the elements. Bear 1-G, by using a pre-existing den complex, opportunistically took advantage of what was already available. The use of previously dug dens is not unusual for several bear species (Judd et al., 1986).

Den structure

Sloth bear dens are smaller than, yet comparable to those excavated by Polar bears *Ursus maritimus* and Brown bears

Ursus arctos (Judd et al., 1986; Durner et al., 2003). However, how typical 1-G's den is when compared to other Sloth bear maternity dens is unknown. It is also not known how much alteration 1-G made after the initial excavation by another bear. Bear 2-K's den was simple, and we surmise that it would probably have been inadequate for giving birth to and rearing cubs in the wild. Sloth bear maternity dens have not been reported as being this shallow, so it is possible that this den choice was influenced by the female's inexperience having been in captivity for a large part of her life.

Recommendations

Our recommendations for breeding captive Sloth bears derived from our observations of these two breeding females are listed below.

1. When possible, facilities should be located in or near the species' natural habitat, or an attempt to approximate that habitat should be made.
2. The mothers and cubs should be kept isolated from other bears until cubs are at least 18 months old.
3. Additional macronutrients and vitamin supplements should be provided for lactating others.
4. If there is no natural cave structure, bedding should be provided for the mother before and after birth.
5. Only familiar staff should conduct observations and

monitoring so as not to disturb the sloth bears.

6. A closed-circuit television (CCTV) camera should be installed in potential dens to make it possible to observe the family unit without disturbing the denning females.

Conclusions

Sloth bears are listed as Vulnerable on the International Union for Conservation of Nature's Red List (Dharaiya et al., 2016), and the species is facing increasing pressures from human- population growth and other factors. Therefore, it is increasingly important to understand the needs of both wild and captive female sloth bears to enable them to breed successfully. With this in mind, it is important to note that pregnant sloth bears at the BBRC continued to eat and drink until the day before or day of giving, although they exhibited a slightly depressed diet during their pregnancy. Additionally, the coats of both pregnant sloth bears appeared shiny and clean. Previous studies of wild sloth bears have suggested that females remain in their dens for up to 2 months after parturition. The BBRC data support the claim that sloth bear mothers do not eat or drink for a substantial amount of time after parturition, although the findings showed the mothers only stayed in the dens for about a month before exiting for food and water. The disparity might be attributed to the differences between a captive and a wild setting, or it may

simply be the product of being able to observe the bears more closely and gather more accurate data.

The initial emergence of the cubs in one of the case studies, 20 days after the mother first emerged, generally supports the findings of field telemetry studies, which suggest that the cubs made their first sojourn from the den roughly 2 weeks after the mother first emerges for food and water. However, the total time, as with the amount of time the mother remained in the dens without eating or drinking, was roughly a month less at BBRC than in the wild study. This c. 2-week time span – when the mother leaves the den for food and water while leaving the cubs behind – is a very vulnerable time for the cubs, when human poachers and other predators may target the cubs.

This rare opportunity to observe the denning behaviour of two female Sloth bears in natural surroundings in a large free-ranging area at BBRC has provided valuable information for those working to conserve this threatened species.

Acknowledgements

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Art – 168. PREVAILING HUMAN - HYENA CONFLICT IN AGRA DISTRICT, UTTAR PRADESH, INDIA AND CONSERVATION STRATEGIES

S. Ilayaraja, A. Sha. Arun and **M.V. Baiju Raj**

Abstract

The striped hyena *(Hyaena hyaena)* is the smallest of the true hyenas listed by the IUCN as near - threatened. Wildlife SOS (a non-profitable Non-Governmental Organisation) with assistance from Forest department has been dedicated to work on rescue and rehabilitation of these animals as a part of conflict mitigation and concentrating to create awareness by conducting various wildlife awareness education programs in schools and villages. The human wildlife conflict involving elephant, tiger and leopard is well documented, but that of hyena is not much explored. The striped hyena (*Hyaena hyaena* L. 1758) is a near threatened large carnivore. However, it possesses a wider distribution range than other hyena spp., the available information about its status and ecology is very limited. This study recorded six such cases encountered during 2015 to 2017. In total, seven animals were rescued and four hyenas (2M: 2F) were released after treatment, two females were dead due to massive injury and

one female hyena is still under care as it became blind. The study revealed that conflict increases in winter season and females are more victimized than males.

Keywords: Anthropogenic pressure, human-wildlife conflict, striped hyena, wildlife rescue

Introduction

Over the years, increased anthropogenic pressure coupled with the expansion of agriculture lead to the depletion of the territory and prey base of wildlife species residing in these environments (Mills and Hofer, 1998; Alam et al., 2015). As a result, animals such as hyenas are forced to venture into human settlements in search of food and water for survival. Most often they prey on domestic animals and livestock. Wildlife is accountable for the loss of 3% of livestock per year (Jackson and Nowell, 1996). A number of different wild animals are involved in such conflicts, but it is aggravated when large carnivores are involved (Dickman, 2008). Man, wildlife conflict primarily involves inter specific competition for resources which automatically jeopardizes the lives of the local people and leads to economic losses as well (Sillero- Zubiri and Laurenson, 2001). These conflicts mainly occur at the forest edges and in those areas where the predators have easy access to the livestock without getting

noticed (Woodroffe and Ginsberg, 1998). Occurrence of conflicts has increased in recent decades. Anthropogenic activities have escalated in almost every eco-zone owing to gradual elevation in need for daily subsistence (Vitousek et al., 1997). In fact, it is this unrestricted desire for space and subsistence that have initiated fragmentation of forests and habitats and augmented conflicts over available resources even at the national level (Laurance and Bierregaard, 1997; Mishra, 1997).

Carnivores mostly attack livestock that are grazed in forest lands and in human settlements which also poses a risk to human lives. The damage inflicted on humans or on their livelihood often infuriates the herd owners who resort to measures to avenge the loss (Conforti and de Azevedo, 2003). The striped hyena (*Hyaena hyaena L.* 1758) is a near threatened large carnivore with a wider distribution range than other hyena species. Hyaenidae family consists of four species around the globe such as striped hyena, spotted hyena, brown hyena, and Aardwolf (Mills and Hofer, 1998). They are mostly a scavenger by habit (Prater 1971). Some hyena species are considered as proficient hunters. They seek their food by scent (Prater 1971, Kruuk 1976). These carnivores play an important role in maintaining forest and grassland ecosystems (Millls and Hofer, 1998; Abi-said and Abi-said, 2007). Of the four extant hyena species, only the

striped hyena is found in India. The striped hyena is categorized as Near Threatened by the IUCN (Arumugam et al. 2008) and placed in schedule-III.

The total Indian population estimate is around 1000 to 3000 individuals representing around 18-20 per cent of the total world population (Mills and Hofer, 1998). The populations are generally declining throughout their geographical range due to persecution, poisoning and hunting for meat or medicinal purpose, besides depletion of prey populations and wildlife diseases (Singh et al., 2010; Akay et al., 2011; Jnawali et al., 2011; Dejene et al., 2016). Other ecological factors such as scarcity of food and shelter may also be contributing to the decline, including diminishing food stocks and competition with other carnivores over shelter (Alam, 2011, Akay et al., 2011, Khorozyan et al., 2011). Assessment of the status and distribution of animals to monitor population trends, especially in the case of rare or endangered species are the key ecological parameters for understanding the ecology and conservation stat us of a species (Williams et al., 2002).

Keeping view of this, conflict between human and the hyena was studied in and around Agra district of Uttar Pradesh, India to support the conservation strategies and record the fact involved in human-hyena conflict.

Materials and Methods

The study was conducted in Agra district of Uttar Pradesh at the Wildlife SOS managed rescue center, where the rescue calls were received from the local forest department or villagers. Six rescue calls were received during the study years 2015 to 2017. The Wildlife SOS rapid response team responded and rescued the animals from a brutal fate due to the rash and violent action of the frightened and angry villagers, the details are as under.

Case I: A female hyena was rescued on 15 December 2015 from Naroli village (27°27'24.59"N 77°39'58.39"E) in a comatose condition with swollen face, wounds on the body and protrusion of the left eyeball (Fig. 1). The detail radiographic examination revealed a fractured zygomatic bone on the leftside.

Fig. 1. Hyena recovered after treatment and lost vision in right eye.

Case II: A female hyena was rescued from a village Kaveesha, Sarvathpur (N 27° 09.19' E 078° 08.837') on 22 March 2016 with a swollen face and multiple injuries all over the body (Fig. 2). The radiographic examination did not reveal any major injuries to the bones except soft tissue swelling.

Fig. 2. Hyena recovered from multiple injuries and released

Case III: A female hyena was rescued from a mud cave near an agricultural field from village Nagalakeso, Daukethana on 05 April 2016 (Fig. 3). The angry mob started destroying the cave with JCB to kill the hyena. After huge difficulty, the hyena was safely rescued without any injury. One Nilgai carcass was also recovered from the mud cave. The animal's abdomen was bulged and mild enlargement in nipples also noticed.

Initially, pregnancy was suspected. Later, the detail radiographic examination revealed that there were partially digested feed material in the stomach and no evidence of

pregnancy thereof. Since there was no pyometra, it can be taken as pseudo pregnancy.

Fig. 3. Healthy non pregnant hyena released after confirmed with radiographic examination

Fig. 4. Hyena rescued with paralytic condition due to multiple fractures

Case IV: An adult female hyena was rescued in a recumbent condition with facial swelling from Nagla Parasukh,

Etamadpur Range, Agra on 25 February 2017 (Fig. 4). The animal was dragging its body as it was unable to stand and walk. The detailed radiographic examination revealed severe fracture, in dorsal lumbar spine and in the proximal end of femur bone. There were lacerated injuries also on the body.

Case V: One female hyena was rescued from Jainpura village, Fatehpursikari (N27°06.926'E077°40.492') on 05thFebruary 2017 (Fig.5). The animal was badly chased by the villagers including stone pelting. The animal fell into the drainage channel, which was marshy with dirty stagnated water. The animal was rescued with the help of nets and dog catcher without any chemical immobilization. The examination revealed that the animal was unable to bear weight on its hind limbs and bleeding from the inguinal region. The detail radiographic examination revealed a fracture at the right hip joint and rupture of the rectum leading to escape of faecal matters. Ultrasound examination revealed that the urinary bladder was abnormally distended.

Case VI: Two adult male hyenas were rescued from 15 feet dry well from Pinahat (26° 53' 11.91" N 78° 22' 22.37" E) on 01 November 2017 (Fig. 6). Fortunately, there was no fracture except minor injuries. The animals were highly stressed, dehydrated and hungry as well. So after rescue, they were put in a separate covered cage, fed with water mixed with ORS

and kept under observation. Anti-inflammatory pills administered orally by hiding it in the meat pieces, then released successfully (Fig. 6).

Fig. 5. Hyena rescued with massive hip joint fracture receiving treatment

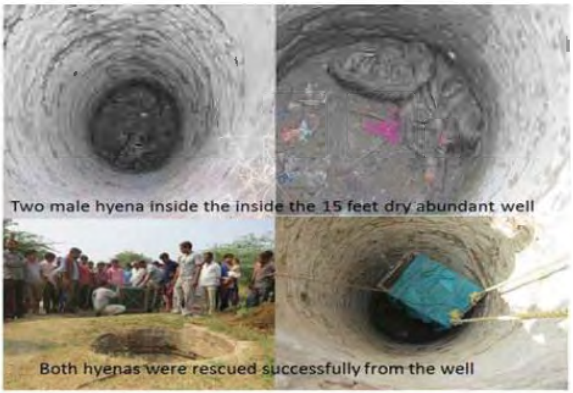

Fig. 6. Two male hyenas rescued from the well and released.

Results and Discussion

The treatments administered to the injured animals and

further steps taken are described in the following.

Case I: The left eyeball protruded excessively with hemorrhage. Surgical correction was tried, but it was unsuccessful and had to be extirpated (Venugopalan, 2002). The animal was kept in RT (the small enclosure to restrict movement) if not it could have hampered the healing of the wound. Subsequent treatment was given to stabilize the condition. The animal recovered well from all injuries except the vision of the right eye.

The brutal head injury might have led to damage of the optic nerve which could have resulted in permanent blindness of the right eye, which made the animal unfit for releasing back in the wild (Fig. 1). Case II: The animal released back into the wild after it recovered (Fig.2).

Case III: The animal was kept under observation and released back into the wild (Fig. 3)

Case IV: Subsequent treatment was given, but animal succumbed to injuries that it had sustained (Fig.4).

Case V: The urinary catheter was fixed to empty the bladder; blood mixed urine was noticed. Necessary critical care was given, but the animal succumbed to injuries. Postmortem examination revealed a ruptured peritoneal cavity filled with blood and feces (Fig.5).

Case VI: After observation period of days, the animals recovered from stress and were fit for release. We suspected

both the males were fighting over territory and accidentally fell in the open well at night and stone pelting by the villagers started by next morning onwards. So, the decision was made to release the animals in two separate locations in order to avoid further infighting as they happen to be solitary animals. Out of seven animals rescued, two (females) passed away, one female was kept in our custody as the animal became blind and rest (2 males and 2 females) were released in a suitable habitat. This was the clear evidence of high intensity conflict between humans and hyenas. Winter season had more cases. Female hyenas were more commonly involved in conflicts with humans as compared to males. (Fig. 7).

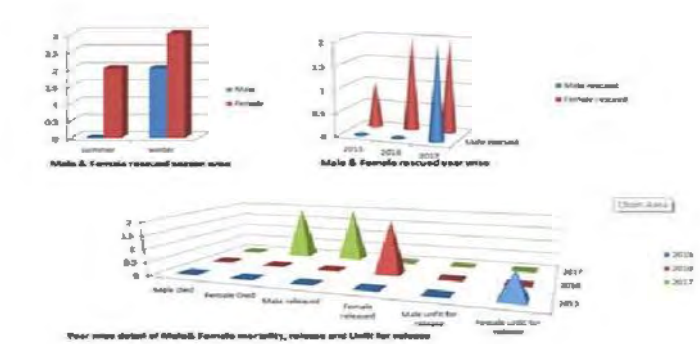

Fig. 7. Hyena rescued, released and mortality details summary

Thus, keeping in view the importance of the issue, there is an imminent need to educate the public to increase tolerance towards wild animals in an attempt to mitigate human-wildlife conflicts. The factors such as the nocturnal habit of the hyena, solitary behavior and occurrence in low densities

are further increasing the complication, the assessment of current status and population trends of the striped hyena. Questionnaire surveys, extrapolation, Lincoln index, identification of individuals and tracks, signs and vocalizations (Mills, 1998) and capture-recapture method using photo camera trap (Karanth, 1995) may be used for striped hyena.

Conflict Mitigation Strategies

The Human-Hyena conflict can be reduced by developing certain precautionary measures to minimize the risk, such as effective nocturnal livestock management and herding during daytime. The strategies that could be implemented in an attempt to lower livestock loss may include enhanced guarding and construction of predator- proof pens. Encouragement of better breeds of guard dogs could greatly reduce depredation or avoid predator form the livestock. The Livestock Insurance Scheme should be introduced in this area to compensate the poor villagers.

Further studies need to be initiated to assess the current status and population trends of striped hyena in India to develop an effective conservation modality for this species.

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Art – 169. COMPARATIVE STUDY ON THE RHYTHMIC CHANGES IN HAEMATOLOGICAL PARAMETERS BETWEEN CAPTIVE AND FREE RANGING WILD SLOTH BEAR *(Melursus ursinus)*

Attur Shanmugam Arun, Prasad Mishra Sidharth, Rasha Mol VP, Veerasamy Sejian b and Raghavendra Bhatta

Abstract

A study was conducted to assess the rhythmic changes in the haematological variables between captive and free ranging wild sloth bear *(Melursus ursinus).* The animals were distributed into two groups, Group I (Captive sloth bear; n = 15) and Group II (Free ranging wild sloth bear; n = 15). The blood samples were collected from 30 Sloth bears. In wild condition, the mean (±SD) of WBC and monocytes developed highly significant ($p < 0.01$) difference than captive whereas in captivity the RBC, haemoglobin and PCV revealed a significantly ($p < 0.05$) higher value than that of wild habitat. Based on sex, the mean (±SD) of RBC, haemoglobin and PCV were significantly ($p < 0.05$) higher in captive female than male whereas the MCH was significantly ($p < 0.05$) higher in male than female. Based on age, wild adult in mean (±SD) of WBC and captive adult in mean (±SD) of RBC, PCV and MCH showed significantly ($p < 0.05$) higher value as

compared to their respective counterpart. Therefore, this comparative analysis of all hematological parameters of sloth bear based on habitat, gender and age would be evidenced enough for protection and conservation of the animal by better disease diagnosis and management in both captivity and free ranging wild habitat.

Introduction

Sloth bears are mostly found in the tropical and subtropical regions of the Indian subcontinent. Their inhabitation ranging from the foothills of the Himalayas to the southern end of the Western Ghats mountain range in India as well as in the island of Sri Lanka (Prater 1965). The number of sloth bear distributed in the Indian peninsula was estimated to be approximately 7600. Two subspecies of sloth bear, namely *Melursus ursinus ursinus* and *Melursus ursinus inornatus*, were distributed in this region. *Melursus ursinus ursinus* were found in the Indian peninsula whereas *Melursus ursinus inornatus* relatively smaller in size and more hairy than previous one was found in Sri Lankan peninsula (Pocock 1933). It was established that around 5–7 million years ago, the sloth bears were diverged from the sun *(Helarctosmalayanus)*, American black and Asiatic black *(Ursus thibetanus)*, brown and polar *(Ursus maritimus)* bears in the ursine phylogenetic tree (Zhang 1994; Talbot and Shields 1996;

Yu et al. 2004).

Along with the passage of time, the bear populations faced habitat loss and fragmentation which lead them to now survive in small isolated regions caused due to expanding human habitation and agriculture demand (Garshelis et al. 1999). Reduction in bear habitat was evidenced by increase in human–bear conflicts in the encroached buffer regions (Rajpurohit and Krausman 2000; Bargali et al. 2005). Depletion in sloth bear habitat and numbers leads to classify the specific bear as vulnerable in the International Union for the Conservation of Nature and Natural Resources Red List of Threatened Species in 1990 (International Union for the Conservation of Nature and Natural resources (IUCN) 1990) and was considered to be protected under Schedule I of The Indian Wildlife Protection Act (IWPA) 1972.

In spite of the need for conservation and protection of sloth bears, few standardized physiological information are available regarding sloth bear biology (Laurie and Seidensticker 1977). In contrast to other ursids, these bears were medium sized and myrmecophagous in nature. These bears have developed a more nocturnal activity pattern (Sunquist 1982; Yoganand et al. 2005), extended parental behaviour (Laurie and Seidensticker 1977) and smaller home ranges (Sunquist 1982; Joshi et al. 1995). Due to such adaptations, they have lower metabolic rate compared to

brown and polar bears, and sloth bears never undergo for winter hibernation or winter sleep as does by other bears (Mcnab 1992). The differences in sloth bear physiology as compared to other bears were established from their morphologic appearance (Pocock 1933), feeding behaviour (Gokula et al. 1995; Joshi 1997) and physiologic state (Mcnab 1992), which were useful for better understanding and improved veterinary care and management of sloth bear populations. Standardisation of haematological parameters is an important method to assess health of animals and understand the impact of disease on individual and population levels (Geffre et al. 2009; Friedrichs et al. 2012). Various factors may influence the haematological variables. Extrinsic factors include factors that may stress the animal (Bush et al. 1980; Kusak et al. 2005), whereas intrinsic factors are associated with host characteristics (Hissa et al. 1994; Kusak et al. 2005).

In the present study, we have been able to obtain the mean haematological values for Indian sloth bears, which will prove to be a useful data to evaluate health profiles of sloth bears. Blood constituents could be useful to monitor the health, nutritional deficiencies and disease diagnose of animals (Fahlman et al. 2011). All sloth bears sampled either in free ranging wild or captivity were physically and clinically healthy, with normal behavioural responses during examination at the time of

sampling as determined by heart/respiration rate, hydration, body temperature and a detailed external physical examination. Therefore, the research was conducted with the objective to (1) establish haematology reference values for sloth bears at captivity and free ranging wild habitat; (2) compare the differences between the haematological parameters due to age, gender and habitat; (3) compare values of these parameters to existing data on other ursids and (4) determine the multivariate and univariate analysis of haematological parameters to evaluate the relationship between each other.

Material and methods

Free ranging wild sloth bears rescued from human animal conflict situations in the state of Karnataka and captive sloth bears rehabilitated at lifetime care centre of Wildlife SOS at Bannerghatta Bear Rescue Centre were utilised for this study. Thirty sloth bears samples from both free ranging wild sloth bears, and sloth bears at the Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park, Bannerghatta, Bangalore, Karnataka, India. Both these locations are within the documented habitat range for sloth bear species. The sloth bears in the wild were free ranging ones and in the captive one is rehabilitated individuals and maintained on an enriched native diet of local seasonal fruits and grains.

All bears sampled appeared physically healthy, with normal behavioural responses, and were clinically healthy during examination at the time of sampling (as determined by body temperature, hydration, heart/respiration rate and a detailed external physical examination). Each bear was sampled only once for this study. Sloth bears were immobilised using a ketamine–xylazine combination (Page, 1986), ketamine hydrochloride (5 mg/kg body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg/kg body weight; Troy Laboratories Pvt Ltd.). These drugs were administered using a distance projectile drug delivery system. Blood was collected from the jugular vein within 10 min after immobilisation using a 20-ga sterile hypodermic needle in Vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA), with and without ethylene diamine tetra acetic acid for haematology and serology, respectively. Samples were immediately stored on cool packs at 4–8°C and transported to the laboratory. Standard haematology parameters such as erythrocyte count, leukocyte count, platelet count, haemoglobin, packed cell volume (PCV), erythrocyte sedimentation rate, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were analyzed for each sample within 24 h of collection using a haematology analyser. Blood smears

were made and stained using Wright–Giemsa stain. Differential counts evaluating the percentage of each cell type in the smear were done under oil immersion using a light microscope. Absolute differential leukocyte counts were determined by multiplying relative percentages with the total leukocyte count.

Statistical analysis

On the completion of the haematological studies, the data collected were tabulated and descriptive statistical analysis i.e. mean ± SD (standard deviation), sum error mean, range, 95% confidence level of mean were conducted separately for captive, wild and entire population. The significance of difference in the mean value based on habitat was performed by independent t-test for normally distributed data and non-parametric Mann–Whitney U test for variables that violated the normality. The normality test was done by Kolmogorov–Smirnov and Shapiro–Wilk test. The homogeneity of variance of normally distributed variable was conducted by Levene test and for non-parametric data by Kruskal–Willis one-way ANOVA test. Breusch–Pagan and Koenker test were carried out to observe the heteroscedasticity of the data for further multivariate analysis of haematological parameters. Two types of multivariate analysis were conducted PCA (principal component analysis) and multiple regression. Statistical power

of multiple regression analyses is compromised when the number ratio of sample size to independent variables was below five. Bartlett's sphericity test was conducted to test the significance of correlation between each parameter with other then subjected to PCA on the whole haematological variables to reduce them to a smaller number of PCA factors that explained most of the variables in the original data set. Multiple regression analyses were used to obtain the partial correlation coefficients between haematological parameters as well as to determine which correlations were significant. Before multivariate analysis, the non-parametric data were converted to normality form by log transformation. All processing of data was conducted with the software packages Microsoft Excel 2010 for data storage and SPSS version 21 for statistical analysis. The p-values $p < 0.05$ and $p < 0.01$ with an alpha level of 95% were assumed as statistically significant (*) and highly significant (**), respectively.

Result

Basic descriptive statistical analysis on different haematological variables of entire population (Table 1) was determined as a standardised value. Mean value of each haematological parameter based on habitat (Table 2), sex (Table 3) and age group (Table 4) was compared to evaluate

the significance difference between each other. Comparing the mean of these haematological parameters, it was found that WBC, MCHC, monocyte and granulocytes were higher in wild population as compared to captive whereas RBC, Hb, PCV, MCV, MCH, lymphocytes and platelets were higher in the captivity as compared to wild population (Figures 1 and 2). Mean (±SD) of WBC was found to be highly significantly higher in wild habitat (19.8 ± 7.22) as compared to captivity (11.8 ± 1.96) (Table 2). Mean (±SD) of RBC was found to be significantly higher in captivity (6.28 ± 0.64) as compared to wild habitat (5.42 ± 1.05) (Table 2). Mean (±SD) of Hb was found to be significantly higher in captivity (16.3 ± 1.35) as compared to wild habitat (14.0 ± 3.65) (Table 2).

Mean (±SD) of PCV was found to be significantly higher in captivity (47.5 ± 4.93) as compared to wild habitat (40.8 ± 11.11) (Table 2). Mean (±S.D) of monocytes was found to be significantly higher in wild habitat (6.14 ± 3.39) as compared to the captivity (1.07 ± 1.03) (Table 2).

Table 1. Statistical description of haematological parameters of entire SB population in both captivity and free ranging wild habitats.

				Range		95% CL of Mean	
Parameters	Sample size	Mean ± SD	SEM	Minimum	Maximum	LCL	UCL
WBC	30	15.8 ± 6.63	1.21	7.10	36.2	13.5	18.0
RBC	30	5.85 ± 0.96	0.176	3.60	8.03	5.56	6.24
Hb	30	15.2 ± 2.93	0.53	5.60	20.6	14.1	16.2
PCV	30	44.2 ± 9.11	1.66	19.5	57.5	41.0	47.6
MCV	30	75.7 ± 8.85	1.61	51.0	91.0	71.9	78.2
MCH	29	26.0 ± 3.25	0.60	14.7	31.5	24.6	27.0
MCHC	29	34.4 ± 2.43	0.45	28.6	39.9	33.5	35.3
Lympho	30	22.8 ± 14.8	2.69	1.80	60.0	17.6	28.3
Mono	30	3.60 ± 3.56	0.65	0.000	12.0	2.34	4.77
Granulo	30	73.3 ± 15.1	2.77	29.6	96.6	67.8	78.4
Platelet	30	3.88 ± 1.52	0.27	0.90	6.70	3.29	4.33

SD: Standard deviation CL: confidence level; LCL: lower confidence level UCL upper confidence level.

Table 2. Comparison of haematological parameters of entire SB population based on habitat.

		Free ranging wild			Captivity			
			95% CL of mean			95% CL of mean		
Parameters	Sample size	Mean ± SD	LCL	UCL	Mean ± SD	LCL	UCL	*p*-Value
WBC	15	19.8 ± 7.22	16.1	23.6	11.8 ± 1.96	10.7	12.5	[b]0.00**
RBC	15	5.42 ± 1.05	4.95	6.02	6.28 ± 0.64	6.00	6.66	[a]0.012*
Hb	15	14.0 ± 3.65	12.0	16.1	16.3 ± 1.35	15.7	17.0	[b]0.016*
PCV	15	40.8 ± 11.1	34.9	47.1	47.5 ± 4.93	45.4	50.5	[a]0.041*
MCV	15	75.6 ± 12.1	68.4	80.8	75.8 ± 3.87	73.9	77.7	[b]0.461
MCH	14	25.9 ± 4.69	23.3	28.1	26.0 ± 0.86	25.5	26.4	[b]0.331
MCHC	14	34.4 ± 3.17	32.7	35.9	34.3 ± 1.59	33.5	35.1	[a]0.96
Lympho	15	17.0 ± 14.4	10.4	25.8	28.5 ± 13.2	22.3	35.1	[a]0.09
Mono	15	6.14 ± 3.40	4.47	8.01	1.07 ± 1.03	0.54	1.53	[b]0.00**
Granulo	15	76.8 ± 16.2	66.5	84.3	69.8 ± 13.7	63.1	76.5	[a]0.215
Platelet	15	3.81 ± 2.00	2.63	4.63	3.94 ± 0.88	3.49	4.35	[a]0.822

*$p < 0.05$; **$p < 0.01$. SD: Standard deviation; CL: confidence level; LCL: lower confidence level; UCL: upper confidence level. [a]Parametenc independent *t*-test to analyse the difference between mean (±SD) of haematological parameters based on habitat.

[b]Non parameteric Mann–Whitney *U* test to analyse the difference between mean (±SD) of haematological parameters based on habitat.

However, other haematological parameters MCV, MCH, MCHC, lymphocytes, granulocytes and platelets were found to be not significantly different between both the habitats (Table 2). Comparing the mean of these haematological parameters, it was found that WBC, RBC, Hb, PCV, MCV,MCH and granulocytes were higher in female wild population as compared to male wild population whereas MCHC, lymphocytes, monocytes and platelets were higher in male wild population than female wild but the differences were not significant (Table 3). However, in the captive condition, the WBC, RBC, Hb, PCV, granulocytes and platelets were higher in female than male and rest of the parameters were higher in male. In the captivity, mean (±SD) of RBC was found to be highly significantly higher in female (6.71 ± 0.69) as compared to male (5.77 ± 0.33) (Table 3). Mean (±SD) of Hb in captive condition was found to be significantly higher in

female (17.0 ± 1.63) as compared to male (15.3 ± 0.89) (Table 3). Mean (±SD) of PCV was found to be highly significantly higher in captive female (50.1 ± 5.67) as compared to captive male (43.5 ± 2.41) (Table 3). Mean (±SD) of MCH was found to be significantly higher in captive male (26.5 ± 0.91) as compared to captive female (25.3 ± 0.586) (Table 3). Mean (±SD) of platelets in captive condition was found to be significantly higher in female (4.23 ± 0.59) as compared to male (3.69 ±1.03) (Table 3). Moreover, other parameters were found to be not significantly different from each other based on gender in captivity.

In the wild habitat, the age group 2 (individuals lying within the age of 5–10 years) showed higher WBC, Hb, PCV, MCV, MCH and granulocyte as compared to age group 1 (individuals lying within the age of 1–5 years) as shown in Table 4. However, RBC, MCHC, lymphocytes, monocytes and platelets were found to be higher in age group 1 than age group 2 (Table 4). Only WBC with mean (±SD) value of 24.0 ± 7.58 in wild habitat age group 2 as compared to age group 1 (16.3 ± 5.14) was found to be significant and only monocytes with mean (±SD) value of 8.06 ± 2.54 in wild habitat age group 1 as compared to age group 2 (4.67 ± 3.47) whereas all other parameters were not found

Table 3. Comparison of haematological parameters of entire SB population in both captivity and free ranging wild habitats based on gender.

		Male			Female			
			95% CL of mean			95% CL of mean		
Parameters	Sample size	Mean ± SD	LCL	UCL	Mean ± SD	LCL	UCL	p-Value
Free ranging wild habitat								
WBC	6	20.9 ± 7.67	14.8	25.9	21.8 ± 7.57	17.2	27.8	[d]0.84
RBC	6	5.36 ± 1.43	4.35	6.42	5.67 ± 0.69	5.08	6.14	[c]0.64
Hb	6	13.7 ± 2.06	12.2	15.3	15.4 ± 3.95	12.5	18.2	[c]0.37
PCV	6	40.1 ± 9.83	33.4	47.3	44.6 ± 11.7	36.2	52.3	[c]0.48
MCV	6	76.8 ± 10.4	68.7	83.5	78.7 ± 12.9	68.5	87.3	[c]0.79
MCH	6	26.6 ± 3.95	23.4	28.9	27.1 ± 3.79	24.4	29.7	[c]0.79
MCHC	6	34.7 ± 3.72	32.1	37.3	34.6 ± 2.24	32.8	36.1	[c]0.98
Lympho	6	14.6 ± 7.73	9.03	20.3	9.03 ± 4.98	5.31	12.8	[c]0.17
Mono	6	5.78 ± 3.92	3.15	8.78	5.68 ± 2.85	3.60	7.80	[d]0.96
Granulo	6	79.4 ± 8.80	73.2	85.8	85.3 ± 7.64	79.6	90.9	[c]0.24
Platelet	6	4.47 ± 1.86	3.10	5.82	4.01 ± 2.01	2.55	5.46	[c]0.68
Captive habitat								
WBC	6	12.0 ± 2.96	9.65	14.0	12.3 ± 1.55	11.2	13.5	[c]0.82
RBC	6	5.77 ± 0.33	5.52	5.99	6.71 ± 0.69	6.33	7.31	[c]0.01**
Hb	6	15.3 ± 0.89	14.7	16.0	17.0 ± 1.63	16.0	18.4	[c]0.04*
PCV	6	43.5 ± 2.41	41.6	45.2	50.1 ± 5.67	46.4	53.9	[d]0.00**
MCV	6	75.3 ± 3.02	73.2	77.5	74.7 ± 5.19	71.9	78.9	[c]0.83
MCH	6	26.5 ± 0.91	25.8	27.2	25.3 ± 0.586	25.0	25.8	[c]0.02*
MCHC	6	35.2 ± 1.01	34.4	35.9	34.0 ± 1.76	32.6	35.1	[d]0.13
Lympho	6	24.2 ± 8.45	17.0	30.5	21.5 ± 10.5	14.0	29.2	[c]0.63
Mono	6	1.50 ± 1.22	0.67	2.33	1.16 ± 1.17	0.33	2.00	[d]0.69
Granulo	6	73.3 ± 9.56	66.3	81.5	77.3 ± 11.5	69.0	85.3	[c]0.52
Platelet	6	3.69 ± 1.03	2.94	4.29	4.23 ± 0.59	3.82	4.68	[d]0.05*

*p < 0.05; **p < 0.01.SD: Standard deviation; CL: confidence level; LCL: lower confidence level; UCL: upper confidence level. cParameteric independent t-test to analyse the difference between mean (±SD) of haematological parameters based on sex.dNon-parameteric Mann–Whitney U test to analyse the difference between mean (±SD) of haematological parameters based on sex.

to be significantly different from each other (Table 4). In the captivity, it was determined that WBC, monocytes, granulocyte and platelets were found to be higher in age group 1 as compared to age group 2 and other parameters namely RBC, Hb, PCV, MCV, MCH, MCHC and lymphocyte were found to be higher in age group 2 as compared to age group 1 (Table 4). In the captivity, mean (±SD) of RBC was found to be highly significantly

higher in age group 2 (6.61 ± 0.711) as compared to age group 1 (5.80 ± 0.386) (Table 4). Mean (±SD) of PCV in captive condition was found to be significantly higher in age group 2 (49.6 ± 5.77) as compared to age group 1 (43.6 ± 2.09) (Table 4). Moreover, mean (±SD) of MCH was found to be significantly higher in captive age group 2 (26.7 ± 1.12) as compared to captive age group 1 (25.6 ± 0.509) (Table 4). To conduct test of significance of each parameter, normality test was done. It was illustrated that the WBC, Hb, MCV, MCH, lymphocyte and monocyte were significantly violating the normality principle based on habitat. These data were transformed by log transformation for further univariate and multivariate statistical analysis. On analyzing the homogeneity of variance, it was found that sex, age and combined effect of habitat and sex have no significant effect on the haematological parameters (Table 5). Parameters like WBC, RBC, Hb, PCV, MCHC, monocytes and platelets were found to be significant homogeneity of variance with respect to habitat while other parameters were found to have no significant effect.

Table 4. Comparison of haematological parameters of entire SB population in both captivity and free ranging wild habitats based on age.

Parameters	Sample size	Age group 1 Mean ± SD	95% CL of mean LCL	UCL	Age group 2 Mean ± SD	95% CL of mean LCL	UCL	p-Value
Free ranging wild habitat								
WBC	7	16.3 ± 5.14	12.6	19.7	24.0 ± 7.58	19.1	29.0	[a]0.04*
RBC	7	5.53 ± 0.92	4.96	6.20	5.30 ± 1.31	4.40	6.11	[a]0.70
Hb	7	13.7 ± 1.99	12.4	15.0	14.4 ± 5.19	10.7	17.6	[a]0.76
PCV	7	40.2 ± 7.52	35.4	45.2	41.6 ± 15.2	31.3	51.7	[b]0.82
MCV	7	74.1 ± 13.0	65.3	82.8	77.6 ± 12.8	68.1	84.8	[a]0.63
MCH	7	24.4 ± 3.28	22.1	26.7	27.0 ± 5.85	22.8	30.2	[a]0.32
MCHC	7	34.5 ± 2.13	32.9	35.8	34.4 ± 4.18	31.7	37.3	[b]0.96
Lympho	7	16.9 ± 7.51	12.4	22.4	16.2 ± 20.5	5.13	31.0	[a]0.26
Mono	7	8.06 ± 2.54	6.46	9.87	4.67 ± 3.47	2.47	7.06	[b]0.05*
Granulo	7	74.9 ± 8.77	68.8	80.3	79.0 ± 22.9	61.6	90.7	[b]0.21
Platelet	7	4.54 ± 2.36	2.80	6.00	3.04 ± 1.57	1.98	4.05	[a]0.19
Captive habitat								
WBC	7	11.7 ± 1.72	10.7	13.0	11.4 ± 1.75	10.2	12.6	[a]0.775
RBC	7	5.80 ± 0.386	5.53	6.08	6.61 ± 0.711	6.22	7.13	[a]0.022*
Hb	7	15.5 ± 0.78	14.9	16.0	16.9 ± 1.58	16.0	18.1	[a]0.051
PCV	7	43.6 ± 2.09	42.1	45.1	49.6 ± 5.77	46.1	53.7	[a]0.017*
MCV	7	75.1 ± 4.85	72.2	78.6	75.2 ± 3.08	73.2	77.3	[a]0.985
MCH	7	25.6 ± 0.509	25.3	25.9	26.7 ± 1.12	25.9	27.4	[a]0.043*
MCHC	7	34.2 ± 1.79	33.0	35.3	35.5 ± 0.967	34.8	36.2	[a]0.073
Lympho	7	27.0 ± 11.1	19.1	34.0	28.3 ± 7.43	22.9	33.7	[b]0.803
Mono	7	1.86 ± 0.90	1.29	2.43	1.57 ± 1.61	0.71	2.71	[a]0.383
Granulo	7	71.1 ± 11.8	63.7	79.4	70.1 ± 1.61	64.3	76.1	[b]0.847
Platelet	7	4.19 ± 0.38	3.95	4.43	3.46 ± 1.19	2.68	4.24	[a]0.15

*p < 0.05; **p < 0.01.

SD Standard deviation; CL: confidence level; LCL: lower confidence level; UCL: upper confidence level.

e Parameteric independent t-test to analyse the difference between mean (±SD) of haematological parameters based on sex.

f Non-parameteric Mann–Whitney U test to analyse the difference between mean (±SD) of haematological parameters based on sex.

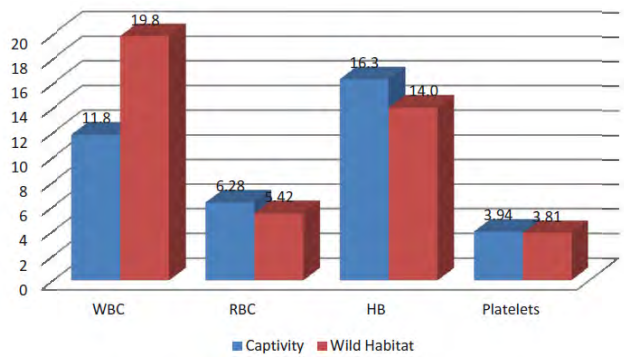

Figure 1. Comparison on mean value of WBC, RBC, Hb and platelets of sloth bear in captive and free ranging wild habitat.

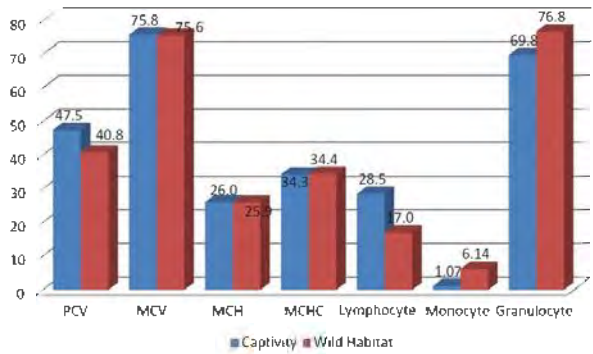

Figure 2. Comparison on mean value of PCV, MCV, MCH, MCHC, lymphocyte, monocyte and granulocyte of sloth bear in captive and free ranging wild habitat.

Table 5. Analysis of homogeneity of variance of haematological parameters w.r.t. habitat, age and gender.

	Habitat	Sex	Age	Habitat × gender	Habitat × Age
Parameters	*f*-Value	*f*-Value	*f*-Value	*f*-Value	*f*-Value
WBC	[b]0.00**	[b]0.81	[b]0.36	0.90	0.03*
RBC	[a]0.035*	[a]0.11	[a]0.17	0.39	0.41
Hb	[b]0.018*	[a]0.10	[b]0.78	0.99	0.35
PCV	[a]0.01**	[a]0.10	[a]0.52	0.75	0.28
MCV	[b]0.44	[a]0.85	[b]0.45	0.74	0.64
MCH	[b]0.31	[a]0.81	[b]0.02*	0.46	0.54
MCHC	[a]0.018*	[a]0.52	[a]0.53	0.56	0.48
Lymphocyte	[a]0.10	[a]0.31	[a]0.96	0.67	0.83
Monocyte	[b]0.00**	[b]0.95	[b]0.14	0.91	0.09
Granulocyte	[a]0.84	[a]0.22	[b]0.49	0.81	0.64
Platelets	[a]0.00**	[a]0.94	[a]0.06	0.42	0.51

*$p < 0.05$; **$p < 0.01$.
[a]Parameteric Levene test to analyse the homogeneity of variance.
[b]Non-parameteric Kruskal–Willis one way ANOVA test to analyse the homogeneity of variance.

However, the combined effect of habitat and age showed that the homogeneity of variance of WBC had significant effect and all other parameters had no significant effect (Table 5). By heteroscedasticity test it was observed that all the variables were not significant ($p < 0.05$) as mentioned in Table 6. The homoscedasticity normality distributed variables and log

transformed non-parametric variables were subjected to PCA and multiple regression analysis. According to the result of Bartlett's sphericity test, the null hypothesis was rejected that the correlation matrix of all haematological variables was an identity matrix and that all correlations were zero ($p < 0.01$), so further proceeded for PC analysis. PCA resulted in three factors, which accounted for cumulative total variation of 80.16% (Table 7). PC1 was positively correlated with PCV, Hb and MCV; PC2 was negatively correlated with lymphocytes and positively correlated with granulocytes and PC3 was positively correlated with platelet. The eigenvalue of PC1, PC2 and PC3was found to be 4.53, 2.87 and 1.41, respectively (Table 7). The variable showed a factor loading of more than 0.785 was taken into consideration into that particular principal component. The factor loadings developed by PCV, Hb and MCV in PC1 were 0.98, 0.92 and 0.82 and were most closely correlated to each other to produce variation by PC1 of about 41.19% of total variation. Similarly, lymphocyte and granulocyte developed a factor loading of about −0.93 and 0.89 to produce variation by PC2 of about 26.14% of total variation. Moreover, platelet developed a loading factor of 0.90 to produce variation by PC3 of about 12.82% (Table 7).

Potential effects of habitat on the different parameters were also investigated; simple bivariate plots were constructed and the fit for a regression model was tested for each parameter (Table 8). The correlation between WBC and lymphocyte was also conducted as presented in Figure 3 represented highly significant ($p < 0.01$) negative relationship with the regression coefficient value of ($R2 = 0.46$). Correlation between WBC and granulocyte has been presented in Figure 4 which showed statistically positive significant ($p < 0.01$) relationship between these two parameters with the value of regression

Table 6. Linearity analysis of haematological parameters by heteroscedasticity test.

Parameters	Breusch–Pagan	Koenker test
WBC	0.942	0.496
RBC	0.066	0.051
Hb	0.232	0.708
PCV	0.004*	0.533
MCV	0.035*	0.381
MCH	0.219	0.583
MCHC	0.185	0.271
Lymphocyte	0.310	0.417
Monocyte	0.118	0.135
Granulocyte	0.685	0.221
Platelet	0.153	0.509

*$p < 0.05$.
All the parameters were found to be homoscedasticity, so were subjected for univariate and multivariate analysis.

Table 7. Principal component analysis of haematological parameters.

	Variables				
Principal component	Parameter	Factor loading	Per cent variation	Cumulative variation	Eigenvalue
PC1	PCV	0.984	41.2	41.2	4.53
	Hb	0.915			
	MCV	0.815			
PC2	Lymphocyte	−0.929	26.1	67.3	2.88
	Granulocyte	0.892			
PC3	Platelet	0.903	12.8	80.1	1.41

Positive and negative signs preceding variables indicate their orientation on PC axis.
Variables with factor loading above 0.785 were considered important factors in each component.

coefficient as R2 = 0.26. Similarly, moreover, correlation between WBC, monocyte and platelet was also carried out but no significant ($p < 0.05$) relationship was found between each other. Similarly, correlation between RBC and Hb was checked found to be highly positively significant ($p < 0.01$) with regression coefficient of about R2 0.56 as mentioned in Figure 5. The correlation between RBC and PCV was also established to find a positively highly significant ($p < 0.01$) resulted with R2=0.67 as shown in Figure 6. However, relationship between RBC, MCV, MCH and MCHC was found to be not significantly correlated with each other. Furthermore, correlation between Hb and PCV (Figure 7) was evaluated. The value of regression coefficient (R2 = 0.84) depicted highly significant ($p < 0.01$) correlation.

Table 8. Multiple regression analysis of haematological parameters with each other.

Parameters (y)	Correlation with other parameters (x)	Regression equation	R^2-value	p-Value
WBC	Lymphocyte	$y = 3.60 - 0.32x$	0.46	0.000**
	Monocyte	$y = 2.67 + 0.06x$	0.02	0.51
	Granulocyte	$y = 1.74 + 0.01x$	0.26	0.004**
	Platelets	$y = 2.84 - 0.04$	0.03	0.41
RBC	Hb	$y = -2.33 + 3.04x$	0.57	0.000**
	PCV	$y = 2.01 + 0.09x$	0.67	0.000**
	MCV	$y = 1.33 + 0.05x$	0.02	0.47
	MCH	$y = 4.11 + 0.55x$	0.01	0.67
	MCHC	$y = 8.95 - 0.09x$	0.05	0.23
Hb	PCV	$y = 1.63 + 0.02x$	0.84	0.000**
	MCV	$y = -2.91 + 1.30x$	0.46	0.00**
	MCH	$y = -1.13 + 1.18x$	0.49	0.00**
	MCHC	$y = 2.24 + 0.01x$	0.02	0.50
PCV	MCV	$y = -160 + 47.2x$	0.43	0.00**
	MCH	$y = -58.0 + 31.5x$	0.24	0.007**
	MCHC	$y = 76.0 - 0.92x$	0.06	0.20
Platelet	Lymphocyte	$y = 3.79 + 0.03x$	0.00	0.93
	Monocyte	$y = 3.89 - 0.004x$	0.00	0.97
	Granulocyte	$y = 2.70 + 0.02x$	0.03	0.40
Lymphocyte	Monocyte	$y = 21.0 + 0.15x$	0.00	0.96
	Granulocyte	$y = 92.2 - 0.95x$	0.95	0.00**
Monocyte	Granulocyte	$y = 2.22 + 0.02x$	0.06	0.30

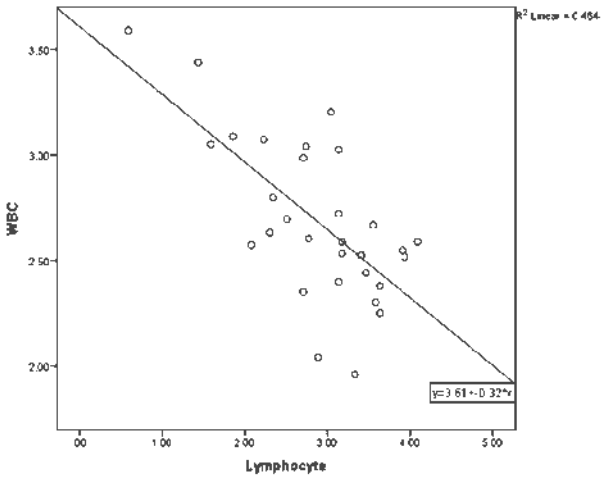

Figure 3. Relation between WBC and lymphocyte.

Relationship between Hb and MCV was represented in Figure 8 which revealed a highly significant correlation between them with regression coefficient of R2 = 0.47. As for as correlation between Hb and MCH is concerned (Figure 9), it was found to be statistically highly significant as the value of regression coefficient was (R2 = 0.49). A similar correlation between MCHC and Hb and between MCHC and PCV were observed which were found to be non- significant. In Figure 10, correlation between PCV and MCV is presented that also showed highly significant ($p < 0.01$) relationship as the regression coefficient value is (R2 = 0.43). The correlation between the PCV and MCH was determined in the Figure 11 with regression coefficient of R2 = 0.24 which was found to be highly Significant ($p < 0.01$).

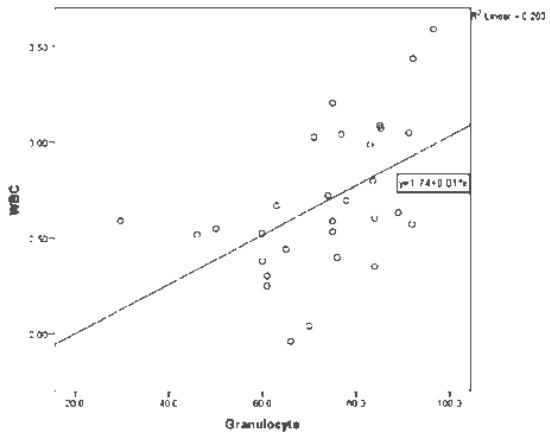

Figure 4. Relation between WBC and granulocyte

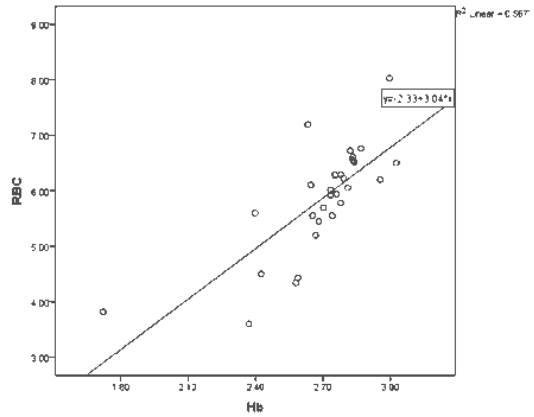

Figure 5. Relation between RBC and Hb.

Finally, the correlation between lymphocyte and granulocyte was carried out which were presented in Figure 12. The regression coefficient value (R2 = 0.95) showed highly significant ($p < 0.01$) relationship between

these two parameters. On contrast to the above, correlation between lymphocyte and monocyte and between monocyte and granulocyte showed non- significant relationship with each other, respectively.

Discussion

In studying free ranging animals, the great challenge faced by the researcher was the variation arise due to capture and immobilisation procedure, blood collection and analytical

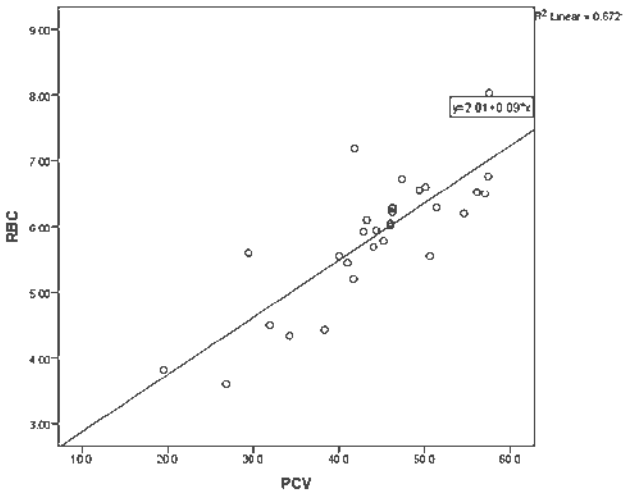

Figure 6. Relation between RBC and PCV.

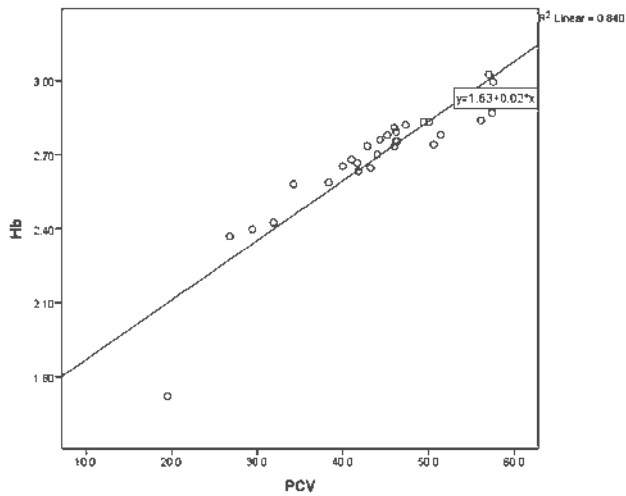

Figure 7. Relation between Hb and PCV.

procedures that might affect the results. Various factors might affect the haematological variables and should be considered when establishing and using reference intervals (Cattet et al. 2003b; Kusak et al. 2005). Results of haematology values were determined for 30 sloth bear including both captive and free ranging wild sloth bear as standardised value as shown in Table 1. All haematological parameters were presented as mean for the entire population of either of the habitat based on age and sex (Tables 2 and 3). The haematological parameters were compared to the previously, established average value of other bears like black bear (Svihla et al. 1955; Youatt and Erickson 1958; Hellgren et al. 1993), brown bear (Kusak et al. 2005) and

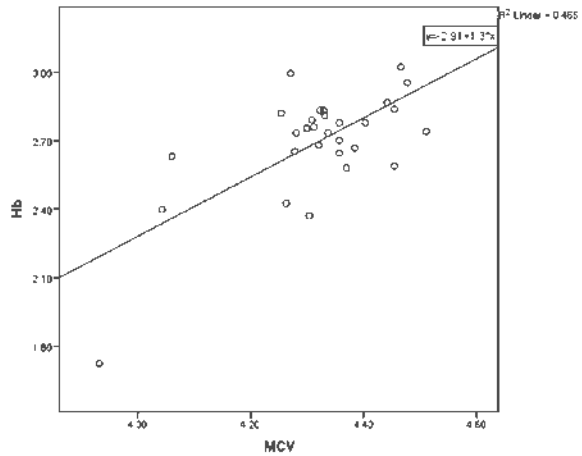

Figure 8. Relation between Hb and MCV.

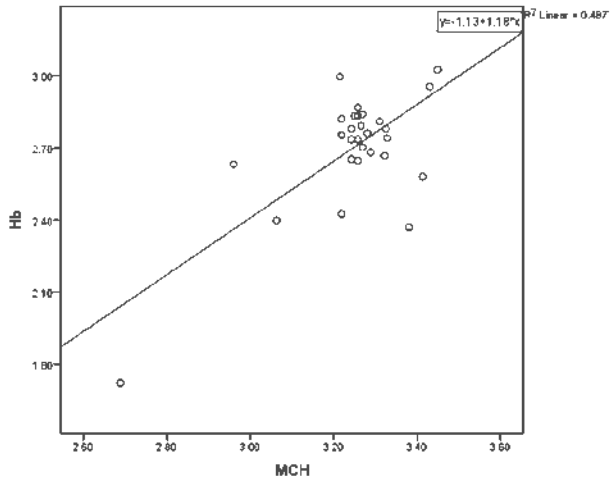

Figure 9. Relation between Hb and MCH.

grizzly bear (Cattet et al. 2003a). The higher WBC in the wild population of sloth bear than captivity as shown in Figure 1 indicates a potential physiological leukocytosis. Potential

leukocytosis in wild sloth bear indicates that higher circulating neutrophils and monocytes to provide immunity to the body may be associated with some inflammatory reactions to potential pathogens or parasites similar to the previous findings established by Swenson (1984). While the captive population showed a normal WBC and monocytes indicates a low-level stressor factors inherent with the animals newly introduced to captive environment. Also, monocyte was found to be very high in the wild condition as compared to captive animal which point out that the wild sloth bears were may be prone to some infectious pathogenic or parasitic diseases as previously proposed by Graesli et al. (2014).

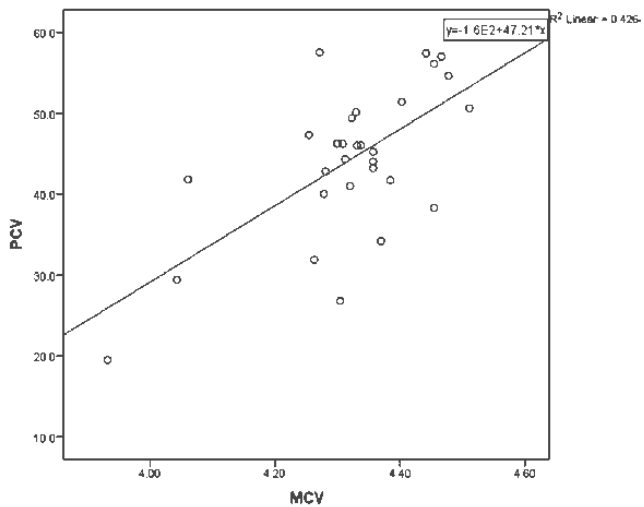

Figure 10. Relation between PCV and MCV.

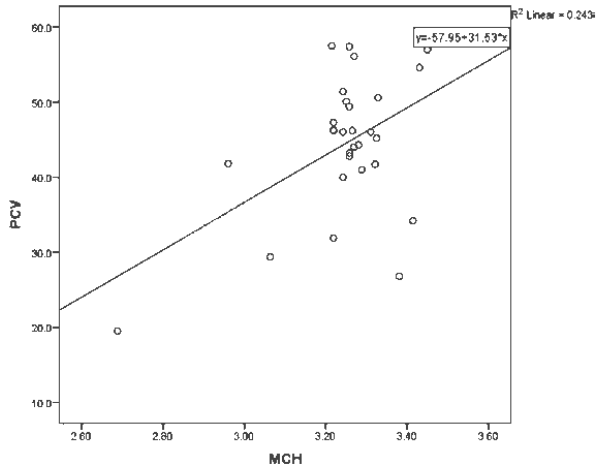

Figure 11. Relation between PCV and MCH.

Usually, the erythrocyte count, Hb and other associated parameters change in concurrence with changes in altitude, climatic condition (i.e. atmospheric oxygen concentration, environmental temperature), habitat quality, nutritional status of animal etc. (Sealander 1964; Swenson 1984). The study revealed that the RBC, Hb and PCV were higher in the captive condition than the free ranging wild habitat that means wild sloth bears were in a microcytic anemic condition than the captive ones (Figures 1 and 2). The higher RBC in the captive population of sloth bear might be due to higher activity, aggressiveness or greater interaction in captivity as compared to wild habitat. That resulted may be because of environment and climatic differences between the two habitat or might be due to high

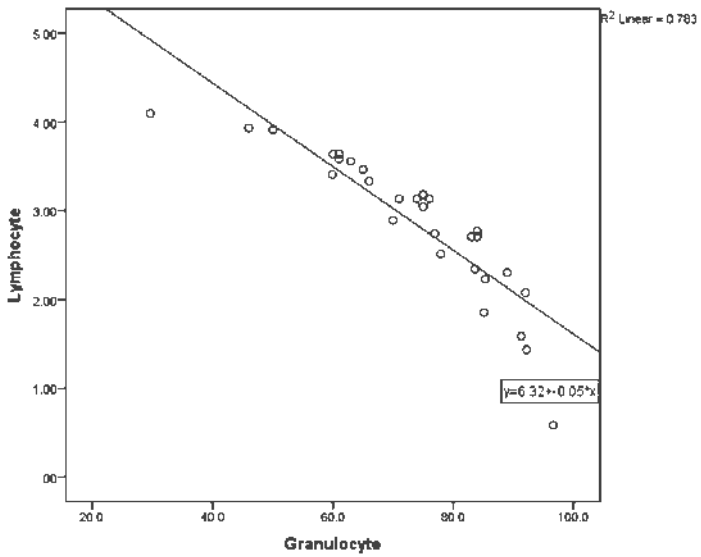

Figure 12. Relation between lymphocyte and granulocyte.

iron concentration diet provided in captive condition than available in natural wild environment (Seal et al. 1967; Matula et al. 1980). Typically, the RBC, Hb and PCV are found to be higher in infant and geriatric individual than adult is evident regardless of sex or species (Seal et al. 1967; Pearson 1972; Fahlman et al. 2011). So, it could be predicted that in studied population the captive animals were of either infant or geriatric animal than the wild population. MCV and MCH were found to be higher in captivity than the wild condition might be due to higher oxygen demand but the difference in the mean value was not significantly different (Figure 2). MCHC was found to be almost same in both the habitats as presented in Figure 2 with no significant difference

(Table 5). But lymphocyte was found to be higher in captive animals than wild ones whereas granulocyte was higher in the wild than captive animals as shown in Figure 2 with no significant difference (Table 5).

In both the habitats (captive and wild), the female sloth bear RBC was found to be higher than the male counterpart contrary to the findings as established for European brown bears (Kusak et al. 2005). The MCV and MCH values were found to be higher in captive male than female whereas the wild females have higher MCV and MCH values as compared to the wild male. However, the MCH value was found to be significantly different between males and females in captive condition satisfying the previous finding revealed by Shanmugam et al. (2008), but not in the wild habitat. WBC was found to be higher in female sloth bears as compared to male bears in both wild and captive habitats satisfying to the previous findings by Villiers and Jk (1998). In brown bear, it was reported that the males had a higher neutrophil count compared to females (Kusak et al. 2005). In wild condition, the platelets were discovered to be not significantly higher in male as compared to female whereas the captive females had significantly higher platelets as compared to captive male similar to the previous finding where female sloth bears also had a higher platelet count compared to male bears (Graesli et al. 2014). A sexually dimorphic functional difference on

platelets was studied in mice where platelets secreted from females were more responsive to stimuli than those derived from males due to the involvement of estradiol in triggering pro-platelet formation.

In both the habitats based on age, some of the haematological parameters were found to be significantly different from each other between age group 1 and 2 (Table 4). In wild habitat, adult sloth bears were revealed to be had significantly higher WBC as compared to cubs and subadult individual in contrast to that of previous findings where cubs showed had higher neutrophil and overall higher leukocyte count as compared to adult and subadult bears (Hissa et al. 1994). However, in captivity, the WBC was found to be higher in cubs and subadult than adult but not significantly. This indicates a potential physiologic leukocytosis due to higher activity in cubs and subadult compared to adult bears in captive condition (Swenson 1984) whereas in the wild habitat, the adults were more active than cubs. The variation in white blood cell differential count in different age groups could reflect maturation of the immune system, because younger animals are known to have a higher lymphocyte count than adult animals (Villiers and Jk 1998). In wild, the cub and subadult RBC was found to be higher than adult but not significantly. In captivity, the erythrocyte count was discovered to be significantly higher in adults as compared to cubs

and subadult satisfying to the previous findings by Pearson (1972), Fahlman et al. (2011) and Seal et al. (1967). The findings were also satisfied according to the measures of haemoglobin concentration, PCV, MCV and MCH, all of which were higher in adult individuals of captivity than that of the cubs and subadult bears (Pearson 1972; Shanmugam et al. 2008). Similar findings on haemoglobin, PCV and MCV were also evidenced in the wild individuals. Lower values of RBC in captive cubs than captive adults might be due to plasma expansion associated with rapid development and body growth in neonates, thereby production of the rate of red blood cell increases. Similar observations have been reported for other neonatal mammals in several other studies. The lower haemoglobin, PCV, MCV and MCH in captive and wild cubs and subadult compared to adult bears indicated that the cubs may evidence microcytic anaemia during the rapid growth phase similar to the findings reported on black bear cubs (Matula et al. 1980). It has also been established on brown bears that young bears had a lower erythrocyte, haematocrit and haemoglobin value as compared to older bears (Pearson 1972). The MCHC was found to be almost similar in both captive and wild habitats, as well as, there was no significant difference established between neither based on gender nor age in both the habitats.

By multivariate analysis, it was determined that PCV, haemoglobin, MCV, lymphocyte, granulocyte and platelets were

important components to establish correlation between each other (Table 7). Linear regression analysis was established between the various haematological parameters (Table 8). The analysis revealed that the WBC and granulocyte were significantly negatively correlated with the lymphocyte (Figures 3 and 12) means if the value of lymphocyte increases leads to decrease in the value of WBC and granulocyte, whereas WBC and granulocytes were positively correlated (Figure 4). Similarly, RBC, haemoglobin, PCV, MCV and MCH were significantly positively correlated (Table 8) with each other means if any of these parameters' increases leads to the simultaneous increase in the other parameters.

Conclusion

This study is the first of its kind to establish the differences in the rhythmic changes in the haematological variables between captive and wild sloth bear. The study developed a standardised mean value for haematological variables which can be useful in health assessments of captive and free-ranging sloth bears. The WBC, monocytes and granulocyte were found to be higher in wild whereas other parameters like RBC, haemoglobin, PCV, MCV, MCH and lymphocyte were revealed to be higher in captive individual. However, when these parameters were studied between male and female or between cubs, subadult and adult, a significant

difference developed between some of the parameters deviating from the findings of the captivity and wild individual. Therefore, this comparative analysis of all haematological variables of sloth bear based on differences in habitat, gender and age would be very useful for protection and conservation of the animal by better disease diagnosis and management under both captivity and wild conditions.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Art – 170. MOLECULAR DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF ESCHERICHIA COLI INFECTION IN CAPTIVE SLOTH BEARS (*Melursus ursinus*)

M. Palanivelrajan, **A. Sha. Arun,** M.G. Jayathangaraj, K. Vijayarani, Bhaskaran Ravi Latha and P. Sridevi

Abstract

Escherichia coli can also be a reservoir of intestinal microbiota. As there are no data on E.coli strains from the intestinal microbiota of the sloth bear *(Melursus ursinus)*, the aim of this study was to investigate and characterize fecal E.coli from sloth bears reared at Wildlife SOS, Bannerghatta Bear Rescue Centre (BBRC), Bangalore, Karnataka, Pathogenic Escherichia coli strain from faeces of sloth bears was screened by using culture, Gram's stain, biochemical tests and polymerase chain reaction(PCR). The results showed that out of 30 samples collected, 29 samples (96.67%) were cultured on EMB agar and Gram's stain revealed rod-shaped Gram-negative organism with green metallic sheen-like colonies. The biochemical tests of 15 cultured samples (s1.72%) revealed positive to indole production, positive to glucose, negative to H2S production and negative to urea production.

The fun C gene was amplified by PCR assay for the pathogenic Escherichia coli and positivity was found to be 46.67% among the sub adults and 53.33% among the adults. In the sub-adult sloth bear group, four males and three females harboured the infection (57.14% and 42.86%, respectively) and in the adult sloth bear group one male and seven females harboured the infection (12.50% and 87.50% respectively). Antibiotic Sensitive Test (ABST) was carried out and was found be sensitive for Enrofloxacin (EX), intermediate for Azithromycin (AZM), Cefotaxime (CTX) and Gentamicin (GEN). Similarly, it was found to be resistant for Amoxyclav (Amoxiciline/Clavulanic acid) (AMC), Clindamycin (CD), Methicillin (MET), Streptomycin (HLS) and Tetracycline (TE). This study may provide useful information for development of strategies related to the control of Escherichia coli infections of captive Sloth Bears, in the future.

Art – 171. FIRST REPORT ON MOLECULAR IDENTIFICATION OF BAYLISASCARIS TRANSFUGA INFECTION IN SLOTH BEAR *(Melursus ursinus)* IN SOUTH INDIA

M. Palanivelrajan, Bhaskaran Ravi Latha, **A. Sha Arun**, M. G. Jayathangaraj, K. Vijayarani and P. Sridevi.

Abstract

A round worm (189mm of length) was collected from rescued sloth bear *(Melursus ursinus)* reared at Bannerghatta Bear Rescue Centre (BBRC), Bangalore, Karnataka (South India). Morphometric findings of the worm revealed distinctive morphological features which included three well developed triradiate lips (one dorsal and two sub ventral) presence of cervical alae and filariform oesophagus in adult parasites of *Baylisascaris transfuga*. The caudal ends of the male parasites were slightly curved with presence of large number of precloacal papillae (48 – 70), with prominent cloacal opening and tail knob, whereas female posterior end revealed the presence of a blunt tail. DNA was extracted by using Blood and Tissue DNA extraction kit for molecular identification by Polymerase Chain Reaction to amplify the Cytochrome c Oxidase subunit I (COI) gene and sequenced.

The sequence obtained was 99% identical to the COI segment of Baylisascaris transfuga after a BLAST comparison to the GenBankTM database. Based on the morphometrical findings and molecular characterization, this study is the first record on the identification of *Baylisascaris transfuga* by molecular as well as morphological identity related techniques, from sloth bears in South India.

Art - 172. INFRARED THERMOGRAPHY (IRT): NOVEL REMOTE EXAMINATION TOOL IN CAPTIVE ELEPHANT HEALTH CARE.

Illayaraja S and **Arun A. Sha.**

Introduction with historical perspective

Infrared thermography (IRT) is a safe, modern non-invasive, non-contact thermal profile visualization technique using thermographic scanning equipment. Thermal or Infrared energy is a part of electromagnetic spectrum with high wavelength that is over and above the visible range of the human eye. Instead, we perceived it as heat. Electromagnetic radiation is ubiquitous and maybe classify according to its frequency or wavelength. The radiation restricted to the wavelengths from 760nm to 1nm is refer to as Infrared radiation or "thermal radiation". Unlike visible light, everything with the temperature above absolute zero emits heat. The higher the temperature of an object, the greater the amount of Infrared radiation it emits. Even very cold objects, such as ice cubes, emits Infrared radiations.

Sir William Herschel, the famous astronomour, who discovered the planet Uranus discovered Infrared in his laboratory in Bath. He directed sunlight through a glass prism

to create a spectrum and then measure the temperature of each color. He noticed that all of the colors (violet, blue, green, yellow, orange and red light) had temperatures higher than the controls and increased from the violet to the red part of the spectrum. He found that the rejoin just beyond the red portion of the spectrum, where no sunlight was visible at the highest temperature of all. His discovery of a form of light beyond red light is known as Infrared radiation (Infra means below). Herschel demonstrated that there were types of light that we cannot see with our eyes. His son produced the first evaporagraph as a result of this discovery. It's interesting to note that they were contemporaries of Charles Babbage, who originated the concept of programmable computer.

The modern use of Infrared imaging cameras with computer technology has been vital to the advancement and practical use of the thermal imaging in all areas of imaging. It was actually Hippocrates was the first to obtain "Thermogram". His method, which is consider the first for measuring body temperature, involved covering the patient's thorax with an earth-soaked cloth. As the Warner areas dry faster the pattern of enlargement of the dry area shows that temperature distribution. He also immersed his patients in mud, with the same objective.

The thermal imaging camera is not like an ordinary camera

because it is specially designed to measure heat and only images that immitted Infrared radiation from an object. This type of camera allows the temperature of an object to be measured and recorded and to create a thermal image i.e. a thermogram. It makes no difference if it is too dark to see the object with the naked eye: the thermal image will be unaffected. Thermographic method has found numerous applications not only in industries but also in human and veterinary medicine, primarily for diagnostic pursposes for many years in many developed foreign countries. The first thermographic image of man was done by Czerny in 1928 in Frankfurt. In the year 1952, Germany reported the first medical use of thermography. Because of its huge cost, lack of expertise and sufficient awareness, it is still an unfamiliar technique in India.

Application of thermography in the field of veterinary

Infrared thermography has been used primarily as a veterinary diagnostic tool in horses and can be expanded for use in other species like elephants. In veterinary diagnostics of farm and wild animals, thermal imaging is used to determine the causes of lameness, injuries, and inflammations of the locomotor system; to diagnose infectious diseases, oestrus, and pregnancy; and to assess stress levels. Hurnik et

al. (1984) studied the suitability of IRT for the detection of health disorders in Holstein Friesian dairy cattle and in 1985 studied the relationship between differences in body surface temperatures and the oestrus in Holstein-Friesian dairy cows, and a possibility of using this technique to determine the onset of oestrus. Kozumplik et al. (1989) used IRT in the diagnosis of inflammatory processes on the sex organs of breeding bulls. Spire et al. (1999) used IRT to detect inflammation caused by contaminated growth promoting ear implants in cattle. Cockcroft et al. (2000) described the use of IRT as an aid in the diagnosis of septic arthritis of the right metatarsophalangeal joint of Friesian heifer. Schaefer et al. (2003) used IRT for identifying calves with bovine viral diarrhea. They found that increases in eye temperature were mosre consistent than other anatomical areas. There were also significant changes in eye temperature several days to one week before other clinical signs of infection. Nikkah et al. (2005) used IRT to observe hooves of dairy cows. Images of hooves were taken to determine temperature of the coronary band. Sabec and Lazar (1990) and Loughmiller et al. (2001) used IRT to study about osteoarthrosis tarsi deformans in Swedish Landrace boars and febrile responses in pigs following inoculation with Actinobacilluspleuro-pneumonia respectively.

Thermography applications have been successfully implemented in wildlife for a long period for disease diagnosis and other studies related to the reproductive process, animal behavior thermoregulations and estimation of population size, without compromising their welfare as the observed subject does not have to be restricted or sedated, which significantly reduces stress levels (Lavers et al. 2005). Speakman and Ward (1998) used IRT for a heat transfer study under various ambient temperatures (from "25 to 33o C) in the red fox *(Vilpesvulpes)*, the Arctic fox *(Alopexlagopus)*, and the kit fox *(Vulpesnacrotas)*. Thermal images reveal that the nose, lower legs, paws and the front of the ear are important thermoregulatory surfaces in all fox species. Dunbar and MacCarthy (2006) used IRT to detect the science of rabies infection in raccoons *(procyonlotor)*. Dunbar et al. (2009) used IRT to diagnose foot-and-mouth diseases by detect temperature changes in mule deer *(odocoileushemionus)*. Nakayana et al. (2005) and McCafferty (2007) documented changes in fecal temperature in rhesus monkeys *(macacamulatta)* due to stress in threatening situations and changes in blood flow of dolphins during their perceived of tuna fish, respectively, by using IRT. Michael et al. (2016) used IRT to evaluate the relationship between apparent thermoregulatory behavior and the environment as well as

the skin and core temperatures for tame savanna elephants *(loxodontaafricana)* that were free ranging in the hot parts of the day, in the natural environment.

Preparations for veterinary Thermal Imaging

- Ideally, the animal should be dry and as clean as possible to eliminate artifacts on the image. For example, water or respiration on the animal typically appears as cool zones. Depending on amount, it could hamper the ability to view and area of importance. This may not always be possible to achieve. Therefore, establishing baseline images is critical and follow up studies at a later time period help to determine the thermal landmarks of importance that are associated with the particular condition.
- Have the animal stabilized in a barn (with the horse) or a grooming area, if possible. For small pets make sure they haven't been resting in the sun or in front of a heat source prior to the imaging procedure.
- The mane on a horse should be kept clear of the neck region being viewed. For a small animal, it may be preferable to have any clipping perform at least 24 hours prior to the test. However, there is a study that shows clipping has relatively minimal effort on the relative temperature differences.

- Try not stimulate the skin surface with anything that may contribute to increase blood flow to the area. This is usually somewhat transitory and will dissipate within 10 to 15 minutes, typically. It is depending on the sensitivity of the thermal imager being used, this may have minimal effect on evaluating a fundamental issue with the animal. The relative thermal variation should still appear, if its associated with the physiological conditions.
- As much as possible, within the comfort zone of the animal, remove any bandages, wraps, blankets or coverings that may restrict the view.
- Avoid coat conditioners, sprays, liniments, adhesives, creams at the time of imaging.
- Take note of any scars, medications or naturopathic remedies and supplements in the event and unusual anomaly or pattern appears on the image, that may have an effect on a blood flow to an area.
- Avoid direct sunlight and significant drafts on the animal.
- In the summer months, taking images earlier in the day is preferable, or later in the evening.

Health issues of captive elephants in India captive elephant's health issues in India

Elephants in captivity are highly prone to various health

problems such as degrative joint diseases, stifle joint hygroma, foot pad inflammation, toenail abscess, shoulder bursitis, temporal adentitis etc. the unfavourable tethering area, lack of exercise and hygiene, ignore foot care, ill treatment on part of keepers and malnourishment further enhanced their health problems under captivity. Hence, soon after being rescued, the thorough examination of the rescued elephants becomes an absolute must to evaluate its health status. The infrared thermography technique plays a vital role in this initial evaluation, even though the animal doesn't cooperate due to its unfamiliar surroundings and a new mahout.

Wildlife SOS is an NGO actively involved in wildlife care and conservation in India. The organization manages an elephant conservation and care centre at Mathura, Uttara pradesh and houses 20 rescued elephants, suffering from various ailments, the centre has fully equipped diagnostic facility that processes equipments for performing infrared thermography, thus enabling a field veterinarian in improvising the yearly diagnosis and providing suitable treatments.

Imaging protocol in Elephants

Typical exam procedures with the thermal imager for veterinary applications may involve controlled environmental conditions. The imager used should stand up to consistent

measurement under fairly extreme environments. We used FLIR- E 60 thermal imaging camera for the study and conducted the examination from 3-meter distance. To measure temperature accurately, it is necessary to compensate for the effects of several different radiation sources. This is done automatically by the camera. However, the following object parameters must be supplied from the camera: the emissibity of the object, the reflected temperature, the distance between the object and the camera and relative humidity. Since the skin possess high emissibity (0.98), the effect of reflected temperature will not affect the thermal measurement. Hence, it can be ignored. We used digital temperature and humidity meters (HTC-2) for recording the environmental temperature and humidity. An important concept in the "color palette". A color palatte is the set of colors that is used in a thermal image, with specific colors varying with temperature. Thermal cameras allow a white choice of color palettes. It is important to select a palette that is easy to interpret when examining animals. We used, 'high rainbow' as it has easily distinguishable colors- a palette displaying the coldest area in blue and hottest area in white, with red and yellow in between.

Factors such as wet skin, skin contamination due to durt, moisture in the furr, windy locations, direct sunlight and other

heat sources will affect the appearance of the thermal images and can lead to an error in thermal measurements. Thus, care should be taken to avoid errors due to the above- mentioned the factors.

Benefit of IRT in elephant

A healthy organism is characterized by a balanced distribution of temperature between different parts of the body. The same anatomical parts can be compared to identify warmer spots, and the causes of thermal deregulation can be determined based on our knowledge of a given species (Bowers et al. 2009). Heat may be transferred by conduction, convection and radiation. The last option is used by thermohraphic imaging systems. All organic activity generates heat. If there are changes on the organic activity, there will also be changes in the amount of heat that is emitted. Thermography can detect many conditions that change the normal thermal pattern. It can show differences in thermal symmetry and abnormally warm or cold areas in patients. IF the thermal pattern is not symmetrical or is asymmetrical of 1C or more, it is often significant and indicates possible pathology such as infection, soft tissue injury, joint problem, nervous dysfunction etc. Elephant thermal imaging is a non-invasive diagnostic tool to detect minute differences in the

animal's thermal and neural condition. All the body parts of the elephant such as the foot, limbs, shoulder region, hind quarters, dorsal spine, neck, ear head and trunk can be scanned easily to find out hot or cold spots. It allows us to quickly and efficiently identify trauma/injury in animals. By identifying locations of injury, we can prevent further damage. A hot spot indicates inflammation or increased blood flow. Cold spots indicate a decreased blood flow, usually the result of swelling, nerve damage or scar tissue. With the help of thermography, we have successfully diagnosed toenail abscesses, compensatory leg lameness, cutaneous inflammation and hygroma in early stages and provided treatment accordingly. Thermography is a suitable tool to evaluate as well as implement proper foot care in elephants.

Conclusion

Thermal imaging cameras are a great tool to find out whether an animal is suffering from pain as in inflammatory conditions. Elephants are ideal models for thermal imaging studies as their skin is scarcely covered with hair. As a physiological diagnostic tool, thermography makes it possible 'to see the unseen' before anatomical changes have developed. The diagnosis of localized inflammation would not have been possible without thermography. Since it is portable, easy to

use/learn, not stressful to the animal as it is non-contact, safe remote sensing method and less in cost when compared to digital radiography; it can be considered as an efficient diagnostic tool in the health care of captive elephants.

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Art - 173. SUCCESSFUL WOUND MANAGEMENT IN WILD CAUGHT INDIAN CRESTED PORCUPINE FROM A CONFLICT SITUATION

Ilayaraja Selvaraj, Acharya PR., Pradeep R., Arun A. Sha., Yaduraj khadpekar

Abstract

The Indian Crested Porcupine is a large rodent that possesses nocturnal behaviour. It has the ability to adopt wide range of habitat and food types, as a result of urbanization, infrastructure development, and pesticide use. Along with this, the type of habitat suitable for the porcupine is currently declining. The Indian Crested Porcupine is protected under Schedule IV of the Indian Wildlife Protection Act of 1972 and is listed by the IUCN as 'Least Concern' as of 2008. Porcupines are widely hunted for both human consumption and medicinal use. As a result of its destructive nature in regard to vegetation and agricultural crops, the porcupine is considered an agricultural pest, which leads to frequent situations of human-porcupine conflict. Wildlife SOS is an NGO working to mitigate wildlife conflict situations, as well as rescuing and rehabilitating wildlife in many states within India. On the 24th of February 2018, an injured porcupine was rescued by the

Wildlife SOS team from a well in the Etmadpur range (27˚1'6 24.2'' N 78˚11'' 24.8 E) receiving a call from the UP forest department. The porcupine was brought to the Wildlife SOS veterinary hospital in a semi-conscious state. The animal was in lateral recumbency, with a massive, actively bleeding wound on its neck and forehead paired with facial swelling. The skin of the forehead region was hanging, as were both ear flaps. The dorsal aspect of the neck and body was devoid of quills, and a diffuse serrated injury was also noticed. Radiographic examination of the skull revealed a fracture on the left lateral aspect of the skull bone. The animal was kept under general anaesthesia using an isofluorane mask. The wound was thoroughly cleaned with an antiseptic solution of povidone-iodine and metronidazole at a ratio of 1:10, followed by a wash consisting of ringers lactate solution to sterilize the wound and eliminate any old, dried clots. After debridement, the hanging skin flap was opposed with PGA absorbable suture material (2-0) up to the base of the ear. However, there was not enough skin to oppose the wound on the dorsal aspect of neck region; therefore, we applied pull-over suture to bring the wound edges as close to each other as possible without compressing the neck. This was done to minimize the exposed area of the wound, and also for the purpose of uninterrupted quick healing. The wound was

dressed with Piperacillin powder topically, as well as fly repellent “Skin Heal” spray. The porcupine was housed in an observation enclosure for further treatment and monitored care. Piperacillin was mixed with normal saline and applied topically, followed by the application of a collagen ointment applied and counter dressing with fly repellent spray. This was repeated daily until healed. Oral medication of Melonx and Rhutoheal, and supplemental ‘Multistar Pet’ were injected inside fed fruits and vegetables and supplied for 7 days. Progression of healing was assessed using thermography. The animal completely recovered by day 55 and was released back in its natural habitat successfully on day 70.

Keywords: porcupine, wound management, thermography, rescue, conflict

Art - 174. PREVALENCE AND MOLECULAR DIAGNOSIS OF ESCHERICHIA COLI IN CAPTIVE SLOTH BEARS

(Melursus ursinus)

M. Palanivelrajan, **A. Sha Arun**, M.G. Jayathangaraj, K. Vijayarani, Bhaskaran Ravi Latha, P. Sridevi and K. Porteen

Abstract

Pathogenic *Escherichia coli* strain from faeces of sloth bears was screened by using culture morphology, Gram's staining, biochemical tests and polymerase chain reaction. Our results showed that out of 60 samples collected, 59 samples (98.33%) were cultured on EMB agar and Gram's stain revealed rod-shaped Gram-negative organism with green metallic sheen - like colonies. The biochemical tests of cultured samples revealed positive to indole production, positive to methyl red test, negative to Voges- Proskauer test, negative to Simmon citrate utilization test, positive to glucose, negative to H2S production and negative to urea production. All *E. coli* isolates were found sensitive to azithromycin, streptomycin and tetracycline. They were found intermediate to enrofloxacin and gentamicin. The *fimC* gene was amplified by PCR for the pathogenic *Escherichia coli* and were found to be positive of 53.33% among the juveniles, 46.67% among the

sub-adults, 53.33% among the adults and 33.33% among the geriatrics were found to be positive. This study may provide information for developing strategies in the future in the control of *Escherichia coli* infections in sloth bears. **Keywords:** Antibiogram, *Escherichia coli*, *fim*C gene, Sloth bear, *Ursidae*

Introduction

Ursidae have a complex gut microbiota resulting from a dynamic interplay among diet, host and commensal bacteria, which play an important role in the maintenance of health and disease modulation. The composition of the gut microbiota depends on the physiology of the gut as well as the type of diet and varies among the hosts (Ley *et al.,* 2008). Ursids including Sloth bear *(Melursus ursinus)* are an enigmatic family. *Escherichia coli* are part of the normal intestinal microbiota and coexist with its host in mutual benefit. Captive animals, especially those acquired from the wild can be asymptomatic carriers of pathogens. Some of these pathogens have also caused morbidity and mortality in animals within zoological gardens. Escherichia coli are often a long-term commensal in animals, a part of their normal intestinal microbial community. Escherichia coli can cause a variety of diseases including dysentery, haemorrhagic uremia

syndrome, bladder and kidney infections, septicemia, pneumonia, and meningitis in humans and animals (Cambre et al., 1980). The intestinal microbiota can also be a reservoir of extra intestinal pathogenic *Escherichia coli* and strains from the intestinal microbiota of the Sloth Bear are scanty. The aim of this study was to investigate and characterize faecal *Escherichia coli* from sloth bear *(Melursus ursinus)*.

Materials and Methods

In this study, fecal samples were collected from Bannerghatta Bear Rescue Centre (BBRC), Wildlife SOS, Bangalore, Karnataka. During the sampling period in 2017, the faecal samples from 15 juvenile, 15 sub-adult, 15 adult and 15 geriatric captive sloth bears were collected in nutrient broth using sterile swabs and kept at 40C until further processing. The swabs were incubated at 370C for 24 hrs. A loop-full of culture was then spread on Nutrient Agar plate and incubated further at 37^{o}C for 24 hrs. Eosin Methylene Blue (EMB) media was used as a selective and differential culture media. A loop-full of culture was spread on Eosin Methylene Blue (EMB) agar and incubated at 37^{0}C for 24 hrs. to obtain of *Escherichia coli* colonies. The isolated colony was stained with Gram's stain and biochemical tests (HiIMViCTM Biochemical Test Kit, TSI agar and urea agar) and

was confirmed by Polymerase Chain Reaction (PCR).

Antimicrobial resistance pattern of pathogen E. coli isolates were studied by Modified Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2006) for the following antibiotics: amoxicillin/clavulanic acid (30mcg), azithromycin (15mcg), cefotaxime (30mcg), clindamycin (2mcg), gentamicin (10mcg), enrofloxacin (10mcg), methicillin (5mcg), streptomycin (300mcg) and tetracyline (30mcg). For the PCR reaction, the DNA was extracted from the isolates by using boiling method (Medici et al., 2003). One ml of the pre-enriched culture was transferred to a 1.5ml micro- centrifuge tube. The cell suspension was centrifuged for 10 min at 10,000rpm. The supernatant was discarded carefully. The pellet was resuspended in 300µl of DNase- RNase- free water by vortexing. The tube was centrifuged at 10,000rpm for 5 min, and the supernatant was discarded carefully. The pellet was resuspended in 200µl of DNase- RNase-free water by vortexing. The microcentrifuge tube was incubated for 15 min at 100°C and immediately chilled on ice. The tube was centrifuged for 5 min at 10,000 rpm at 4°C. The supernatant was carefully transferred to a new microcentrifuge tube and incubated again for 10 min at 100°C and chilled immediately on ice. Further, it has stored at -20^{0}C. An aliquot of 5µl of the

supernatant was used as template in the PCR. The molecular weight of the fimC gene corresponding to the avian pathogenic Escherichia coli was 477bp (Janben et al., 2001). The following primers were used to amplify the fimC gene (Forward Primer: 5'– GGGTAGAAAATGCCGATGGTG–3' and Reverse Primer: 5'- CGTCATTTTGGG GGTAAGTGC– 3'). The PCR was performed in a 25µl reaction mixture consisting of 12.5µl of 2X PCR mastermix, 1µl of each primer, 2µl of extracted DNA and finally volume was adjusted with nuclease free water. Amplification was carried out in thermocycler with initial denaturation 94°C for 2 min followed by 25 cycles each of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, extension at 92°C for 90 sec with a final extension period of 7 min at 72°C. 10µl of each PCR products was electrophoresed on 1.5% agrose gel containing ethidium bromide in the presence of 100bp ladder. The presence specific amplicon of 477bp was viewed under UV transilluminator. The PCR amplifies the fimC gene of the one of the isolate was sequenced using commercial sequencing service.

Results and Discussion

Out of 60 samples collected, 59 samples (98.33%) were cultured on EMB agar and green metallic sheen - like colonies

(Figure 1) were picked for Gram's staining and revealed Gram-negative rod-shaped organism. Usage of biochemical tests in HiIMViCTM Biochemical Test Kit, TSI agar and urea agar revealed that were positive to indole production, positive to methyl red test, negative to Voges-Proskauer test, negative to Simmon citrate utilization test, positive to glucose, negative to H2S production and negative to urea production in cultured samples. All E. coli isolates which were studied have been found sensitive to azithromycin, streptomycin and tetracycline. They were found intermediate to enrofloxacin and gentamicin. Further, based on the PCR assay, among 53.33% juvenile, 46.67% sub-adult, 53.33% adult and 33.33% geriatric screened for the pathogenic Escherichia coli were found positive. In the juvenile sloth bear group, three males and five females harboured the infection (60.00 and 50.00%, respectively), in the sub-adult group, four males and three females harboured the infection (66.67% and 33.33%, respectively), in the adult group one male and seven females harboured the infection (20.00% and 70.00%, respectively) and in the geriatric group three male and two females harboured the infection (27.27% and 50.00%, respectively) (Figure 2 and Table 1).

The fimC gene was chosen for amplification since presence of this gene indicates the *E. coli* as a pathogenic gene. All the

isolates from the sloth bears were positive for the presence of fimC gene indicating the pathogenic nature.

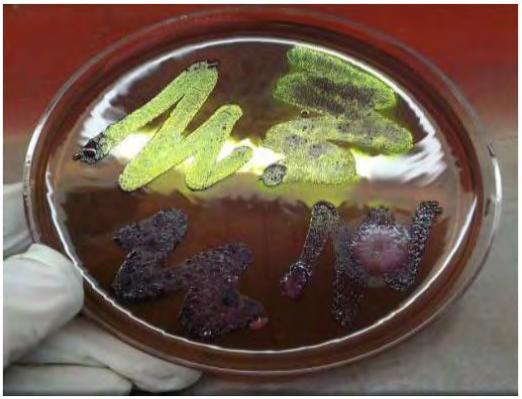

Fig.1 *Escherichia coli* on Eosin Methylene Blue (EMB) Agar

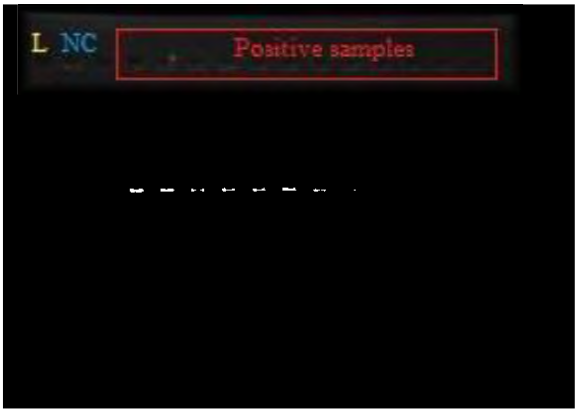

Fig.2 Amplification of *Escherichia coli* by PCR

1.5 % agarsoe showing PCR products of *fimC* gene Lane L – DNA ladder (500bp)

Lane NC – Negative control Lane positive samples – *fimC* gene

amplification (477bp)

Supplement: Partial nucleotide sequence of *fim*C gene from *E. coli* isolated from sloth bear (*Melursus ursinus*)

>ConsensusTTTGGGTAGAAAAATGCCGATGGTGTAAAGGATGR
KCGKTTTATCGTGACGCCTCCTCTGTTTGCGATGAAGGGAAAAA
AAGAGAATACCTTACGTATTCTTGATGCAACAAATAACCAATTG
CCACAGGATCGGGAAAGTTTATTCTGGATGAACGTTAAAGCGAT
TCCGTCAATGGATAAATCAAAATTGACTGAGAATACGCTACAGC
TCGCAATTATCAGCCGCATTAAACTGTACTATCGCCCGGCTAAA
TTAGCGTTGCCACCCGATCAGGCCGCAGAAAAATTAAGATTTCG
TCGTAGCGCGAATTCTCTGACKCTGATTAACCCGACACCCTATT
ACCTGACGGTAACAGAGTTGAATGCCGGAACCCGGTTCTTGAAA
ATGCATTGGTGCCTCCAATGGGCGAAAGCACGGTTAAATTGCCT
TCTGATGCAGGAAGCAATATTACYTWCCGAACAMTAAATGATT
ATGGCGCACTTACCCCAAAATGACGAA

IUPAC nucleotide code	Base
A	Adenine
C	Cytosine
G	Guanine
T (or U)	Thymine (or Uracil)
R	A or G
Y	C or T
W	A or T
K	G or T
M	A or C

Table.1 Prevalence of *Escherichia coli* in Sloth bears (*Melursus ursinus*) by PCR

Group of animals (n=30)	Sex	Samples collected	Positive for pathogenic *Escherichia coli*	Prevalence (%)
Juvenile (2-7 years) (n=15)	Male	5	3	60.00
	Female	10	5	50.00
	Both	15	8	53.33
Sub-adult (8-13 years) (n=15)	Male	6	4	66.67
	Female	9	3	33.33
	Both	15	7	46.67
Adult (14-18 years) (n=15)	Male	5	1	20.00
	Female	10	7	70.00
	Both	15	8	53.33
Geriatric (above 19 years) (n=15)	Male	11	3	27.27
	Female	4	2	50.00
	Both	15	5	33.33

The sequence results were compared with that of the pathogenic *coli* isolate sequences using nBLAST (Nucleotide local alignment service tool) available at https://blast.ncbi.nlm.nih.gov/Blast.cgi ?PAGE_TYPE=BlastSearch. The sequence data is provided as supplement. The prevalence of 46.67% of pathogenic *Escherichia coli* confirms the presence of infection and ampleness of the bacteria in the Bannerghatta Bear Rescue Centre (BBRC). The isolated pathogenic *Escherichia coli* which could be either enteropathogenic, enterohaemorrhagic or commensal are the possible cause of zoonotic infection in wild animals and in humans. The sources of infection could not be revealed. The possible sources could be food, water, birds, visitors, rodents, lizards and bats (Oludairo *et al.,* 2016). The sloth bear are omnivores animal and has yield a 46.67 % rate of pathogenic *Escherichia coli* infection and which is in agreement with the findings of Gopee *et al.,* (2000) who reported isolation of *Escherichia coli* from omnivores,

herbivores, and carnivores at the rate of 87.2%, 70.0%, and 57.3%, respectively, regardless of animal class, were significantly different. Most (99.6%) of the *Escherichia coli* isolates tested for antibiotic sensitivity exhibit resistance to one or more of the antimicrobial agents used. Resistance was generally high to cephalothin (99.2%), ampicillin (62.4%), tetracycline (58.2%), and streptomycin (36.0%) but low to gentamicin (9.6%), chloramphenicol (1.6%), and norfloxacin (0.4%). Captive wildlife in zoo enclosures is potentially exposed to strains of *Escherichia coli* through contact with animal handlers or through the microorganism in their diets.

Acknowledgement

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Art -175. A REPORT ON THE ANTHELMINTIC EFFICACY OF FENBENDAZOLE IN CAPTIVE SLOTH BEARS

(Melursus ursinus)

Arun A. Sha, J. Afreen Fatima, S. Gomathinayagam, M. Palanivelrajan, G.M. Arpitha and M.G. Jayathangraj

Abstract

The effectiveness of Fenbendazole in the treatment of ascarid infection was evaluated in sloth bears of Bannerghatta Bear Rescue and Rehabilitation Centre, Bannerghatta Biological Park, Bengaluru. The Faecal Egg Count Reduction Test (FECRT) was done on faecal samples collected from 32 Sloth bears and all the faecal samples were found positive for ascarid eggs. Pre and post treatment egg counts indicated that there was reduction of only 36% infection after administration of Fenbendazole at the recommended dose.

Key words: Anthelmintic efficacy, Fenbendazole, Sloth bear

Introduction

Bannerghatta Bear Rescue and Rehabilitation Centre (BBRC), Bannerghatta Biological Park, Bengaluru is the world's second largest rescue centre with its mission to conserve and rehabilitate the Sloth bears in captivity. The samples for this

present study were taken from the BBRRC. Since the treatment for helminthic infections was not known, a study was undertaken to note the efficacy of fenbendazole a commonly available and used drug for treatment of domestic animals.

Materials and Methods

The study was conducted in 32 sloth bears of BBRC by the Faecal Egg Count Reduction Test (FECRT). Faecal samples were collected immediately after observed defecation and each sample was stored at 4˚C in a sealed plastic bag (Nielsen et al. 2010) and transported to the laboratory. Samples were subjected to faecal examination for detection of parasitic eggs (Soulsby, 2012). The faecal samples were positive for ascarid eggs. Sloth bears were treated with fenbendazole at the recommended dose rate of 10mg/kg body weight, orally for 3 consecutive days (Bowman, 2009). A Modified Mc Master egg counting technique as per Zajac and Conboy (2013) using Sheather's sugar solution was used to assess the severity of the infection in the pre and post treated faecal samples. Three replicate chambers were counted for each sample resulting in an analytical sensitivity of 40 eggs per gram (epg) of feces per individual.

Results and Discussion

Pre and post treated faecal samples were subjected to FEC test. The data was analysed statistically for finding out the per cent reduction in egg counts using a Reso' FECRT analysis program. It indicated that there was only 36% reduction on day 14 post treatment, indicating that the Ascarid sp. infection in Sloth bears were not controlled by fenbendazole treatment at the recommended dose. This finding was in accordance with Moudgil et al. (2014) who reported the molecular identification and fenbendazole resistance against *Baylisascaris transfuga* infection in *Melursus ursinus* (Sloth bear). They were treated with fenbendazole at 10mg/kg body weight for three consecutive days, associated with complete disinfection of the enclosures. But the treatment was effective for the rest few days with the reduction of epg of feces from 2800 (0 day) to 400 (14.28%), 700 (25.00%), 800 (28.57%) and 2400 (85.71%) on day 1, 4, 7 and 21 post treatments, respectively.

Summary

The efficacy of using Fenbendazole as dewormer to ascarid infection in sloth bears of Bannerghatta Biological Park, Bengaluru has been found to be only 36 per cent.

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Art – 176. EX-SITU CONSERVATION- AN EFFECTIVE METHOD AND SUPPORT TOWARDS IN SITU CONSERVATION

Baiju Raj and **Dr. Arun A. Sha**

Abstract

Wildlife SOS (WSOS) has set up India's first Private Public Partnership model rehabilitation center for sloth bears in collaboration with state forest departments. Sloth bears have been used for performing (dancing) on the streets for centuries; thus, this project has become historic in the field of conservation. All of the "dancing bears" were voluntarily surrendered to the forest department by the Kalandars (bear dancers) and a rehabilitation package was provided to each family. Over 600 bears were rescued and surrendered. The bears are kept under quarantine for 90 days, conditioned to solar power fence and then provided access to large, forested enclosures. High quality balanced and nutritious diets are provided to improve their health. The bears slowly start showing their natural behaviors like climbing, digging and foraging, as well as change their activity pattern to being more active in the late evening. With the state forest departments, WSOS has set up four such rehabilitation centers in the

country. Today these centers not only take care of the existing population but also help the forest departments to mitigate human-animal interactions across the country; many wild bears are rescued and released back into the wild. WSOS also helps the Indian government to minimize the poaching of bears from various regions. A well-managed ex- situ conservation project is always considered as insurance for in-situ conservation. The bears which are rescued from human-bear interactions are radio collared and released back to their natural habitats. The radio collars help us to understand the bears' movement patterns as well as the reasons behind human- interactions. These centers which have been set up in collaboration with the forest departments have not only ended the dancing bear trade but have also reduced the poaching of bears within the country so that wild populations are less exploited.

Art – 177. NEONATAL CARE OF SLOTH BEAR CUBS

Dr. Arun A Sha and **Baiju Raj**

Abstract

Sloth bears are classified as vulnerable under the IUCN Red List of Threatened Species and protected under Schedule of The Indian Wildlife (Protection) Act, 1972. Their unique feeding ecology includes frugivory and myrmecophagy, making them specialists in their ecological niche. In last two decades Wildlife SOS has hand reared over 100 sloth bear cubs of different age groups (2 weeks to 2 years) that were confiscated or rescued from various situations such as forceful, early weaning due to illegal poaching. Orphaned cubs have not always received their mother's colostrum milk, making them vulnerable to various nutrition- based health issues on top of the physiological and psychological stress they have endured. Since sloth bear cubs will stay with their mothers until three years of age, depending on their gender, we will attempt to reunite the cubs with their mother if possible by tracking the mother or looking for an injured female caught in a snare around the same area as the cubs were found before we attempt to hand rear the abandoned cubs. If a reunion is not possible, the procedure of quarantine

and socialization (if it is more than one individual) will be followed. The successful hand raising of sloth bear cubs involves multiple challenges such as bottle feeding, stimulating the cubs to defecate and urinate, and providing them warmth, comfort and security as the mother would provide. In this presentation, the authors describe the protocol followed for raising neonate cubs in terms of suitable commercial milk replacers, feeding schedule and frequency, precautions, hygiene, routine body weight checks and assessment of body growth rate , safe and minimal handling of cubs depending on the rehabilitation plan, veterinary care, common health issues, preventive care, behavior and health monitoring of the cubs in addition to providing desired micro and macro captive habitats for housing the cubs.

Art – 178. OPERANT CONDITIONING THROUGH POSITIVE REINFORCEMENT OF CAPTIVE SLOTH BEARS *(Melursus ursinus)* FOR IMPROVED HUSBANDRY PRACTISES AT WILDLIFE SOS

Dr. Ilayaraja Selvaraj, **Dr. Acharya PR** and **Dr. Arun A. Sha**

Abstract

Unlike domesticated animals, providing care and treatment for captive wild animals requires additional precautions to ensure the safety and comfort of the animals as well as the personnel involved. Hence, it is mandatory to adapt techniques to achieve this while causing minimal stress to the animals and personnel. In developed countries, animal care staff in zoos train animals to voluntarily participate in certain procedures using operant conditioning with positive reinforcement. This allows for better provision of stress-free healthcare to animals. However, in India, the technique of operant conditioning through positive reinforcement is yet to be developed and practiced due to lack of knowledge and lack of manpower in Indian zoos. Wildlife SOS took immense efforts to provide better care for the rescued dancing sloth bears by establishing a unique rescue and rehabilitation facility for their welfare. This, in turn, created an opportunity for the animal care staff to learn

operant conditioning techniques and provide continuous encouragement to practice the same. By using positive reinforcement, we achieved voluntary blood collection, oral cavity examination, nail trimming, wound dressing and abdominal ultrasonography examination with sloth bears. Though the task is time consuming and sometimes demanding, it is worth practicing, especially for animals that are unfit for chemical immobilization and those who need regular monitoring.

Art – 179. GERIATRIC CARE OF CAPTIVE SLOTH BEARS

(*Melursus ursinus*)

Dr. Arun A Sha and **Dr. Ilayaraja Selvaraj**

Abstract

Sloth bears are one of the four bear species in India and one which has been highly exploited by people for several centuries. They have been poached since time immemorial for their body parts and cubs are taken away after killing the sow for use in street performances. Several literatures quote the lifespan of sloth bear sin the wild to be between 25 to 30 years, however the rescued bears very rarely reach this age due to their appalling previous life. As per the current understanding and authors experience over 15 years of sloth bear work, captive sloth bear age classes are being standardised as Neonate (< week), Cub (< year), Juvenile (1-2 years), Sub-adult (3-4 years), Adult (5-20 years) and Geriatric (>20 years). It is mandatory to perform a detailed clinical examination on every animal at the time of rescue and estimation of approximate age of the animal is of utmost importance to provide the necessary treatment and post—rescue management. It has been observed that the behaviour of younger animals towards a geriatric individual is of

dominance, and hence management of these aged animals requires a complete hierarchy-based approach. Such animals need to be housed in systems that encourage minimal activity patterns along with compatible social interaction. Geriatric animals in captivity are more prone to problems such as cancers, claw avulsion, impaired vision and hearing, gallbladder diseases, locomotor disorders, muscle atrophy, arthritis, pyometra, skin infections, and other bacterial and viral diseases. Periodic chemical immobilization of such animals must be reduced, in favor of behavioral restraint for medical exams through the use of operant conditioning with positive reinforcement. A geriatric based protocol in terms of customized diet, feeding regime, and enrichments should also be strictly followed.

Art – 180. FIRST REPORT ON PROBIOTIC - LACTOBACILLUS PLANTARUM FROM SLOTH BEARS (*Melursus ursinus*) IN INDIA

Dr. M. Palanivelrajan, **Dr. Arun A Sha**, M.G. Jayathangaraj, K. Vijayarani, Bhaskaran Ravi Latha, P. Sridevi,

Abstract

The intestinal microbiota composition depends on the gut physiology and diet of animals. Previous reports said that the facultative anaerobes *Enterobacteriaceae* and *enterococci* were dominant in wild bear feces. In general, the most common microorganisms used as probiotics are lactic acid-producing *lactobacilli* and *bifidobacteria*. There is no data on probiotics from the gut microbiota of sloth bears *(Melursus ursinus)*. The main purpose of this study was to investigate and characterize the probiotics in feces from individual sloth bears (n=60) reared at the Wildlife SOS, Bannerghatta Bear Rescue Centre (BBRC), Bangalore, Karnataka. Fecal samples were grown on MRS agar and the growth morphology of Lactobacillus bacteria was observed as creamish white circular shaped colonies. Phenotypically, the isolates were Gram positive stained rods and negative for catalase, oxidase, indole test, methyl red test, Voges-Proskauer test, citrate

utilization test and carbohydrate fermentation. DNA was extracted by boiling method and Lactobacillus spp., 16S rDNA primers were used for the detection of Lactobacillus spp. by PCR. The amplified PCR product was sequenced, and the isolates were identified as *Lactobacillus plantarum*. For the characterization, the growth tolerance of *Lactobacillus plantarum* was observed in Na Cl and bile salt. The probiotic bacteria in faeces from sloth bears was isolated and identified as *Lactobacillus plantarum*. Naturally, probiotics prevent gastrointestinal diseases and modulate the intestinal immune response. Hence, this study provided the information on *Lactobacillus plantarum* in sloth bears and suggested for further research on the importance of the identified probiotics in sloth bears.

Art – 181. NON-CARDIAC TRANSTHORACIC ULTRASONOGRAPHY IN SLOTH BEAR (*Melursus ursinus*); BEAR'S-BLUE

Ilayaraja Selvaraj, Arun A Sha, Nithin K, Acharya PR and Pradeep R

Abstract

Like the trans-abdominal ultrasonography examination technique used to examine the visceral organs in the abdominal cavity, the non-cardiac trans-thoracic ultrasonography technique will help to examine the lungs. The lungs are located within the thoracic cavity of all mammals and are the only organs in the body to be filled with air under normal conditions. In the past, the belief was that the lung is not optimal for ultrasonography imaging because the aerated lungs creates insurmountable obstacles; therefore, people concentrated more on trans-abdominal, trans- rectal and trans-thoracic echocardiography. These methods became well established in human medicine and were gradually adopted into veterinary medicine as well. However, the ultrasonography examination of pulmonary system remained a grey area. Recent advancements and continued development in medical science has surmounted

the previously thought impossible and has proven that ultrasonography is an excellent diagnostic model in regard to the anatomy of all mammalian species. Rantanen 23 was the first veterinarian to use the ultrasonography to diagnose pulmonary disease in horses, and successfully diagnosed pneumothorax in 1986. Following this discovery, awareness in the field increased and resulted in the establishment of the Bed side Lung Ultrasound Examination (BLUE) protocol in human medicine and the Vet-BLUE protocol in small animal veterinary medicine. However, these advanced technologies are not as well established or effectively utilized in wild animal medicine as they are in small animal veterinary practice globally, except for in a few research institutions and teaching universities. Therefore, adopting this advanced diagnostic procedure in wildlife is pertinent for providing the utmost care in these species. During the study, we were able to clearly document the examination of the pulmonary system of sloth bears by adopting the non-cardiac, trans-thoracic ultrasonography technique. This technique includes patient preparation, positioning, probe selection, and recording of the ultrasonography image to compare and interpret normal versus abnormal lung and its radiographs. Thus, we developed the BLUE protocol for bears (Bear's-BLUE). The aim of this article is to disseminate the knowledge of sloth

bear lung ultrasonography to wildlife veterinarians at field level, and to encourage the neophytes to adopt this practice and improve their diagnostic ability to provide better care to the animals and develop a more effective therapeutic plan.

Keywords: Bear's-BLUE, Lung ultrasonography, Transthoracic ultrasonography, Sloth bear *(Melursus ursinus)*, Wildlife, Pulmonary system.

Introduction

The classical belief in both human and veterinary medicine was that the lung is not an optimal organ for ultrasonography imaging, which led to a delay in its use of the pulmonary system for many years. Therefore, medical fields focused more in trans-abdominal, trans-rectal, and trans-thoracic echocardiography until recent years. With the recent advancements in medical technology, those within medicine have proven that ultrasonography is an excellent diagnostic tool in all species. Lung ultrasound possesses a greater sensitivity than lung auscultation and supine chest radiography for many acute and potentially life-threatening respiratory conditions in both humans and animals alike [2, 5, 13, 24, 28]. Rantanen [23] was the first veterinarian to utilize ultrasound to diagnose pulmonary and pneumothorax in

horses in 1986. Following this discovery, the same techniques became an integral part of human medicine as well. In 1988, with remarkable foresight, Roy Philly dubbed the ultrasound probe as the 'modern stethoscope'. In 2011, Moor dubbed the ultrasound probe as the 'visual stethoscope,' as artifacts within the lung ultrasound are clearly distinguishable independent of patient or ambient noise. In 2008, Thoracic FAST, also known as TFAST, became the first standardized ultrasound exam of the thorax in small animals that included a chest tube site for lung surveillance and detection of pneumothorax (PTX) [17]. More recently was the establishment of Vet-BLUE [19, 20], the regional-based lung ultrasound protocol for small animals, which further enhanced the utilization of lung ultrasound to examine the complete pulmonary system. This allowed for the finding of numerous types of lung pathology, such as lung oedema, consolidation, pleural effusion and pneumothorax. The Vet-BLUE protocol covers 8 regional sites, 4 on each side, which includes the caudodorsal lung lobe region (Cd), the perihilar lung lobe region (Ph), the middle lung lobe region (Md), and the cranial lung lobe region (Cr). Although this lung ultrasound procedure is well established in small animals and equine medicine, it is not significantly utilized in wild animal medicine due to various issues such as difficulty of restraint

and lack of knowledge in regard to implementation of the same system in different species. As well, little information about lung ultrasonography in wild animals is documented [22], in particular for sloth bears

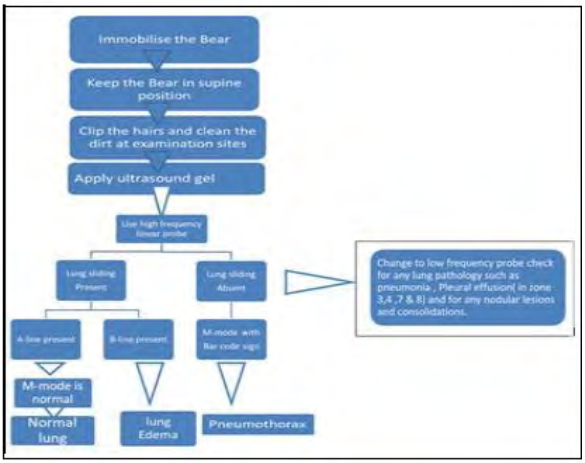


Fig 1: Bear's Bedside Lung Ultrasound Examination (*Bear's* –BLUE) Protocol

(family *Ursidae*). Due to this, we underwent numerous efforts to implement the existing advanced lung ultrasonography techniques in sloth bears in order to examine the normal lung and pathology; known as *Bear's*-BLUE protocol (Fig. 1).

Materials and Methods

This study was conducted at the Agra Bear Rescue Facility (ABRF), which is managed by the NGO Wildlife SOS in Agra, India, and is home to 186 dancing sloth bears undergoing

rehabilitation. As the sloth bears were rescued from street performers and are burdened with poor health and disease, being able to provide the maximum possible veterinary care by adopting recent advancements in the field of veterinary medicine is an absolute must to diagnose and treat the conditions as well as stabilize the health of those animals. The sloth bears are very prone to developing various kinds of pulmonary disorders, ranging from pneumonia to pulmonary tuberculosis. Therefore, due to its superior technique and time-saving ability in comparison to chest radiography, we adopted the lung ultrasonography procedure for the evaluation of the pulmonary system in sloth bears.

Patient preparation

Although they have been in captivity for much of their lives, the sloth bears are still wild animals and as a result their behaviour is highly unpredictable. Therefore, choosing the proper restraining method plays a pivotal role in implementing any diagnostic procedures within this species. Safety of the veterinarian, animal keepers, and various other staff is of the utmost importance. Chemical immobilization of the bear is achieved by using a mixture of xylazine and ketamine, which results in hassle-free restraint and the ability to implement any diagnostic procedures effectively. The

immobilized bear is kept in a supine position on the table. The hairs on the thorax were trimmed using hair clippers, as long fur and dust will create issues with penetration of the ultrasound beam. To get a better-quality image, ultrasound gel is applied to the shaved skin to create an optimal interaction between the probe and the desired area. Four zones, on each side of the thorax, are used for examination (Fig. 2).

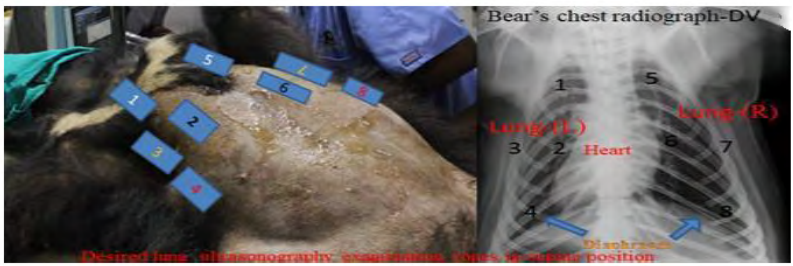


Fig 2: Bear kept in supine position. Hear clipped and gel applied to all eight zones.

Organ anatomy

A precise knowledge of organ anatomy and its topography is essential for the sonographer to utilize the diagnostic techniques quickly and efficiently within the stipulated time. In the absence of such anatomic knowledge or past experience, identification of pathologic changes may be inaccurate or not possible. The lung anatomy of sloth bears resembles that of the human. The multi-lobulated lungs – 3 lobes on right, 2 lobes on left, and one mediastinal lobe (ML) or accessary lobe – are located inside the thoracic cavity and

possess well developed visceral and parietal pleura. In the healthy lung, the interstitial thickening ranges from 5.76µm to 7µm (Fig. 3). This technique of lung ultrasonography examination and its nomenclature was previously described in both human [15] and small animal [19, 20] studies, and therefore worked well for sloth bear research with slight modification.

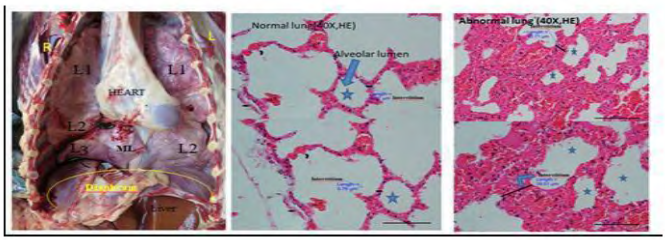


Fig 3: Lung lobes location and histology of normal & abnormal lung tissue.

Instrument & Probe selection

Selection of a good quality ultrasound machine, which has compatibility with the phased array, linear, and curved linear probes, is a prerequisite for achieving enhanced performance (Figure 4. In this case, a GE logq-E machine and the above-mentioned probes were utilized, and the images were recorded to better understand the anatomy. Image quality differs based on the probe that is used. The high frequency linear probe is used to evaluate the pleural line and visceral-parietal pleural interface (VPPI), as well as pneumothorax.

This is because the linear probe has a higher resolution in comparison to the others. Both the low frequency curved linear and phased array probes are used to diagnose alveolar interstitial syndrome (CHF/ARDS), pleural effusion, consolidation, pulmonary oedema, and pneumonia, due to their having a greater penetration in comparison to the higher frequency linear probe.

Fig 4: Different kinds of Ultrasound probe used in *Bear's* – BLUE.

Probe orientation

The standard orientation of the probe is generally cranio-caudal, with the probe marker towards the head. This orientation allows for the observation of the previously described "gator sign," which is likened to a partially submerged alligator peering over the water at the sonographer [3, 19] (Fig. 5). In this way, the "gator sign" is represented by two rib heads and an interposed intercostal space – demarcated as the gator's eyes and the bridge of the

gator's nose, respectively.

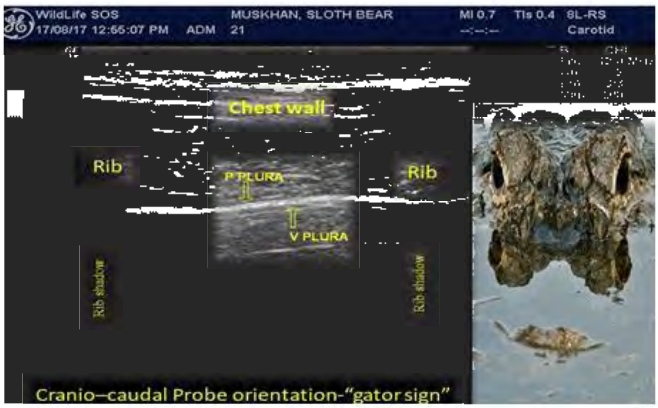


Fig 5: Cranio-Caudal probe orientation-'Gator Sign'.

Ultrasound modes

The commonly used ultrasound modes in lung ultrasonography are the "B-Mode" and "M-Mode". Both modes are described below.

B-mode, or 'Brightness Modulation', is the display of a 2D map of B-Mode data and is the most common form of ultrasound imaging. Unlike A-Mode, B-Mode is based on the image brightness and the absence of vertical spikes. Therefore, the brightness depends upon the amplitude or intensity of the echo.

M-mode, or 'Motion Mode' (also known as 'Time Motion' or TM-mode), is able to display a one- dimensional image

and is commonly used in cardiac and foetal cardiac imaging to analyse moving bodily organs. This can be accomplished by recording the amplitude and rate of motion in real time, by repeatedly measuring the distance of the object from the single transducer at a given moment. A single sound beam is transmitted, and the reflected echoes are displayed as numerous dots of varying intensities, thus creating lines across the screen.

The typical M-mode imaging of a healthy aerated lung follows the image of a sky, ocean, and beach, with linearity appearing on the top of the image and a more granular appearance to the bottom of the image (Fig. 6). However, a lung suffering from pneumothorax or consolidation will instead reveal a 'barcode' sign, which is seen by complete linearity in both the top and bottom of the image, or as a 'stratosphere" appearance (Fig. 7). M-mode is also important in recording the 'lung point,' [10] which is the area along the thorax where the collapsed lung re-contacts the chest wall (Fig. 8). Finding of the 'lung point' increases the diagnostic certainty of pneumothorax.

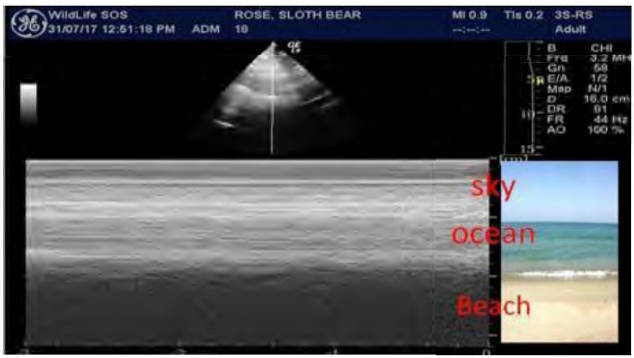


Fig 6: M-mode view of normal lung.

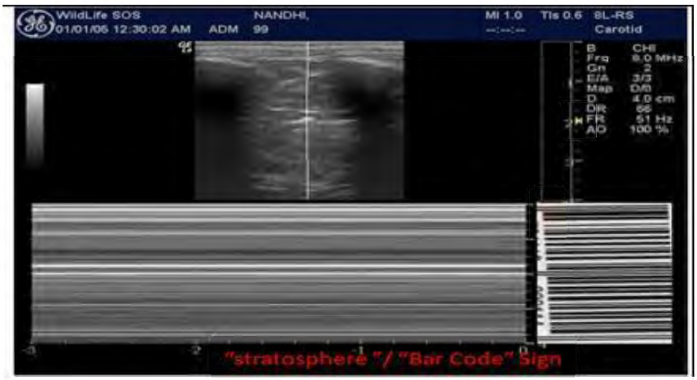


Fig 7: M-mode view of Pneumothorax.

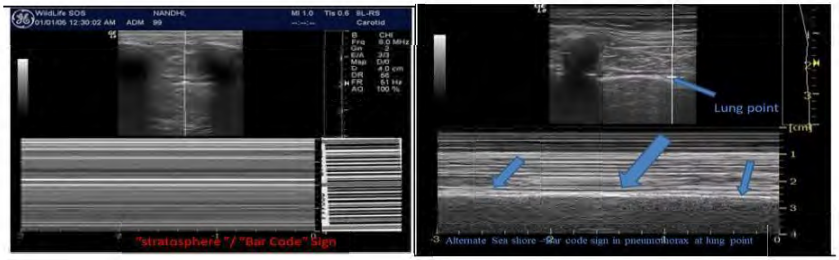


Fig 8: M-mode view Pneumothorax with 'Lung-Point'.

Artifact analysis and interpretation

The basic principles of lung ultrasonography in acute respiratory conditions generally centre around the observation of ultrasonographic artifacts based on the dry lung. This is seen as 'A-lines' with a 'glide sign' or lung sliding, in comparison to the wet lung which shows characteristic ultrasound 'lung rockets' or 'B-lines'. At the surface of the lung, the prominent element is air. The acoustic impedance of air is 0.0004 3 105 gp/cm2 s [26], which is very different from that of bone (7 3 105 gp/cm2 s), parenchyma (1.65 3 105 gp/cm2 s), and water (1.48 3 105 gp/cm2 s). The movement of the lung toward the chest wall is distinctive. The ribs are identifiable by their acoustic shadow. Between two ribs is a hyper-echogenic line that is always visible, and behind which only airy artifacts are present [14]. The inability of ultrasound to penetrate air filled structures emphasizes the importance of its use during the examination of normal lungs. Thus, ultrasound imaging is herein exclusively composed of artifacts. However, in disease states where the normally air-filled lung is collapsed, consolidated, or replaced by fluid or lesions, ultrasound waves can penetrate the thorax and display an image. This form of examination may provide valuable information to complement any radiographic findings.

Skill in lung ultrasonography is superior to the need for that in most radiographs and CT scans, and is pertinent in allowing physicians and veterinarians to diagnose pleural effusion, interstitial syndrome, and pneumothorax [7]. The diagnosis of alveolar consolidation has been in practice for many years, and the signs within an ultrasound are now standardized (Fig. 9) [9]. Various demarcations and measurements of ultrasonography are described below.

A-lines: Equidistance, horizontal, repetitious lines below the pleural line. The normal aerated lungs always produce A-lines (A = air, artifact = normal lung). A-lines are horizontal reverberation artifacts indicating a normal lung surface.

Glide sign or lung sliding: A to-and-fro dynamic of the pleural line. It may also be known as the 'lung -wall interface,' [14] indicating movement of the visceral pleura past the parietal pleura from the respiratory cranio-caudal excursion of the lung.

B-lines : Vertical, hyper-echoic rays projecting from pleural line are also known as the 'ring down artifact.'1 These B-lines extend to the bottom of screen without fading, similar to a laser beam, and oscillate in a synchronized fashion with both inspiration and expiration [3, 8, 13, 18, 19, 23, 28]. Presence of these B-lines indicates sub-pleural interstitial edema, or 'wet lung [6, 12, 18]. B-lines, also termed 'lung rockets,' are

vertical reverberation artifact or 'comet-tail artifact' [29]. Ultrasound lung rockets are thought to be the radiographic equivalent of Kerley B- lines [12, 13, 25]. The number of ultrasound lung rockets (ULRs), and the distance between each lung rocket, is directly correlated with the degree of lung edema in humans [8, 11, 12, 21, 25, 28]. 'White lung' is the term used when ULRs blend into one another and become confluent, as is mentioned as 'infinity-∞.'

Z-lines: Vertical, comma-tail artifacts that differ from B-lines. Z-lines are less echogenic than the pleural lines, are ill defined, and usually taper off at after two to four centimetres. However, they do not erase the A-lines, and do not move during lung sliding [12].

O-lines: The possible pattern of the O-line is the absence of any horizontal or vertical artifact. A slight movement of the probe often brings out O-lines. This pattern should be considered having the same meaning as of A-line [12].

Air bronchograms: These are punctiform or linear hyper-echoic artifacts within the consolidation. 'Dynamic air bronchogram' is the term used for the centrifugal inspiratory dynamic of air bronchograms where movement of 1 mm is required. Alveolar consolidation is defined as an image yielding two signs. The first sign is a tissue-like image arising from the pleural line or lung line, as well as tissue-like

behavior of the image with no dynamic in the depth-surface axis. The second sign is the 'shred' sign, which is a shredded deep border of the tissue image, as in a connection with aerated lung [13]. Both signs yield 90% sensitivity and 98% specificity regarding the diagnosis of alveolar consolidation (Fig. 10) [9].

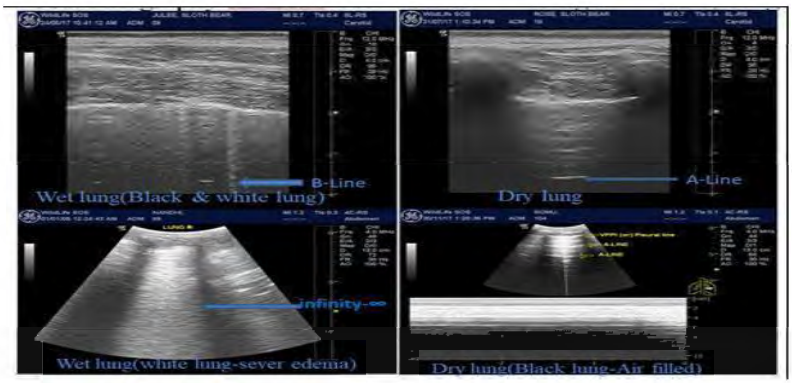


Fig 9: Ultrasonography artefacts based on the Wet lung versus Dry lung

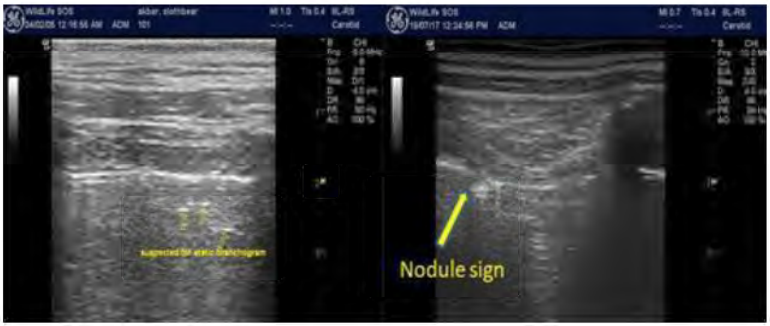


Fig 10: Lung consolidation and infiltration.

Result & discussion

Zones 1, 2, 5 and 6 are suitable areas for examination of pneumothorax in supine position, while zones 3, 4, 7 and 8 are suitable for plural effusion diagnosis. This is due to the fact that air is lighter than fluid, and therefore air will always rise to the top while fluid will settle at the bottom. All the zones are suitable for examination of lung pathologies such as oedema, consolidation, pneumonia and other nodular lesions due to carcinoma or tuberculosis (Fig. 11, 12 & 13). Small surface probes of 3.0 and MHz are most suitable for this application, but 2.5, 5, and 7.5- MHz probes are equally effective. Our findings of the normal lung pattern, which is characterized by horizontal and parallel A- lines, and those findings of alveolar interstitial syndrome, which yield parallel and vertical B-lines, coincides with the findings of Lichtenstein [4]. In the over 5400 mammalian species that currently exist, a wide variety of anatomic features have evolved, making knowledge of one species not necessarily applicable to another in a straightforward manner. In an ideal setting, attempts at ultrasonography should be utilized by those having a clear anatomic picture of the species involved, but this is still lacking for many species. The vast differences between species dictate the selection of the ultrasonography technique to be applied, and the quality of the images that are obtained [22].

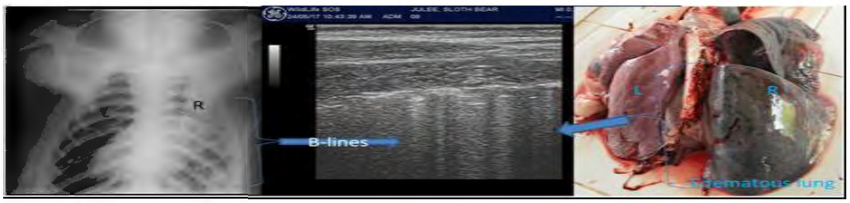
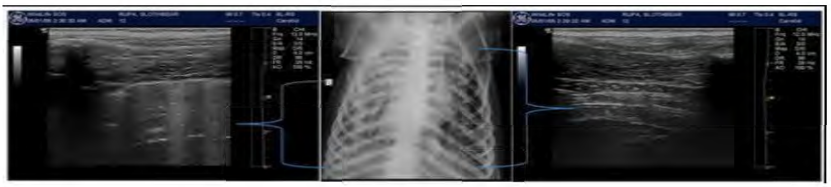


Fig 11: Radiographic, ultrasound image and Postmortem lesion of lung edema.

Fig 12: Radiographic & Ultrasonographic image of sloth



Bear's with lung edema and consolidation due to Pulmonary tuberculosis.

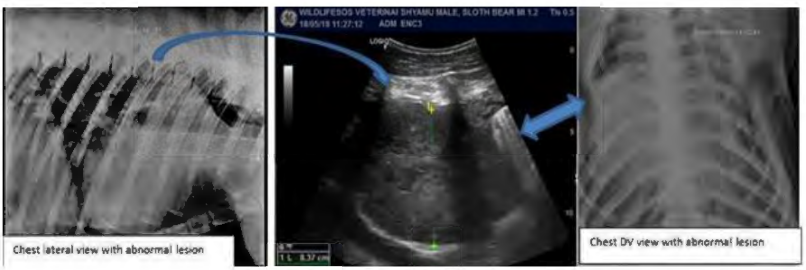


Fig 13: Radiographic & Ultrasonographic image of sloth Bear's lung Space occupying lesion.

Conclusion

The simplicity and high feasibility of ultrasonography makes it an attractive and easy-to-use diagnostic tool at the bedside for lung examination. Therefore, it should be incorporated as an integral part of diagnosis for the veterinarian, as well as in routine health evaluation protocols in bears. Unlike other regions, such as the heart and intra-abdominal organs, the surface of the lung is able to be easily visualized using ultrasound, and artifact can be quickly detected. A simple portable unit without Doppler is also enough. The skills required to recognize any artifact can be easily learned. The utility of lung ultrasonography has been confirmed by a growing number of researchers and their studies, and is simple to perform, provided one thinks differently [27].

Acknowledgements

The authors wish to acknowledge the Wildlife SOS management, staff of the Agra Bear Rescue Facility (ABRF), and the Utter Pradesh Forestry Department for their kind support and cooperation.

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Art – 182. SUCCESSFUL REMOVAL OF FISHHOOK FOREIGN BODY FROM TURTLES

Ilayaraja Selvaraj, Acharya PR, Pradeep R and **Arun A. Sha**

Abstract

Freshwater turtles, and their eggs, are commonly used by humans as a source of food, as well as exploited as a source of profit. Alterations to the turtle's natural habitat – by the construction of dams and barrages, cultivation along riverbanks, and pollution – are also major threats to the survival of the freshwater turtle. During two different occasions, the rescue team at Wildlife SOS has been able to save two freshwater turtles that were burdened with an entangled fishhook inside the oral cavity. Treatment and care of the two turtles is described below on a case-wise basis.

Case 1. An Indian Flap Shell turtle *(Lissemys punctata)* was rescued and brought to the Wildlife SOS veterinary hospital on 17.03.18. On close examination, it was found the turtles mouth had been punctured by a fishing hook that penetrated into the lower jaw. This injury was accompanied by a necrotic lesion that surrounded the site of puncture. Radiographic examination confirmed the diagnosis. During treatment, the

mouth was opened with forceps and lidocaine spray was applied to the affected area. The fishing hook was removed using a backwards motion and the simultaneous application of gentle downward pressure. Following removal of the hook, the ulcer was cleaned and rinsed with chlorohexidine solution, and oral meloxicam drops were applied. On day 3 of treatment, the turtle was released back to its natural habitat.

Case 2. A Spotted Black Terrapin *(Geoclemys hamiltonii)* was rescued on 25.04.18 with thread hanging from its mouth and mild bleeding of the oral area. On open mouth examination, it was revealed that the thread was attached to a metal object. Following radiographic examination, it was confirmed that a long fishing hook had severely impaled the throat region. This injury caused significant pain, as well as difficulty of elimination of the hook without general anaesthesia. General anaesthesia was achieved using a mask and 4% isofluorane. Once the animal was anesthetized, the mask was removed and a 2.1mm endotracheal tube was fixed in order to maintain anaesthesia with 2% isofluorane. After careful consideration of the position of the hook and barb, the decision was made to avoid any current attempts to pull the string and hook from the oral cavity directly, as this may have led to severe damage to the caudo-ventral bony structures and soft tissue. The

mouth was kept open with the help of forceps during the procedure. The visible end of the hook was held with needle forceps and gently pushed into the oesophagus, which allowed veterinarians to locate the cutaneous bulging on the lateral aspect of the neck caused by the barb end of the hook. A minute incision was made at this area after disinfection, allowing the barb to be pulled outwards from the neck and cut by wire cutters. Following removal of the hook, oral meloxicam and an injection of enrofloxacine was given. The turtle was able to recover well and was released into its natural habitat on day 5.

Keywords: Anaesthesia, spotted black terrapin, Indian flap shell turtle, fishhook.

Art – 183. SUCCESSFUL MANAGEMENT OF A SKIN TEAR INJURY IN PEAFOWL *(Pavo cristatus)*

Ilayaraja Selvaraj, Acharya PR., Pradeep R, Arun A. Sha

Abstract

Indian Peafowl is the largest breed among the pheasants and is protected under Schedule I of the Wildlife Protection Act of 1972. Indian Peafowl *(Pavo cristatus)* is one of India's national birds and is of religious importance. It also plays an important role in regulating the ecosystem balance. During a recent dust storm occurring on 03.05.18, a peafowl weighing 3.2 kilograms was found to have a large wound on its neck and was brought to the Wildlife SOS veterinary hospital by a forest guard of Uttar Pradesh Forestry Department, Mathura range. On examination, the bird was dull, refused to eat, and was unable to extend its neck. A long, vertical skin laceration was noted on the medial aspect of the neck, which caused the exposure of the trachea and oesophagus. The bird was kept under isofluorane general anaesthesia at 2% via a mask. Radiographic examination of the neck region did not reveal any abnormalies on the cervical vertebrae; therefore, the decision was made to oppose the skin and close the wound in order to allow uninterrupted quick healing and avoid

further contamination. The wound was cleaned and rinsed with an antiseptic solution of povidone-iodine and metronidazole at a 1:10 ratio. 4-0 PGA absorbable suture material with an atraumatic needle, “mersuture,” was used to oppose skin using a simple continuous suture pattern. Calandula dressing powder was mixed with negasunt powder and applied to the wound. Fly repellent spray was applied gently around the periphery of the wound, avoiding application directly into the injured area. Both meloxicam at 0.2mg/ Kg BWT and long- acting enrofloxacin 0.5ml were injected intramuscularly. The animal was kept in an observation enclosure for further treatment and care. Oral meloxicam drops were continued for another 5 days. Initially after treatment, the bird was still anorexic and dull, and was hanging its head. It did not show interest in food until the second day of post-operative care. By the third day, the bird was showing significant improvement and began to hold its head and neck in an erect position. On day 5, the peafowl was examined again to ensure proper healing and an intact suture line. By day 10, the bird had completely recovered and was released back to its natural habitat.

Keywords: Indian pea fowl, Anaesthesia, wound

Art – 184. USE OF SMALL EXPLOSIVES ARE A THREAT TO SLOTH BEARS IN INDIA

Attur Shanmugam Arun, Shanmugavelu Swaminathan, Thomas Sharp, Yogaraj Panerselvam, Kartick Satyanarayan, Geeta Seshamani

Abstract

For 6 months in 2017, the field research team of Wildlife SOS had been tracking a radio-collared wild sloth bear *(Melursus ursinus)* in the forests of Bannerghatta National Park on the outskirts of Bangalore in Karnataka. The female bear was not originally from the park but from an area roughly 30 km away. She had been translocated there because she was a problem bear, believed to be responsible for attacking and killing a local person. Before she was released into the park, Wildlife SOS had received permission to put a very high frequency, Global Positioning System (VHF/GPS store-on-board) collar on her. After about 6 months she seemed to be doing very well and settling into a specific area based on the data we were collecting through tracking her by VHF triangulation. And she rarely (though occasionally) entered the border areas outside the park where there were farms

and small villages. We were happy to see that she was staying out of trouble and spending the vast majority of her time within the park. We were even able to get some camera-trap photos of her on December 6, 2017. She looked great—healthy and active! The WSOS researchers were all encouraged by how she was doing, and we were eager to see the finer detail GPS data being collected and stored in her collar.

Known sloth bear fatalities caused by baited explosives in Karnataka, India.

Incident	Date	District / Division	Location	Sex
1	Aug 26, 2014	Tumkur	Tumkur	Female
2	Mar 11, 2015	Ramnagara	Ramnagara	Female
3	Jun 13, 2016	Ramnagara	Sathanoor	Female
4	Jul 31, 2016	Ramnagara	Sathanoor Range	Male
5	May 1, 2017	Chikkamangalur	Kaddur	Female
6	May 19, 2017	Chikkamangalur	Kaddur	Male
7	Jan 5, 2018	Ramnagara	Ramnagara	Female
8	Jan 21, 2018	Bannerghatta National Park	Kodihalli Range	Female

On January 21, however, the signal location from this bear had not moved all day. She was eventually found dead. We conducted a full necropsy, but this was unnecessary, as the cause of death was immediately evident. She had bitten into an explosive device (a “country-made bomb”), hidden in a food bait. Her entire tongue, roof of the upper mandible, and skull were severely damaged. As if this was not enough, during the necropsy we discovered that she had been pregnant with two fetuses. The only bit of good news was that we were able to salvage and recover the collar from the dead

bear, which enabled us to retrieve the GPS data of her movements.

(above) A problem sloth bear translocated to Bannerghatta National Park, near Bangalore, Karnataka, India, was tracked with a VHF/GPS collar and camera-trapped in December 2016, 6 months after being moved. She was settling in the area and rarely ventured out of the park. (below) The sloth bear monitored by Wildlife SOS in Bannerghatta National Park was killed by an explosive bait after she wandered outside the park.

These home-made explosive devices are known to be used throughout Southeast Asia by farmers to remove depredating wildlife, and poachers to obtain certain wildlife parts; but they are unselective and kill any other unfortunate wild animal whose curiosity got the better of it. We were aware that these explosive devices were being used in southern India and had documented several sloth bear deaths over time, however the issue never really grabbed our attention until now. The death

of this valuable radio-collared bear indicated to us that this was potentially a real threat and prompted us to investigate further and dig into our records. Our investigations also revealed local witnesses who confirmed many other cases. Since August 2014, we documented 8 sloth bear fatalities in the southern Indian state of Karnataka alone. Six of the 8 bears were females confirming that this is indeed impacting the population.

The making, possession and use of these explosives are illegal in India, yet they seem to be commonly used in southern India, particularly Karnataka. They are generally made from mining supplies and used to kill Indian boars *(Sus scrofa cristatus)* for their meat. The people who use them tend to put them out during the night and pick them up in the morning before cattle or humans can be injured, which could get them into a great deal of trouble with local authorities as that would be a non-bailable offence and a heinous crime. Unfortunately, despite these incidents being reported to the authorities, the threat to wildlife is not taken as seriously and action is yet to be taken against these offenders, which likely emboldens them further. Sloth bears and wild boars are not the only species threatened by these explosives: we are aware of reports of elephants that lost a limb, trunk or jaw to these baited explosives.

Moving forward, Wildlife SOS will continue investigations into the use of these explosives for poaching wildlife in India, while pushing for law enforcement to control this serious threat to India's wildlife.

Art – 185. AN ATTEMPT TO ESTABLISH A STANDARD OPERATING PROCEDURE (SOP) FOR COMPLETE PHYSICAL EXAMINATION OF LEOPARD *(Panthera pardus fusca)* CUBS RESCUED IN MAHARASHTRA

Ajay B. Deshmukh, Arun A. Sha, Ilayaraja S and Bahar S. Baviskar

Abstract

During 2009-2018, twenty-six leopard rescue operations involving fifty leopard cubs were conducted in three districts, namely Ahmednagar, Nashik and Pune, in Maharashtra by Wildlife SOS. During the rescue operation, the following non-invasive parameters were studied which includes, thorough physical examination, estimation of age, gender, body weight, rectal temperature, respiratory rate, heart rate or Pulse rate.

The approximate age of the rescued leopard cubs was falling between 15 days to 120 days with an average of 47 days old. Out of 50 rescued leopard cubs, 24 were male and 26 were female. Body weight of the rescue leopard cubs were ranged from 0.4 – 3.8 kg with an average 1.9 kg. Rectal temperature of the cubs ranged from 100°F – 102°F with an average

100.73^0F. Respiration rate was found within the range of 16-40 breaths per minute, with an average of 32.61 breaths per minute. Heart rate was found within range of 120-140 beats per minutes with an average of 132.6 beats per minute, pulse rate was falling between 109 - 126 with an average of 117.5 nos. All the parameters studied during the rescue operation helped to monitor and assess the health of the rescued cubs till its reunion with respective mothers. It is vital to record all the parameters correctly to decide the health status of the rescued animals especially cubs when we are planning for their reunion, soft release or rehabilitation. Any deviation from the normal values of the clinical parameters may indicate the specific or non- specific illness or disorders or disease condition with or without any clinical manifestation.

Art – 186. COPROLOGICAL ANALYSIS FOR ESTIMATING PREVALENCE OF GASTROINTESTINAL PARASITES IN CAPTIVE SLOTH BEARS (*Melursus ursinus*)

V. Manjunatha, M. Rout, **Arun. A. Sha**, S.M. Byregowda and **T. Joseph**

Abstract

The aim of the present study was to investigate the gastrointestinal parasitic infestations in captive bears maintained in Wildlife SOS, Bannerghatta Biological Park, Bengaluru. A total of 85 fecal samples were collected over a period of 12 months from apparently normal/healthy captive bears and examined. The fecal samples were analysed using sedimentation and floatation techniques followed by microscopic identification of parasitic eggs. It revealed the prevalence of 51 (60%) *Hymenolepis diminuta, 20 (23.52%) Toxocara sp ova, 3 (3.52%) Capillaria sp, 2 (2.35%) Trichuris sp ova, 2 (2.35%) Eimeria sp* oocysts and 8 (9.41%) Ancylostomatid eggs. The study suggested that among different parasitic infections, the prevalence of cestodes was extremely higher, since insects were the intermediate hosts for the Hymenolepis sp. The study is suggestive of periodic anthelmintic therapy in the said species under captivity so as

to maintain a sound health.

Keywords: *Ancylostoma, Capillaria, Eimeria, Hymenolepis diminuta*, Sloth bear, *Toxocara, Trichuris.*

Introduction

Biological parks and zoos maintain wild animals for aesthetic, recreational and conservation purposes (Varadharajan and Pythal, 1999) with the goal of preserving rare and endangered species (Parsani et al., 2001). The health of animals under captivity depends on many factors like feeding, keeping conditions, management practices and environmental conditions. Again, parasitic infestation in wild animals housed in zoos varies in accordance with the type of husbandry, diagnosis, choice of drug and type of treatment. Animals maintained under captivity often remain under considerable stress that further deteriorates their resistance to parasitic infections. With regular practice of deworming, they usually do not show severe signs of parasitism (Parsani et al., 2001). When animals are moved from one enclosure to another without proper treatment, then there remains a possibility of parasite transmission. Intermixing of different species within the same area also poses further risk of parasitic infections. Environmental change and living conditions of freedom to captivity have influence upon the

animal ecology thereby increasing their sensitivity to parasitic infections (Goossensa et al., 2005).

Captive carnivores (under the *families Canidae, Felidae*, *Mustelidae*, *Ursidae* (bears), *Procyonidae, Viverridae* and *Hyaenidae*) are susceptible to a wide array of infectious and parasitic diseases. The sloth bear *(Melursus ursinus)*, also known as the labiated bear, or Stickney bear is an insectivorous bear species native to the Indian subcontinent. Knowledge of ursine parasites has expanded considerably after the subject review by Stiles and Baker in (1935). Impaired health condition resulting from parasitic infections may also have a negative impact on health. As zoos are opened to the public, close contact with humans, it increases the risk of development of anthropozoonosis (Panayotova - Pencheva, 2013). Investigations regarding endoparasitic fauna pave the way for estimating the prevalence, distribution and biology of parasites (Zasityte and Grikienciene, 2002). With this background, this pilot study was carried out during 2015-2016 to obtain a preliminary baseline data or to establish the profile of gastrointestinal parasite fauna of sloth bears in Wildlife SOS, Bear Rescue Centre, Bannerghatta Biological Park (BBP), Bengaluru.

Materials and Methods

Sloth bears in Wildlife SOS, Bear Rescue Centre, BBP, Bengaluru were examined for parasitic infections during 2015 - 2016. Eighty-five fresh bear fecal samples (approximately 4 gm) were collected from each animal at random from the floor of the enclosure during daylight, placed in a polythene bag, marked properly, and temporarily stored at 4°C. Only scats deposited within the past 6 hours were collected. All samples were sent to Wild Animal Disease Diagnostic Laboratory for processing. The intestinal parasites were assessed through coprological analysis using prescribed methods. Centrifugal sedimentation and floatation technique were followed for qualitative analysis with few modifications in the protocol of Thawait et al. (2014), using 2% formalin solution instead of water, followed by microscopic identification of parasitic eggs with Olympus light microscope. Every sample was checked under the microscope at 10X and 40X magnifications (Dryden et al., 2005). The ova, cysts and oocyst of different parasites were identified according to the morphology (Soulsby, 1982).

Results and Discussion

The prevalence for gastrointestinal parasites in our study was 60% for *Hymenolepis diminuta* (Fig 1) 23.52% for Toxocara sp., 3.52% for Capillaria sp. (Fig 2), 2.35% for

Trichuris sp. (Fig 3) and Eimeria sp. and 9.41% for Anchylostomatid ova (Fig 4) (Table 1). Most of the Ancylostomatid eggs are in morula stages. Nematode eggs can often only be identified to the genus level using microscopy. Thus, it could not be established morphologically whether the Ancylostomatid eggs found were species of Ancylostoma or of Uncinaria. Ancylostoma caninum and A. tubaeforme have earlier been reported from black bears (Crum et al., 1978; Foster et al., 2004), while U. yukonensis and U. rauschi have been reported from black and grizzly bears (Olsen, 1968; Choquette et al., 1969). Researchers from the former USSR also documented U. stenocephala in brown bears in 1953 and 1962 (Rogers and Rogers, 1976). Although the genus Eimeria is generally monoxenic and broadly distributed in wild animal populations (Barret and Dau, 1981), its low prevalence in our study could be presumed to have occurred incidentally through contamination of fields with rabbit feces as rabbit enclosure is in close proximity or might be due to some other reasons. The oocysts being unsporulated (but with a thin, white grey double oocyst wall and no micropyle), were not sufficient enough for morphological species identification. Intervention of molecular diagnostics using polymerase chain reaction may be useful for further identification. Notably, only three Eimeria species have been described from bears, E.

albertensis and E. borealis from black bears (Hair and Mahrt, 1970) and E. ursi from brown bears (Yakimoff and Matschoulsky, 1935). Among nematodes, Toxocara canis and T. mystax were reported in captive brown bears in Germany (Couturier, 1954). Thawait et al. (2014) reported single infection of Toxocara sp. in bears under captivity at Nandan Van Zoo, Raipur, Chhattisgarh. Four hookworm species e.g. Ancylostoma brasiliense, A. malayanum, A. ceylanicum, and A. caninum, were reported from captive sloth bears in India (Baylis and Daubney, 1922).

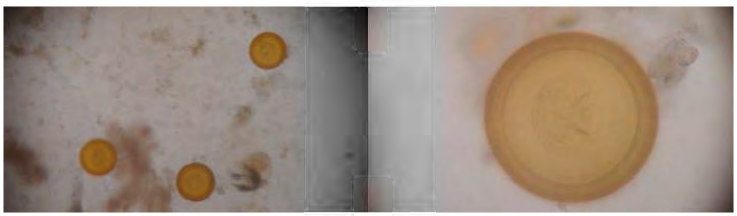

Fig 1: *Hymenolepis diminuta* **ova in fecal sample from sloth bear** (10X and 40X).

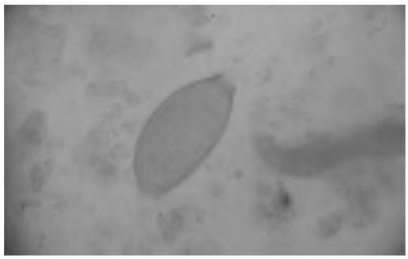

Fig 2: Ovum of *Capillaria sp*. **in fecal sample from sloth bear.**

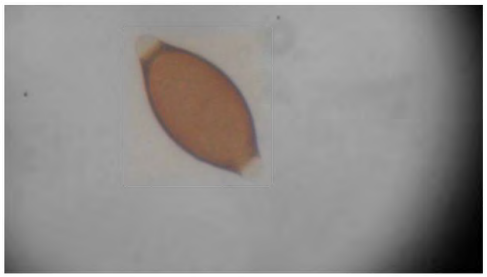

Fig 3: Egg of Trichuris sp. **identified in feces of sloth bear.**

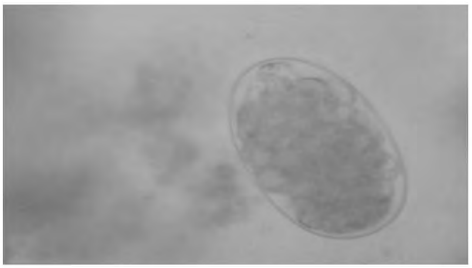

Fig 4: Ancylostomatid egg identified in feces of sloth bears.

A. malayanum also was reported from Himalayan black bears from India and Ceylon (Lane, 1916) and from a captive sun bear from India (Baylis and Daubney, 1922). Thus, five nematodes (Baylisascaris transfuga, B. melursus, Ancylostoma malayanum, A. brasiliense and A. caninum) have been reported from sloth bears in India. In fact, in wildlife species, natural resistance against parasitic diseases and a state of equilibrium between host and parasite normally prevent the clinical disease, unless in stress conditions (Mir et al., 2016). Kumar et al. (2013) were of opinion that parasite infections increase with the animal load or stocking density per square surface. The same situation applies to wild animals in captivity that are normally kept in the same enclosure for prolonged periods of time, with space limitations and under constant stress, leading to immunosuppression and further susceptibility to parasitic infection (Mir et al., 2016). Therefore, parasitic infections and interspecies transmission are common in zoos posing immense negative effects. Upon examination of 94 European brown bear *(Ursus arctos)* fecal samples collected in three counties of Croatia, Aghazadeh et al. (2015) reported parasites from five genera including the nematodes B. transfuga and Syngamus sp. and the protozoan and the protozoan Cryptosporidium sp., Eimeria sp., Giardia sp. and Ancylostomatid eggs. Surprisingly, during the

coprological study of gastrointestinal parasites of captive animals at Rangpur recreational garden and zoo in Bangladesh, Khatun et al. (2014) could find no gastrointestinal parasites in bears. The results of the present study demonstrate that gastrointestinal parasites are common in sloth bears. This pilot study was undertaken to provide preliminary baseline data on the parasite fauna of ursids under captivity at Wildlife SOS, Bear Rescue Centre, BBP, Bengaluru. The results have revealed that this species may be infected by a range of parasites. An additional parasite survey of bears in the region may be called utilizing genus-specific PCR in addition to fecal flotation and sedimentation methods. Based on the prevalence of gastrointestinal parasites and administration of desired anthelmintic to the captive wild animals periodically coupled with better sanitary measures, one would be able to reduce the parasitic infection in the zoos. Such routine monitoring of parasites and regular deworming along with hygienic measures are necessary to prevent gastrointestinal infections in captive animals. Confined areas in zoo enclosure make captive animals more prone to different parasitic infections (Kashid et al., 2002). Moreover, the timing of fecal sample examination is also important since periods when particular parasites shed eggs vary greatly. More studies of parasitic infections are essential

to understand the epidemiology of parasitism and its prevention.

Acknowledgment

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Art – 187. STANDARDIZATION OF TOE-NAIL BLEEDING TIME (TNBT) IN CAPTIVE SLOTH BEARS *(Melursus ursinus)*

Arun A. Sha, Ilayaraja S, Tista Joseph, Nithin K., and Govind S.

Abstract

Bleeding time is defined as the time from the moment that incision is made to the point where bleeding ceases. Bleeding time (BT) is the oldest and simplest test for assessing the platelets function. BT is affected by diet, body temperature, age gender, platelet function, certain vascular disorders. Buccal mucosal bleeding time (BMBT) test is most widely accepted test for bleeding time but which is impractical option in case of wild animals especially captive wild animals, hence Toenail bleeding time (TNBT) was performed on sloth bears. On the other hand, TNBT can also be achieved through positive conditioning method. The benefits of this test are that it is quick and facile. As opined by M.E. Mylonakis *et al.* (2005), that there is no significant difference in BT before and after immobilization in dogs. The aim of this study is to standardize BT in clinically healthy Sloth Bear (*Melursus ursinus*) and the health status of the bears was confirmed through normal

platelet count, both physical and behavioural observations. Establishing the normal range of BT can help us to assess platelet function and the body's ability to form a clot, bleeding disorder, aspirin resistance, and to check liver disorders. The other important role of finding the bleeding time is to rule out the venomous snake bite. An attempt was made to establish normal BT by using TNBT in Sloth Bears. In this study the BT was checked in 35clinically healthy captive Sloth bears over a period of 24 months during their routine general health examination after immobilization. Out of 35 bears 20 were females, and 15 were males. In the study, it was observed that the BT ranges between 0.45- 4.70 min in both the genders. The BT range in females was between 0.45- 4.70 min whereas in males, it ranges between 0.40-4.60 min. In this article, the BT values were correlated with Prothromvbin time and the platelet count. The stepwise procedure and the precautions during the entire practice will be explained in detail in this article.

Art – 188. MITOCHONDRIAL DIVERSITY AMONG CAPTIVE SLOTH BEARS *(Melursus ursinus)* AND THEIR PROVENANCE

Arun A. Sha, Anjali Verma and K. Praveen Karanth

Abstract

Sloth bears *(Melursus ursinus)* are endemic to the Indian subcontinent and are found in variety of habitats, including wet and dry tropical forests, savannas, scrublands and grasslands. Historically these bears were tamed by street performers and used as "Dancing bears". However, with the banning of use of animals in performance this practice has stopped, and the bears are being rehabilitated in rescue centres like BBRC. Here we characterize the mitochondrial genetic variation of these rehabilitated bears at rescue centres to better understand their wild origins. Mitochondrial ND4, Cyt b and COX 3 were PCR amplidied and sequenced from 74 samples (rescued and wild). A total of 17 mitochondrial haplotypes were found in the samples. Haplotypes diversity of 61 sequences was +_ 0.915 and nucleotide diversity was +_ 0.00403. These numbers were comparable to those from other bear species sampled from the wild. Assuming their most studies bears have been sampled from across their

range, this result would suggest that the Sloth bears in India have tamed from multiple populations from across India. We had limited sequence information from wild populations, and these were also included in the analysis. The origin of at least 4 animals could be deciphered from this dataset, these animals have all been captured in the state of Karnataka. However, sampling from the wild for the rest of the country is very limited at this point. Our dataset can be compared with sequences from samples collected from the wild to better understand the origin of these currently captive animals.

Art – 189. ONE OF THE BEST STRATEGIES TO MINIMIZE HUMAN- LEOPARD CONFLICT IS TO REUNITE RESCUED LEOPARD (*Panthera paradus fusca*) CUBS WITH MOTHER - A SUCCESS REVIEW.

Ajay B. Deshmukh, Kartick Satyanarayan, Geeta Seshamani, Arun A. Sha, Illayaraja S, Bahar S. Baviskar

Abstract

Loss and fragmentation of habitat, intensifying anthropogenic pressure due to ever-increasing human population and changing agricultural practices have changed the breeding biology of the leopard (*Panthera paradusfusca)* to a large extent. Based on our observations and documentations, the leopards living around the forest fringe areas or human dominated landscape mostly prefer the agricultural land for breeding, especially sugarcane fields, the chances of survival of the cubs are more considering that the sugarcane field remains undisturbed over a year till harvesting which enables the mother leopard to raise the young ones with least disturbances. Wildlife SOS, in this study witnessed the rescue and reunion of fifty leopard cubs from twenty-six rescue operations accomplished during 2009-2018

in three districts of Maharashtra state namely Ahmadnagar, Nashik and Pune. Relocating the rescued leopard cubs from the place of rescue with minimal handling as early as possible on the same day during the late evening or night eventually increases the success of all fifty reunions, except two cases. The first case, it took three days to reunite them and in second case it took six days to do the same. Rest of the forty-eight (48) cases, reunion was successful within 45 to 360 minutes with an average of 220 minutes. The post reunion survey confirms that no conflict situation was reported in the locality after reunion of rescued leopard cubs which necessitates the importance of reuniting the rescued leopard cubs with its mother, as early as possible. Resulted, we are in the process of establishing a standard leopard cub reunion protocol for easy operation and sharing the knowledge to other places where they faced the similar problem more often. The authors discussed the stepwise protocol in detail for better understanding and practicality to avoid any mishaps or failure.

Art – 190. STUDIES ON PHYSIOLOGICAL AND HEMATO-BIOCHEMICAL CHANGES DURING XYLAZINE ZOLAZEPAM + TILETAMINE AND DEXMEDETOMIDINE - ZOLAZEPAM + TILETAMINE ANESTHESIA IN SLOTH BEARS

M.M. Anitha, L. Ranganath, H.S. Shwetha, **Arun Sha**, B.P. Shankar, V. Mahesh and R.B. Srinivas

Abstract

The physiological and hemato-biochemical changes were recorded at 15, 30, and 60 minutes after immobilization with XZT (group A) and DexMZT (group B) in six sloth bears of each group. Significant physiological difference in heart rate between the groups at 30 minutes was observed which could be due to α 2 agonist mediated biphasic cardiovascular response causing bradycardia. Significant decrease in hemoglobin concentration at 30 minutes in group B could be attributed to α 2 agonist influencing shifting of fluids from extracellular to intracellular compartments. Significant difference in neutrophil count can be attributed to DexM showing very less stress response. No significant changes were observed in biochemical values.

Introduction

Sloth bears are classified as Vulnerable in IUCN red list of

Threatened species in 2008. Although bears are not difficult to anesthetize, it is better to be cautious as they are monogastrics and prone to vomiting on induction or regurgitation during anesthesia (Caulkett and Cattet, 2002) which could be dangerous.

In the present study, the complications of anesthesia including the physiological, hemato-biochemical assessments and side effects were considered for a safer immobilization in captive sloth bears.

Materials and Methods

Twelve sloth bears of either gender were randomly selected for immobilization in Wildlife SOS, Bannerghatta Bear Rescue Centre at Bengaluru. The bears were divided into two groups A and B of six each. Group A and B were immobilized with XZT and DexMZT respectively. Physiological parameters (Rectal temperature -^{0}F; Respiratory rate – breath/minute; Heart rate- beats/ minute) were recorded using OT patient Monitor as suggested by Ozeki et al., (2014) at 15, 30, 60 minutes after immobilization. 0 minute/Normal values were referred as per book standards (Fowler, 1978). Hematological and Biochemical parameters were analyzed at 15, 30, 60 minutes using Mindray BC-2800 Vet auto analyzer and Thermo scientific Konelab-20 fully automated biochemical analyzer

respectively. 0minute/Normal values were referred as per book standards (Miller and Fowler, 2003). The Mean and Standard error of the data were analyzed by t-test using computer based statistical programme.

Results and Discussion

Mean values of rectal temperature recorded at 15, 30, 60 minutes were 99.48 ± 0.19, 100.1 ± 0.21, 99.82±0.14 0F respectively. Respiratory rate were 20.50 ± 2.34, 16.83 ± 0.75, 23.50±1.69 breaths / minute respectively and Heart rate were 66.17 ± 6.26, 76.50 ± 5.92 and 77.83±4.11 beats / minute respectively.

Although all the values were within the normal range a non-significant rise in rectal temperature could be due to vasoconstriction of peripheral vessels caused by α 2 agonist, it did not lead to hyperthermia as stated by Cattet et al., (2003a) and Cattet et al., (2003b). Since α 2 agonist induce hypoxemia regular monitoring is needed and if necessary, oxygen supplementation can be provided as suggested by (Cattet et al., 2003c., Rai, 2009). The respiratory values in group B were within the normal range similar to reports of by Neto, (2009); Teisberg et al., (2014) and Jin et al., (2016) and DexM had organ protective effect against ischemic and hypoxic injury as stated by Alfonso and Reis, (2012). A significant difference in

heart rate (P<0.05) was found at 30 minutes although the values were within normal range, similar reports were indicated by Ansah, (2004) Neto, (2009) and Teisberg et al., (2014) where mild bradycardia induced by DexM can be tolerated by healthy animals with no cardiovascular disease. Alfonso and Reis, (2012) stated that DexM provides Reno protection, Neuro protection and Cardio protection.

The Mean ± SE of TEC in group A increased slightly at 30 minutes which could be attributed to the effect of α 2 agonist causing splenic contractions as reported by Ganong, (2002).

TLC count in group B consisting two cubs and a subadult bears were higher compared to group A which could be due maturation of immune system as stated by Arun et al., (2008).

At 30 minutes after immobilization there was a significant (P<0.05) decrease in hemoglobin concentration and (P< 0.01) decrease in PCV values in group B. This could be due to α 2 agonists influencing shifting of fluids from extracellular to intracellular compartments to maintain cardiac output and the difference is more in group B as DexM has a property to preserve blood flow to vital organs as reported by Rafee et al., (2015).

Differential leucocyte counts are varied in both groups, this could be due to the different age groups and sex as stated by (Arun et al., 2008; Graesili et.al. 2014). A significant difference

in neutrophil count at 30 minutes between the groups may be attributed to very less stress response in group B due to faster and smoother induction of DexM causing less physical exertion which is similar to reports of Rafee et al., (2015) (Table 1–4).

Table.1 Mean ± SE values of Hematological parameters of sloth bears in Group A and B

Parameters	(Miller and Fowler, 2003)	Group A			Group B		
	0 min/Normal range	15 minutes	30 minutes	60 minutes	15 minutes	30 minutes	60 minutes
TEC (× 10^6 / µl)	5.58 – 14.70	5.56 ± 0.23^a	5.92 ± 0.17^a	6.11 ± 0.07^a	5.82 ± 0.18^a	5.97 ± 0.17^a	6.11 ± 0.08^a
TLC (× 10^3 / µl)	5.90 - 24.0	12.92 ± 0.91^a	12.28 ± 1.10^a	13.28 ± 0.85^a	14.63 ± 0.76^a	13.45 ± 0.97^a	14.60 ± 0.80^a
Hemoglobin (g / dl)	8.5 – 20.4	14.50 ± 0.35^a	13.92 ± 0.27^a	15.27 ± 0.18^a	14.33 ± 0.51^a	13.88 ± 0.59^a	15.10 ± 0.21^a
PCV (%)	35.0 – 54.0	42.98 ± 2.44^a	42.57 ± 2.59^a	43.72 ± 0.91^a	38.87 ± 1.87^a	36.32 ± 1.37^a	41.07 ± 0.87^a
DLC (%)							
Neutrophils (%)	65.76 – 71.25	66.17 ± 1.54^a	67.00 ± 0.68*	65.50 ± 1.18^a	61.17 ± 4.27^a	60.83 ± 2.24*	58.17 ± 3.63^a
Lymphocytes (%)	5.97 – 25.75	25.33 ± 1.45^a	23.17 ± 0.79^a	24.83 ± 0.79^a	28.83 ± 4.29^a	28.17 ± 2.74^a	31.50 ± 3.59^a
Eosinophils (%)	2.14 – 21.31	2.83 ± 0.3^a	2.50 ± 0.3^a	2.67 ± 0.21^a	3.00 ± 0.3^a	3.00 ± 0.3^a	2.50 ± 0.22^a
Monocytes (%)	0 – 5.45	5.33 ± 0.42^a	6.67 ± 0.4^a	6.33 ± 0.67^a	6.00 ± 0.26^a	6.83 ± 0.5^a	7.17 ± 0.48^a
Basophils (%)	0 – 2.64	0.50 ± 0.22^a	0.67 ± 0.21^a	0.67 ± 0.21^a	0.50 ± 0.22^a	0.67 ± 0.21^a	0.67 ± 0.21^a

Table.2 Mean ± SE values of Biochemical parameters of sloth bears in Group A and B

Parameters	(Miller and Fowler, 2003)	GROUP A			GROUP B		
	0 min / Normal range	15 minutes	30 minutes	60 minutes	15 minutes	30 minutes	60 minutes
ALT (IU / L)	6 – 60	14.73 ± 1.50[a]	13.93 ± 0.77[a]	12.60 ± 0.82	15.70 ± 2.56[a]	14.00 ± 2.35[a]	13.17 ± 2.24
AST (IU / L)	63 – 234	81.00 ± 5.13[a]	79.50 ± 6.93[a]	75.83 ± 5.62	74.50 ± 7.11[a]	72.50 ± 4.43[a]	70.83 ± 4.62
BUN (mg / dl)	8 – 38	14.57 ± 1.65[a]	14.43 ± 1.84[a]	14.28 ± 1.77	12.08 ± 1.73[a]	11.60 ± 1.70[a]	11.51 ± 1.65
Serum Creatinine (mg/dl)	0.5 – 3.0	1.70 ± 0.21[a]	1.70 ± 0.24[a]	1.62 ± 0.23	1.35 ± 0.11[a]	1.32 ± 0.13[a]	1.23 ± 0.12
Total Proteins (g / dl)	5.4 – 8.6	6.18 ± 0.35[a]	6.25 ± 0.37[a]	6.15 ± 0.37	6.00 ± 0.39[a]	5.92 ± 0.32[a]	5.92 ± 0.29
Glucose (mg/dl)	48 – 166	135.9 ± 10.71[a]	146.6 ± 6.93[a]	143.0 ± 6.05	121.4 ± 9.61[a]	130.7 ± 10.74[a]	128.3 ± 10.22

Mean value with superscript ([a]) differ non-significantly and superscript (*) differ significantly between columns in Table 1 and 2

There was no significant difference observed in ALT and AST values indicating the anesthesia having minimum effect on liver cells during biotransformation of drugs similar to reports by Grimm et al., (2011). No significant difference were observed in BUN and Serum Creatinine values although the lesser values in group B could be because of the faster and smoother induction of DexM. It could be also because it is normal for younger animals to have lower BUN and Creatinine values as stated by Graesli et al., (2014). Mean ± SE of glucose in group A was non-significantly higher at 15 and 30 minutes causing urination in group A. This could be because of hyperglycemia caused by Xylazine, as it causes decrease in insulin release from β cells and / or increased glucogon from α cells and that Medetomidine cause decrease in Insulin without resultant increase in glucose as reported by Posner and Burn, (2009).

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Art - 191: GROSS ANATOMY OF BONY ORBIT AND EYEBALL OF SLOTH BEAR *(Melursus ursinus)*

N.M. Rajashailesha, R.V. Prasad, **Arun. A. Sha,** K.V. Jamuna, M.L. Satyanarayana and S. Ganga Naik

Abstract

The sloth bear is originally thought to be a bear-like sloth due to its sloth like looks and behaviour (Joshi et al., 1995). Vision plays a major role for the movements of animal for hunting and for identify inmates. The orbit is the bony fossa that separates the eye from the cranial cavity and protects it along with orbital fascia, fat and extraocular muscles of the eyeball, and provides several pathways through foramina for the various blood vessels and nerves involved in eye function. The size, shape and position of the orbit are closely associated with visual activity and feeding behaviour. Due to paucity of available literature on the bony orbit and eyeball of the sloth bear (Kalita et al., 2006) the present research was planned.

Key words: Bony orbit, Orbital index, Eyeball, Tunics, Sloth bear

The sloth bear is originally thought to be a bear-like sloth due to its sloth like looks and behaviour (Joshi et al., 1995).

Vision plays a major role for the movements of animal for hunting and for identify inmates. The orbit is the bony fossa that separates the eye from the cranial cavity and protects it along with orbital fascia, fat and extraocular muscles of the eyeball, and provides several pathways through foramina for the various blood vessels and nerves involved in eye function. The size, shape and position of the orbit are closely associated with visual activity and feeding behaviour. While the depth of the orbit contribute to protection, appearance and governs the expanse of the visual field and degree of depth of the field for a given species. Due to paucity of available literature on the bony orbit and eyeball of the sloth bear (Kalita et al., 2006) the present research was planned.

Materials and methods

The eyeball with intact extra ocular muscles of six adult sloth bear of both sex were collected during post mortem which died due to natural causes. The eyeballs were removed according to the methods of Keller (1975). Briefly, the eyelids were sutured together with continuous suture pattern from the medial to lateral canthus; the lids were then held and pulled out with an artery forceps and a scalpel knife was used to cut around the periorbital sinus as traction was applied to the lids. This exposed the globe and further incision was made

around the orbit, to reach and severed the optic nerve. The eye was then put on the dissecting board and the eyelids, periorbital fat, extraocular muscles and connective tissue were removed to free the eyeball before it was put into neutral buffered formalin for 48 hours to prevent artificial deformation. After enucleation three heads were subjected for maceration to study the bony orbit in accordance with the method described by Simoens et al. (1994) and Onar et al. (1997). Photographs were taken by Cannon T6I and Stereo zoom microscope, Lawrence and Mayo® with Nikon Coolpix digital camera. Ocular Dimensions were recorded by using Vernier caliper and a thin non absorbable twine with a needle at one end for guidance, measurements were made on the vertical axes, mediolateral and anterior posterior borders.

Orbital parameters measurement: Vernier caliper was used in this study for orbital measurement: 1) Orbital length: The perpendicular distance between the supraorbital and infraorbital margins of the orbit. 2) Orbital width: The horizontal distance between the rostral and caudal margins of the orbital rim. 3) Orbital index = Orbital width / Orbital length X 100. 4) Orbital axis was recorded by using straight bicycle spokes and metal scale. 5) Inter-orbital distance: i. At rostral level: Distance between the junctions of frontolacrimal sutures of either side at the rostral margin of the orbit. ii. At

middle level: Distance between the supraorbital borders of orbit on either sides. iii. At caudal level: Distance between the junctions of the zygomatic bone at the caudal margin of the orbit on either side (Miller et al., 1964). The results were expressed as Mean±SE. Statistical analysis was carried out using SPSS statistical program.

Results and discussion

The orbits of the sloth bear had incomplete bony rim and oval in shape (Fig.1) The orbital margin outlines the base of the cone and directed more frontally similar to leopard cat (Sarma et al., 2001) in contrast to laterally orbits in dog (Evans and de Lahunta, 2013). The dorsal and medial segments of the orbital margin were formed by the frontal bone, medioventral margin by the maxillary 86 bone and ventrolateral margin by the orbital border of the zygomatic bone. The presphenoidal orbital wing and ethmoid bone forms the caudal part of the medial wall and contains the optic canal and ethmoid foramen. The lacrimal bone contributed to a small portion of the rostroventral medial wall and contained the fossa for the lacrimal sac and the caudal orifice of the nasolacrimal canal (Figs. 2, 3) similar to the findings made by Kalita et al., (2006) in sloth bear. The frontal surface of lacrimal bone towards the orbital margin presents a bony

projection (Fig. 3). The orbital rim showed a wide gap between the frontal process of zygomatic bone which was more pointed than dog and zygomatic process of frontal bone similar to dog (Dyce et al., 2002 and Evans and de Lahunta, 2013). The bony orbital cavity of sloth bear continued ventrally with the pterygopalatine fossa which contained maxillary, sphenopalatine and caudal palatine foramina from dorsal, middle and ventral respectively. Behind it was followed with most dorsal single ethmoid foramen placed 1.2 cm rostral to optic canal which is often paired in dog (Dyce et al., 2002) followed by the optic canal, the foramen orbitorotendum which is divided by a horizontal plate of bone into upper orbital fissure and lower rostral alar foramen (Fig. 2) is in concurrence with the findings in dog (Dyce et al., 2002). The mean orbital axis of both right and left orbit of sloth bear were similar in length 60.16±0.600 mm, with a minimum orbital axis of 59 mm and maximum of 61 mm.

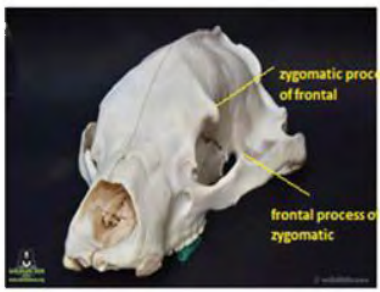
The mean orbital index of sloth bear of both right and left orbit were similar in length 82.11±0.83 mm, with minimum bony orbital length of 38.5 mm and maximum length of 40 mm and minimum width of 31 mm and maximum of 33 mm (Fig. 4). In contrast, orbital index of both orbits was more in Kagani goat measured about 89.12±2.49 mm (Sarma, 2006). According to Archana et al., (2006) the orbital cavity of north-

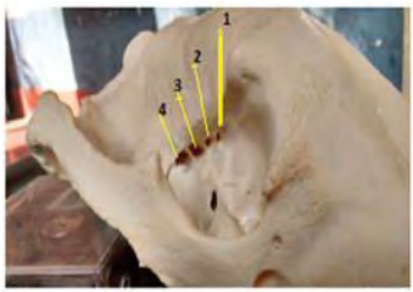
western Himalayan leopard was 6.0 cm in length and 5.0 cm in width. The length and width of bony orbit of sloth bear were more than canine and felines and less than bovine and equine species as observed by Gellat et al. (2013). Saber and Gummow (2015) stated that the Lion (Panthera leo) orbital height were 7.75±0.50 cm with minimum height were 7.00 cm and maximum height were 8.00 cm and orbital width were 5.625±0.48 cm and minimum height were 5.00 cm and maximum height were 6.00 cm. Yahaya et al., (2012) reported in camel orbital horizontal diameter 5.45± 0.06 cm and orbital vertical diameter 5.48± 0.04 cm.

Less than the lion skull as showed by Saber and Gummow (2015). Archana et al. (2006) reported that the inter-orbital distance was7.50 cm in north-western Himalayan leopard. Whereas in kagani goat, rostral, middle and caudal inter-orbital distance were 10.14±0.10 cm, 9.90±0.25 cm and 18.42±0.33 cm, respectively (Sarma, 2006). Mazak (2008) recorded the inter-orbital breadth in the male tiger and female tiger *(Panthera tigris)* with the landmark, the smallest distance between inner edges of orbits were 2.37±0.02 cm in male and in female tiger were2.45±0.02 cm, respectively. The dimensions of interorbital distance in present study and comparing the observations in tiger and lion, the sloth bear skull was comparatively smaller than tiger and lion and larger

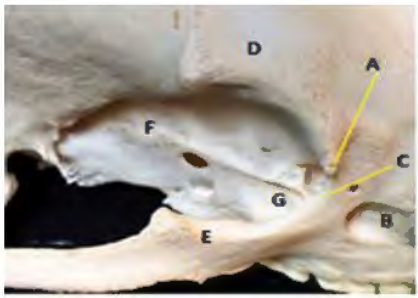
than Himalayan leopard, Kagani goat, dog and cat.

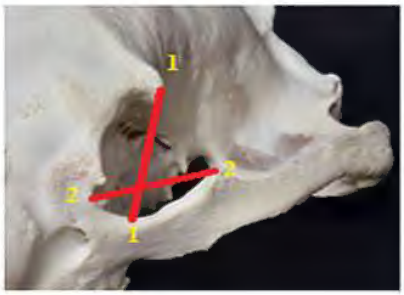
Eye ball: The eyeball of sloth bear in the present study had black colour at corneoscleral rim and shining blackish white colouration at the caudal rim. The shape of the eyeball was almost circular with nearly identical vertical axes (1.48±0.10 cm and 1.5±0.08 cm of right and left eyeball, respectively) and horizontal axes (1.48±0.10 cm and 1.5±0.08 cm of right and left eyeball, respectively). The anterio-posterior axes (2.15±0.15 cm and 2.16±0.16 cm of right and left eyeball, respectively) were being slightly longer than vertical and horizontal axes in dimension with no significant difference. The eyeball of sloth bear was comparatively smaller than the northwestern Himalayan leopard as reported by Archana et al., (2006). With respect to dimension of the eyeball (vertical and horizontal axes), the present findings indicated almost circular eyeball in sloth bear similar to dog and cats, but the anterio-posterior axes dimension was slightly more than the rest to the reports of Gellat et al. (2013).



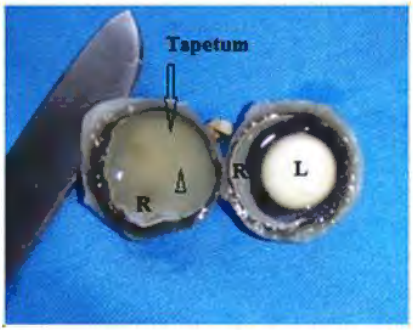


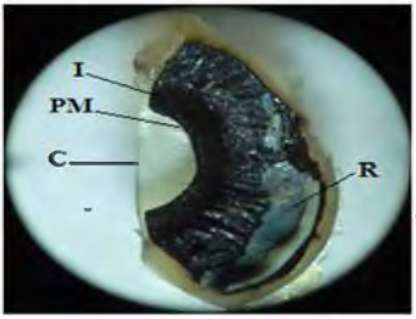
Figs. 1-4. 1. Photograph showing dorso-lateral view of Sloth bear skull; 2. Lateral view of bony orbit showing foramen 1-ethmoid, 2-optic, 3-orbital, 4-rostral alar foramen;





3. Bony boundary of orbit. A-bony projection of lacrimal bone, B-infraorbital foramen, C-lacrimal bone, D-frontal bone, E-zygomatic bone, F-orbital part of ethomoid; 4. Measurement taken for the bony orbit 1-1 orbital length, 2-2 orbital width.





Figs. 5-6. 5. Photograph of transverse section of eyeball of sloth bear at equator. L-lens, I-iris, R-retina. Open arrowhead showing optic papila; 6. Stereozoom microscopic photograph of anterior part eyeball of sloth bear. R-retina, I-iris, PM-pupilary margin, C-cornea.

The internal morphology of eyeball in this study was similar to that of other domestic animals as reported by the other authors (Getty, 1975; Evans et al., 2013 and Gellat et al., 2013). It contained the outer fibrous tunic *(tunica fibrosa bulbi),* the middle vascular tunic *(tunica vasculosa bulbi*), and the inner nervous tunic *(tunica interna bulbi).* Anteriorly sclera merges with the peripheral cornea and the bulbar conjunctiva to form limbus. However, in the present study, the cornea formed the smallest part with similar in vertical and horizontal dimension 1.31±0.03 cm and sclera was the largest part among the fibrous tunic and this was in agreement with the findings of Evans and de Lahunta, (2013) and Gellat et al. (2013) confirming eyeball morphology similar to dog and cat. The present study also revealed that the corneoscleral junction was pigmented black colour as in case of dog (Gellat et al., 2013). Choroid shows presence of tapetum responsible for nocturnal vision which was semicircular in shape with base of the semicircle tapetum Placed further ventral to optic papilla (Fig. 5). Whereas in canines it was roughly triangular in shape (Gellat et al., 2013) and in cat tapetum occupies a rounded triangular area with the base oriented horizontally to level of optic papila and the apex directed upwards as reported by Braekevelt, (1990). Similar to domestic animals

the ciliary body of sloth bear was situated caudal to the lens and made up of radially arranged ciliary processes (Fig. 6), which ring the pupil and grooves to which suspensory ligament of lens were attached. Iris like a diaphragm except at central opening which was circular in fixed eyeball of sloth bear, whereas, it was vertical in bobcat, domestic cat and lynx (Gellat et al., 2013). The retina showed light grey in colour and covered the entire inner coat of the eyeball. Cranially it was restricted by the iris and origin of optic nerve from behind (Fig. 5).

The lens of sloth bear was situated caudal to iris (Fig. 5) showed biconvex in shape in fixed state and was attached to the ciliary body at its circular margin by thin zonular fibres. Sloth bear lens dimension had a central thickness of 5.08±0.15 mm (right lens) and 5.16±0.16 mm (left lens) with equatorial diameter of 8.33±0.33 mm which are comparatively smaller than the central thickness of lens of dog and cat and equatorial diameter was also smaller than dog and cat (Gellat et al., 2013).

The present study in sloth bear revealed more frontally located bony orbit and smaller eyeball with presence of semicircular tapetum. These variations could be structural modifications to suit more of binocular and the nocturnal vision and hibernating behaviour in the wild conditions.

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Art – 192. ROLE OF FLEXIBLE FIBER OPTIC BRONCHOSCOPY IN EARLY DIAGNOSIS OF PULMONARY TUBERCULOSIS IN SLOTH BEARS *(Melursus ursinus)*

Arun A Sha, Ilayaraja S, Yaduraj and **Nithin K**

Summary

Infection by Mycobacterium tuberculosis in Captive sloth bears represents a typical case of spill over infection resulting from human cohabitation, after the animals. Were illegally poached from the wild, as cubs. The stress and hardships that the animal endure, play major role in the development of the infirmity, as their immune system is usually compromised. Around 71% of rescued sloth bears deaths are due to this disease. This population of rescued dancing sloth bears during the illegal custody by nomadic tribes, were exposed to severe stress agents like trauma, various physical and physiological illness, malnourishment etc., and hence making them susceptible to various disease like tuberculosis.

Background

The diagnosis of trachea-bronchial tuberculosis on the grounds of clinical and radiological findings in sloth bears *(Melursus ursinus)* is more difficult due to the lack of

etiological confirmation. Sloth bear belonging to the family Ursidae and is endemic to Indian subcontinent is also protected under schedule 1 of the Indian wildlife protection Act., 1972 and considered vulnerable in IUCN.

Methodology

In the past BALF was performed on an immobilized sloth bear through endotracheal tube with the help of flexible autoclaved long catheters to collect the bronchial alveolar lavage fluid (BALF). Similarly, Flexible bronchoscopy (EVIS Olympus CF, TV-571 1.0-meter endoscope with 9.8mm Día Plug and play system) can be performed efficiently and effectively on captive Sloth bears. It is a safe and easy procedure, the entire length of trachea till the level of tertiary bronchioles viewed and appropriate samples will be collected by using biopsy styles and BALF.

Results and conclusion

This article describes about the application of flexible bronchoscopy (FB) in the diagnosis of tuberculosis (TB) with the involvement of tracheobronchial wall. Pathological changes typically include mucosal and submucosal oedema and hyperemia. It also comprises of tubercular-like nodules, caseous necrosis and fibrous hyperplasia. Acid-fast bacilli staining is done from bronchial alveolar lavage fluid (BALF)

sediment and a positive stain confirms the diagnosis. It also aids in the differential diagnosis of complicated and refractory pneumonia. Furthermore, it can be beneficial in gastric washing and sputum collection. This paper outlines the diagnostic challenges and conservation implications of tuberculosis in Captive wild animals.

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Art – 193. POST DENTAL PROCEDURES: A DIRECT BENEFIT ON WELFARE AND BEHAVIOUR CHANGES IN RESCUED SLOTH BEARS *(Melursus ursinus)*

Attur Shanmugam Arun, S Ilayaraja, K Yaduraj, Lisa Milella, Paul Cassar and **K Nithin**

Summary

The street performing Sloth bears were rescued from various part of India were housed in rescue and rehab centres of Wildlife SOS which were established with the help of state forests department. These Sloth bears were basically from traditional performing bears which were used for public entertainment. Such Sloth bears were rescued and housed in near natural habitat in four different rescue centres. Around 450 such sloth bears were rescued and rehabilitated by the organisation. One of the cruel practices by the gypsy community was to brutally knock out all the four canine teeth for the safely of the handlers. Between 35th to 45th weeks of the sloth bear cubs age, the permanent canine teeth would erupt. Over 200 such performing Sloth bears were undergone various dental procedures like endodontic treatment and extraction procedure at various level of their illness. Based on their dental radiography examination, the decision was made

to opt an appropriate dental procedure under gaseous general anesthesia by using isoflurane.

Background

The Sloth bear *(Melursus ursinus)* is endemic to the Indian sub-continent and found in India, Sri Lanka, Nepal, Bhutan and Bangladesh. In India, Sloth bears are distributed from the Southern tip of the Western Ghats to the foothills of the Himalayas (Prater, 1965). The sloth bear population now confined to small isolated regions of Indian sub-continent due to expanding human habitat and agricultural demand (Garshelis et al. 1999). One of the threats for Sloth bears in the natural habitat are cub poaching for dancing bear practice. The oral health of any animal is directly related with the overall health of the animal, especially the dental and gum diseases are very common in captive wild animals due to their poor feeding habitat and hygiene.

Methodology

Over a period of 9 years, 81 sloth bears were undergone root canal treatment and 216 Sloth bears were undergone canine teeth extraction by using a standard protocol established by a team of veterinary dentists at two centres namely Agra Bear Rescue Facility and Bannerghatta Bear

Rescue Centre, India. From each category of treated sloth bears, 20 adult sloth bears were selected with equal gender ratio (10M:10F) and observed for pre and post treatment behaviour observation based on the standard data sheet prepared for various parameters including feeding behaviour, compatibility, socialization with other sloth bears, aggression, weight gain and stereotypy on daily basis.

Results

All the 40 Sloth bears observed for the post dental treatment were showed remarkable improvement from the original observation of the same parameters prior to treatment for a period of a month. The overall result showed that the dental treated bears were more social, the feed consumption was improved, the style of eating become normal, aggression has gone down drastically due to less or no pain, the weight gain has become adequate and the stereotypy has reduced from 90% to 27%.

Conclusion

By analyzing various physical and behavioural parameters pre and post dental treatment, the improvement noticed in terms of health was very well in connection with the welfare like utilisation of enclosure enrichments and behaviour of the

individual sloth bear. So ultimately oral health is the best reflection of the general health of the animal. Dental procedures should be extended in the practice of wild rescued animals which, many at times fail to get released due to dental issues, so that the chances of them getting released into the wild would increase and it might also increase the rate of survivability in the wild.

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Art- 194. INTRA- ABDOMINAL LAPAROSCOPIC STERILIZATION OF MACAQUES - AN ATTEMPT TO MITIGATE HUMAN-PRIMATE CONFLICT THROUGH AN EFFECTIVE ANIMAL BIRTH CONTROL TECHNIQUE

Ilayaraja Selvaraj, Arun. A. Sha, Baiju Raj and **Abhishek Kumar Singh**

Summary

Using humane methods of troop capturing, 830 individuals belonging to 38 troops from 11 different locations were captured, out of which vasectomy and tubectomy were performed on 190 & 310 individuals, respectively. The rest of the animals were not fit for surgery as they were too young, underweight, senile or pregnant. After surgery, the animals were kept under observation and post-operative care for a day and rereleased along with the same troop, in the same location from where they were caught. The one-year continuous monitoring of the sterilized monkeys at the respective release sites revealed that they had adapted to their routine activity pattern and no mortality was reported from the sterilised population.

Background

Rhesus macaques *(Macaca mulatta)* are protected by schedule III of Wildlife Protection Act. However, they are losing conservational support with the rapid increase in their population as well as nuisance activities. Hence, conflict mitigation becomes a huge challenge for the city administration and the forest department as well. Wildlife SOS, an NGO, took the initiative of a laparoscopic sterilization project to mitigate the Human Primate conflict caused by rhesus macaques in Agra district, Uttar Pradesh; by adopting humane methods of troop capturing, transportation, animal selection, endoscopic surgical procedure, creating permanent identification and re-rerelease. It was funded by Agra Development Authority and the district administration.

Methodology

Documentation of the regions having maximum human - primate conflict was carried out and an extensive study of macaque troops was conducted for their behaviour, identification and selection of target macaque troops. Based on the study, suitable sized conditioning macaque trap cages were deployed to the selected target location. Macaque troops were conditioned to enter the large trap cages through regular

feeding. Entire troops were captured after conditioning. After successful trapping of a troop, individuals were separated by a filter cage, then shifted into the vehicle to avoid any stress or injuries due to infighting during transportation to the hospital site for endoscopic sterilization. Soon after the animals reached the hospital, an initial examination and selection procedure was carried out to segregate the senile, juvenile, underweight/week and possibly pregnant animals for further care and feeding. Only the selected healthy individuals were starved for the sterilization procedure. Xylazine Hydrochloride (2mg/kg BWT) & Ketamine Hydrochloride (6mg/kg BWT) were used for induction once the animal was placed inside the squeeze cage. The sedated individual was prepared aseptically for the operation by shaving and disinfecting the abdominal region, followed by antibiotic & anti-inflammatory injections. The animal was kept in dorsal recumbency in a slightly inclined position by lifting its hip region with a folded clean cloth covered cushion. General anesthesia was maintained by applying an isoflurane (2%) face mask. 3mm laparoscopic surgical equipment were used to minimize the trauma and the standard three-port technique was utilized for vasectomy/ tubectomy (One midline and two lateral pre-umbilical surgical ports were created. A telescope

was inserted through midline port while cutting and grasping forceps were inserted through the lateral ports). Vas deferens (in males) or fallopian tubes (in females) were located and gently grasped by forceps. A 3-4cm long fragment of vas deferens/ fallopian tubes was removed by cutting and cauterization, using an atraumatic thermo-cautery forceps and scissors connected to an electrocautery unit. The midline port below the umbilicus was closed with 4-0 absorbable suture material using simple interrupted suture and the skin was opposed with tissue adhesive glue. The other two lateral incision ports were also opposed with tissue adhesive glue. After the operative procedure, permanent tattooing and tagging of each animal was done by a tattoo artist for identification in case of re-capture. The macaques were vaccinated against rabies and Tetanus toxoid. A day after post-operative care, the entire troop was re-released at the same area it was captured from.

Results

830 individuals, belonging to 38 troops were captured from 11 different locations out of which 500 individuals were sterilized (190 Vasectomy & 310 Tubectomy). The rest of the animals were not fit for surgery as they were either too young

(221 are juveniles), underweight (68 individuals), senile (2 individuals) and pregnant (39 individuals). After surgery, the animals were kept under a days observation and postoperative care; and re-released along with their troop in the same location from where they caught. Further one-year continuous monitoring of the sterilized monkeys at the respective released sites revealed that the monkeys had adapted to their routine activity pattern without exhibiting any abnormalities. Hence, laparoscopic tubectomy/vasectomy is preferred over other surgical techniques because it can usually be carried out without affecting an individual's social position, as the endocrine axis that drives behaviour remains intact. This method was found to be simple, easy and a faster method of vasectomy/tubectomy in the male and female rhesus macaques. The macaque sterilization program which was initiated by the Agra Development Authority and the District Administration was a successful venture and there shall be a visible change when we attain sterilization of at least 75% of the captured population. When the females do not carry young ones, their aggressiveness will reduce to a significant level as they will only be in search of their own food, and not under duress to provide for their young or to protect them. Our studies revealed that macaques are

aggressive during the mating season and when they are with the babies.

Conclusion

Considering the faster patient recovery period due to smaller surgical sites; no post-operative morbidity because of lower infection rates; and less post-operative pain and stress, this minimal invasive laparoscopic sterilization technique of macaques can be considered as a successful tool for animal birth control, thereby reducing the human primate interface, like in the case of stray dogs, wherein birth control programs have proved successful in reducing their dependency on humans.

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Art – 195. CASE DETECTION OF LEPTOSPIROSIS IN CAPTIVE SLOTH BEARS *(Melursus ursinus)* IN INDIA

Karikalan Mathesh, Sabarinath Thankappan, Sujith K. Behera, **Ilayaraja Selvaraj**, **Arun A Sha**, Yosef Deneke, Syed Atif Ali, Chandra Mohan Siddappa, Med Ram Verma, Pallab Chaudhuri and Anil Kumar Sharma

Summary

In this present study sloth bears serum samples, which were submitted to wildlife center IVRI suspected for leptospirosis in different time periods were found positive for leptospirosis utilizing plethora of test. It signifies leptospirosis may be a risk for the sloth bear and the zoo personal as well.

Background

Sloth bears are classified as vulnerable in the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species in 1990 (IUCN, 1990) and are protected under Schedule I of The Indian Wildlife (Protection) Act (IWPA), 1972 (IWPA, 1972). Infectious diseases such as tuberculosis and leptospirosis and noninfectious diseases such as Biliary Adenocarcinoma

(BACA) have created havoc to the health of sloth bears (Gosselin and Kramer 1984; Kingston and Wright 1985; Veeraselvam et al., 2013; Khanna, 2014; Hedau and Kamdi, 2016; Anderson et al., 2018; Veerasami et al., 2018).

Material and Methods

In this study, a multi-pronged approach has been adopted in order to diagnose leptospirosis in sloth bears based on antigen (PCR based on G1/G2 primers and LigBF/LigBR primers) and antibody detection (MAT and rLigB based LAT), serum biochemistry profile (SGOT, SGPT, Creatinine and BUN) and gross/histopathological findings of liver and kidneys. Serum samples collected from eleven sloth bears which succumbed to disease suspected of leptospirosis in two different sloth bear rescue centres in India were tested for the presence of agglutinins against different serovars of Leptospira interrogans by icroscopic Agglutination Test (MAT). Further, a sero-diagnostic marker specific for pathogenic leptospira such as LigB was employed in a Latex Agglutination Test (LAT) to further confirm the veracity of test results obtained with MAT.

Results

It showed that MAT titres of serum samples were in the range of 1:400 - 1:800 and showed disproportional exaggerated elevation in SGOT (AST) levels. Further, Creatinine and BUN levels were also elevated in all the dead sloth bears. The liver and kidneys of dead sloth bears showed gross/histological lesions suggestive of leptospirosis which were confirmed by PCR using two sets of primers viz. G1/G2 and LigBF/LigBR primers. The sloth bear sera samples showed presence of agglutinins against serovars Pyrogenes, Javanica, Canicola and Tarassovi. The serum biochemistry of all the dead sloth bears revealed that both SGOT and SGPT levels were elevated. The Mean ± Standard Error for SGOT and SGPT in apparently healthy bears (both sex combined) is 125± 14 IU/L (Bush et al 1980) and 36.78± 7.52 (Chandra Mohan et al., 2018) respectively. However, all the dead sloth bears showed SGOT levels on the higher range of 276-348 IU/L while SGPT levels ranged from 36-48 IU/L. One hallmark feature observed in serum biochemistry in all the sloth bears which succumbed to leptospirosis was the significantly higher SGOT/ SGPT ratio during the terminal stages of the infection. The average SGOT/SGPT ratio in apparently normal sloth bears is approximately 3.4. The SGOT levels elevated

progressively without a concomitant change of SGPT during the acute disease course resulting in death of all sloth bears. Our experience with regard to SGOT/SGPT levels in terminal stages of leptospirosis suggests that SGOT/SGPT ratio can serve as a valuable prognostic parameter for leptospirosis with a ratio of indicative of a grave prognosis for the disease in sloth bears. We observed SGOT/SGPT ratio as high as 8.09-8.75 in five sloth bears which succumbed to leptospirosis. Chang et al (2005) also observed this disproportional exaggerated elevation in SGOT levels and high SGOT/SGPT ratio in human patients who died due to leptospirosis. All the dead sloth bears showed BUN levels on higher range of 26-40.5 mg/dl whereas the normal range of BUN in sloth bear is 19.08± 0.55 (95% confidence Interval 18.75-19.41) (Veeraselvam et al., 2014; Veeraselvam et al., 2018). Blood urea nitrogen (BUN) is used to evaluate kidney function and elevations in BUN level are often a result of a decrease in Glomerular Filtration Rate (GFR) (Macedo and Mehta, 2014). This clearly suggests kidney damage which resulted in decreased glomerular filtration of urea resulting in decreased excretion of urea in urine. All the dead sloth bears showed creatinine levels on slightly higher range of 1.6-2.1mg/dl whereas the normal range of creatinine in sloth

bear is 1.24 ± 0.09 (95% confidence Interval 1.19 - 1.29) (Veeraselvam et al., 2014; Veeraselvam et al., 2018). An elevation in the serum creatinine concentration usually reflects a reduction in the glomerular filtration rate (GFR) suggestive of kidney damage (Samra and Abcar, 2012).

Conclusion

The recombinant LigB based LAT (rLigB based LAT) gave congruous results (sensitivity 89.66%, specificity 100 %, Accuracy 93.28%) in comparison to MAT and proved to be an ideal penside diagnostic test for testing leptospirosis in sloth bears in field conditions. It can be concluded, based on serology, (MAT and rLigB LAT), PCR (using G1/G2 primers and LigBF/LigBR primers), serum biochemistry (SGOT, SGPT, BUN and Creatinine), as well as gross/histopathological evidences, that sloth bears can harbor and shed leptospires and can serve as a potential source of infection for other wild animals in captivity as well as for zoo personnel.

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Art - 196. TRANS- ABDOMINAL ULTRASONOGRAPHY IN SLOTH BEARS *(Melursus ursinus)*

Ilayaraja Selvaraj and Arun. A. Sha

Summary

Ultrasonography is an unique non-invasive diagnostic tool which is successfully implemented and currently in practice in human medicine as well as animals. The trans abdominal ultrasonography technique is the well-established non-invasive diagnostic approach in canines and felines for examining the visceral organs those are located inside the abdominal cavity for varies disorders such as hepatic carcinoma, cholecystitis, cholelithiasis, splenomegaly, hydronephrosis, cirrhosis, pancreatitis, urolithiasis, ascites, abnormal condition associated with adrenal, prostrate in male, ovaries & uterus in females and presence of foreign bodies etc. But such information is rarely available for wild animals, especially in sloth bears. We used the trans abdominal ultrasonography examination approach toexplore the abdominal organs as described by the previous authors in domestic canines for various disorders.

Background

There are eight bear species recognized till date around the world. In India there are only four bear species which includes sloth bear. The sloth bear is listed as vulnerable in IUCN and protected under schedule I of the Indian wildlife Production Act of 1972. Sloth bears are listed under Appendix I of CITES. Sloth bears are also commonly kept under captivity in many zoos and therefore knowledge of normal ultrasonographic anatomy is useful not only for disease diagnose but also to make adequate preventive care for captive sloth bear populations. However, diagnostic imaging in wildlife medicine is still a challenge owing to the lack of normal parameters that can guide the veterinarian. Ultrasonography may prove to be a valuable diagnostic screening tool for multiple disease processes in ursids. This study aims to document the normal sonographic anatomy of the visceral organs within the abdominal cavity and few pathologic conditions occurred in sloth bears.

Methodology

Over 50 captive rescued sloth bears were subjected for abdominal ultrasonographic examination. The group includes both adults male and females from the Agra bear rescue

facility and Bannerghatta bear rescue facility after the sloth bears were anaesthetized by using a ketamine-xylazine combination, ketamine hydrochloride (5 mg/kg body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg/kg body weight; Troy Laboratories Pty Ltd.). The ultrasound examinations were performed during the mandatory periodic health check-up. Health status was determined by long-term history, clinical and laboratory examinations. Blood sampling was performed by jugular venepuncture. Complete blood count, kidney and liver function tests were performed. Immobilized sloth bears were placed in dorsal recumbency, the abdomen was prepared, and sonographic coupling gel was applied 5-10minutes before scanning to percolate the skin to get the better imaging. A scan ultrasonographic (Logiq F6, General Electric Company, USA) device, with a multifrequency micro convex, curve linear and linear array probes was used. The animals were examined in clock wise diaphragmatic-hepatic view, spleen- renal view, cysto-colic view and hepato-renal view. Abdominal images of anatomical structures were evaluated for size, shape, position, echotexture and echogenicity. The bladder was visualized in transverse and longitudinal planes. Bladder wall thickness was measured dorsally. Kidney images were obtained in

frontal planes, sagittal and transverse, to fully evaluate the anatomical position, length, shape, echotexture, echogenicity and relationship between the cortical and medullary region. The liver was visualized in the sagittal and transverse planes with the transducer positioned at the ventral midline caudal to the xiphoid, directing it anteriorly. Images with the transducer between the last intercostal spaces were also obtained. Stomach wall thickness was measured between rugal folds. After B-mode examination, colour Doppler and pulsed waved Doppler were used with linear array probe to evaluate renal vasculature and locate the abdominal aorta and vena cava.

Result

However, the size and shape of the abdominal visceral organs vary from the domestic canine species the echotexture and echogenicity of the organs remains almost as same as like as the canines as described by the previous authors. In sloth bears the kidney is lobulated. sloth bear possesses well developed liver with 5 lobes and gallbladder with bile ductus. Spleen is sickle in shape. We diagnosed gallbladder abnormality such as increased wall thick ness, heavy sludge, gall stone, splenomegaly, hepatitis, ascites from the adult bears. bears are more prone for hepatic carcinoma in captive

condition, so the periodic evaluation of the hepatic system with this non-invasive trans abdominal ultrasonography will help to make the required treatment and preventive measures to ensure the quality life of those animals under captive conditions.

Conclusion

The trans abdominal ultrasonographic examination procedure is an absolute diagnostic modality, easy to practice, free from the ionizing radiations; it can be implemented without any doubt in routine health examination of the sloth bears in the captive facility to diagnose the disease as well as evaluate the abdominal organs especially the hepatic & gall bladder diseases.

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Art - 197. MORPHOLOGICAL IDENTIFICATION OF AMBLYOMMA INTEGRUM TICK IN SLOTH BEARS

(Melursus ursinus)

Dr. Adhithyan, Dr. Arun A Sha, and **Dr. Riya Bakde**

Abstract

Infestation with ectoparasites is a widespread finding in wild animals. Sloth bears provide a suitable environment for ticks owing to their dense long hair and large body surface area. Previous surveys undertaken in Karnataka state documented a total of 41 tick spp from wild animals whereas 12 species of ticks had a higher prevalence along the Western Ghats. Infestation with ectoparasites can lead to deterioration of the immune system of the bear attributed to several tick-borne diseases. The present study was conducted to identify the tick spp affecting the Sloth bears at Wildlife SOS, Bannerghatta. Tick samples were collected from a total of 50 bears under captivity in 70% ethanol and were subjected to morphological examination. The most commonly occurring tick spp was found to be Amblyomma integrum identified on the basis of longirostrate mouth parts and typical ornate scutum. Rhipicephalus spp ticks were less frequently observed. Transmission to sloth bears housed at Wildlife SOS

can be accredited to their close settlement with Sus Scrofa (wild boar) and Axis Axis (Spotted dear) who are known to harbor these ticks. Nymphic stages of Amblyomma integrum have been associated with otoacariasis in humans and therefore is of zoonotic importance necessitating its awareness among veterinarians working with wild animals on daily basis.

Art - 198. PLASMA MINERAL PROFILES OF SEMI-CAPTIVE AND FREE RANGING WILD SLOTH BEARS *(Melursus ursinus)*

Attur Shanmugam Arun, S. Karthik Bhat, P M Sidharth, Partha Sarathi Swain, Lyju Jose, N K S Gowda, V. Sejian and R. Bhatta

Abstract

A study was conducted to establish the normal physiological plasma mineral profiles of free-ranging and semi-captive Indian sloth bears and to determine if habitat influenced the physiological plasma mineral profiles in sloth bears. The Sloth bear (Melursus ursinus) is endemic to the Indian sub-continent and found in India, Sri Lanka, Nepal, Bhutan and Bangladesh. In India, Sloth bears are distributed from the Southern tip of the Western Ghats to the foothills of the Himalayas (Prater, 1965). The wild population of sloth bears has declined by 30 – 49% in the last 30 years (Garshelis et al., 1999). Plasma samples from 28 sloth bears were analysed for their mineral concentrations. A significant change in Co ($P<0.05$), Cr ($P<0.01$), Mg ($P<0.01$), Na ($P<0.01$) were observed between free range and captive category of sloth bear with higher values in the free ranging animals. The difference could be attributed to the availability of feed

resources and the selective feeding behaviour of free ranging category animals. This indicates the advantages of maintaining the Sloth bear in their natural habitat. Sloth bears were immobilized using a ketamine-xylazine combination, ketamine hydrochloride (5 mg/kg body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg/kg body weight; Troy Laboratories Pty Ltd.). The 28 Sloth bear samples were from both free ranging wild Sloth bears and Sloth bears at the Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park, Bannerghatta, Bangalore, Karnataka, India. The Sloth bears in the wild were free ranging ones and the ones in captivity were rehabilitated individuals kept under captivity for more than 7 - 10 years. The concentration of microminerals (mg/L) namely Zn, Co, Fe, Mn, Cr, Cu respectively, were 1.56±0.90; 0.85±0.42; 3.85±1.11; 0.29±0.27; 0.45±0.01; 1.00±0.85; and macro minerals (mg/dL) like Mg, Ca, Na, K, were 2.06±0.72; 8.89±2.28; 42.3±5.96;and 10.7±2.04;, respectively. It was found that plasma of free ranging bears showed higher concentrations of Zn, Fe, Mn, Cu, Mg, Na, K whereas samples of bears in semi captivity showed higher Co, Ca, and Cr levels., which signifies that free ranging animals have a choice over their feeding but in semi-captivity, it is controlled and man-made.

Art - 199. ESSENTIALS OF GERIATRIC MANAGEMENT IN WILDLIFE PRACTICE WITH SPECIAL REFERENCE TO CAPTIVE SLOTH BEARS (*Melursus ursinus*)

Dr Arun A Sha, Dr. Ilayaraja, Dr. Nithin, Dr Adhithyan NK, Dr Riya Bakde

Abstract

The geriatric phase is where an animal will show increased vulnerability to infections owing to weakened immune systems and reduced bodily functions. Considering their life span, geriatric bears above 20 years of age, need special attention and care in overall veterinary and husbandry practice. The present study was conducted on senile bears in Bannerghatta Bear Rescue Centre (BBRC), Bangalore. The study mainly focused on individual dietary needs and common health problems in geriatric Sloth bears. Specific nutritional and managemental modifications with emphasis on vitamins and mineral supplementations and liver and kidney protectants were undertaken for selected Sloth bears more than 22 years of age. They were provided with a special diet of required quantity containing 70g of dietary fibre (20% more than the regular food), 5% less protein, low sodium and low fat diet which was rich in Ca, vitamin D3, Vitamin B12,

Vitamin C and potassium. The food was supplemented with probiotics (*lactobacillus sp*. and *bifidobacterium sp*.) and joint support medications containing chondroitin sulphate and glucosamine. Managemental practices like housing the Sloth bear with more compatible age groups and minimizing human interaction were adopted to provide stress-free environment to these Sloth bears. The effectiveness of these changes was assessed by their behavior and fecal score which were monitored regularly. The combined effect of calorie and protein restriction, proper supplementation including probiotics, joint supports were found to be very useful in managing the older Sloth bears in optimum health condition. The understanding and adoption of individual medical and managemental needs in geriatric patients are essential for their welfare in captivity to enhance the quality of life.

Keywords: geriatric phase, sloth bears, management, dietary

Art - 200. CHEMOTHERAPY AND MANAGEMENT OF TUBERCULOSIS IN RESCUED "DANCING" SLOTH BEARS

(Melursus ursinus)

Dr. Arun Sha, Dr. Ilayaraja, Dr. Nithin K, Dr. Adhithyan NK

Abstract

Tuberculosis caused by Mycobacterium tuberculosis (MTB) is a highly contagious bacterial disease with reverse zoonotic potential. It is the major cause of death in dancing sloth bears rescued from the Kalandar community owing to their unhygienic handling and close congregation with humans most often acquired by a spill over transmission. Cubs weaned from their mothers at an early age may further aggravate their chances of infection due to weak immune system, compromised environmental upbringing and diurnal stress. The present study was conducted on the rescued dancing sloth bears rehabilitated at Bannerghatta bear rescue center, Bangalore which was diagnosed with MTB. The Sloth bears diagnosed with MTB were therapeutically managed using four drugs namely, Isoniazid, Rifampicin, Ethambutol and Pyrazinamide that were available in a tablet form and administered orally with honey, fruits, dates, porridge, milk and egg. However, the feed was refused by the bears and this

drawback was overcome by replacement with syrup formulations. Administration of Isoniazid further affected the synthesis and metabolism of pyridoxine (vitamin B6) which was dealt by parallel supplementation of Vitamin B6 and liver protectants. The anti-TB regimen consisting of Isoniazid and Rifampicin syrup combination was given at a dose rate of 10mg/kg bodyweight for every alternate day for a period of 90 treatment days. Regular monitoring of the bears under treatment was done to ascertain the possible adverse effects of the medications like staggering, wobbling and incoordination. Preventive measures were aimed at feeding nutritious feed and isolation practices along with therapeutic management for prolonged sustenance of the bears under captivity.

Keywords: Tuberculosis, Therapeutic, Isoniazid, Rifampicin

Art - 201. URINE ANALYSIS AS AN EARLY DIAGNOSTIC TOOL IN SLOTH BEARS *(Melursus ursinus)*

Dr Arun A Sha, Dr Adhithyan NK, Dr Riya Bakde

Abstract

Urine Analysis is an important screening tool for early diagnosis of renal diseases. In the present study, urine samples from 69 sloth bears (25 males and 44 females) housed at Bear Rescue and Rehabilitation Centre, Bannerghatta, Bangalore were collected and analyzed to evaluate the constituents of urine in Sloth bears over a period of three months during July to August 2019. Commercially available Idexx urine analysis strips and Vetlab urine analyzer were used to measure pH, glucose, protein, urobilinogen, ketone bodies, erythrocytes and leucocytes in urine. The average pH of bear urine was found to be 7.86 ± 0.65. Out of the 69 sloth bears screened, 84.05% had alkaline urine while acidic urine was observed in 5.79% of the bears. Trace amounts of glucose (50mmol/L) and protein (<30mg/dl) was present in 7.24 % and 4.30% of the bears screened. Leukocytes in urine were observed in 81.15 % of the Sloth bears out of which 41.07% showed leukocyturia (500/µl) without any clinical signs. An abnormal number of

erythrocytes in urine without showing any clinical signs was observed in 72.46% of the bears tested whereas 27.54% had a reasonable amount of erythrocytes in urine (0-10 cells per microlitre). Urobilinogen was detected in the urine of only one (1.40%) Sloth bear. Ketone bodies and bilirubin were not detected in the urine samples in any of the bears examined. Presence of abnormal constituents in the urine of bears may indicate early affections of the renal system that can be further diagnosed and therapeutically managed to aid in curbing fatalities.

Keywords: Sloth bear, Urine Analysis, Leukocyturia

Art - 202. INFLUENCE OF HABITAT ON HORMONAL PROFILES OF SLOTH BEARS *(Melursus ursinus)* IN SEMI-CAPTIVITY AND FREE RANGING AREA

Attur Shanmugam Arun, Partha Sarathi Swain, P M Sidharth, Lyju Jose, V. Sejian and R. Bhatta

Abstract

Change in habitat as well as interference of human activity tends to alter the hormonal profile of the wild animals. In the present study, Plasma T3 and Cortisol levels were significantly altered, whereas hGH, IGF-1, T4 and Leptin are found statistically similar in both free ranging and semi-captive sloth bears due to change in their habitat and diet. Sloth bears (Melursus ursinus) have been listed as Vulnerable by the IUCN red list with the estimates of about 20,000 or fewer individuals and less than 10,000 adults existing in the wild. In India, Sloth bears are distributed from the Southern tip of the Western Ghats to the foothills of the Himalayas. The total number of 28 Sloth bear plasma from both free ranging wild Sloth bears and semi-captive Sloth bears at the Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park, Bannerghatta, Bangalore, Karnataka, India were used in the study. Both these locations are within the documented

habitat range for Sloth bear species. All Sloth bears sampled were adults and appeared physically healthy, with normal behavioural responses, and were clinically healthy during examination at the time of sampling (as determined by body temperature, hydration, heart/respiration rate, and a detailed external physical examination). The Sloth bears in the wild were free ranging ones and the ones in captivity were rehabilitated individuals kept under captivity for more than 7 - 10 years. The plasma samples were collected after the Sloth bears were immobilized using a ketamine-xylazine combination, Ketamine hydrochloride (5 mg/kg body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and Xylazine hydrochloride (Xylazil, 2 mg/kg body weight; Troy Laboratories Pty Ltd.). The plate reader Multiskan Go (Thermo Scientic) was used to measure all the readings as per the standard kit protocol. In free ranging sloth bears hGH, Cortisol, IGF-1, T3 and Leptin were higher than that of the bears kept under controlled conditions i.e. semi captivity. However, T4 was higher in semi-captive sloth bears. Lesser ($P<0.001$) cortisol under semi-captivity indicates lesser stress which may be due to availability of food or they were acquainted to the human interference in their habitat. The results obtained in the study indicated that change in habitat affects serum T3 and Cortisol level significantly,

whereas hGH, IGF-1, T4 and Leptin are comparable between free ranging and semi-captive sloth bears.

Art - 203. IMMUNORESPONSE OF C-REACTIVE PROTEIN (CRP) TO INFLAMMATION AND INFECTION IN INDIAN SLOTH BEAR *(Melursus ursinus)*

Attur Shanmugam Arun, S. Ilayaraja, P M Sidharth, **K Nithin** and **Ajay Deshmukh**

Abstract

Blood serum samples of 57 Indian sloth bears were analyzed to determine the C-reactive Protein (CRP). CRP level is a best biomarker for inflammation and infection. The study was conducted to determine the CRP level difference based on sex, age, health status and seasonal variation of Indian sloth bear. The sloth bear population now confined to small isolated regions of Indian sub-continent due to expanding human habitat and agricultural demand (Garshelis et al. 1999). There number has been reduced by 30–49% in last 3 decades in wildlife (Garshelis et al., 1999). During their habitation they were prone to some infection and inflammation. That is measured by CRP profile which is an acute-phase protein synthesized by the liver (Welinder et al., 2016). The blood samples were collected from both free ranging wild sloth bear and sloth bears at the Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park,

Bannerghatta, Bangalore, Karnataka, India. The blood samples were collected from different age group [age group I (1-6 years), age group II (7-12 years), age group III (13-18 years), age group IV (19-24 years)], sex (male and female), and seasonal variation (winter, summer, rainy and spring) of these sloth bears in 8-9 different months all around the year at different health status (healthy and sick) for analysis. During the blood collection the bears were anaesthetized by using a ketamine-xylazine combination, ketamine hydrochloride (5 mg/kg body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg/kg body weight; Troy Laboratories Pty Ltd.). These drugs were administered targeting each animal through a distance projectile drug delivery system. Using a 20-gauge sterile hypodermic needle in Vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA), blood was collected from the jugular vein within 10 min after immobilization along with heparin vial. Samples were immediately kept on a ice cool packs at 4˚C and transported to the testing laboratory. From each animal samples were collected once for this particular study time period. All processing of data was carried out by using the software packages i.e., Microsoft Excel 2010 for data storage and SPSS version 22 for statistical analysis. The P-values $P<0.05$ with an

alpha level of 95% were assumed as statistically significant (*). The standardised mean CRP concentration of sloth bear was found to be as 0.136 ± 0.055 mg/L. The male and female CRP level ranges from 0.03-0.21 mg/L and 0.04-0.22 mg/L respectively with no significance difference. The mean CRP (mg/L) concentration of healthy (.137±0.057 mg/L) and sick (0.134±0.052 mg/L) individual was calculated to be almost same with no significance differences. The range of mean CRP concentration of age group I, age group II, age group III and age group IV were demonstrated to be 0.06-0.21 mg/L, 0.03-0.21 mg/L, 0.04-0.22 mg/L and 0.07-0.21 mg/L respectively. The mean CRP (mg/L) concentration based on season was illustrated to be significantly ($P<0.05$) different between seasonal group I (Winter- Dec to Feb), seasonal group II (Summer- Mar to May), seasonal group III (Rainy- Jun to Aug) and seasonal group IV (Spring- Sep to Nov) with mean value as 0.113±0.061 mg/L, 0.164±0.046 mg/L, 0.158±0.044 mg/L and 0.126±0.056 mg/L respectively. These sloth bear had highest CRP concentration during summer season followed by rainy and spring season, and lowest in winter. The increased immune response in summer than winter in response to infection and inflammation may be due to highest secretion of testosterone in summer than winter. The highest testosterone level is responsible for activation of sex hormone binding

globulin (SHBG) receptor by increasing the cAMP production which result into further activation of protein kinase A towards immunoregulation activity against infection and inflammation independent of nutritional status and social activity. So, further detail study on this aspect is important to observe how SHBG is responsible for immunoregulation.

Art - 204. PREY SELECTION AND DIET COMPOSITION OF WILD LEOPARD *(Panthera pardus)* IN ARASIKERE, KARNATAKA, INDIA

Yogaraj, Swaminathan, Reegan, Ajay Deshmukh and **Arun A Sha**

Abstract

The present study focused on the Arasikere, which is famous for its faunal diversity, especially the leopards and sloth bears. The study was conducted in Arasikere, a small, isolated, prey-poor protected area abutting Hassan district. The food habits of the leopard *(Panthera pardus)* were studied by scat analysis between February to September in the year 2016. These leopards feed on a variety of wild and domestic prey animals. However, the data regarding their diet composition is scanty. Two methods of analysis were used in quantifying the leopard's diet, percentage frequency of occurrence of prey hair or claws in the scats, and estimation of standard error taken by the leopard using a regression equation. Scat samples were collected for seven months, from 5 transects that were 3 km long across the footpath approximately once a month which resulted in a collection of a total of 54 scat analysis revealed that the wild animals (45.00%) was their

preferred diet, and domestic species including with dog and livestock were more frequently consumed (52.00%) while Sambar deer (23.70 %) and Goat (21.30 %) was most commonly used as diet during seasonal variations. Other species include porcupine, wild boar, hare, monkeys, squirrel, peafowl, spurfowl and mongoose, sheep, goat, cow, domestic dog, and some unknown species. Furthermore, habitat analysis, questionnaire, human-carnivore conflicts and season with innerves for cattle watchers were also assessed.

Keywords: Arasikere, Leopard, scat analysis, livestock, prey, conflict.

Art - 205. ELEPHANT ENRICHMENT: WHAT, HOW AND WHY?

Yaduraj Khadpekar, Pramod Rajput, Rahul Prasad, Ilayaraj Selvaraj, Arun A Sha, Swaminath Chaurasia, Naresh Kumar, Ajay Deshmukh, Kartick Satyanarayan, Geeta Seshamani.

Abstract

Asiatic elephants *(Elephas maximus)* are probably the most popular attraction for visitors to the zoos in India and abroad. Many zoos in India housed one or more Asiatic elephants. However, elephants in captive situations tends to develop abnormal behaviours due to psychological stress or boredom that are not observed in wild counterparts. These behaviours are called stereotypic behaviours and may lead to health problems. In such elephants its always a challenge for veterinarians, elephant keepers and captive elephant managers to reduce these stereotypic behaviors in the elephant under their charge. The enrichments are aimed at encouraging the natural behaviours of the elephants in the available space. They should be designed so as to give the optimum psychological and physical stimuli and exercise to elephants, which generally lack in the captive environment.

Some simple additions or modifications in the enclosures of the elephants may very efficiently provide these enrichments. Elephant Conservation and Care Centre (ECCC) at Mathura, Uttar Pradesh was established by Wildlife SOS under the guidance of Uttar Pradesh Forest Department in 2010 to rescue and rehabilitate captive elephants that were being kept illegally and or being abused by their owners. The elephants that come at ECCC have gone through chronic physical and psychological stress for years and thus exhibit severe stereotypic behaviors. The team at ECCC puts in their best efforts to design and install various types of enrichments for these elephants which successfully reduce their stereotypies. This presentation discusses the enrichments that are being used for elephants at ECCC and now they are helping the elephants.

Art - 206. ENTANGLED JAW TRAP INJURY AND IT'S SURGICAL MANAGEMENT IN INDIAN STRIPPED HYENA

(Hyaena hyaena)

Ilayaraja S, Acharya P R, Baiju Raj M V., Arun A Sha,

Abstract

The Indian Stripped hyena *(Hyaena hyaena)* is a commonly occurring species of hyena in Indian subcontinent. It is near threatened large carnivore having wider distribution range than other hyenas and they are predominantly scavengers. Stripped hyenas are cave animals and possess typical nocturnal habits. As it only emerges in complete darkness and is quick to return to its lair before sunrise. Since attack on humans rarely occur still interaction between them is very frequent. Hyenas either get severely injured or killed by direct interaction with humans for defending themselves or indirect interaction with man made structures which accidentally or intentionally leads to cause of fatal injury or death. It has been a major emerging conflict between the striped hyena and humans, compare to the other wild animals in Uttar Pradesh. The significant reason would be that of hyenas having their feeding focusing towards human lifestyle and mainly their children at some instants. This increases the hunting habits of

humans towards the stripped hyenas, beyond the reason of meat. Since January 2018 to till date the Wildlife SOS rescue team was rescued three stripped hyenas with entangled jaw traps from different part of Agra as a part human animal conflict mitigation. Surgical intervention was required for two cases and rest was managed successfully without any surgery. Out of these three, two animals were treated and released back into wild and remaining one though it fully recovered from the jaw trap injury; kept under observation due to blindness because of massive hit on the head by the villagers at the time of conflict. The surgical management and post-operative treatment care is discussed in this article.

Keywords: Stripped hyena, jaw trap, human animal conflict.

Art - 207. AN ATTEMPT TO STANDARDIZE THE PRELIMINARY MILK COMPOSITION IN SLOTH BEARS

(*Melursus ursinus*)

Dr. Arun A Sha, Dr. Ilayaraja, Dr. Nithin K, and **Dr. Adhithyan NK.**

Abstract

Milk composition of Ursidae is one of the least explored areas in wildlife physiology. There have been few studies on milk composition and lactation pattern in American black, American brown and Polar bears but there is a shortage of information regarding the same in Sloth bears. Anatomically, Sloth bears has three pairs of mammary glands, and treats are similar to that of canine. The quantity and quality of milk vary according to the face of lactation, diet of the mother in prolactin levels. The peak lactation period of sloth bear is about 180 days wherein the cub stays with mother for over a period of 2-3 years and continues to nurture the bear even after one year of age. The present study attempted to determine the composition of milk from 9 lactating sloth bears under semi captive environment housed at bear rescue and rehabilitation centre of Wildlife SOS. As a part of general health examination, the lactating Sloth bears' milk was

collected aseptically in sterile vials by massaging the teats after immobilization. The milk was analysed using indiFOSS Milkoscreen™. The result showed that milk of Sloth bears contained an average of 9.76% of fat, 11.57% SNF and 8.5 % Protein. The understanding about the composition of milk of sloth bear is essential for hand rearing bear cubs and providing a better nutrition plane to orphan wild rescued cubs.

Keywords: Sloth bear, milk composition, lactation

Art - 208. COMPARISON OF GUT MICROBIOME COMPOSITION BETWEEN FREE RANGING WILD AND CAPTIVE SLOTH BEAR *(Melursus ursinus)* AND FROM SCAT SAMPLES BY USING BOTH CULTURE DEPENDENT AND INDEPENDENT BIOTECHNOLOGICAL TOOLS

Dr. Arun A Sha, Dr. Lyju Jose, Dr. Sejian V, Raghavendra Bhatta

Abstract

The present study presents the comparison of gut microbiota composition in sloth bear in their wild and captive state. The prime aim of this study was to characterize and compare the faecal bacterial community composition and abundance of the microbe in the wild as well as captive sloth bears. Since the gut microbes play essential role in provision and conservation of nutrients available to the animals, the following sections are concentrating only on the analysis of gut microbes composition in the wild and captive animals. The nutritional contributions of gut microbes depend on the diet, habitat and other environmental factors. Thus any variations in food or habitat can result in differential diversity in gut microbes leading to an imbalanced production of nutrients. While this relationship has been explored much

among domestic animals, it has not been explored among wild animals. Due to the lack of economic interest and difficulties in obtaining samples. In this study, we have used metagenome- based approach. To examine the complexity of microbial community and their interactions within the niche. The collected data will be used to understand the wildlife conservation and management of captive animals. The importance of this study holds because any change in gut microbiota composition in the wild and captive animals could be an essential resource to implement measures for the protection and maintenance of these species in a captive state.

Art - 209. OPTIMIZING A CAGE DESIGN FOR LEOPARDS DURING WET-WELL RESCUE OPERATIONS IN HUMAN DOMINATED LANDSCAPE

B. Deshmukh, Arun A. Sha, B. S. Baviskar, P.B. Baviskar**,** Y.R. Khadpekar and V. D. Khose

Abstract

Rescue operation of Leopard *(Panthera pardus)* from a wet well presents a great challenge every time however it is more challenging in human-dominated landscape where any small mistake can turn into mishap. Also, there is always uncontrolled mob around the well where escape of the distressed animal during the rescue operation can cause havoc. Particularly in the wet-well rescue operation the animal cannot be chemically immobilized due to chances of drowning of the animal. Therefore it was obligatory to design the rescue cage with minimal weight and automatic locking door system where animal cannot escape after entering the rescue cage during the operation. Keeping all these things and previous experiences in mind, a four feet long and 3 feet wide fiber cage weighing 120 kg was designed. The height of the rescue cage was kept 3.7 feet with ventilation on the one fixed side of the cage.

Automatic locking door system was provided to prevent the opening of the door once the animal enters the cage. Additionally, 1.5 inches space has been provided on the side where the door opens to avoid crushing of the tail after the animal entered into the cage. Side rings were provided to lift the cage easily without approaching the cage from close distance.

This cage has been utilized for more than 70 leopard rescue operations from the wet well from the human dominated landscape in Western part of Maharashtra and hence proves helpful during the wet-well rescue operation in human-dominated landscape.

Art - 210. CAPACITY BUILDING OF FRONTLINE STAFF OF FOREST DEPARTMENT FOR 'WILDLIFE INVESTIGATION THROUGH FORENSIC TECHNIQUES'

B.S. Baviskar, P.B. Baviskar**, A. B. Deshmukh** and
A. B. Shrivastava

Abstract

Wildlife forensic is an emerging branch which requires the immediate attention from the conservation point of view. Forensic techniques help in the analysis of the samples/evidences collected during the investigation and proves helpful in legal matters. Wildlife investigation for research and legal matters is gaining momentum all over India. In Central India, there is an urgent need of strengthening the frontline staff for practicing all the techniques including sample collection, sample processing, investigation of the crime scene for supportive evidences, labeling of the samples, transportation of the sample using appropriate preservatives and all other related techniques.

Considering the need of the frontline staff, the module was designed by Wild-CER in such a way that it introduces field staff to the forensic science and its significance in the investigation process with practical demonstration. Educating

the frontline staff of forest department and field veterinarians about the significance of wildlife forensic techniques and about practical implementation of the techniques during research, crime investigation and postmortem are the main objectives of this training program.

In all, four training programs on 'Wildlife investigation through forensic techniques' were conducted in Central India including at Navegaon-Nagzira Tiger Reserve and Brahmapuri division, Maharashtra. We allowed the participants to assess their knowledge level by filling up the pre-training evaluation form and post training evaluation form for the designed module. The comparison of the individual scores pre training and post training was done. Also, scores for different modules pre-training and post-training were collected and analyzed.

It was evidently found that training like 'Wildlife investigation through forensic techniques' increases the knowledge level of the field staff significantly and needs to be conducted on regular basis.

Art - 211. ESTROGEN PROFILES AND RELATED BEHAVIOUR DURING BREEDING SEASON IN FEMALE SLOTH BEARS.

Yaduraj Khadpekar, Barbara Durrant, John Whiteman, Megan Owen, Srinu Maharana, Sant Prakash

Abstract

Sloth bear (*Melursus ursinus*) is a common wildlife species housed in the zoos in India as well as abroad. It is one of the four Ursids found in India. Inspite of being common in zoos and having the widest distribution among all Indian bear species, there is a severe lack of scientific knowledge about the basic biology of sloth bears. Understanding of their reproductive physiology and behaviour is lacking and the scant data available are from the anecdotal observations of the wild populations.

A research project was initiated at the Agra Bear Rescue Facility in Uttar Pradesh in 2015 to study the reproductive endocrinology and related behaviour in sloth bears using noninvasive approach. Urine samples were collected non-invasively from 37 captive female sloth bears over a period of three years covering three breeding seasons. Samples were collected daily during April to July and twice a week for rest of the year.

Data related to changes in vulva visibility and behavioural changes were also collected through keeper feedback check sheet, continuously throughout the study period. Estradiol levels in the urine samples from the breeding seasons were quantified using enzyme immunoassays. The behaviour and vulva visibility data collected were correlated with the urinary estradiol levels.

Preliminary results showed that the changes in vulva visibility overlap and correlate well with the increased estradiol levels. The increasing estradiol levels appear to have triggering effects on the reproductive behaviour rather than the maintenance effect. This is for the first time reproductive behaviour and its temporal correlation with estradiol levels is being described in sloth bears.

Art - 212. SURGICAL IMPLANTATION OF RADIO TRANSMITTERS IN RUSSELL'S VIPERS AND KING COBRAS

Shantanu Kalambari, **Arun A. Sha,** Xavier Glaudas and Gerald Martin

Objectives

The surgical implantation of radiotransmitters in Russell's Vipers (*Doboia russelii*) and King Cobras (*Ophoiophahis Hannah*) for radiotelemetry studies.

Animals

The surgical technique was conducted to one adult male King Cobra and 6 adult Russell's Vipers, 4 males and 2 females.

Methods

Following immobilization and sedation with Isoflurane gas, an incision was made on the line of the 2nd row of the dorsal scales 2/3rds of the length of the body anterior to the gonads. The incision was made through the skin and underlying inferior costo cutaneous and lateral *squamoscutali* muscles. An incision was then made through the ventral abdominal muscles and transverse abdominal muscles, ventral to the

costal cartilages.

The radio transmitter was then interested through the incision into the peritoneal cavity. A sterilized artificial insemination tube/thin material tube was then pushed under the skin anterior to the incision along the length of the body in the line of second dorsal row of the scales, and the small nick was made at the point at the length of the transmitter antennae. The antenna was pushed through the tube and the tube was then gently withdrawn through the cut in the skin, leaving the antennae positioned along the length of the snake's body. The peritoneal cavity was flushed with metronidazole iv fluid. A single stay suture was placed around the head of the GPS transmitter, attaching it to the peritoneal surface through the intercostal muscles. 4 to 5 samples interrupted sutures were used to close the incision in the abdominal muscles using 2 - 0 vicryl and the skin was sutured using nano absorbent sutures. The wound was then closed around the sutures using sterile cyanoacrylate glue. A single suture was used to close the cut from which the antenna tube was withdrawn which was also sealed using cyanoacrylate glue. The snakes were then kept under observation for 24 hours, following which they were released back into the wild.

Results

No adverse behaviour was observed in any of the snakes

following the surgeries. The antenna was found to have protruded through the skin of the King Cobra due to an external injury on the snake, hypothesized to be due a bite from a carnivore's mammal. The individual was recaptured and the GPS transmitter was reimplanted following which no further problems were observed.

Conclusion

Surgeries were performed over the last year, following which all of the snakes were kept under observation and followed by biologists and volunteers. The method of implantation was not associated with major complications. No changes or aberrations in feeding, or foraging behaviour. In one case of a female Russell's viper under study the individual was found to have successfully mated and reproduced within a few months of the surgery.

Art - 213. CHEMICAL IMMOBILIZATION OF PLAINS ZEBRA *(Equus borchelli)* USING DIFFERENT CHEMICAL IMMOBILIZING DRUG AND ITS EVALUATION; A CASE STUDY.

Dr. Manjunatha V., Nirupama Jaisingh, Dr. Umashankar, K S, **Arun A Sha,** Dr. Kshama L M, Dr. Sripad K, Dr. Byregowda S M.

Abstract

Zebras belonging to the Equidae family, there are three different species of Zebras seen around the world, they are plains Zebra *(Equus borchelli)*, Mountain Zebra (*Equus zebra*) and Grevy's Zebra *(Equus grevyi*). These Zebras are found naturally only in the African component. The Plains Zebra occur widely in Eastern and Southern Africa, with more than half a million animals in the Serengeti Masai Mara ecosystem of Tanzania and Kenya. The other two species are found in small numbers in Kenya, Ethiopia, Namibia and South Africa. Zebras are commonly used in game reserves, zoo exhibitions and safari parks in several parts of the world including India. Zebras attract many visitors in zoo. Bannerghatta Biological Park received 4 Plain Zebras from Zoological center Tel AVIV – Ramat Gan Safari Israel. Since the animals were imported they were kept under the quarantine office Bangalore,

animals were kept in Bannerghatta Biological Park quarantine facility for 2 months, advised to collect the blood samples, serum samples and preputial swabs and vaginal swabs for screening of various equine diseases, our study is to immobilization of the Zebras for the collection of these samples for every 7 days for 4 times. Many chemical anesthetics available for the immobilization and used in captive and free ranging/wild zebras, we used Etorphine hydrochloride 9.8 mg/ml (M99), 4.8mg Etorphine hydrochloride combined with Acepromazine hydrochloride, Xylazine 100mg/ml and Ketamine 100mg/ml for the immobilization of the Zebras in different combinations. In this 3 combinations we found that Xylazine and Ketamine combination is not suitable for immobilization of zebras, the Etorphine hydrochloride 9.8mg/ml (M99), 4.8mg Etorphine hydrochloride combined with Acepromazine hydrochloride can be used safely, the induction is very smooth within 1 to 2 min and smooth reviving in 1 to 2 min using Diprenorphine (Revivion).

Art - 214. REUNION OF RUSTY SPOTTED CATS *(Prionailurus rubiginosus)* FROM THE HUMAN DOMINATED AGRICULTURAL LANDSCAPE IN WESTERN MAHARASHTRA, INDIA

Ajay B. Deshmukh, Bahar S. Baviskar, and Priya B. Baviskar

Abstract

The Rusty-spotted Cat *(Prionailurus rubiginosus)* is the smallest wild cat species that occurs only in India and Sri Lanka. Although it is classified as near threatened species by IUCN red list, its population dynamics, density, reproductive behavior is poorly documented.

In the span of 5 years from 2014-2019, 26 kittens (15 male and 11 female) were rescued from 18 incidences from the human dominated agricultural landscape of the different villages from Pune district, Maharashtra, India. The rescued kitten was physically and clinically examined before release.

The body weight of the rescued kitten ranged from 0.02 - 0.07 kg with an average of 0.54 kg. Rectal temperature was recorded in all incidences which ranged from 100-1020F with average 101.10F. The respiratory rate ranged from 16-40 per minute with an average of 30 per minute, while heart rate ranged from 130-180 with an average 155. As all of them

were found to be healthy and fit for release, they were released on the same day. The reunion was confirmed on the basis of direct sighting of mother taking kitten away or from the pugmarks of both mother and kitten.

All the rescue and reunion operations of Rusty-spotted Cats were conducted in the sugarcane field, particularly at the time of sugarcane harvest. The agriculture field has high density of rodents, and this may be the reason that Rusty spotted cat prefer such landscape for breeding throughout the year.

Conservation planning for Rusty spotted cat must include its ability to adapt to the human dominated agricultural landscape in the near future.

Keywords: Rusty spotted cat, Reunion, Sugarcane field, Human dominated the landscape, Western Maharashtra

Art - 215. SITUATIONAL ADAPTATION OF ANESTHETICS DOSAGE FOR VARIOUS LEOPARD RESCUE OPERATIONS IN HUMAN DOMINATED LANDSCAPE, WESTERN MAHARASHTRA, INDIA – A DECADE REVIEW

A.B. Deshmukh, Arun. A. Sha, B.S. Baviskar and P.B. Baviskar

Abstract

Habitat fragmentation and intensifying anthropogenic pressure is causing regular man-animal conflict situations with leopards in Western Maharashtra, India. Chemical immobilization in different emergency situations has found to be a practical challenge for any wildlife veterinarian in the field. The wildlife veterinarian should opt for the safest method of drug delivery system and an aesthetic dose for both the animals and human. The authors present the different combination of the anesthetics drugs used during the forty eight leopard rescued during the period from 2009 to 2019.In all operations we used the Xylazine (X) and Ketamine (K) as the anesthetic drugs which is very commonly accessible and available in India. Out of forty eight wild leopards rescued, Twelve leopards were rescued from dry well using 1:4 (X:K), Thirteen leopards were rescued from house/school using 1:5(X:K), Twelve Leopards were rescued from Jaw Trap or

Snare Trap using 2:5 (X:K)and Eleven Leopards were rescued from village or sugarcane field using 2:6(X:K) ratio. This also included free ranging leopards in human dominated landscape with possibilities of causing man- animal conflict situation. All the animals showed smooth induction and safe recovery during the entire operation. It was evident from this study that different combination of Xylazine and Ketamine proved precise without any side effects or complications. No anesthetic emergency or causalities were observed during the chemical immobilization. These reports illustrate the practical significance of using different dose combination of the anesthetics depending on the situation in the study location.

Keywords: Xylazine, Ketamine, Leopard, Conflict, Chemical Immobilization

Art - 216. FORMULATING STRATEGIES FOR HANDLING NEGATIVE INTERACTIONS INVOLVING LARGE CATS IN CENTRAL INDIA

Bahar S. Baviskar, Priya B. Baviskar, **Ajay B. Deshmukh**

Abstract

In Central India, loss of habitat, habitat fragmentation resulting into disturbed corridors and tremendous anthropogenic pressure on the forest cover is resulting in more frequent negative interactions of large cats with human being. High occurrence of these negative interactions is changing the attitude of the people towards large cat conservation resulting in confrontation and conflict with the authority. Confronting conflict situation on regular basis necessitates formulation of different strategies to deal the negative interactions involving large felids.

Taking into consideration the long term planning for conservation of large cats, we formulated few strategies which involves Capacity building of frontline staff for conducting rescue operations, investigation of death involving large felids using forensics and molecular techniques, training veterinarians for chemical immobilization, deploying well equipped team and support team to handle the situation,

conducting immunization drives for cattle, domestic and feral dogs throughout the year in buffer villages of tiger reserves, conducting education and awareness programs in the villages in and around the protected areas etc. Involvement of local communities and all stakeholders in conservation planning will always facilitate long term conservation of large cats in Central India.

Art - 217. HISTOLOGICAL AND HISTOCHEMICAL DETAILS OF EXTRAOCULAR MUSCLES OF SLOTH BEAR (*Melursus ursinus*)

N.M. Rajashailesha, R.V. Prasad, **Arun. A. Sha**, K.V. Jamuna, M.L. Satyanarayana, S. Ganga Naik and P. Ravi Kumar

Abstract

The eye ball with intact extraocular muscles of six adult sloth bears were collected during post mortem examination of bears that had died due to natural causes at the Wildlife Save Our Soul (WSOS), Bannerghatta Bear Rescue facility Centre (BBRC), Bengaluru and were utilized for the present study. Individual muscles were fixed in 10% neutral buffered formalin and processed for various histological and histochemical stains. The EOMs were oriented unidirectional. Each muscle fiber was separated by endomysium with large number of blood vessels and few nerve fibers. Between the fascicle nerve fiber bundles and capillaries were also seen. In cross section muscle fibers appeared oval/round/irregular/polygonal with peripherally located nuclei with prominent nucleolus. Around the muscle fibers reticular fiber network was seen, which was continuous with perimysium. Based on the diameter, muscle fibers can be

divided into large, medium, and small. Large muscle fibers shave homogenous cytoplasm, which were few in number can be considered as slow twitch while, medium and smaller ones have granular cytoplasm are considered to be fast twitch. The medium and small muscle fibers were irregular in shape. In the present study all EOMs showed strong PAS positive activity uniformly suggested that the higher concentrations of glycogen in tissues account in providing energy. Strong PAS positive reaction observed around the individual muscle fiber indicating more of mucopolysaccharide in the connective tissue. All EOMs were negative for acidic and sulfated mucopolysaccharide. Based on these morphological feature's animal can be classified having fast twitch ocular muscle.

Art - 218. BASIC ANATOMICAL STUDIES OF THE PELVIC LIMB IN AN ASIAN ELEPHANT (*Elephas maximus*)

Arun. A. Sha, **Nithin**, M H. Girish, K. Manjunathan, M. L. Bharat Kumar & K. V. Jamuna

Abstract

Asian elephants not only have cultural and ecological significant in Indian history and heritage but are crucial element of the wild ecosystem. In spite of their ancient lineage, studies related to their anatomy are limited, and research is restrictive due to religious constrains and stringent wildlife protection loss. The objective of the present study was to understand the anatomical features of the pelvic limb specimen obtained from a adult wild tusker elephant scumbled due to orthopedic problem. Based on the permission issued from Chief Wildlife Warden, Karnataka, the pelvic limb was secured from Magadi Forest Division. The specimen was carefully preserved immediately with ice bars and subsequently, 230 liters of 10% formalin saline was injected into the hind limb through the saphenous and femoral artery. Radiographic examination of the limb was performed, and radiographic parameters were standardized. Skin separation of the hind limb was done to reveal the musculature. Average skin thickness was measured and

appropriate needle size and length to be used in intramuscular injection procedures were determined. A total of nine major muscles of the pelvic limb, glutes, gluteus maximus, rectus femoris, biceps femoris, semitendinosus, soleus, popliteus, vastus intermedius, vastus medius and gastrocnemius were dissected and weighed. The origin and insertion of the individual muscles were subjected to detailed anatomical exploration. The osteometric parameters of the long bones were determined to ascertain their length and girth. The findings of the study are limited owing to the various challenges faced in terms of samples size, field radiography, restricted access by vets and dearth of research resources. The study highlights the importance of anatomical studies in wild animals for improving the quality of veterinary service in the wildlife sector for field operation.

Art - 219. OSTEOLOGY OF THE SKULL BONE OF ADULT SLOTH BEARS (*Melursus ursinus*) AND ITS CLINICAL IMPORTANCE IN DENTAL INTERVENTIONS

Arun A. Sha, Ilayaraja S., Prajakta, Vineet Kumar Pal, Adhithyan and **Riya Bakde**

Abstract

The sloth bear (*Melursus ursinus*) is an insectivore bear species native to Indian subcontinent. The sloth bear evolved from ancestor brown bear and shares features found in insect-eating mammals. The sloth bear lag the central maxillary incisors except in new born cubs of two to three months old, irrespective of the gender. Dancing sloth bears rescued from kalandars show more dental issues like carries, tooth decay, cracked or broken teeth, gingivitis due to their inhumane act of breaking down the canines for their safety reasons. Since there is a lack of information on anatomical measurements of the skulls of sloth bears and its clinical value during regional anesthesia, the present work was taken. The total of six skulls were used to make the morphometric indices. The skull length, cranial length, nasal length and cranial width were 27.5 cm, 20.6 cm, 5cm and 13.2 cm respectively. Also, the cranial index was 64.07. In addition, the

distance from the route of premolar tooth to infra-orbital canal and from the latter to the route of the canine tooth were 4.5cm and 5cm, respectively. The length and height of the mandibles were 20.2 cm and 11.3 cm, respectively. Furthermore, the distance from the lateral alveolar route to mental foramen and from the mental foramen to caudal mandibular border were 4.8cm and 15.4 cm, respectively. In the current work, the distance from mandibular foramen to the base of mandible as well as from caudal border of mandible to ventral to the mandibular foramen were 3.5cm and 4.5 cm, respectively. Also, the distance from the base of mandible to condyloid fossa and from the latter to the maximum height of the mandible were 6.2cm and 5.2cm respectively. Finally, the distance from caudal border of the mandible to the mandibular foramen and from the latter to mandibular angle and 10.9 cm and 2.3 cm, respectively. These data, as essential landmarks are discussed with regard to their application to clinical maneuvers around the head of the adult sloth bears such as local or regional anesthesia like nerve blocks during treating head injury, extraction and endodontic dental treatments.

IV b) LIST OF AUTHORS AND THEIR AFFILIATIONS

Author's Name	Affiliation
Abhishek Kumar Singh	Wildlife Veterinary Officer, Wildlife SOS, Gurgaon. India
Acharya P. R	Wildlife Veterinary Officer, Wildlife SOS, Agra, India
Adhithyan N. K.	Wildlife Veterinary Officer, Wildlife SOS, Bangalore
Afreen Fathima J.	Research Scholar, Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai
Ajay Deshmukh	Wildlife Veterinary Officer Manikdoh Leopard Rescue Centre, Wildlife SOS, Maharashtra, India
Akshaya Kundu	Assistant Professor, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Bhubaneshwar, OUAT, Odisha
Anitha M.M.	Research Scholar, Institute of Wildlife Veterinary Research, Doddaluvara, Kodagu Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries, Sciences University, Bidar, India
Anjali Verma	Research Assistant, IISC, Centre for Ecological Science, Bangalore
Arpitha G. M.	Research Scholar, Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai
Arun A. Sha	Director - Research and Veterinary Operations, Wildlife SOS, Bangalore
Arunmozhivarman K.	Scientist, Translational Research Platform for Veterinary Biologicals (ADBT-TANUVAS Partnership Programme), Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
Arzoo Malik	Research Assistant, Wildlife and Conservation Biology Research Lab HNG University, Patan (Gujarat) India
Bagath M.	Scientist, ICAR - National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore
Bahar S. Baviskar	Executive Director, Wild-CER (Society for Wildlife Conservation, Education and Research), Nagpur
Baviskar P.B.	Assistant Professor, Department of Veterinary Parasitology, Nagpur Veterinary College, Nagpur, Maharashtra.
Baiju R.	Director of Conservation Projects, Wildlife SOS, India
Barbara Durrant	Director of Reproductive Sciences, San Diego Zoo Global, Escondido, CA, USA
Bharat Kumar M. L.	Research scholar, Dept of Veterinary Anatomy, Veterinary College, Hebbal, Bangalore, Karnataka.

Bhaskaran Ravi Latha	Professor and Head, Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, TN, India
Bhatta R.	Director, ICAR - National Institute of Animal Nutrition and Physiology, Adugodi, Hosur Road, Bangalore
Byregowda S.M.	Director, Institute of Animal Health and Veterinary Biologicals, Hebbal, Bengaluru, Karnataka, India.
Chandra Mohan S.	Scientist, Centre for wildlife conservation, Management and Disease Surveillance
Chitra Karuppannan	Scientist, Translational Research Platform for Veterinary Biologicals (A DBT-TANUVAS Partnership Programme), Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
Dave Garshelis	Co-Chair, Bear Specialist Group Minnesota Department of Natural Resources Grand Rapids, USA
Devaraj C.	Scientist, ICAR - National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore
Ganga Naik S.	Associate Professor, Dept of Veterinary Anatomy, Veterinary College, Hebbal, Bangalore, Karnataka.
Geeta Seshamani	Co-Founder, Wildlife SOS, India
Gerald Martin	Founder, Gerry Martin Project, Karnataka.
Girish M H.	Faculty, Dept of Anatomy, Bangalore Veterinary College.
Gochalan Elango	Junior Veterinary Officer, Wildlife SOS, New Delhi, India
Gomathinayagam S.	Professor and Head, Madras Veterinary College, Chennai, Tamil Nadu
Gopal Dhinakar Raj	Director, Translational Research Platform for Veterinary Biologicals (A DBT-TANUVAS Partnership Programme), Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
Govind S.	Wildlife Veterinary Officer, Wildlife SOS, BBRC, Bengaluru, Karnataka, India.
Gowda N K S	Principal Scientist, ICAR – National Institute of Animal Nutrition and Physiology, Adugodi, Hosur Road, Bangalore, Karnataka.
Harikrishnan C. P	Wildlife Veterinary Officer, Wildlife SOS, Bangalore, Karnataka
Ilayaraja S.	Deputy Director Veterinary Services, Wildlife SOS
Jamuna K V.	Department of Veterinary Anatomy, Veterinary College, Hebbal, Bangalore, Karnataka.
John P. Whiteman	Assistant Professor, Institute of Conservation Research, San Diego Zoo Global, San Diego, USA
Jayathangaraj M. G.	Director, Department of Veterinary Clinical Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Kamalanathan M.	Wildlife Veterinary Officer, Wildlife SOS, Bangalore
Karikalan M.	Scientist, Indian Veterinary Research Institute
Karthik Bhat S.	Senior Research Fellow, ICAR – National Institute of Animal Nutrition and Physiology, Adugodi, Hosur Road, Bangalore, Karnataka.
Kartick Satyanarayan	Co-Founder and CEO, Wildlife SOS, India
Khose V. D.	Innovative entrepreneur in Wildlife
Krishnan G.	Senior Scientist, ICAR -National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore
Kshama L M	Veterinary Officer, Bannerghatta Biological Park.
Laura Maillard	Vet Research Scholar,WSU CVM, Pullman, WA, USA
Lisa Milella	Veterinary Dentists, International Animal Rescue, United Kingdom
Lyju Jose	Research Scholar, Department of Biotechnology, Jain University, Bangalore, Karnataka, India
Mahendrajit Singh	Associate Professor, Department of Epidemiology & Public health, Central University of Tamilnadu.
Mahesh V.	Assistant Professor, Institute of Wildlife Veterinary Research, Doddaluvara, Kodagu Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries, Sciences University, Bidar, India
Mallepaddi Chand Prudhvi	Research Associate, Genomix Molecular Diagnostics (P) Ltd, Kukatpally, Hyderabad, AP, India
Manjunathan K.	Faculty, Dept of Anatomy, Bangalore Veterinary College.
Manjunath V.	Scientist I, Wild Animal Disease Diagnostic Laboratory, Institute of Animal Health and Veterinary Biologicals, Bannerghatta Biological Park, Bannerghatta.
Maroudam Veerasami	Scientist, Translational Research Platform for Veterinary Biologicals (A DBT-TANUVAS Partnership Programme), Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
Martin Vordermeier	Professor, Animal and Plant Health Agency (APHA), Addlestone, New Haw, Surrey, UK
M Ram Verma	Asst. Professor, Department of Epidemilogy &Public health, Central University of Tamilnadu, Thiruvarur
Megan Owen	Global Director, Institute for Conservation Research, San Diego Zoo Global, Escondido, USA
Michelle Mousel	Research Geneticist, USDA ARS ARDU, Pullman, WA, USA
Mohana Subramanian B.	Scientist, Translational Research Platform for Veterinary Biologicals (A DBT-TANUVAS Partnership Program), Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
Mukul CG	Wildlife Veterinary Officer, Wildlife SOS, Agra, India

Nagalingam R S.	Asst. Professor, Department of Microbiology and Cell Biology, IISC, Bangalore
Nandita Patel	Faculty, Wildlife and Conservation Biology Research Lab HNG University,Patan (Gujarat), India
Naresh Kumar	Project Co ordinator, Wildlife SOS, ECCC
Nilmani Rabha	Field Biologist, Wildlife Trust of India
Nirupama Jaisingh	Veterinary Officer, Bannerghatta Biological Park.
Nishith Dharaiya	Co-Chair, Sloth Bear Expert Team, Bear Specialist Group Hemchand Acharya North Gujarat University Patan (Gujarat) India
Nithin K.	Wildlife Veterinary Officer, Wildlife SOS, Bangalore
Palanivelrajan M.	Faculty, Department of Wildlife Science, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India
Pallab Chaudhuri	Principal Scientist, Department of Epidemilogy & Publlic health, Central University of Tamilnadu.
Partha Sarathi Swain	PhD, Animal Nutrition, National Dairy Research Institute, Bangalore
Paul Cassar	Dentist, International Animal Rescue, United Kingdom
Polavarapu Rathnagiri	President & CEO, Genomix Molecular Diagnostics (P) Ltd, Kukatpally, Hyderabad, AP, India
Porteen K.	Asst. Professor, Department of Veterinary Public health and epidemiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India
Pradeep R.	Wildlife Veterinary Officer, Wildlife SOS, New Delhi, India
Prajakta Bhole	Wildlife Veterinary Officer, Wildlife SOS, Agra
Pramod Rajput	Wildlife Veterinary Officer, Wildlife SOS, Mathura
Prasad R V	Dean, Department of Veterinary Anatomy, Veterinary College, Hebbal, Bangalore, Karnataka.
Praveen Karanth K.	Associate Professor, IISC, Centre for Ecological Science, Bangalore
Pushpendra K Sing	Junior Veterinary Officer, Wildlife SOS, India
Rajashailesha, N. M.	Assistant Professor, Department of Veterinary Anatomy, Veterinary College, Hebbal, Bangalore.
Rahul Prasad	Wildlife Veterinary Officer, Wildlife SOS, Mathura
Ranganath L.	Head of the Department, Department of Veterinary Surgery, Bangalore Veterinary College
Rashamol V P	Research Scholar, ICAR-National Institute of Animal Nutrition and Physiology, Academy of Climatic Change Education and Research, Kerala Agricultural University, Thrissur, Kerala.
Rashmi S. Gokhale	Wildlife Veterinary Officer, Wildlife SOS, Bangalore
Reegan	Wildlife Biologist, Wildlife SOS, Bangalore
Riya Bakde	Wildlife Veterinary Officer, Wildlife SOS, Bangalore

Rout M.	Scientist, ICAR-Directorate of Foot and Mouth Disease, IVRI Campus, Mukteswar, Nainital, Uttarakhand, India.
Sabarinath Thankappan	Scientist, Division of Bacteriology and Mycology, ICAR- Indian Veterinary Research Institute, Izatnagar UP
Sant Prakash	Prof & Head, Department of Zoology, Dayalbagh Educational Institute, Agra, UP, India
Satyanarayana M L.	Prof. & Head, Veterinary Pathology. Veterinary College, Hebbal, Bangalore, Karnataka.
Sejian V.	Senior Scientist, Animal Physiology Division, ICAR – National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore
Shankar B.P.	Institute of Wildlife Veterinary Research, Doddaluvara, Kodagu Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries, Sciences University, Bidar, India
Sharma A. K.	Principal Scientist, Centre for wildlife conservation, Management and disease surveillance
Sharma M. V.	Wildlife Veterinary Officer, Wildlife SOS, Agra
Shwetha H.S.	Assistant Professor, Institute of Wildlife Veterinary Research, Doddaluvara, Kodagu Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries, Sciences University, Bidar, India
Shantanu Kalambari	Wildlife Veterinarian, CUPA, Bangalore
Shrivastava A. B.	Head, Wildlife Health Division, Madhya Pradesh, Society for Wildlife Conservation, Education and Research, Nagpur, Maharashtra.
Sidharth Prasad Mishra	Research Scholar, Department of Animal Genetics and Breeding, WBUAFS, Kolkata.
Simone Ayoob	Wildlife Veterinary Officer, Wildlife SOS, Bangalore, India
Sneha Mishra	JRF, Department of Microbiology and Cell Biology, IISC, Bangalore
Sridevi P.	Professor, Department of Clinics, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai.
Srinivas R.B.	Institute of Wildlife Veterinary Research, Doddaluvara, Kodagu Veterinary College, Hebbal, Bengaluru, Karnataka Veterinary, Animal and Fisheries, Sciences University, Bidar, India
Srinu Maharana	Senior Research Associate, Wildlife SOS.
Suchitra Sahoo	Research scholar, Department of Veterinary physiology, College of veterinary Science and Animal husbandry, Bhubaneshwar, OUAT, Odisha
Sujith K. Behera	Asst. Professor, Department of Epidemiology & Public health, Central University of Tamilnadu, Thiruvarur

Swagat Mohapatra	Faculty, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Bhubaneshwar, OUAT, Odisha
Swaminath Chaurasia	Enrichment officer, Wildlife SOS, ECCC
Swaminathan S.	Senior Wildlife Biologist, Wildlife SOS, Bangalore
Syed Atif Ali	Research Scholar, Department of Epidemiology & Public health, Central University of Tamilnadu.
Thomas Holder	Researcher, Animal and Plant Health Agency (APHA), Addlestone, New Haw, Surrey, UK
Thomas Sharp	Director, Sloth Bear Expert Team, IUCN Bear Specialist Group, Wildlife SOS, Salt Lake City, Utah; United States.
Thulasi Appoothy	Senior Scientist, Rumen Microbiology Laboratory, Animal Nutrition Division, National Institute of Animal Nutrition and Physiology, Bangalore, India
Tista Joseph	Wildlife Veterinary Officer, Wildlife SOS, BBRC, Bengaluru, Karnataka, India.
Tiushar Jyotiranjan	Research Scholar, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Bhubaneshwar, OUAT, Odisha
Tom Smith	Associate Professor, Brigham Young University – Provo, Utah, United States
Umashankar	ADVS, Bannerghatta Biological Park.
Venkataraman K.	Associate Scientist, Translational Research Platform for Veterinary Biologicals (ADBT- TANUVAS Partnership Programme), Tamil Nadu Veterinary & Animal Sciences University, Chennai.
Vibha Raghuram	Wildlife Veterinary Officer, Wildlife SOS, Bangalore
Vijayarani K.	Faculty, Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India
Vineet Kumar Pal	Wildlife Veterinary Officer, Wildlife SOS, Agra
Xavier Glaudas	Biologist, Gerry Martin Project, Karnataka
Yaduraj Khadpekar	Senior Veterinary Officer, Wildlife SOS, Agra
Yogaraj P.	Wildlife Biologist, Wildlife SOS, Bangalore
Yosef Deneke	Associate Professor, Department of Epidemiology & Public health, Central University of Tamil Nadu, Thiruvarur

Abbreviations:

FLA: Full Length Article

ABT: Abstract

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Editor's Note:

If any of the articles which were published by any of the Wildlife SOS team during their working period with us, is found missing, please intimate the publication department of Wildlife SOS and it will be carried in the next volume.

WILDLIFE SOS

Phone: +91 1146601096
Web: www.wildlifesos.org
Mail: research@wildlifesos.org

Follow Us:
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