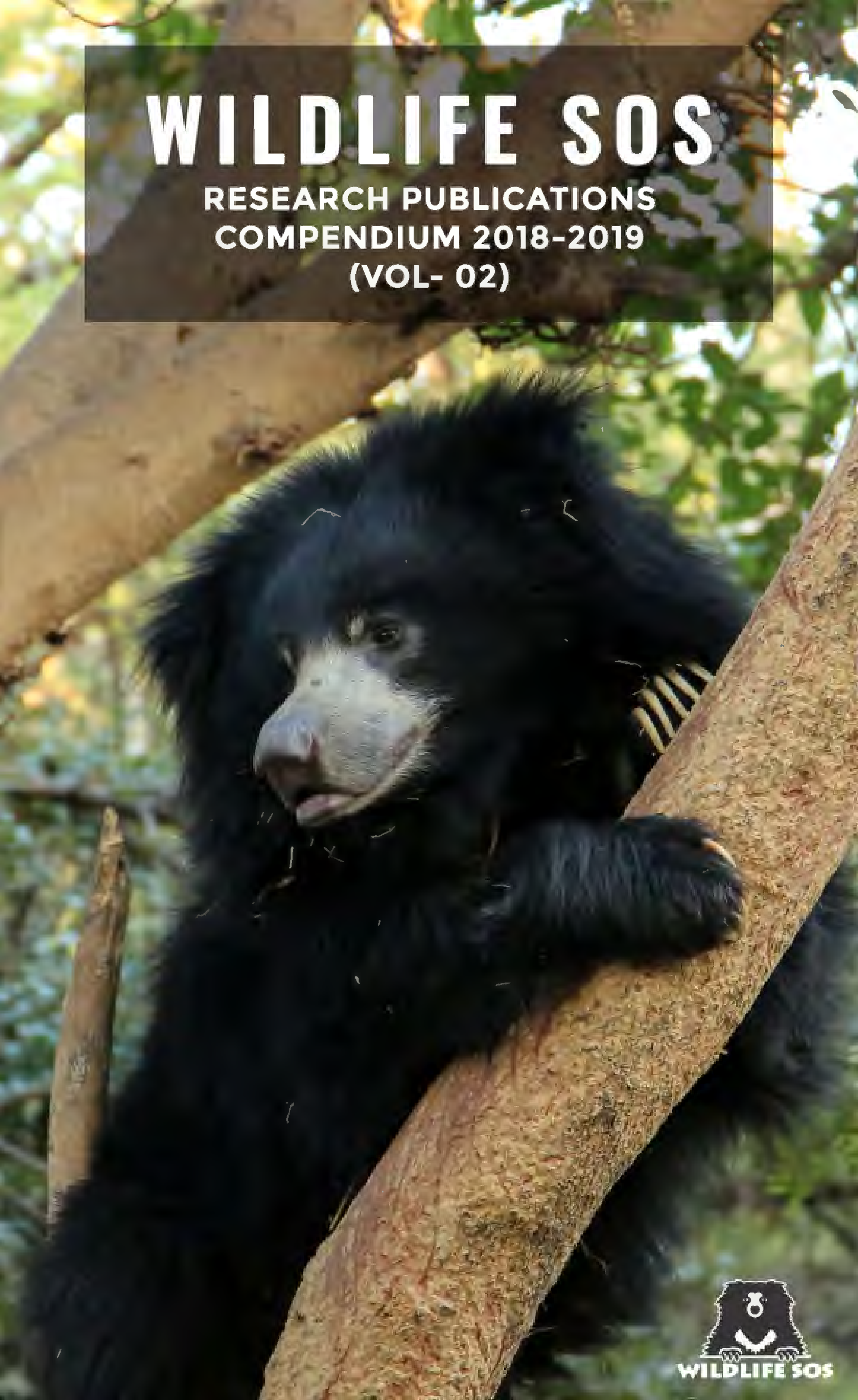
# WILDLIFE SOS

## RESEARCH PUBLICATIONS COMPENDIUM 2018-2019 (VOL- 02)



WILDLIFE SOS

**A COMPENDIUM OF SCIENTIFIC PUBLICATIONS OF WILDLIFE SOS**

**– TWO DECADE REVIEW**

**Volume - II**

An Official Publication of Wildlife SOS(R)



* **Refer Page No: 278**

# NOTE FROM THE CO-FOUNDERS

From 1995 to 2009, Wildlife SOS rescued 628 dancing bears from the streets of India-- bringing an end to a centuries-old tradition that inflicted terrible cruelty on thousands of highly endangered sloth bears. A tribe known as the Kalandars (originally Muslim gypsies with a highly nomadic lifestyle) began 'dancing' sloth bears for the emperors during the Mughal era. Over centuries, as the kingdoms in India disappeared, the 'dancing' bear trade transitioned to become entertainment for villagers and tourists who paid to watch the bears jump in agony.

These bears were poached from the wild as cubs to be sold in perpetuation of this brutal practice. With no anaesthesia, a red hot poker rod would be driven through the muzzle of the baby bear, often at the tender age of six months and the canines would be forcibly removed. A thick rope would then be pushed through the raw wound that controlled their every move.

The organization's '*Dancing Bear Rehabilitation Project'* was supported by The Ministry of Environment and Forests, Government of India and the state forest departments to facilitate the rehabilitation of 628 bears across four rescue centres in India, which are still being run by Wildlife SOS in collaboration with the state forest departments.

We also created alternative livelihoods for the Kalandar community, provided education to their children and introduced women empowerment initiatives, and now have a successful model in place for the rescue and rehabilitation of wildlife. Any conservation effort to be sustainable must empower the community and protect the animals, which is what was successfully achieved in the Dancing Bear Project.

As part of the bear rehabilitation program, our team works with the rescued bears to help them overcome the negative impact on their physical and psychological well-being and teach them to tap into their instincts, in a near natural environment. A majority of these bears have faced physical mutilation, been kept in inhuman conditions, forced to walk for miles on hot tarred roads and suffered a life at the end of a short rope.

At Wildlife SOS, our bears are encouraged to forage, climb trees, and play with various enrichments which not only give them the chance to display natural behaviour but also have the added benefit of keeping them mentally and physically stimulated.

It is with immense pride that we put forth this compendium of 20 years of Veterinary Research Publications compiled by our proficient team of veterinarians and biologists. The aim is to create an effective guide to young wildlife veterinarians when they encounter similar cases.

Geeta Seshamani

**Geeta Seshamani**
**Co-founder**
**Wildlife SOS**

**Kartick Satyanarayan**
**Co-founder**
**Wildlife SOS**

**Dr Rabindra Kumar Singh**
**Former Principal Chief Conservator of Forest (HOFF),**
**Chhattisgarh Forest Department**
**Raipur**

# FOREWORD

In an effort to expand wildlife veterinary and biology research I happily present the second volume of compendium of veterinary research and publications by Wildlife SOS. The guidance that volume I provided to students, researchers, biologists and veterinarians alike has allowed the proliferation of multiple and varied studies and research in the field of veterinary science, animal behaviour, anatomy, surgery, anaesthesiology, pathology, clinical medicine etc. The continuous and unhindered efforts of Wildlife SOS has provided solutions to a plethora of issues faced in the field.

The compendium is a handbook of globally acclaimed publications which are informative, insightful and present detailed accounts of case reports, experiments, articles and conservation studies. The literature discusses cases pertaining to a wide array of species.

Wildlife SOS is passionately and devotedly working for the rescue, rehabilitation and conservation of number of wild animals such as Sloth bears, elephants, leopards, black bears, brown bears, reptiles and other displaced wildlife. Not only this, they are actively assisting forest department, police and law enforcement agencies in investigations and providing legal support against wildlife poaching.

I look forward to the next volume of research studies that the organization brings out and nourishes the tree of learning.

**Dr Rabindra Kumar Singh**

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**Wildlife SOS**
**Director - Research & Veterinary Operations**
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## ACKNOWLEDGEMENTS

The publication of this second volume on sloth bear biology reflects efforts of the continuing focus on research that our organisation is committed to. I would hence like to first convey my gratitude to both the co-founders of Wildlife SOS, Mr. Kartick Satyanarayan & Mrs. Geeta Seshamani for their unflinching belief and generous support to our research projects.

My colleague Dr. S. Ilayaraja has been a constant companion in the pursuit of scientific inquiry and his attention to rigor as well as to the welfare of the animals must surely be acknowledged.

I would certainly be amiss if I did not mention the team of animal keepers at the Bannerghatta Bear Rescue Centre whose dedication ensured that our studies were successful. I would like to specially thank Dr. Nithin K & Dr. Tista Joseph for having helped shape both the volumes from its early stages & been instrumental in their publications.

The untiring efforts of Ms. Srimathi & Ms. Prajakta are responsible for making this book a reality. Mr. Swaminathan's constant source of support & encouragement ensured that we completed the book on schedule.

Last but not least, I would like to thank the support of the forest departments of Uttar Pradesh, Karnataka & Madhya Pradesh for their cooperation & support.

**Happy Reading!!**

(Attur Shanmugam Arun)

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# IV) CONTENTS

## IV a) ARTICLE TITLE INDEX

# Art – 102. A STUDY ON IMPACT & DISTURBANCES OF FLOOD ON THE REPTILIAN HABITAT (SNAKES & MONITOR LIZARDS)

**Baiju R**, Janoo N K, Prerna, **Kartick S** and **Geeta S**

## Abstract

A study was carried out for understanding the impact and the disturbances of flood in Yamuna on the reptilian habitat mainly *snakes* and *Varanus bengalensis*. The study was mainly carried out as base at Sur Sarovar Bird Sanctuary and a 40 km stretch towards upstream and 40 km stretch downstream of Yamuna River. The study was conducted in coordination with the Wildlife SOS Reptile Rescue Unit and Forest Department. The reptile rescue unit is working round the clock to understand the impacts of the flood on the habitat. Due to the regulation of water in Yamuna River from the upstream the high land and the banks were flooded, and the snakes and other reptiles were found straying into higher land. The negative impact of the 2010 flood was more compared to the 2011 flood in this stretch of Yamuna River. August to October 2010 the snakes which were rescued in conflict were mainly near the riverbank houses and trees. The main species rescued were *Python molurus, Ptyas mucosa, Naja naja, Bungarus caeruleus* and *Eryx johnii*. Out of these species maximum rescued species was *Python molurus molurus* N=64 in 2010 and N=42 in 2011. *Varanus bengalensis* were the other reptilian species which were affected badly as it mainly lives in burrows. This were rescued > 25 and <32 during this in 2010 and < 25 were rescued in 2011. Maximum rescues were within a short radius of < 1.5 km from River mainstream.

Also, the snakes were found dead on rescue due to flood and conflict. The dead specimens rescued in 2010 were 11 and in 2011 were 4. With the help and coordination of rescue and awareness through print media and other electronic Medias the conflict between reptiles and Man had been drastically reduced in the city and around.

**Keywords:** Wildlife Biologist, DFO, Student, Forestry & Environmental Management, Co-founders, Wildlife SOS.

## Art – 103. SEXING IN CAIMAN CROCODILES AT BANNERGHATTA BIOLOGICAL PARK

Chittiappa B.C, **Yaduraj,** Murthy V.C, V.K Angadi, K.K.Kiran, M. K Prashanth and R. Hemalatha

### Abstract

As a routine work sexing in Caiman crocodiles *(Caiman crocodilus)* was conducted at Bannerghatta Biological Park. A total number of 30 crocodiles were examined after restraining physically using ropes and wet gunny bags. Mid finger was inserted into the cloaca towards head end and felt the presence of ventrally grooved penis in case of males, which was everted and when no such process was felt confirmed as females. Out of 30 Caiman crocodiles, 17 were found to be males and 13 were confirmed as females. To aid in identification first dorsal scale was cut in case of males and second in case of females, later tincture iodine applied to arrest bleeding.

# Art – 104. TREATMENT OF METABOLIC BONE DISEASE (MBD IN CAPTIVE HEADSTARTED TURTLE JUVENILES (Batagur Spp.)

**Yaduraj Khadpekar,** Sheena Koeth, Gowri Mallapur, Shailendra Singh, and Ashutosh Tripathi

## Abstract

The Red-crowned Roof Turtle *(Batagur kachuga)* and Three-striped Roof Turtle *(Batagur dhongoka)* are large riverine turtles historically found throughout the Ganges river drainage in north India. The best remaining populations are in Chambal River within National Chambal Sanctuary. Both species are protected under schedule I and schedule IV respectively of Indian Wildlife Protection Act, 1972 and are categorized as Critically Endangered and Endangered in the Red List of Nature Conservation Union (IUCN, 2007). In November 2008, 36 juveniles from two consecutive years, 16 months and 4 months old, of these species, from Turtle Survival Alliance – Madras Crocodile Bank Trust, Uttar Pradesh Forest Department's Garhaita Turtle Conservation Centre (GTCC), Uttar Pradesh, India, a head starting facility for these turtles, presented with lethargic movement, weakness and abnormally soft and thin shells. Few of these juveniles were also observed to have partial blindness and gastrointestinal infection. From the available case history, it was noted that the diet may have inadequate amounts of calcium. Extreme cold weather and inadequate exposure to sunlight may have been factors that led to poor calcium metabolism. A tentative diagnosis of Metabolic Bone Disease (MBD) was thus made and treatment was initiated.

The turtles were treated with parenteral calcium gluconate @ 100 mg/kg body weight (to be repeated once a week). Parenteral Vitamin A @ 200 IU/kg body weight was given with calcium. Oral Ciprofloxacin @ 10 mg/kg body weight every 48 hours was added to the regimen of the severely affected individuals for 2 weeks. Modifications were suggested in feeding and holding facilities in order to provide adequate amounts of dietary calcium and optimum exposure to natural sunlight for maintaining a healthy batch of animals.

# Art – 105. ANESTHETIC INDUCTION OF CAPTIVE TIGERS USING ATROPINE SULFATE-XYLAZINE - KETAMINE COMBINATION INTRAVENOUSLY

M. K. Sanath Krishna, Sujay Suresh, M. Karthik,
B. C. Chittiappa and **A. Sha Arun**

## Abstract

Ketamine-xylazine combinations have long been used in large felids for routine surgical procedures. Even though studies have been carried out in multiple species on intravenous usage of this combination, there is a scarcity of reports on safe intravenous usage of the combination in large felids. Over a period of six months, eight captive Bengal tigers of different age categories were anesthetized with atropine sulfate - xylazine-ketamine combination intravenously for either surgical procedures or short distance transportation. Food and water were withheld for 12 hours prior to the procedure in all of the tigers. Tigers were secured in a squeeze cage and intravenous cannulation was carried out using either the dorsal or lateral coccygeal vein. Anesthesia was induced with an intravenous bolus dose of atropine sulfate (0.03 mg/kg and xylazine (1.5mg/kg) followed by ketamine (2mg/kg). Overall, induction time was 1-2 minutes, recumbency time without supplementation of ketamine was 35-49 minutes, and recovery time was 50-66 minutes. Depth of anesthesia was constantly observed with palpebral and pedal reflexes. During 2 surgical procedures when tigers showed recovery symptoms, ketamine was given intravenously (1mg/kg) which prolonged the anesthesia for 20-36 minutes. Heart rate and respiratory rate decreased and remained constant during the entire process, but respiratory rate increased rapidly during the recovery process.

The present study indicates that an atropine sulfate- xylazine-ketamine combination administered intravenously produces a safe and satisfactory anesthesia in captive Bengal tigers. Further studies to investigate various dosages or the substitution of other drugs for the combination are warranted.

**Acknowledgements**

We would like to thank The Executive Director, Bannerghatta Biological Park for his support during the study. We also thank Range Forest Officers, Veterinary assistants, animal keepers and zoo staff for their kind cooperation and support during the procedures involved in the study.

# Art – 106. CAPTURING WILD BENGAL TIGERS *(Panthera tigris tigris)* IN CONFLICT ZONES IN HUMAN DOMINATED LANDSCAPE

Sanath Krishna Muliya, Nagaraju. D. N, **Arun. A. Sha,**
Chittiappa B. C. and Kantraju. H. O.

**Abstract**

Human-tiger conflict has always been a major problem in India. Over a short period of 3 months from December 2013 to February 2014, tigers mauled 17 people in lndia out of which five cases were from the state of Karnataka. The said tigers involved in the conflict were identified, captured and subsequently shifted to a rescue centre. Camera traps were laid at the attack sites, nearby waterholes and probable movement path near the kill sites to establish the identification of the Tiger. Cage traps were setup at probable travel path as well as attack sites with live bait in one case and kill suspected tiger's kill in another as lures. Trained elephants were used to track and subsequently dart the tigers. While one tiger was darted directly after it was sighted near its last human kill, other was trapped in cage with its kill as lure and subsequently darted to minimize capture stress.

Plastic two-chambered compressed gas darts of 5 ml capacity with needle of 2.0 mm diameter and 30 mm length were used for remote injection using CO2 powered tele-injection projector. Xylazine and Ketamine were used at dose rate of 1.25 mg/ kg and 2 mg/kg respectively which provided satisfactory immobilization for further procedures. Physiological parameters were well within normal range during entire procedure. As soon as the tigers were shifted to a protected

environment inside the cages, Yohimbine hydrochloride was given intravenously at dose rate of 2 mg/kg body weight. Details on chemical immobilization of stray wild tigers provided. Since both tigers were incapacitated with loss of canine, injury near shoulder and paw region, they were shifted to a rescue centre for further treatment.

## Art – 107. DO SLOTH BEARS LIVE IN BHUTAN? (supported by an IBA Research and Conservation grant)

Nishith Dhariaya, Dave Garshelis, **Thomas Sharp,** Rob Steinmetz, Yeshey Wangdi, Sonam Wangchuk

Royal Manas National Park, Bhutan, and adjoining Manas National Park, Assam, India. Sloth bears and Asiatic black bears (here- after black bears) are similar-looking species whose ranges overlap in some areas along and south of the Siwalik Range, the foothills of the Himalayas. These two species are still reported to co-occur within some protected areas in Uttarakhand and Assam, India.

Historically, their co-occurrence was likely more widespread, but due to their similar appearances, historical reports of their presence tend to be unreliable. A 1990s survey in Nepal showed no overlap in their ranges sloth bears live only in the lowland Terai and low elevations of the Siwaliks, whereas black bears are in the higher hills (Garshelis et al. 1999a). In Bangladesh, a recent survey, sponsored in part by the IBA, revealed that sloth bears had been extirpated sometime in the mid-1990s (Islam etal. 2013). This occurred without recognition by any government authorities, and with claims that sloth bears probably still persisted in some isolated pockets (Sarker et al. 2006). Purported observations of sloth bears after their extirpation in Bangladesh were actually shaggy-haired black bears.


Following this discovery in Bangladesh, we wondered about reports of sloth bears in Bhutan. Bhutan is a primarily mountainous country. It contains prime habitat for black bears, which are noted to prey on livestock and destroy crops. However, the arc of the Terai also narrowly overlaps the extreme southern border of Bhutan, where it is called the Duars. Since sloth bears occur in the Duars of neighboring Assam, it would seem logical that they would also occur in Bhutan.

Both the 1994 and 1996 Red List of Threatened Animals indicated that sloth bear presence in Bhutan was questionable. However, the 1999 IUCN Status Survey and Conservation Action Plan for Bears mapped two populations of sloth bears in Bhutan (Garshelis et al. 1999b), based on information from the head of the Nature Conservation Section of the Forestry Services Division of Bhutan, and a Bhutanese representative of World Wildlife Fund. Wangchuk et al. (2004) mapped an even more extensive range, covering the southern third of the country.

Given this uncertainty, we initiated a project in 2012 to survey potential sloth bear habitat in Bhutan and determine whether sloth bears exist there (map).We also conducted training for wildlife staff in Bhutan so they can better distinguish sloth bears from black bears, understand their ecology, and establish a monitoring system for sloth bears.

We focused our survey in Royal Manas National Park (RMNP; 1,057 $km^2$, 90°35’– 91°13’ E and 26°46’– 27°08’ N), the oldest national park in Bhutan. Elevations in RMNP range from 80 m along the Indian border to 2,900 m in the north. The park contains a rich array of flora and fauna, including tigers as a focal species. We focused our survey in Royal Manas National Park (RMNP; 1,057 km2, 90°35’– 91°13’ E and 26°46’– 27°08’ N), the oldest national park in Bhutan. Elevations in RMNP range from 80 m along the Indian border to 2,900 m in the north. The park contains a rich array of flora and fauna, including tigers as a focal species. Rai (2006) claimed to have found sloth bear sign at elevations up to 1,100m in RMNP; however, we examined photographs of this presumed sloth bear sign and concluded that it was likely confused with sign from black bears (or at least there was no way to distinguish the two species). Nevertheless, an adjacent sister park in Assam, India (Manas NP [MNP]) is known to contain both black bears and sloth bears (Choudhury 2011, Borah et al. 2012).

**Training Workshops and Interviews**

We conducted two training workshops for staff of the Wildlife Conservation Division of Bhutan. We presented information about sloth bear and black bear ecology, distribution, and identification, and we solicited information about records of sloth bears.

We learned that none of the staff had seen a live sloth bear or sloth bear sign in RMNP or other sites in Bhutan. One RMNP patroller said that he found pieces of a dead bear at a salt lick called Kalikhar (see below) in 1999, and based on the long hair, thought it could have been a sloth bear. One RMNP elephant driver said that there used to be a species of bear that lived primarily in the grasslands along the international border, but increased poaching in this area caused this species to decline; he had not seen one since about 2000– 2002. He indicated that the other species (black bear) is still relatively common.

We spoke to a park guard in MNP who indicated that black bears are more common in the forested areas and sloth bears more common in grasslands, where they eat termites. He said that he had been attacked in 2010 by a sloth bear while he was walking along a trail in the grasslands. He stabbed it with his knife, and it ran off.

**Sign Surveys**

In early December 2012, we searched for sloth bear sign along 8 km of trail through the RMNP lowland forest. Specifically, we looked for excavated termite mounds, which are unmistakable as sloth bear sign (black bears are not known to excavate termite mounds).We went to areas that were previously grasslands to look for sloth bear sign, but much of it had grown up into scrub and small trees. We visited the largest grassland in RMNP, called ‘Specialthang’, but found no termite mounds or sloth bear diggings. By contrast, on a jeep ride through adjacent MNP we observed numerous termite mounds and found one mound in a grassland that had been excavated by a sloth bear.

**Camera Trapping**

We examined photographs of bears from camera trapping efforts in RMNP during 2009-2014. Most camera trapping was directed at tigers, and as such, was focused in lowlands, where we expected to find sloth bears.

One photograph of a sloth bear was obtained at the Kalikhar salt lick (176m elevation), 2.4km from the Indian Border, in February 2009. Although the camera was set at this site for two months that year, and for six months in 2012-2013, this single photograph is the only evidence of a sloth bear visit. No other sloth bear photos were obtained in extensive camera trapping during 2010-2011 (Tempa et al. 2011) and again in 2014 (Table 1). However, six years of camera trapping in the lowlands of RMNP yielded 42 photos of Asiatic black bears. If black bears were also rare in the camera-trapping records, we would have concluded that cameras targeting tigers were not effective in detecting bears. But since so many photos of black bears were obtained, this was clearly not the case.

This camera-trap photo, from 2009 is the only recent evidence of a sloth bear in Bhutan (Kalikhar salt lick, Royal Manas National Park, 2.4 km from Indian border).


**Table 1. Camera-trap photos of Asiatic black bears and sloth bears in the lowlands of Bhutan, 2009–2014.**

| | Royal Manas NP | | Phipsoo WS | |
|---|---|---|---|---|
| **Time period** | Black bear | Sloth bear | Black bear | Sloth bear |
| Feb - Mar 2009[a] | 2 | 1 | 0 | 0 |
| Nov 2010 -Feb 2011[b] | 3d | 0 | 0 | 0 |
| Feb-March 2012[a] | 3 | 0 | 0 | 0 |
| Dec 2012-June 2013[c] | 5 | 0 | 0 | 0 |
| Jan-Jul 2014[b] | 13 | 0 | 16 | 0 |
| **Total** | **26** | **1** | **16** | **0** |

a camera trap set at selected sites; time period over which bears were obtained.
b Time period of systematic camera-trapping effort.
c Camera set at 1 site over this time period
d Tempa et al. (2011) recorer 18 photos of black bears, 3 of which were considered independent events.

## Current Sloth Bear Distribution in Bhutan

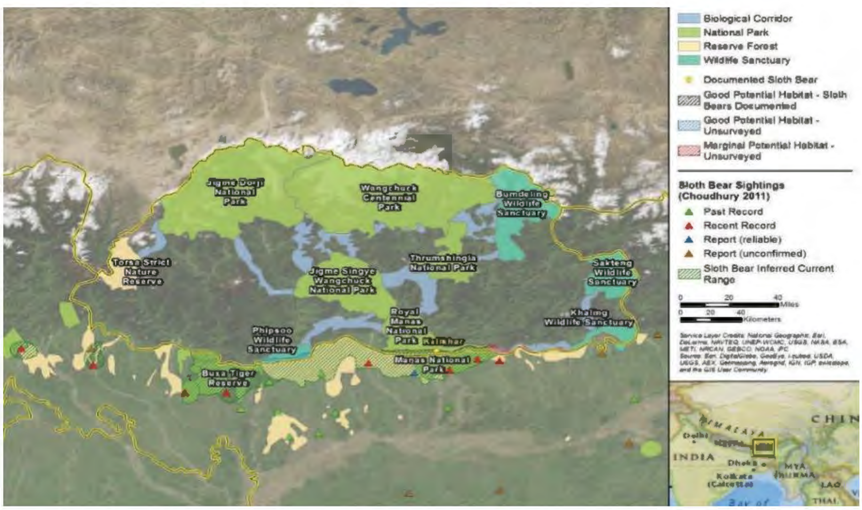
It is evident from our investigation that sloth bears are presently rare in RMNP. There may be a trans-boundary population centred in India (MNP), or the whole population may exist on the Indian side, with occasional vagrants coming across the border. Sloth bears appear to be more common than black bears on the Indians ide of the border, based on the camera-trap records there (J. Borah, pers. comm., 2012).

The grasslands that we surveyed seemed similar in RMNP and MNP, yet we found termite mounds only in MNP. Termites appear to be a critical food resource for sloth bears in the northern part of their range, where fruits are seasonal. Unlike black bears, sloth bears do not hibernate during the non-fruiting season, so their distribution may be limited by rapidly diminishing abundance and diversity of termites with increasing latitude and elevation.

Sloth bear surveys in Nepal, including Chitwan National Park, showed that termite mounds were more abundant within or near sal *(Shorea robusta)*-dominated forest than in open grasslands (Garshelis et al. 1999a). Despite termite mounds being more abundant in forest, sloth bear density was actually higher in the grassland, where they commonly excavated non- mound-building underground termite colonies. It was for this reason, combined with the knowledge that black bears do not use grasslands, that we concentrated our search for sloth bear sign in the small patches of grasslands in RMNP.

Indeed, the few reports of sloth bears obtained during our interviews corroborated the affiliation of this species with grasslands.

Phipsoo Wildlife Sanctuary is the only protected area in Bhutan that has both grasslands and sal forest, so the habitat Our present understanding of the sloth bear distribution along the Bhutan- Indian border.




The only confirmed presence point in Bhutan is one camera trap photo at Kalikhar, RMNP there may be especially suitable for sloth bears. Although Phipsoo is quite small (269km2, it adjoins Manas reserve forest and Buxa Tiger Reserve, on the Indian side, both of which have existing sloth bear populations and or sightings. Sloth bear surveys have not been conducted thus far in Phipsoo. However, recent camera-trapping there documented an abundance of black bears and no sloth bears (Table1).

**Did Sloth Bears Ever Inhabit Bhutan?**

We conducted an exhaustive literature search and found no definitive records of sloth bears in Bhutan. However, whereas many British naturalists wrote about sloth bears in India (Garshelis and Steinmetz 2014, few visited the independent Kingdom of Bhutan. John Claude White, a British political officer of Sikkim, managed to travel and spend time in Bhutan.

In his memoir (White 1909 he noted various wildlife, including bears: “Three species, one inhabiting high altitudes from 11,000 to 12,000 feet; the common black bear, found every here from 6,000 feet downwards; and a third species, also said to be common, inhabiting the lower valleys.” From this it appears that a third species of bear, which we presume was the sloth bear, was common in low elevations of Bhutan at the turn of the 20th century. If true, we do not know why they have become so rare there today, though we hypothesize that factors include poaching (which is relatively easy in the open habitats sloth bears prefer), scarcity of termites (a key food), and perhaps competition with more numerous black bears. We plan to conduct further field work to examine these hypotheses more thoroughly.

**Royal Manas NP Wildlife staff and authors**

**Acknowledgements**

This project was supported mainly through a Research and Conservation Grant from the IBA. Further financial and logistic support was provided by the Wildlife Conservation Division, Department of Forest & Park Services, Bhutan. We thank the wildlife staffs who eagerly participated in the workshops, shared their knowledge of bears, and participated in the sign surveys. We also thank Mr. Nilmani Rabha for accompanying us in the field and the Mr. Anindhya Swargoveri, Field Director Manas Tiger Reserve, India for permission and providing necessary logistical support during our survey there.

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# Art – 108. STANDING SEDATION AS A SAFE TOOL FOR WOUND MANAGEMENT IN SEMI CAPTIVE ELEPHANT - A CASE REPORT

Nirupama J**, Arun A. Sha,** Vaseem M and **Gochalan E.**

**Abstract**

A 29-years old female elephant named Meneka weighing 3500kg body weight was brought to the hospital with the complaint of wound near the umbilical, trunk and base of the tail. Elephant was very restless and every 5 min she was throwing mud in the ventral abdomen and not allowing to examine. Due to her aggressive behavior it was difficult to treat and examine her; the decision was taken to give standing sedation in order to carry out a close examination and treatment. Animal was sedated with 4ml xylazine (100mg/ml, xylazine injection, illus® and 5ml ketamine (100mg/ml, ketamine injection, illus® as total dose. Elephant showed drooping of trunk, reduced motility relaxation of genitalia and involuntary urination after 20 minutes of sedation. After 45 min she was under complete standing sedation allowing doctors to do the routine blood sampling and treatment procedure. The animal was handled safely for the next two hours. All her vital parameters were within the normal range. There were few lacerations near the base of the tail and near the umbilical region a deep wound with missing skin flap was noticed. Wound area was thoroughly cleaned with povidine iodine solution then cleaned with metronidazole. After the area was completely cleaned lorexane ointment was applied and the surrounding area sprayed with topicure spray. Animal was injected with 80ml of long acting Enrofloxicane (100mg/ml) and advised to keep in the kraal area for few days. Alternative days dressing was carried out for 20 days and after a month, wound completely healed.

# Art – 109. MOLECULAR CHARACTERIZATION OF THE PREDOMINANT CULTURABLE AEROBIC BACTERIA FROM FAECES OF CAPTIVE LEOPARDS *(Panthera pardus)*

**A Sha Arun,** Lyju Jose, A. Thulasi, D. Rajendran, M. Chandrasekharaiah

## Abstract

This study was undertaken with the objective of identifying the predominant aerobic bacteria in the faeces of captive leopards. Freshly voided faeces were collected from leopards housed in a captive zoo facility and brought to the laboratory. Serial dilutions were prepared using MacConkey's Agar (contains peptone, pancreatic digest of gelatin, sodium chloride, lactose and bile salts as major ingredients). The plates were incubated at 37$^{o}$C for three days. A dense growth of organisms was observed. Thirty single colonies were transferred to the broth and re-cultured on the afore said medium. Pure isolates were obtained and further characterized using traditional biochemical tests. Twenty-six isolates were found to be catalase positive, gram negative non-motile coccobacilli. DNA extracted from the pure isolates was used as a template for the polymerase chain reaction (PCR). Amplification of a 750bp fragment was obtained with forward (AGAGTTTGATCCTGGCTCAG) and reverse (TACCAGGGTATCTAATCCTGTT) primers. The PCR product was further purified and cloned and electroporated into E. coli. Positive clones were sequenced to identify the bacteria. It was found that twenty-six of these isolates has 97-99% sequence

homology of the 16S rRNA with that of *Acetinobacter baumanii*. Though this bacterium has not been studied in depth in animal faeces, this is a ubiquitous inhabitant of soil, water and other environment surfaces. *A. baumannii* is commonly found on the skin and nasal passages of animals.

# Art – 110. WHOLE METAGENOME ANALYSIS TO IDENTIFY THE RUMEN MICROBIAL AND CARBOHYDRATE ENZYME PROFILE IN CROSSBRED CATTLE

Lyju Jose, **A. Sha. Arun,** A. Thulasi

## Abstract

The microenvironment of rumen harbors a conglomerate of microbes including bacteria, archae, fungi and protozoa that enable breakdown of plant lignocellulosic biomass. A battery of glycosyl hydrolases (GHs) functioning in a concerted fashion is required for the complete hydrolysis of lignocellulose in the rumen. A complex rumen biome mediates the hydrolysis of crop residues and complex polysacchararides and subsequent fermentation into volatile fatty acids. The present study was undertaken to assess the rumen microbial diversity and Carbohydrate Active Enzyme (CAZyme) profile in crossbred cattle fed maintenance level rations of ragistaw, paragrass and concentrate. The total DNA was extracted from the rumen digests of experimental animals and 16S rRNa gene was amplified by using universal primers. The clone library was constructed by using pGEM-T easy vector system and transformed into E. coli. A total of 123 positive clones were sequenced and analysed (BLAST). 65 different Operational Taxonomic Units (OUTs) were identified belonging to 4 different phyla. Whole metagenomic sequencing of DNA was done under Illumina, MiSeq platform. Metagenome data set was uploaded on MG-RAST server and the phylogenetic analysis revealed the predominance of phylum Bacteriodetes followed by Firmicutes. The putative protein-coding regoins

were predicted using FragGeneScan. All ORFs encoding putative Carbohydrate Active Enzymes were identified using CAT tool kit at Carbohydrate Active Enzyme (CAZyme) data base.

# Art – 111. POSITIVE CONDITIONING THROUGH TARGET TRAINING WITH PROTECTIVE CONTACT METHOD AND ITS NECESSITIES FOR EFFECTIVE MANAGEMENT OF CAPTIVE ELEPHANTS.

**Ilayaraja S, Yaduraj K, Baiju R, Kartick Satyanarayan, Geeta Seshamani,** Steve K and **Arun A. Sha**

## Abstract

In India captive elephant population is moderately high and the elephants are used for commercial works, temples and some places as a begging elephant. Existences of human/mahout's encounter also happening due to improper management of captive elephant. This is mainly happening due to the carelessness of the mahouts, their overconfidence and their inhumane way of negative enforcement without understanding the animal behavior. In order to overcome the long standing elephant welfare issue, Wildlife SOS, an NGO started an 'Elephant Care & Conservation Centre' in collaboration with Uttar Pradesh Forest Department and making: effort to setup a model facility for the captive elephant with positive reinforcement by applying enrichment technique, target training and protective contact method. Reinforcing desirable behaviour is one of the cornerstones of animal training. Though it's a very simple concept, but a very complex undertaking; basically, it motivates an animal to repeat the desired behaviours and to stay interested. Trainers and keepers are devoted to the proper care and management of their animals by creating a complex and stimulating environment with lots of variety of enrichments called environmental enrichment. Targets can be a versatile training aid; elephants easily learn to touch a target for a click

and a treat. The protective contact is a safety approaching technique practiced in captive elephants under chain-free condition to ensure the safety of both handlers and the animal for carrying out any procedure on the animal. In this article we discussed in detail about the advantages of above said techniques and how we executed in our elephant care centre to improve the welfare of the elephants.

## Art – 112. AMYLOID HEPATOPATHY IN AN ASIAN PALM CIVET *(Paradoxurus hermaphroditus)*

**Ilayaraja Selvaraj, Arun A Sha, Niraj Dahe,** Karikhalan M

### Abstract

Wildlife SOS runs wildlife rescue helpline in and around Agra city where the team usually gets call from public for any wildlife rescue. Likewise, an injured male Palm civet was rescued and found severe injuries all over the body, so the same was brought to our wildlife hospital for treatment. The animal was highly stressed and had multiple bite marks all over the body. The radiographic examination revealed fracture of maxilla and other bones were intact. The wounds were cleaned and dressed. Necessary antibiotic and anti-inflammatories were administered. We planned the surgery to fix the fracture after stabilizing the health condition. But the animal started showing convulsion and died on second day. Since the animal had multiple bite mark on the body, we wanted to rule out rabies. The Post-mortem examination revealed severe internal hemorrhage and contusion. Kidney and spleen were congested. Liver congested and discolored with mosaic appearance, gall bladder is filled with bile. All representative tissue samples were collected from the visceral organ for histopathological examination. However, no marked abnormalities noticed in brain macroscopically, sample collected for detail histopathological examination to rule out rabies. The histopathological examination of brain tissue not revealed any negri body thus confirmed no rabies. But the histopathology examination of liver under hematoxylin and eosin (H&E) staining

revealed amyloid deposition in the interstitium thus suggesting amyloidosis. Hence the condition confirmed as amyloidosis-induced hepatopathy.

**Keywords:** Amyloid hepatopathy, Asian Palm Civet, *Paradoxurus hermaphroditus*

## Introduction

According to IUCN Red List status and Indian Wildlife (Protection) Act, 1972 Common Palm Civets are placed in lower risk and Schedule II respectively. They are mainly frugivorous, but also eat small vertebrates and invertebrates. They are solitary, nocturnal, and largely arboreal, spending the day in trees and sometimes in buildings. The civet produces a musk (also called civet) highly valued as a fragrance and stabilizing agent for perfume. Both male and female civets produce the strong-smelling secretion, which is produced by the civet's perineal glands. It is harvested by either killing the animal or removing the glands, or by scraping the secretions from the glands of a live animal. The latter is the preferred method today. Common Palm Civets occur in a range of habitats up to 2400 m, including evergreen and deciduous forests (both primary and secondary), plantations, and around human dwellings and settlements [12]. Amyloid is a pathologic proteinaceous substance deposited between cells in various tissue and organs of the body in a wide variety of clinical setting. Over 20 different precursor proteins have been identified in the various forms of amyloidosis. However, amyloidosis is classified based on the difference in the nature of the precursor protein, all amyloidoses have similar homogeneous eosinophilic histologic appearance when stained with HE and share affinity for certain histologic stains such as Congo red [34]. Amyloidosis is reported in horse [9, 10, 16, 20], cow [15,32] sheep [8,17], goats[23], dogs[2,18], felines [3,34,24], birds [6,19,29] and macaques [37] by different authors but such report is not available in Palm civets. AA-amyloidosis is the most common type of amyloidosis in mammals including domestic animals and birds and often results in hepatic or renal failure due to physical disruption of the normal cellular and organ processes [34]. In this article we documented a case of amyloid induced hepatopathy in a Common Palm Civet.

**Materials and Methods**

A 3 kg male common palm civet rescued from Agra was brought to wildlife hospital at Agra Bear Rescue Facility, Uttar Pradesh, India (27°0'N;77°45'E). The general examination revealed multiple bite mark on the body of the animal (Figure-I) and the oral cavity examination revealed severe injury on the upper palate. The Radiographic examination of the animal confirmed longitudinal fracture of maxillary bone, and other bones were remaining intact (Figure 2). The wounds were cleaned and dressed with antiseptic ointment, the oral cavity rinsed with chlorhexidine mouth wash solution. Antibiotic and anti-inflammatory injection were given and kept the animal under observation for further treatment and care and planned for the surgery to fix the fractured upper palate once the health condition stabilized. But on second day morning the animal started showing convulsion and died. The post-mortem examination revealed severe internal haemorrhage and contusion. Kidney and spleen were congested (figure5). Liver congested and discolour with mosaic appearance; gall bladder is filled (figure-4). All representative tissue samples were collected from the visceral organ for histopathological examination in 10% formalin. However, no marked abnormality noticed macroscopically in brain, sample from brain tissue were also collected for detail histopathological examination to rule out rabies at Indian Veterinary Research Institute. lzatnagar, Bareilly, India.

## Result and Discussion

The detail histopathology examination of brain tissue samples not revealed any negri body thus confirmed no Rabies. But the histopathology examination of liver section under HE stains suggested amyloid deposition in the inter stitium and confirmed amyloidosis (figure 6). Other organs got no microscopic lesion were evident.

Several different pathologic mechanisms and conditions underlier various forms and types of amyloidosis although abnormal proteins with similar staining characteristics are deposited in various organs and tissues of the affected animals [37]. Kidney is the main target organ for the deposition of amyloid in familial amyloidosis of the Abyssinian cat and Shar-Pei dogs, while the amyloid is mainly deposited in the liver in Siamese cats [4,26].

Deposition of amyloid in the pancreas of cats, non-human primates and humans can lead to the development of type 2 diabetes mellitus [31,11]. The affected organs are often enlarged, moderately firm, and abnormally discoloured 31]. In AA-amyloidosis, the deposition in most species is in the central organs and tissues such as spleen, liver, kidney and the arterial walls [14,22]. Depending on the extent of the deposition, there may be splenomegaly, hepatomegaly, and renomegaly as spleen, liver and kidneys are the most commonly affected organs in systemic AA-amyloidosis [37]. In animals, at least eight different amyloid precursors have been described [23]. The precursor proteins in amyloid fibrils may be amyloidogenic mutants as in some familial amyloidosis, whereas other precursors are normal wild-type proteins [7,35]. The exact mechanisms through which the proteins are converted into amyloid fibrils in vivo are not well known. Amyloidosis is described in association with different chronic disease in captive cheetah *(Acinonyx jubatus)*, Siberian tigers *(Panther tigris altaica)*, mink *(Afustela vison)*, black-footed cats *(Felies nigripes)*, black- footed ferret cats *(Mustela nigripes)*, Dorcas gazelle *(Gozella darcas)*, mountain gazelle *(Gazella gazella)*, bighorn and Dall's sheep, free-living lioness *(Panthera leo)* and in swan and other anatidae [5,8,17,21,24,25,27,30,34,36]. Johnson [13] and Terio [33] reported that the chronic inflammation and chronic stress as a predisposing factor for amyloidosis in cheetah. Terio [34] reported that amyloidosis is the inherited trait in black-footed cats.

**Figure 1. Multiple bite mark on the body**

**Fig 2: The Radiograph of Civet with longitudinal fracture of maxillary bone**

**Figure 3. Hemorrhage and contusion in thoracic and abdominal cavity**

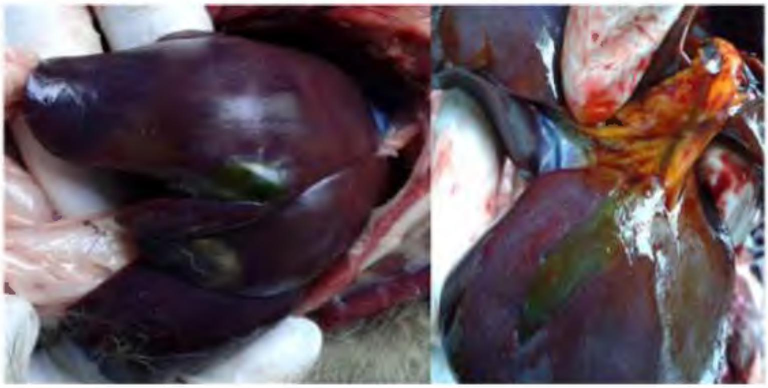

Figure 4: congested and **discolored Liver with filled gall bladder**


**Figure 5. Congested kidney and spleen.**

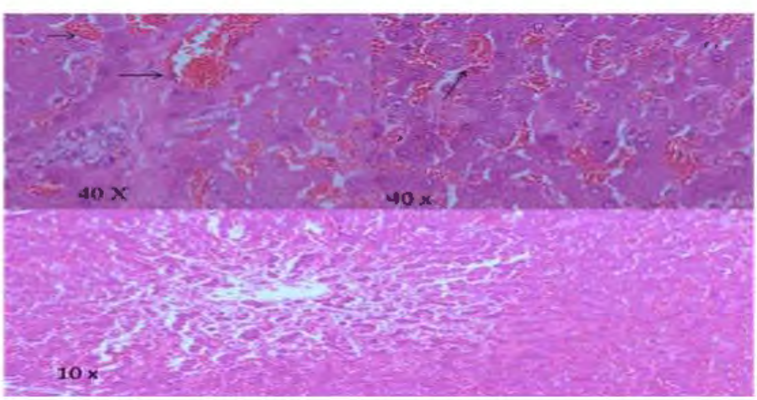


**Figure 6. Amyloid deposition (pinkish deposite) in the intertitum of liver (HE staining)**

## Conclusion

In this case, the initial injuries and associated inflammatory process along with the stress might be the predisposing cause for the Amyloidosis.

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## Art – 113. COMPLETE PULPECTOMY IN AN INDIAN LEOPARD CUB *(Panthera pardus fusca)* UNDER MEDATOMIDINE-KETAMINE ANAESTHESIA

**Arun A Sha,** Sanath Krishna Muliya, Mahendrajit Singh, **Harikrishnan. C** and **Ilayaraja. S,**

### Abstract

An orphan leopard cub of about five-month- old, rescued by Forest department of Karnataka was housed in Bannerghatta Biological Park, for its further management and care. After a period of two weeks, the cub started to show aggressive behaviour and signs of self - mutilation, in addition to severe inflammatory swelling in the facial region. The cub was chemically immobilized with a mixture of Medetomidine hydrochloride and Ketamine hydrochloride (@30 micro grams/kg and 2mg/kg respectively to carry out thorough physical examination. The oral cavity examination revealed fractured lower left canine, with exposed pulp cavity. In general, complicated tooth fractures; the fractures causing pulp exposure often lead to pulpitis, pulp necrosis, and periapical inflammation leading to chronic pain. In this case also the cub was repeatedly clawing the region to relieve the pain due to this. So, it was decided to carryout total pulpectomy and thereby retain the canine tooth. Radiographs were taken with the help of a dental x-ray machine, using bisecting angle technique and using intra-oral occlusal size 4 x- ray films. The canal was filed using 60 mm

hedstrom files and flushed with 5.25% Sodium Hypochlorite to remove the necrosed pulp. Upon complete cleaning and drying, the narrow canal was filled with calcium hydroxide cement using a spiral lentulo, and which was later radiographed to confirm if the cement has reached the apex. A chemical cure glass ionomer was used as an intermediate restoration followed by an amalgam restoration. A small groove was made on the canal to hold the amalgam. A course of Benzathine penicillin antibiotic and analgesics were administered post operatively. The cub had retained the tooth for almost seven months, until it gained its permanent canines.

# Art – 114. SEX DEPENDENT SEASONAL VARIATIONS IN THE CAPTIVE SLOTH BEAR BODY MASS

**Yaduraj Khadpekar, Ilayaraja S, Arun A. Sha, Baiju Raj M.V,** John P Whiteman, **Niraj Dahe, Kartick Satyanarayan,** and **Geeta Seshamani**

## Abstract

Monitoring of body mass is a basic, but one of the important and effective tools for health monitoring and management of captive bears. However, to interpret the body mass data, it is important to know the typical seasonal variations; such variations have not been documented for sloth bears *(Melursus ursinus)*. We hypothesized that female sloth bears lose mass in the summer oestrous period and regain it in the winter which is a normal period of parturition, whereas male sloth bears do not show this pattern. Body mass records of captive sloth bears (10 females: estimated age 9- 19 years during the study, 6-8 years of data from each, none of them were intentionally mated or gave birth; and 6 castrated males: estimated age 9-21 during the study, 3-6 years of data from each) from the Agra Bear Rescue Facility, Uttar Pradesh, India were analysed to test this hypothesis. It was confirmed from the individual bear records and history that they did not suffer a long-term health condition that may affect the body mass. Female sloth bears' body mass fluctuations showed a distinct annual curve with a peak in December (greater than mean annual body mass by 6±2%, 95% CI), and a lowest point in July (less than mean annual body mass by 7±2%). In contrast, males' body mass fluctuations were more erratic and reached smaller extremes in December (greater than mean annual body

mass by 3±3%) and July (less than mean annual body mass by 3±2%). We conclude that substantial annual body mass fluctuations in summer and winter are normal and expected in the healthy female sloth bears and to a smaller extent in males. We suggest that the reduction in the body mass in summer could reflect increased activity due to estrous and mating, while the mass gain in winter, in the case of females, could reflect the physiological preparation for denning and parturition.

# Art – 115. VOLUNTARY MEDICAL PROCEDURES IN CAPTIVE SLOTH BEARS USING POSITIVE REINFORCEMENT TECHNIQUE AS A HUSBANDRY TOOL

**Simone Ayoob, Arun. A. S, Harikrishnan. C** and **Ilayaraja**

## Abstract

Use of positive reinforcement as a tool for voluntary medical procedures in captive sloth bears has been gaining popularity in zoos across the world, but unfortunately not so in India. The utilization of this technique at the Bannerghatta Bear Rescue and Rehabilitation Center and the Agra Bear Rescue Facility of Wildlife SOS resulted in successful voluntary blood draws, claw trims, oral and ocular examination in eight Sloth bears. A multi-step approach was undertaken where in target training was initiated first, followed by training to present paws and forearm of the bear for examination. Dates and honey were used as primary reinforcement feed and the vocal reinforcers such as 'good' served as the secondary reinforcement during the training procedure. The time taken to train each bear was highly variable which appeared to depend on the individual Sloth bear and the skill of the trainer. Blood draws from the cephalic vein was done in four bears and claw trips of the forearms in the other four. There is a growing need to incorporate this practice as part of husbandry measures for captive wildlife in India. It serves as a safe alternative for restraining the animal, cutting the risk of anaesthesia and acting as a stress-free procedure for both the animal care staff and the animal. The prerequisites of

the procedure is a dedicated trainer and a protected contact wall structure which need not be custom made and often can be manipulated from the existing enclosure structures. Additionally, the entire procedure takes a reasonable amount of time warranting a popularization of the practice.

## Art – 116. FIRST RECORD OF FISHING CAT IN SUR SAROVAR BIRD SANCTUARY, AGRA, INDIA

S. Prerna, **Baiju Raj,** Vikramjit Sharma**, Geeta Seshamani and Kartick Satayanarayan**

**Abstract**

The fishing cat *(Prionailurus viverrinus)* is a wetland dependant endangered species. Its population shows a decreasing trend all across its distribution. In the last decade, there have been many records of presence of fishing cat in India. On 18 February 2016 in the morning, a dead fishing cat was spotted on the National Highway 2 in Sur Sarovar Bird Sanctuary, Agra, India. This is the first record of the presence of fishing cat within this sanctuary.

The fishing cat is a wetland dependent endangered species (Taylor et al. 2016, Naidu et al. 2015, Mukherjee et al. 2010, Mukherjee et al. 2012). It is a nocturnal, rare and elusive cat that can swim well and prey primarily on fish and rodents (Mukherjee et al. 2010, Adhya et al. 2011). Its population shows a decreasing trend all across its distribution (Taylor et al. 2016, Mukherjee et al. 2010). It is listed in the Schedule I of the Indian Wildlife (Protection) Act 1972 and as Endangered in the 2010 as-assessment of the IUCN Red List of Threatened Species (IUCN 2015). Major threats to this species are from anthropogenic activities like encroachments, over-harvesting of aquatic resources, pollution from industries and agriculture, poaching of fishes in protected areas and retaliatory killing (Mukherjee et al.

2012, Mukherjee et al. 2010). There have been records of illegal trade of fishing cat skins in India (Sunquist and Sunquist 2002 cited in Mukherjee et al. 2010).

There have been several new records of the presence of the fishing cat in the last decade but these were largely from South Asia, whereas in South-east Asia there were many unsubstantiated records in the past but these are now uncertain (Nekaris 2003, Duckworth et al. 2005, Mukherjee et al. 2010, Anonymous 2014, Taylor et al. 2016). In India, published records are from the Upper Ganges canal in Ghaziabad town of Muradnagar (Uttar Pradesh; Singh 2015), Nagpur (Maharashtra; WPSI 2005 cited in Mukherjee et al. 2010), Keoladeo Ghana National Park, Bharatpur in Rajasthan (Mukherjee et al. 2012), Coringa Wildlife Sanctuary (Naidu et al. 2015, Sankar 2014, Kolipaka 2006) and in Krishna Wildlife Sanctuary plus outside this protected area in Andhra Pradesh (Naidu 2014), Greater Noida in Uttar Pradesh (Ghosal 2014), Howrah and Hooghly in West Bengal (Adhya et al. 2011). Jhala et al. (2015) reported the presence of fishing cat in Corbett Tiger Reserve (Uttrakhand), Dudhwa National Park (Uttar Pradesh), Pilibhit Tiger Reserve (Uttar Pradesh), Valmiki Tiger Reserve (Bihar), Ranthambore Tiger Reserve (Rajasthan; also reported by Sadhu & Reddy 2013), Simlipal Tiger Reserve (Odisha), Orang National Park (Assam) and in the Sundarbans landscape (West Bengal) (also reported by Gupta 2012). These records are based upon evidence through camera trapping, sign surveys, DNA extraction, and interviews with locals, road accidents or snaring. Sur Sarovar Bird Sanctuary (27°14°8.95"N/ 77°51"9.37'E), an Important Bird Area (BNHS 2014) lies in the Indo-Gangetic plains and is situated on the banks of the river

Yamuna (Gupta et al. 2004, Lawania & Trigunayat 2015) and adjoins National Highway 2 NH2. It was declared as a bird sanctuary in 1991 and it occupies a total area of 7,99 $km^2$, including approx. 3 $km^2$ of a manmade lake (made in 1922), fed by the Agra canal channelled from the Okhla barrage (Kumar 2010). It is one of the 115 wetlands identified under National Wetland Conservation Program since 2007 (MoEFCC 2007), now referred as the National Plan for Conservation of Aquatic Eco-systems NPCA (MoEFCC 2011). Mathura refinery (Indian Oil Corporation) pumps water from this lake through the irrigation department. The river and the lake inside the sanctuary provide a suitable habitat and food for many mammals, reptiles and birds. The common species of fishes found here are *Catla Catla catla, Rohu Labeo rohita, Mangur Clarias magur, Tilapia Tilapia* sp., *Bam Anguilliformes* sp., *Singhi Heteropneu-stes fossilis.* The sanctuary has Northern Tropical Dry Deciduous Forest (Champion & Seth 1968). There are few invasive species of plants and shrub found here like Lantana camara, *Parthenium hysterophorus* and *Prosopis juliflora.* The lake and its shore line were invaded by *Eichhornia crassipes* and *Ipomea* sp. These aquatic invasive species were removed and are checked since 2006 (Kumar 2010).

Major anthropogenic disturbances in the sanctuary are lease of land to other departments, widening of the highway (NH2), poaching of fishes, excessive collection of fuel wood, cattle grazing, fluctuation in the water level due to unsupervised extraction of water by the refinery, encroachments, noise pollution by vehicles as the sanctuary is adjacent to the NH2, large gathering of people for cremation and "Bhandara" a religious feast by individuals or institutions attended by a mass gathering of people (Kumar 2010 and personal observations).

On 18 February 2016 in the morning, a dead fishing cat was spotted on the NH2 at 27°14'36.33" N and 77° 49'47.70" E (Fig. 1). The carcass was brought to the Wildlife Hospital of Wildlife SOS Bear Rescue Facility in coordination with the forest department. A detailed examination of the carcass was done by the wildlife biologist and veterinary officer. It was found that its right forelimb and hind limb were fractured, which is possible because of a road accident. The stomach was empty and congested, and the cat had ticks. The carcass was burnt after the necropsy. The possible reason of the death was a traumatic shock due to the road accident. The fishing cat was a male weighing 14 kg (5-16 kg in Menon 2014, 11-15 kg in Prater 2005), body length 66.04 cm, tail 30.48 cm, head 21.59 cm and chest girth under the forelegs 50.8 cm.

There is an urgent need for an intensive survey to collect more evidence of the presence of fishing cats through sign surveys, camera trapping and molecule identification through scats within the sanctuary, in the fragmented patches of forest which are along the river belt towards Mathura (30-40 km), and in the forest patches towards Bharatpur, which is at a distance of 34 km from this sanctuary.


**Fig. 1.** A dead fishing cat found in Sur Sarovar bird Sanctuary, Agra, India on 16 February 2016 (Photo Wildlife SOS).

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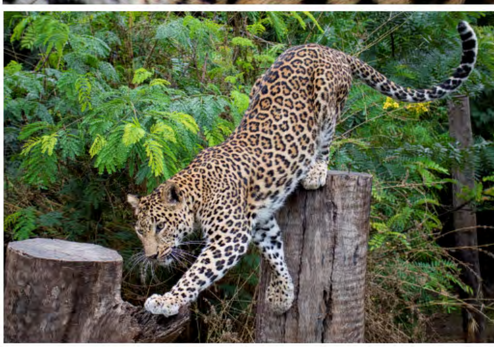
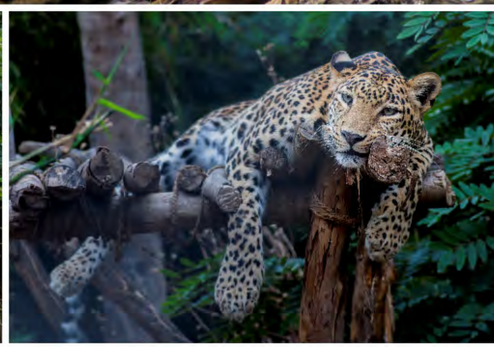
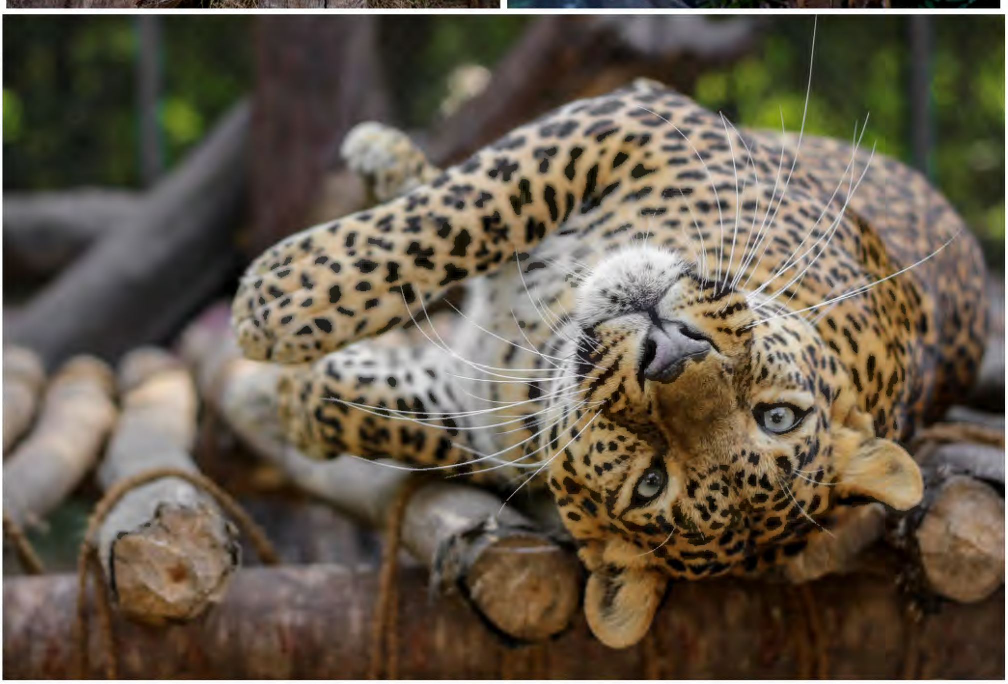
A leopard skin was seized on the outskirts of Delhi in a night long joint operation carried out by Wildlife SOS Anti-Poaching Unit, Haryana Forest Department & Haryana Police.

In a joint operation by Wildlife SOS & the Uttar Pradesh Forest Department, 74 snakes were seized from the illegal custody of snake charmers across Agra and Mathura. A total of 57 cobras, 8 common sand boas, 6 rat snakes & 3 red sand boas were rescued and transferred to the Wildlife SOS rescue facility.

In a night-long joint raid by Wildlife SOS and the Special Task Force of the Uttar Pradesh police, two tiger poachers were arrested and nearly 20 kilos of tiger bones along with tiger skin were seized in Bijnor.

**Art – 117. CHEMICAL IMMOBILISATION OF DHOLE *(Cuon alpinus)*, INDIAN JACKAL *(Canis aureus indicus)* AND INDIAN WOLF *(Canis lupus pallipes)* WITH KETAMINE HYDROCHLORIDE-XYLAZINE HYDROCHLORIDE**

Sanath Krishna Muliya, **Arun A. Shanmugam,** Pa. Kalaignan, **Linto Antony, Harikrishnan Chandranpillai** and Nirupama Jaisingh

**Abstract**

Maintaining wild animals in captivity often requires chemical immobilization to achieve various diagnostic, surgical and management interventions. Four dholes, two Indian grey wolves and four Indian jackals were immobilized using Ketmaine -Xylazine combination for either medical or management interventions. Based on the estimated body weight, canids were darted with upon 6-8 mg kg-1 Ketamine and 0.7-1.14 mg kg-1 Xylazine. Initial signs of drug effect included decreased mentation and progressive ataxia followed by recumbency. The mean +/- SD of induction time was 14.25 +/- 2.75 (range: 11-17 min), 11+/-3.16 (range: 8-15 min) and 15.5 +/- 3.54 (range: 13-18 min) in dhole, Indian jackal and Indian wolf, respectively. Hyperthermia was initially observed in all the jackals and dholes, whereas rectal temperature in wolves remained well within the normal rage for canids. The mean duration of anesthesia was 31 +/- 8.83 (range: 23- 43 min), 32.5+/-5.32 (range: 26-39 min) and 30.5 +/- 7.78 (range 25-36 min) in dhole, Indian jackal and Indian wolf, respectively,

subsequent smooth and uneventful recovery in all the cases. The observations made during immobilisation measures during the procedures in this work suggest that chemical immobilisation of captive dhole, Indian wolf and Indian jackal with 6-8 mg kg-1 ketamine and 1 mg kg-1 xylazine is effective and safe for routine management and medical interventions in these species provided body temperature is closely monitored and corrected as appropriate.

## Introduction

Many procedures that are routinely accomplished in domestic animals with minimal restraint may require anesthesia in wild animals, in order to ensure the welfare and safety of the animals and personnel involved. When appropriately used, chemical immobilisation can be employed to safely restrain and capture many species, thereby minimising stress and the risk of injuries associated with other restraint methods (West et al. 2008). Hence, it is of utmost importance for wildlife veterinarians to know the suitable anaesthetic drugs and the combinations and dosage that provide safe and satisfactory immobilisation for adequate periods of time in different species (Ferreras et al. 1994). Reports on dosage and anaesthetic agents for safe and effective immobilisation of most wild species occurring in in India are very limited (Belsare & Vanak 2013). Furthermore, chemical immobilisation is challenging in India because available field immobilising drugs are limited to xylazine hydrochloride and ketamine hydrochloride due to several legal restrictions. Hence, there is an urgent need to document existing safe immobilisation protocols for many species using this combination.

This will facilitate management interventions in captivity and also serve as a baseline for research and medical interventions in wild counterparts.

Three wild canid species are commonly found in Indian zoos. Among them, Asiatic wild dogs or dholes *(Cuon alpinus)* are pack hunting social canids native to South and Southeast Asia. Due to various anthropogenic threats, this species is classified as endangered in IUCN Red list of Threatened species, version 2010.1. (Kamler et al. 2015). The captive management of this species therefore assumes importance for maintenance of an insurance population, as well as for possible reintroduction of surpluses in suitable habitats. Indian jackal *(Canis aureus indicus)*, a subspecies of golden jackal, are present in all protected areas of India except for those in the high altitude regions of the Himalayas. The IUCN considers golden jackals as a 'species requiring no immediate protection' with the caution that the population throughout its range are likely declining (Jhala & Moehlman 2008). Despite having a large population in captivity that is maintained in almost all Indian zoos, there is a scarcity of reports on captive management of this species. The Indian grey wolf *(Canis lupus pallipes)*, one of two wolf subspecies found in India, inhabit throughout the arid and semiarid plains of peninsular India. Present populations of this species are estimated to be close to 2000–3000 individuals (Jhala 2003). Even though this wolf species has been classified as a species of 'Least Concern' by the IUCN, the *pallipes* subspecies is protected under Schedule 1 of the Indian Wildlife (Protection) Act of 1972, which prohibits their hunting, trapping or killing. In the last few decades, wolf protection and conservation efforts were initiated by setting up Indian wildlife preserves and captive breeding programmes in zoological parks.

Ketamine–xylazine (KX) combinations have been widely used for immobilisation of wild canids (Kotwol 1981; Fuller & Kuehn 1983; Grassman et al. 2006; Gerardo et al. 2010; Belsare & Vanak 2013). Ketamine is a dissociative anaesthetic that is used either as a sole anaesthetic agent or in combination with α-2 adrenergic agonists like xylazine and medetomidine. Xylazine is a potent alpha-2 central nervous system depressant with anxiolytic, muscle relaxant and analgesic properties that help counter-act the undesirable side-effects or ketamince such as convulsions and catalepsy. This manuscript reports safe and effective use of KX in three wild Indian canid species under captive conditions.

## Methods

Four dholes (three males, and one females), two Indian grey wolves (two males) and four Indian jackals (one male and three females) held in captivity in an Indian zoo were immobilised for either medical or management interventions. Based on estimated body weight, each (Ketamil,100 mg mL-1, Troy Laboratories Private Ltd., Smithfield, NSW, Australia) and 1mg kg-1 xylazine (Xylaxin, 20 mg mL-1, Stanex Drugs & Chemicals Private Ltd., Hyderabad, India), projected using n CO2 powered dart projector (Rifle Model IM, DAN-INJECT). Only the animal attendant and veterinarian were allowed to approach canids for visual estimation or their body weight prior to immobilization as a means of minimising excitement prior to drug administration. The induction time (time between administration of immobilising agents and attainment of non-responsive state for safe handling) was recorded for each canid. The actual body weight was measured immediately after immobilisation with an electronic digital scale (Model CS-PF, Canon Services, Bangalore, India) followed by the intended procedure. Opthalmic solution (CIPLOX D®, Cipla Ltd., Mumbai, India) was applied to keep the eyes moist, and canids were blindfolded to cease visual stimuli. Catheters were placed in lateral saphenous veins for administration or emergency medication or additional doses of ketamine as needed.

The heart rate and the respiratory rate were monitored manually by a chest auscultation and chest excursions. The rectal temperature was monitored via patient monitoring units (MEC-1200 Patient Monitor; MINDRAY, Shenzhen, China). Physiological parameters were monitored immediately after induction, and thereafter at 10 min intervals. The total duration of anaesthesia (time from when the animal became motionless until the first voluntary movement) was also recorded. The range and mean ± SD were calculated for all the data recorded, using Microsoft office Excel version 2007 (Microsoft, Redmond, Washington, USA).

## Results

As accurate body weights could not be obtained before drug administration, the mean administered dosages were calculated after obtaining actual body weights, as tabulated in Table 1. Induction of anaesthesia was rapid and smooth in all the animals, with the induction time ranging from 8 to 18 min. Initial signs of drug effect included decreased mentation and progressive ataxia followed by recumbency. Hyperthermia was initially observed in all the jackals and dholes, whereas the rectal temperature in both the wolves remained well within the normal range for canines during the entire procedure (Malmsten 2007). The mean with SD and range of physiological parameters observed are tabulated in Table 2. Hyperthermia was successfully treated by spraying ethanol on groin, abdomen region and foot pads. In case of persistent hyperthermia (≥39°C, 20 min post initiation of procedures) as observed in three dholes, 10 mL kg −1 i.v. bolus dose of lactated ringers' solution was administered.

The duration of anaesthesia ranged from 23 to 43 min. No supplemental ketamine was given to any animal, as the intended procedures were carried out well within this period. All recoveries were smooth and uneventful. All canids took more than 45 min to completely recover from the effects of the anaesthetic agents.

**Discussion**

In general, KX combination exhibited smooth and rapid induction and induced adequate plane of anaesthesia, safe enough to perform intended procedures in all the animals. Effective doses of the KX used in dholes by us were less than those recommended for free- ranging dholes (Grassman et al. 2006; Acharya et al. 2010). We could not compare the results with similar work on captive dholes aspursual of literature did not yield any such study.

**Table I. Mean drug dosage, induction time and recovery time +/- SD used in dholes *(Cuon alpinus)*, Indian jackals *(Canis aureus indicus)* and Indian grey wolves *(Canis lupus pallipes)***

| Species | Actual weight (kg) | Ketamine ($mg\ kg^{-1}$) | Xylazine ($mg\ kg^{-1}$) | Induction time (min) | Recovery time (min) |
|---|---|---|---|---|---|
| Dhole (*n* = 4) | 13.4 ± 1.34 | 8.68 ± 0.42 | 1.08 ± 0.05 | 14.25 ± 2.75 | 31 ± 9.93 |
| Indian jackal (*n* = 4) | 7.38 ± 0.93 | 8.18 ± 0.97 | 1.02 ± 0.12 | 11 ± 3.16 | 32.5 ± 5.32 |
| Indian wolf (*n* = 2) | 26.5 ± 1.13 | 6.8 ± 0.78 | 0.8 ± 0.09 | 15.5 ± 3.54 | 30.5 ± 7.78 |

**Table 2. Mean +/- SD and range for physiological parameters observed in dholes *(Cuon alpinus)*, Indian jackals *(Canis aureus indicus)* and Indian grey wolves *(Canis lupus pallipes)* chemically immobilized with ketamine and xylazine**

| Species | Temperature (°C) | | Respiration (breaths $min^{-1}$) | | Heart rate (beats $min^{-1}$) | |
|---|---|---|---|---|---|---|
| | Mean ± SD | Range | Mean ± SD | Range | Mean ± SD | Range |
| Dhole (*n* = 4) | 39.61 ± 0.64 | 38.77–40.66 | 24.88 ± 7.54 | 15–37 | 67.69 ± 18.76 | 42–96 |
| Indian Jackal (*n* = 4) | 39.27 ± 0.49 | 38.55–40.16 | 32.06 ± 6.15 | 21–40 | 98.94 ± 12.27 | 80–116 |
| Indian wolf (*n* = 2) | 38.34 ± 0.90 | 37.22–39.66 | 15.25 ± 2.49 | 12–19 | 83.38 ± 10.77 | 69–98 |

KX was also very effective and safe in immobilising jackals, although the doses we used were higher than those mentioned in literature for captive jackals (Senthilkumar et al. 2011). Effective doses of the KX used in wolves here were same as those recommended for wolves in captivity (Larsen & Kreeger 2007). The dosage used provided good muscular relaxation and sufficient analgesia for management procedures with adequate handling time and acceptable recovery time in all three species.

Stress and overheating are always the major concerns when capturing large canids. We believe that the hyperthermia that was initially documented in all dholes and jackals was due to the animal's state of excitation at the time of darting. Most canids dissipate heat through panting, but this is often compromised during immobilisation (Larsen & Kreeger 2007). Additionally, canids immobilised with KX have been frequently observed to have poor thermoregulatory ability (Fuller & Kuehn 1983; Larsen & Kreeger 2007). As there was no holding house for dholes and jackals, they had to be darted in a large open enclosure. In contrast, Indian wolves were confined inside a small holding house and quickly darted without development of excitement. It is thus recommended to encourage captive canids to run into a den or other confined area, such as a holding house, wherein they can be darted upon without much excitation. Further, materials to deal with hyperthermia, such as cool water, wet towels, ice packs, ethanol and lactated ringers' solution, should be kept handy, before attempting immobilisation in canids.

Prolonged and rough recoveries have been reported as disadvantages or KX immobilisation in canids due to residual ketamine effects. Consequently, reversal or xylazine and other alpha-2 adrenergic agents with administration of antagonists is not advised until 45 min after the last injection of ketamine (Larsen & Kreeger 2007). Adherence to this recommendation minimises the odds of rigidity, excitement and other adverse residual ketamine-related signs during recovery. As all procedure and recovery were completed within 45 min of initial administration of ketamine, reversal of xylazine with use of yohimbine hydrochloride was not necessary.

Even though the complete recovery period was relatively prolonged, it was smooth and uneventful in all canids. No immobilisation-related mortality occurred during or within one month after the immobilisation procedure. Observations made during chemical immobilisation procedures suggest that immobilisation protocols followed in present study are effective and safe for carrying out routine management procedures and medical interventions in captive dhole Indian wolf and Indian jackal provided body temperature is closely monitored and corrected as appropriate.

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**Conflicts of interest**

The authors disclose no conflict of interest.

**Contributions**

SKM and AAS conceived and designed the procedures. SKM. AAS, PK. LA, HC and NJ carried out the work and compiled data. SK conducted the statistical analysis. SK and AAS prepared the draft manuscript. All authors reviewed and agreed to the content of the final manuscript.

**Reference**


- Acharya B.B. Johnsingh A.J.T. & Sankar K. (2010) Dhole telemetry studies in Pench Tiger Reserve, Central India. Telemetry in Wildlife Science 13, 69-79

# Art – 118. A REPORT ON THE BENZIMIDAZOLE RESISTANCE IN SLOTH BEARS

**Arun A Sha,** J. Afreen Fathima, G.M. Arpitha, M. Palanivelrajan, M.G. Jayathangaraj and S. Gomathinayagam

## Abstract

The effectiveness of anthelmintic fenbendazole was evaluated in 32 sloth bears of Bannerghatta Bear Rescue and Rehabilitation Centre, Bannerghatta Biological Park, Bengaluru by the use of faecal egg count reduction tests (FECRTs). Faecal samples were collected from all 32 sloth bears and subjected to routine faecal examination. The parasitic fauna observed in faecal samples of these bears were Ascarid eggs and cysts of protozoan Isospora spp. Among these, Ascarid eggs were the predominant (52%). Pre- and post- Treatment faecal egg count indicated that there was reduction of only 36% in egg counts after administration of Fenbendazole at recommended dose i.e., 25 mg/kg body weight. It was concluded that Ascarids were able to resist Fenbendazole treatment and alternate strategies for management of parasitic fauna were discussed.

**Keywords:** Febendazole resistance, Ascarids, Sloth bear, Bengaluru

# Art – 119. PREDOMINANT CULTURABLE BACTERIA ISOLATED AND CHARACTERIZED FROM FAECES OF CAPTIVE SLOTH BEARS *(Melursus ursinus)*

**A Sha Arun,** L Jose, A Thulasi, D Rajendran and M Chandrasekharaiah

## Abstract

Studies were undertaken to isolate and characterize the predominant culturable bacteria from bears kept in a captive facility. Bears are terrestrial mammals belonging to the family Ursidae that inhabit wide variety of habitats. With a paucity of information on microbes inhabitating Indian Sloth bears under captivity, this study was undertaken to enumerate the culturable bacteria in captive bears scat samples. Twenty bears were selected for the study and were fed with a maintenance level ration comprising of ragi porridge, cooked vegetables, honey, watermelons etc. Freshly voided faeces were collected from the bears in a container flushed with carbon dioxide, brought to the laboratory and enriched in 98-5 medium. After four days, serial dilutions were prepared and used as inoculums for culturing the bacteria by Hungate's roll tube method. The colonies on the roll tube were purified by alternate transfers from broth to medium and medium to broth. The purified isolates obtained were characterized using routine microbiological and biochemical tests. The 16S rRNA region was used as a phylogenetic marker for the identification of the bacterial isolates. The DNA was extracted from the purified isolates, the 16S rRNA region was amplified using the 27F and 1492R forward and reverse

primers, amplicons cloned into pGEMT easy vector and used for E. coli transformation. Positive clones were sequences and these sequences were used for the identification of the bacteria using BLAST. Among the facultative anaerobes enterobacteriaceae and enterococci were dominant in faeces obtained from all the bears. The major genera of the bacteria isolated under this category includes Escherichia coli, *Proteus vulgaris* and Streptococcus. The E. coli cells were rod shaped and were able to grow under aerobic and anaerobic condition. Proteus vulgaris was found to be oxidase negative but catalase, urease and nitrate positive. Among the strict anaerobes, Prevotella and Clostridium perfringens could be isolated from most of the animals. In three bears Candida was the lone aerobic fungal genera that could be enumerated. The other organisms that were confirmed by 16S rRNA sequence homology are *Acinetobacter baumannii, Proteus mirabilis, Pseudomonas aerogenosa.*

# Art – 120. COMPREHENSIVE ANALYSIS OF RUMEN MICROBIAL AND CARBOHYDRATE ACTIVE ENZYME PROFILE IN INDIAN CROSS BRED CATTLE

L Jose V, A Thulasi and **A Sha Arun**

## Abstract

The bovine rumen harbours a complex and diverse microbial ecosystem which is highly specialized for the deconstruction of plant cell wall polysaccharides present in the diet. Rumen microbiome provides a unique genetic resource of microbial enzymes degrading recalcitrant plant cell wall components. Present study provides a comprehensive assessment of rumen microbiota and Carbohydrate Active Enzymes (CAZymes) profile in Indian crossbred steers. 16s rRNA gene clone library was constructed from the total DNA extracted from rumen contents of experimental animals. A total of 138 positive clones containing almost full length (~1450bp) were subjected for sequencing and analyzed by using suitable bioinformatics tools. 16s rRNA gene clone library analysis revealed the predominance of phylum Bacteroidetes (45%) followed by phylum Firmicutes (40%) in cattle rumen. High throughput sequencing of total DNA, under lllumina-MiSeq platform and has generated 1.9 gigabases of sequences, with an average read length of 871 nucleotides. The phylogenetic analysis by Metagenomics Rapid Annotation using Subsystem Technology (MGRAST) indicated the abundance of phylum Bacteroidetes (38%) followed by phylum Firmicutes (32%) and Proteobacteria (9 %) in the sample. All contigs which passed quality check were converted into 2,10,435 Open Reading

Frames by using FragGeneScan. pfam based CAT (CAZyme Analysis Toolkit) analysis showed that ~8% ORFs in the metagenome dataset, were coding for putative carbohydrate active enzymes. Genes coding for putative proteins belonging to all six functional classes of CAZyme families - Glycoside Hydrolases (GHs), Glycosyl Transferases (GTs), Carbohydrate Esterases (CEs), Carbohydrate-Binding Modules (CBMs), Polysaccharide Lyases (PLs) and Auxiliary Activities (AAs), were all well represented. The majority (~45%) of the putative CAZymes in this study were belonging to GHs which are the efficient degraders of plant polysaccharides.

# Art – 121. EFFECTIVE REVERSIBLE IMMOBILIZATION OF CAPTIVE HIMALAYAN BLACK BEARS *(Selenarctos thibetanus laniger)* WITH MEDETOMIDINE-TILETAMINE- ZOLAZEPAM AND ATIPAMEZOLE

**Attur Shanmugam Arun,** Sanath Krishna, **Linto Antony, Harikrishnan Chandran Pillai,** Manjunatha Venkataramanappa, and Sujay Suresh

## Abstract

We used a combination of medetomidine and tiletaminc- zolazepam to immobilize the Himalayan black bears *(Selenarctos thibetanus laniger)* in Bannerghatta Biological Park, Bangalore, India. Medetomidine and tiletamine-zolazepam were administered at 0.01 mg/kg and 0.5 mg/kg, respectively. We describe procedures and observations recorded during immobilization.

A 1:1 mixture of tiletamine and zolazepam is the drug of choice for the chemical immobilization of bears (Stirling et al. 1989; Taylor et al. 1989; Gibeau and Paquet 1991). Although it offers advantages such as relatively short induction time and predictable immobilization, it has a long recovery period and fails to induce analgesia for potentially painful procedures (Cattet et al. 1997). Addition of selective α2-adrenoceptor agonist drugs such as medetomidine or xylazine can overcome these limitations by significantly reducing the amount of tiletamine-zolazepam used (Cattet et al 2003). Additionally, these drugs have a combined sedative-analgesic-aesthetic effect, which can be completely antagonized with the α2 antagonist atipamezole.

Medetormidine and tiletamine-zolazeparn mixture (MTZ) has been used for chemical immobilization in American black bear (*Ursus americanus*; Caulkett and Cattet 1997), brown bears (*Ursus arctos*; Fahlman et al. 2011), polar bears (*Ursus maritimus*; Cattet et al. 1997), and sun bears (*Helarctos malayanus*; Manabu 2003). However, there is a paucity of data for safe usage of this combination in other bear species including Himalayan black bears *(Selenarctos thibetanus laniger).*

Between January 2014 and June 2014, five adult Himalayan black bears at Bannerghatta Biological Park, Bangalore, India, were immobilized with MTZ for management activities (e.g., physical examination, sample collection, surgical intervention). A day prior to the procedures, animals were secured in holding houses and fasted for 12 h. To avoid stress to bears prior to immobilization, only the veterinarian and an animal keeper were allowed to approach them for visual estimation of weight.

Based on estimated weight, the bears were intramuscularly injected with medetomidine (MEDETOR, ® Virbac Animal Health, Burgdorf, Germany) and tiletamine-zolazepam (ZOLETIL 50, ® Virbac) at 0.01 mg/kg and 0.5 mg/kg, respectively, in a single 3-mL dart with a 1.2.X38-mm needle (DAN-INJECT ApS, B¢rkop, Denmark), using a 1.25-m blowpipe (Zoo model, DAN-iNJECT). Once the animals were completely immobilized, the eyes were covered with a blindfold and the mouth was secured with a muzzle. Heart and respiratory rate were monitored manually by chest auscultation and thoracic movements, respectively. Rectal temperature and percentage of haemoglobin oxygen saturation (SpO2) were recorded by a monitor system

(MEC- 1200 Patient Monitor, Mindray, Shenzhen, China).

After completion of clinical procedures, atipamezole (Atipam, ® Eurovet Animal Health B.V., Bladel, The Netherlands) was administered intravenously at 0.0.5 mg/kg, five times the administered dosage of medetomidine, to ensure that circulating medetomidine was fully antagonized. Observations made during the procedure are shown in Table 1. The mean induction time was 11.2 min (SD=2.28; range=9-14). After the drug was administered, bears exhibited a sequence of consistent behaviors such as yawning, lip-licking, progressive uncoordinated movement, inability to support body weight, head relaxation, lack of palpebral and corneal reflexes, and lack of response to physical stimuli. Convulsions were not observed in any of the bears. The mean recumbency time was 63.2 min (SD=8.47; range=55-77). No supplemental drugs were needed because bears were adequately sedated, and none showed recovery signs during the procedures. Heart rate, respiratory rate, and SpO2 were well within the normal range throughout the procedure. The mean recovery time was 7.2 min (SD=3.27; range=4-12). The first recovery signs after administration of atipamezole were spontaneous eyelid blinking, paw movenents, head lifting, and eventually standing. Advantages of MTZ observed in bears included small drug volume for darting, short and smooth induction, short recovery period, and absence of sudden arousal during recovery.

**Table 1. Name, sex, and body weight with induction, recovery, and recurnbency time for Himalayan black bears** *(Selenarctos thibetanus laniger)* **immobilized with medetomidine- tiletamine-zolazepam, Bangalore, India, 2014. Means with standard errors are also reported for physiologic parameters recorded during the study**

| Name of Himalayan black bear | Sex | Body Weight (kg) | Induction Time (min) | Recum bency Time (min) | Recovery Time (min) | Respiratory Rate (breaths/min) | Pulse rate (beats/min) | $SpO_2$ (%) | Rectal temperature (C) |
|---|---|---|---|---|---|---|---|---|---|
| Ramesh | Male | 106 | 11 | 5 | 77 | 14.8±l.17 | 97.2±0.75 | 96.8±0.41 | 37.9±0 08 |
| Mohan | Male | 113 | 9 | 12 | 62 | 16.5± l.05 | 97.0±0.63 | 96.0±0.00 | 38.1±0.10 |
| Rajesh | Male• | 124 | 13 | 6 | 55 | 11.5±0.84 | 96.8±0.14 | 90.8±0.75 | 37.9±0.04 |
| Rarnya | Female | 112 | 9 | 4 | 64 | 11.3±0.52 | 96.2±0.75 | 98.8 ±0.41 | 38.1±0. 1.5 |
| Lakshmi | Female | 150 | 14 | 9 | 58 | 14 0±0 6:3 | 95.70±0.82 | 96.8±0.41 | 37. 8±0. 53 |

a time between administration of the drug and loss of consciousness

b Time between administration of reversal drug and proper standing

c Time between induction and recovery

d $SpO_2$ = Percentage of haemoglobin oxygen saturation

In Japanese black bears *(Ursus thibetanus japonicas)* immobilized only with tiletamine- zolazepam, recovery was often prolonged, ranging up to few hours (Makoto et al. 2007). The short recovery time in our study can be attributed to antagonism of medetomidine by atipamezole and the reduced amount of tiletamine-zolazepam used (Cattet et al. 1997). Our observations in Himalayan black bears are consistent with those made in sun bears (14.2±7.9 min; Makoto ct al. 2007) and polar bears (2.4±2.5; Cattet et al. 1997).

A common adverse effect reported during anesthesia with MTZ in sun bears was vomiting during the induction, despite previous fasting (Manabu 2003). In our study, bears neither vomited nor showed any signs of retching. Spontaneous palpebral reflex was the first clinical sign of recovery after injecting atipamezole, an observation reported in brown bears where an increased intensity of the palpebral reflex was observed as an early indicator of a light plane of anaesthesia (Fahlman et al. 2011). In addition, nystagmus was not observed in any of our bears, contrary to observations made in polar bears (Cattet et al. 199,). Although polar bears anesthetized with MTZ occasionally had hind-limb ataxia, lasting for 0.5-1h (Cattet et al. 1997), no such observations were made in our study. All bears stood up in normal gait and displayed well-coordinated movement. None showed resedation within 5 h of recovery.

Cardiopulmonary effects of this combination were minimal, similar to reports in polar bears (Cattct et al. 1997) and black bears (Caulkett and Cattct 1997). Contrary to reports of hypothermia and hyperthermia in sun bears and brown bears, respectively, all bears immobilized in our study rnaintained normal rectal temperature (Wallach 1978). Our data indicate that the combination of medetomidine (0.01 mg/kg) and tiletamine- zolazepam (0.5 mg/kg) is a reliable, safe anesthesia in captive Himalayan black bears without adverse effects.

We thank Range Gowda, Executive Director, and Chittiappa Balladi Chanda, Assistant Director (Veterinary Services), Bannerghatta Biological Park, for valuable suggestions and guidance during the study. We also thank Wildlife SOS® and the staff of Bannerghatta Biological Park for technical assistance.

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# Art – 122. A STUDY ON DISTRIBUTION OF SWEAT GLANDS AT THE INTERDIGITAL REGION IN POLAR AND SLOTH BEARS

Sunilkumar Patil, K.V. Jamuna, Shruti P, V.R. Annie, **A. S. Arun** and V. Ramkrishna

**Summary**

Sweat glands present at the pedal skin play a pheromone like chemical communicator at different seasons, which has been observed in polar bears located at polar region. When pedal scent samples collected from paw region by passing sterile cotton swabs between the toes of polar bear were subjected to estrus female exhibited Flehmen's reaction. However to investigate similar observation in sloth bear located in the terrestrial region, the present study was undertaken to detect the presence or sweat glands in the pedal skin region of sloth bear.

**Keywords:** Sweat glands, Pheromones, Chemical communicator, Sloth bear

**Introduction**

Chemical communication *(Pheromones)* plays a central role in regulating social and reproductive behavior and is especially prominent in solitary, wild-ranging species (Wyatt, 2003). Chemical communication is important for assessing reproductive readiness, locating and choosing mates. Chemo-signalling is used as a source for study of sexual behavior with the help of pheromones released by scent glands present at various parts of the body. Biological sources of scent signals may include urine, feces, body odour, apocrine gland and scent gland secretions (Wyatt, 2003). Behavioral mechanisms for scent deposition may be overt (e.g. scent marking, body rubbing, and urination) or passive e.g. scent trails (Clark, 2007).

Interdigital sweat glands are one of the sources for release of chemo signals, in the form of pheromones in mammals. Histological study was conducted in polar bear, to understand the distribution of sweat glands in the interdigital space. So, presence of a row of sweat glands in dermis layer close to the hair follicle in the interdigital area of skin was observed. Along with this other chemical analysis confirmed their evidence as scent glands (Owen, 2014). However, no such sweat gland in sloth bears has been studied.

## Materials and Methods

Skin samples were collected from the dorsal and ventral interdigital region, footpad and the ventral region just proximal to the footpad at the time of postmortem of sloth bears in Bannerghatta National park, Bengaluru, Karnataka. Skin samples collected were fixed in 10% neutral buffered formalin, processed routinely for histology, sections at 5 microns were stained with haematoxylin and eosin stain. Histological examination of the skin of sloth bears, to understand the distribution of sweat glands was performed to investigate the anatomical capacity for sloth bears to produce pedal chemo signals.

## Results and Discussion

Compared to polar bear, sloth bear interdigital skin had very few prominent apocrine sweat glands (Fig. I). Also, the skin of side digit of sloth bear showed very few apocrine sweat glands around the hair follicles (Fig.2) Rows of prominent apocrine sweat glands as observed in polar bear (Owen, 2014) could not be observed in sloth bear, indicating that the sexual behavior in sloth bear could be communicated by other source, such as use of body or urine. The polar bears habituated rubbing, anal glands secretions, in the polar region had the environment comprised of sea ice, lack of permanent vertical vegetation like trees, rocks which are used routinely for targeted scent markings by other group of bears (Clapham et al., 2012; Nie et al., 2012). So, the nature might have provided natural adaptation for polar bears to have chemical communication between the bears. Since sloth bears are residing in the terrestrial environment which have plenty of vertical vegetation might not have developed such prominent apocrine sweat glands near hair follicle in interdigital skin and skin of side digit as compared to polar bears.

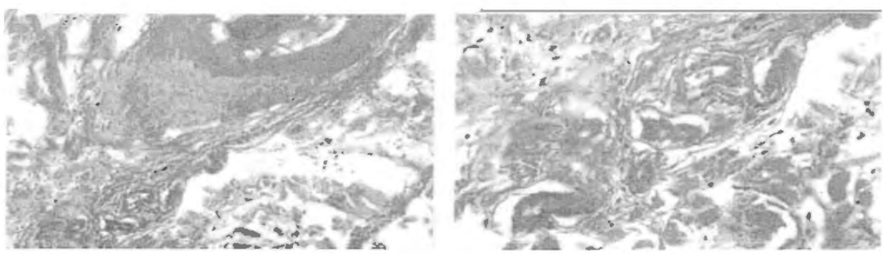

Fig 1 Fig 2

**Fig. 1. lnterdigital skin of sloth bear showing hair follicle (A) with few sweat glands(B), H & E x100**
**Fig. 2. lnterdigital skin of sloth hear showing few sweat glands (B), H & E.x 100**

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# Art – 123. MOLECULAR DIAGNOSIS OF RABIES INFECTION IN A WILD SLOTH BEAR *(Melursus ursinus)* FROM TUMKUR DISTRICT; A CASE REPORT

Manjunatha V, **Arun A Sha,** Manjunatha Reddy G B, Giridhara, Byregowda S M

## Need for the Study

Rabies is zoonotic disease, transmitted by infected animal bite to humans. The present case study describes the diagnosis of rabies in a wild bear. A male wild Sloth bear attacked two persons. Killed one person and severely injured another person at Chilanahalli, Madhugiri range, Tumkur in November 2014. The bear was shot dead by a police officer to avoid further human causalities. Upon our request and permission from the Chief Wildlife Warden of the state, the carcass was subjected for detailed post-mortem examination and all the internal organs were collected including brain samples.

## Results

The post-mortem examination revealed the presence of human flesh with skin inside his stomach. The cause of death was concluded due to bullet injury. PM examination of brain and meninges revealed severe congestion. The brain samples were sent for National Institute of Veterinary Epidemiology and Disease Informatics for rabies diagnosis and found positive for rabies virus antigen by direct fluorescence antibody test (dFAT) and positive for rabies virus nucleic acid by reverse transcriptase polymerase chain reaction (RT-PCR). The rabies virus was reconfirmed by sequencing of partial nucleoprotein gene.

The phylogenetic analysis of N gene sequence from present case with published sequences showed, close relationship with domestic and human rabies virus isolates reported from India.

**Conclusion**

Rabies might be prevalent in wild animals and safe handling and necessary prophylaxis advised always to avoid occupational hazard.

# Art – 124. AMPUTATION OF FORELIMB IN A WILD LEOPARD *(Panthera pardus)*

Nirupama Jaisingh, Vassem Mirza, P.A. Kalignan, Sanath Krishna, L. Ranganath and **Arun A. Sha**

**Abstract**

Leopard (*Panthera pardus)* is one of the five big cats in the genus *Panthera*. It is a member of the family Felidae with a wide range in the Sahara Africa and parts of Asia. Leopards have the ability to adapt to various habitats ranging from the rain forest to the steppe. Due to increase in the urbanization, the habitat of wild animals is getting fragmented and destroyed, which is leading to increase in the frequency of mean-leopard conflicts. During the conflict situation, either the man or the leopard gets injured. There were three leopards which were rescued in different year with wound on the body.

Case 1: an adult female leopard rescued from Mysore was presented with deep wound on the neck region. Animal was immobilized, wound was cleaned thoroughly with normal saline and povidone iodine muscles and skin was opposed. Animal was kept under antibiotic and analgesia for a week. Animal recovered uneventfully after 20 days.

Case 2: an adult male leopard rescued from Hassan was presented with lacerated wound around the thoracic cavity measuring 7 cm wide dorsally exposing the thoracic

vertebrae and 2cm wide ventrally. Animal was immobilized using xylazine and ketamine, radiography was taken which couldn’t reveal any abnormalities. Wound was thoroughly cleaned with povidone iodine and normal saline. Wound was freshened up using BP blade ventral area was sutured using monofilament polyamide 1 and treated as open wound. Animal was kept under antibiotic and analgesia for a week. Wound was cleaned and dressed daily. Wound healed in a month.

Case 3: an adult male leopard rescued from Kaglipura was presented with a wound to the left paw. Radiography of paw revealed fracture of the left phalange. After a week, animal showed self-mutilation and necrosis of the same limb. Animal was subjected to amputation of the limb as per standard surgical procedures. Animal was kept under antibiotic and analgesic for a week. Animal recovered uneventfully after a month.

# Art – 125. TRANSFIXATION OF HUMERUS IN A SPOTTED DEER - A CASE STUDY

Nirupama Jaisingh, **Arun A. Sha,** P.A. Kalingan


## Abstract

A spotted deer *(Axis axis)* female aged around 4 years weighing 30kg rescued from Bangarpet district presented with a complaint of right forelimb swelling and not able to bear weight. Animal on physical examination revealed crepitation, pain and swelling of the limb. Radiography of the right forelimb with factor 60 KV and 13 mV revealed multiple fractures in mid shaft of humerus warranting surgical intervention. Animal was immobilized using Xylazine @ 1mg/kg bwt i/m and ketamine @ 3mg/kg bwt i/m and maintenance with ketamine. A skin incision was made on the lateral aspect of the forelimb, muscle bundles were reflected to expose the fracture ends. Fragments of bone were removed and transfixation of fracture fragments a was done by inserting intramedullary pins transversely through each of the proximal and distil end. Syringe plungers were inserted at both side of pins to support fracture ends. Muscle bundle were opposed with chromic catgut 0, subcuticular suture was applied using chromic catgut 1, skin edges were opposed using monofilament polyamide 1 and limb was bandaged. Post- operatively animal was administered with Inj. LA Enrofloxacin @ 5mg/kg bwt and Inj. Melonex @ 0.5mg/kg bwt i/m for three days. Animal was returned back to forest department and local vets care.

# Art – 126. COMBINATION OF ETORPHINE (M99) AND XYLAZINE AS SUCCESSFUL IMMOBILIZATION AGENTS FOR HOOF TRIMMING IN GAYAL *(Bos frontalis)*

Nirupama Jaisingh, **Arun A. Sha,** and Vaseem Mirza

**Abstract**

The gayal *(Bos frontalis)*, also known as Mithun, is a large semi domesticated bovine distributed in Northeast India, Bangladesh, Northern Burma and in Yunnan, China. A female mithun weighed approximately 500 kg had difficulty in walking, limping and wound in all hoof regions. Animal was immobilized using Etorphine 2ml and xylazine 1ml as total dose intramuscularly to carryout hoof trimming. Animal was completely immobilized after seven min of darting. Close examination of animal revealed overgrown hoof and dewclaws which was led to difficulty in locomotion. Vital parameters like temperature, heart rate, respiratory rate and capillary refill time was monitored every five min. After making the animal into lateral recumbency, overgrown hoofs were trimmed using gigli wire saw without using any damage to the hoof and cleaned with normal saline. Wound area was dressed with Povidone iodine and Lorexane veterinary cream. Animal was administered with Inj. Melonex @ 0.2 mg/kg bwt i/m and revived with diprenorphine 2ml i/v and yohimbine 1ml i/v. Animal recovered uneventually. Animal was observed next day and found to be having proper gait and no sign onlimping.

# Art – 127. DIFFERENTIATING SLOTH BEARS FROM ASIATIC BLACK BEARS IN CAMERA-TRAP PHOTOS

**Thomas Sharp,** Dave Garshelis, Rob Steinmetz, Nishith Dhariya

## Abstract

Camera traps are increasingly used to assess the distribution of wildlife, including bears, throughout the Indian subcontinent and Southeast Asia. Accordingly, researchers must be able to determine with near certainty the species and in our case the specific species of bear that has been photographed. This can be difficult in areas occupied by sympatric, similar- looking bears, especially when some photographs are unclear, at a bad angle, or of poor quality (McLellan 2012, Ngoprasert and Steinmetz 2012). Asiatic black bears and sun bears may be similar looking in photographs because the size of the animal is difficult to ascertain; since these 2 species overlap on a fine scale across Southeast Asia (Steinmetz 2011), this can lead to

*(left) Camera-trap photo of a sloth bear, recognizable by the general dome shape, debris stuck in the fur, and whitish snout. (right) Camera-trap photo of a sloth bear, identifiable from length of its back claws.*

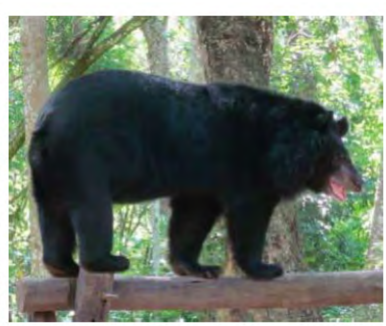

***(left) Camera-trap photo of an Asiatic black bear, recognizable by the smooth, clean-looking coat as well as a short, dark snout. Note that the ruff on the neck is common in both Asiatic black bears and sloth bears. The large ears are not visible in this photo due to the bear's posture. (right) Camera-trap photo of an Asiatic black bear, readily distinguishable by the large ears.***

confusion in species identification. More at issue, though, is the case of Asiatic black bears and sloth bears because these 2 species are even more alike: they are of similar size, nearly-always black with a crescent white chest marking and a ruff of longer hair around the neck. Also, because their zone of overlap is narrow, typically 1 of the 2 species is locally far more common, so a few mistaken identifications could yield an incorrect perception of the status of the rarer one. Perhaps the most notorious example of this occurred in Bangladesh, where the widespread presence of Asiatic black bears masked the complete extirpation of sloth bears (Islam et al. 2013). Likewise, the historical and present distribution of sloth bears in Bhutan has been uncertain due to the presence of the far more common Asiatic black bear (Garshelis et. al. 2015). In recent surveys in northern and eastern India, species- specific population trends could not be discerned because these 2 bears were not identified to species (Jhala et al. 2011).

Here we present several helpful criteria for distinguishing sloth bears and Asiatic black bears in camera-trap photographs, based on our examination of hundreds of such photos. The characteristics used for telling these 2 species apart involve 4 body parts: the coat, ears, snout, and claws. Sloth bear coats appear very shaggy relative to Asiatic black bears, often giving the sloth bear's body a dome-shaped look or the appearance of 2 humps. Also, due to the shaggy coat, sloth bears in the wild—and in captivity for that matter—often have debris such as leaves, seeds, and dirt stuck in the fur. This debris is often very noticeable in camera-trap photographs, and if present, is a definitive characteristic of this species.

Due to the sloth bear's shaggy coat, the ears often appear quite small and can be lost in the upright fur around the head. In stark contrast, the ears of an Asiatic black bear are typically prominent on the head and appear large and round (like that of "Mickey Mouse") in many photographs. The sloth bear's snout tends to be creamy white and longer than that of an Asiatic black bear, whose snout is usually dark brown or black. Finally, the sloth bear's claws are whiter and larger than those of an Asiatic black bear.

**Distinguishing characteristics of sloth bears and Asiatic black bears trait**

| | Sloth Bear | Asiatic Black Bear |
|---|---|---|
| |  |  |
| Coat | 1. Shaggy, unkempt appearance<br>2. Dome or humped shape<br>3. Debris in fur | 1. Glossy, groomed appearance<br>2. Not dome shaped<br>3. Generally clean fur |

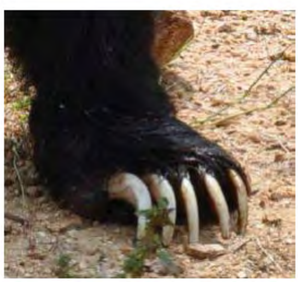
Ears

Appear small: lost in the shagginess around the head.

Typically, prominent, large and round.

Snout

Claws



Long and whitish

Short and usually dark

Invariably, some camera-trap photographs of these 2 species will remain difficult to distinguish. We recommend working through each of the characteristics, and then consulting with others if identification is not certain. We have done this among ourselves, highlighting our interpretations and the rationale behind them. We have come across a number of mistaken identities in photos sent to us by others, which is what motivated us to provide these guidelines. Notably, these guidelines can be useful not only for camera-trap photos, but also for distinguishing sightings or hand-held photos of these 2 looks- alike species.

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# Art – 128. IN-SITU REHABILITATION AND MANAGEMENT OF RESCUED CAPTIVE ELEPHANTS

**Gochalan E, Yaduraj Khadpekar, Ilayaraja S, Arun A. Sha, Baijuraj M. V, Akshay Mohan, Kartick Satyanarayan and Geeta Seshamani**

**Abstract**

The Asian elephants *(Elephas maximus)* is an endangered species throughout its range. It is threatened on account of pressures of poaching for ivory, loss of habitat and ever-increasing incidents of human-elephant conflicts. In February 1992, government of India launched Project Elephant- a major initiative for elephant conservation. However, until recently, domesticated elephants have not received due attention from the conservationists. As a result, many pachyderms have been suffering under captivity due to various management and health issues such as malnutrition, stereotypies, joint disorders, metabolic disorders, foot issues and various infections.

Even after rescue, such elephants from captivity are difficult to rehabilitate or released in the wild. This situation demanded the availability of – In-situ elephant rehabilitation, treatment and conservation facility. Wildlife SOS, a charity wildlife conservation and welfare organization in India, thus took an initiative in 2010 and established 2 captive elephant rehabilitation centres in India for providing proper and scientific long term veterinary care and management to such elephants, and establishing humane methods of elephant husbandry by providing proper training to the elephant keepers (Mahouts). In this presentation, we discuss the process of rehabilitation of rescued captive elephants and the Mahouts training at these centres.

# Art–129. CAPTIVE ASIATIC ELEPHANT *(Elephas maximus)* RESCUES BY WILDLIFE SOS: BACKGROUND AND CHALLENGES

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

## Abstract

Wildlife SOS, a charity wildlife conservation and welfare organization in India, runs two elephant rehabilitation centres in the country. Captive elephants 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 these centres. Between the two centres, total of 22 elephants have been rescued and rehabilitated so far. These include both males and females.

In India, rescue and transport of such elephants is a very complicated process. There are many factors involved such as financial, cultural, legal, political and veterinary, that can play a major role in making the rescues difficult or easy. Thus, almost every elephant rescue so far has been unique for the team of Wildlife SOS. Some of these rescue operations presented with the challenges that were quite difficult or unexpected and the team on the field had to think quickly to overcome them. We hereby explain the procedure for the rescue of elephants by Wildlife SOS and the various challenges faced during these rescues.

## Art – 130. LUMBAR SPONDYLOSIS IN A LIONESS

Nirupama Jaisingh, Sujay C.S, Ranganath L, **Arun A. Sha,**
Nikita and Manjunath V.

### Abstract

Lumbar spondylosis is a degeneration of the spinal column from any cause. In the narrower sense it refers to spinal osteoarthritis, the age-related wear and tear of the spinal column, which is the most common cause of spondylosis. The degenerative process in osteoarthritis chiefly affects the vertebral bodies, the neural foramina and the facet joints (facet syndrome). If severe, it may cause pressure on nerve roots with subsequent sensory or motor disturbances, such as pain, paraesthesia and muscle weakness in the limbs. One of such case was noticed in Bannerghatta Biological Park in one lioness aged 13 years which showed difficulty in bearing weight in hindlimb and reluctant to move. Animal's appetite, urination and defecation were normal. Animal was treated with inj meloxicam@ 0.5 mg/ kg bwt, inj neurobion 8 ml intramuscularly for two weeks and inj methyl prednisolone @ 30 mg/kg bwt epidural weekly once. Consecutive week animal showed arching of back, stiffness of tail, bending towards front and standing only for a minute. Animal was subjected for radiography and blood examination. Lateral radiography of lumbar vertebrae was taken using the factor 69 kv and 24 mA revealed spur formation and fusion of L1L2, L2L3, L3U leading to pressure on the nerve. Blood report was in the normal range. Animal was diagnosed with lumbar spondylosis deformans. Lioness has been treated palliative with infra-red therapy, painkiller and neurobion injections.

# Art – 131. WILDLIFE HUMAN CONFLICT – A VET PERSPECTIVE

**Arun A Sha and Ilayaraja S**

## Introduction

I strongly feel that this article is very eco-friendly, because the words used here are recycled. Human wildlife conflict is not a new area to discuss about but still our level of understanding towards the problem is inadequate. Human-wildlife conflicts have been occurring throughout prehistory and history. The history of human wildlife conflict was recorded in many fossils.

Human-wildlife conflict refers to the interaction between wild animals and people and the resultant negative impact on people and/or their resources, or wild animals and/or their habitat. The said negative impact may disturb the social, physical, cultural, psychological or economic life of humans, the conservation of wildlife populations, or the environment. The reasons are very simple, reduction or loss of natural habitat, exponential human population, demand on resources, encroachments, development projects and poor understanding of the ecology and behaviour of wildlife. Basically, when an animal or a population is at risk of shortage of food, water, habitat, health issues and disturbances, it ended up in one or other way of human wildlife conflict. In other ways, it can be simply defined as a severe mismatch between the goals of humans' vs the needs of wildlife.

In a broader sense, it is an exploitation of natural resources that would cause human wildlife conflict especially when humans attempt to harvest natural resources such as firewood, plants,leaves, honey, fish, bush meats and grassland pasture leads to disturbance or break in food chain or food web. The immunity levels/vulnerability in wild animals to various diseases when compared to domestic animals is different. Hence, spread of disease from wild animals to the domestic livestock as well as vice versa is quite possible. E.g. KFD, FMD, rabies etc. One of the initiators of the concept of man-animal conflict was Das and Guha. They described the two-sided impacts of this conflict. From one side, the source of conflict is the restriction on the local people to access forest resources. On the other side, the source of conflict is the damage incurred to them by wild animals. In India, we face conflict with elephants, leopards, bears, vultures, peacocks, nilgai, olive ridley turtles, wild boars, tigers, reptiles etc. depending on the region and their distribution status.

From 1990 to 2000 (145,000 hectares/year), FAO finds India was the fifth largest gainer in forest coverage in the world; while from 2000 to 2010, FAO considers India as the third largest gainer (304,000 hectares/year) in forest coverage. However, India has lost 367 square kilometres of forest cover in the past two years. According to the India State of Forest Report, 2011, released by the Forest Survey of India (FSI) on February 7, the total forest cover in the country is now at 6,92,027 sq km. These account for 21.05 % of the total geographical area of India. Wildlife corridors that allow seasonal movement of wild animals are also being cut-off by roads, railway tracks and border fencing.

For E.g. between 2010-11 and 2013-14, Karnataka has lost around 5 tigers, 17 leopards and 62 elephants to man-animal conflict while almost 129 people have died during the same conflict due to conflict issues. Between 2000 and 2015, Uttarakhand has lost almost 90 tigers, 800 leopards and 280 elephants to man-animal conflict while almost 400 people have died during the same conflict due to conflict issues.

**Outcomes of conflict**

Human-wildlife conflict causes various negative results. People lose their crops, livestock, pets, property, and sometimes their lives. The animals, many of which are already threatened or endangered, are often killed in retaliation or to 'prevent' future conflicts.

I. The major outcomes of human-wildlife conflict are:

- Injury and loss of life to humans, domestic animals and wildlife.
- Crop damage, livestock and pet depredation, predation of managed wildlife stock
- Damage to human property, trophic cascades, destruction of habitat.
- Collapse of wildlife populations and reduction of geographic ranges.

II. Dos and don'ts in case of wildlife straying into human dominated area

- Keep calm and do not panic, allow the animal enough open space to escape back to its habitat.
- Do not stone, scare, chase or create an unfriendly or stressful environment for the animal; do not corner the animal by igniting a fire or by gathering a crowd.
- Have a backup plan in case of one strategy to capture the animal fails.

III. Conflict management strategies

The need of the hour is to establish sustainable long-term strategies to mitigate human-wildlife conflict, based on scientific fact rather than emotional conjecture. To achieve this, many different strategies are employed depending on the availability of resources, scientific knowledge about the species in question, region and knowledge about the individual that has been identified as a cause.

Conflict management strategies earlier comprised of lethal control, translocation, regulation of population size and preservation of endangered species. Recent management approaches attempt to use scientific research for better management outcomes, such as behaviour modification and reduced interaction. As human-wildlife conflicts inflict direct, indirect and opportunity costs on society. The mitigation of human-wildlife conflict is an important issue in the management of biodiversity and protected areas.

Management techniques for wildlife are of two types. The first type includes the traditional techniques which aim to stop, reduce or minimize conflict by controlling animal populations in different ways. The second type that are less costly in terms of life, include translocation, regulation and preservation of animal populations etc.

<u>The following are a few conflict management strategies:</u>

V. Cleanliness

a. Maintain a clean environment. Do not throw edible waste around.

b. Less dependence on forest produce

Depleting the forest natural resources causes food scarcity for the wildlife inside the forests

c. Payment for environmental services

This concept relates to the financial compensation that is paid to compensate for the exploitation of natural resources for developmental activities. Such funds can then be used for conservation measures. E.g. CAMPA funds.

d. Eco-tourism, community-based natural resource management Successful eco-tourism ventures that are managed or staffed by the local community encourage the conservation of wildlife due to the tangible economic benefit that it holds for the villagers.

e. Compensation/ Insurance

Compensation against crop or livestock loss and death or injury due to man-animal conflict is an important measure that could ease the loss of humans affected. All efforts must be made to encourage insurance companies to offer viable policies to vulnerable population to at least financially protect against man animal conflict. Land-use planning. Shrinking habitats due to ever increasing human population is one of the main reasons for conflict, hence proper planning and clear demarcation of forest land is very important. Similarly, land must also be demarcated in agricultural use, for industrial and infrastructure use including mines and quarries as well as provision to expand without encroaching on forest land. Cropping patterns must also be planned keeping in mind factors such as water table, soil fertility, economic value. Corridors connecting different forested belts must be preserved and where large tracts of native forest are disconnected, efforts must be made to setup corridors that can guide animals safely from one to another.

f. Barriers

Barriers such as moats, trenches, metal railing and power fencing may be useful in controlling straying of wild animals into human habitation. These fences are now being further fortified with bee colonies, oil mixed with chilli powder, tobacco etc to deter animals more effectively.

g. Disguise

In an interesting experiment in a village in India, it was found that over a 3 year period, people who wear a mask on the back of the head suffered no attacks while 29 people who did not wear any such mask had been attacked.

h. Global positioning system

Animals such as elephants could be radio-collared and their movements can be tracked via GPS. The collar can be configured to send out an SMS to the forest department if the elephant strays outside a predefined area.

V. Rapid action force and Rehab centres

The creation of a rapid action force for every district/state consisting of trained veterinarians, para-vets and volunteers to help with crowd control would greatly help in providing timely assistance with minimal damage to man or animal. Adequately equipped rescue and rehabilitation centres to treat injured animals should also be established.

j. Awareness

It is important to generate awareness about not just the significance of wildlife and the environment but also about the steps to be taken when one comes into contact with wildlife. Such awareness programs would cover concepts like minimum distance to be maintained from such an animal, whether an animal is nocturnal or diurnal, emergency first aid in case of animal attack, safe water points for domestic chores, to avoid traveling at night through forests, food web and food chain etc. can be very helpful.

The declaration of one week or day every year as Man-Animal conflict week/day could emphasize the significance of the issue and can be used as an opportunity to concentrate on the issue and spreading the message of peaceful coexistence between man-wildlife.

VI. Environmental Impact Assessment (EIA)

EIAs of any proposed developmental project must be made in an honest manner and the results of the assessment must be given serious consideration and the whole exercise must not be treated like a mere formality.

m. Subsidized cattle fodder

Providing cattle fodder at a reasonable rate or for free for villages at the fringes of forests in the drier areas/ drier seasons of the country. This ensures that domestic cattle don’t graze in forest meadows- reducing human- wildlife and livestock-wildlife conflict.

VII. The role of vets in conflict mitigation

The proper veterinary aid is a major part of conflict situation management.

1. Response time

The team involved in any kind of human- animal conflict mitigation/ rescue operation of a wild animal in distress should always be prepared and react immediately to any such situation, without much delay in the process.

2. Manpower mobilization

At any conflict situation, the rescue team should work along with the forest department staff or any other volunteer rescue team in a joint effort to rescue the animal in distress. Any additional help can be sort safely by the general public in terms of carrying cages etc.

3. Understanding the situation

Assessing the conflict situation in order to design a plan of action is of utmost importance as soon as the team reaches the spot, failure of which could be detrimental to the safety of the animal in distress or the team involved or the local public.

4. Knowledge about the problem animal

It is important to positively identify the species of animal involved, to know the general behaviour of the animal so as to plan the mitigation accordingly.

5. Preparation of rescue kit and checklist:

A rescue kit should be always prepared and ready-to- go along with the rescue team. All essentials including darting equipment's and accessories, nets, ladder, pliers, medical emergencies like tranquilizers, pain medication, antidotes, dressing materials, intravenous fluids, stethoscope, thermometer, blind fold, muzzle, vital monitors etc should be available in the kit. A detailed checklist of the same should be in the kit and prepared well in advance.

6. Selection of capture methods:

After the situation assessment, animal identification, plan of action, division of duties among the team, the animal will be captured either via chemical immobilization or physical immobilization.

7. Selection of suitable anaesthetics and dose:

Choice and dosage of anaesthetic depends on the species of animal, age, general health status of the animal such as extent of injury/ disease/ dehydration status and availability etc.

8. Treatment and release:

Once the animal is immobilized, a thorough physical examination of the animal should be conducted. In case the animal is found to be apparently healthy without any or much injury, it can be certified for release into a protected area. In case the animal is gravely injured or needs veterinary assistance, the animal can be quarantined, temporarily housed at a recognized centre/zoo and released at the earliest post recovery with due protocols.

9. Disease control: The vet involved in rescue operation should assess the health status and rule out the symptoms of any nfectious and communicable diseases to decide upon the release.

VIII. Rescue and rehabilitation

a. Capture techniques

Depending on the species in question, it is to be decided whether to adopt physical or chemical capture techniques. As we all know, physical capture may be possible for smaller mammals, reptiles, birds, turtles and tortoises. At the same time, if other land mammals such as elephants, big cats, bears, wild dogs, wild boars, deer and gaur with severe physical injuries and the extent of damage does not permit to use chemical immobilization procedure, physical capture may be a better option. Physical capture methods can be achieved using the following tools depending on the species. Each method has got its own pros and cons. Factors to be considered when using physical capture method include the health status, the stress, time limit etc.

b. Equipment used in physical capture techniques

- Trap cages/ barrel traps
- Boma technique
- Tongs/hooks
- Drop nets/Spread nets
- Snares/dog catchers
- Chute

c. Chemical capture

This can be achieved by using different varieties of immobilisation drugs that are delivered using distat projectile equipment such as Dan inject, Tele inject, Dist inject etc. The commonly used drugs are inj xylazine, ketamin, zoletil, medetomidine etc. The dose of different drugs would depend on the species involved and their respective body weights as well as its health status at the time of immobilisation etc.

d. Suitable vehicle and transportation method

After the successful capture of an animal, it is important to transport the rescued animal in an appropriate transportation cage according to the species and size of the animal. Care should be taken to address the safety and behavioural needs of the animal while selecting a vehicle. CZA has already published standards for the safe transportation of all different species.

e. General health examination before release

If the animal is fit to be released, it is mandatory to check the health status of the said animal by a qualified wildlife vet, adequate sample collection and check before releasing them back into wild. Necessary quarantine measures have to be followed in the event of soft release. In case of any permanent damage or irreparable physical damage to any rescued animal, it needs to be treated and housed in a lifetime rehabilitation centre.

IX. Vet's challenges in human animal conflict mitigation

Though there are very predominant managemental and administrative challenges exists in mitigating human wildlife conflict such as lack of conflict data, prioritization of developmental activities over environment protection, lack of understanding/awareness about avoidance behavior and safety precautions, financial constraints for implementation of mitigation measures, rehabilitation of villagers and tribals from protected area, the wildlife veterinarian faces different set of challenges altogether such as,

a. Lack of safety gears and protocol
b. Lack of crowd control management
c. Lack of availability of new generation anesthetics
d. Lack of adequate man power
e. Lack of logistics/ time limit
f. Incomplete information about problem animal
g. Lack of coordination among various government agencies
h. Pressure from media and local politicians
i. Lack of enforcement of laws

j. No dedicated release program addressing the above points will help the wildlife vet to better empower and handle any wildlife human conflict successfully.

X. Contribution of Wildlife SOS in mitigating man-animal conflict Sloth Bears

Wildlife SOS was founded to bring to an end the age-old exploitation of sloth bears (dancing bears) by the Kalandar community. This was put to an end by WSOS by rescuing the bears from the kalandars while simultaneously providing alternate livelihoods for these kalandars and promoting education amongst their children. The elimination of this practice has brought poaching of bear cubs to a negligible number.

**Leopards**

In Maharashatra, Wildlife SOS runs a rescue centre for leopards that have been injured in man-animal conflict. These leopards are often rescued from sugarcane fields where they have given birth to their cubs and feel threatened when harvest of the sugarcane begins and this leads to many cases of conflict with farmers. Sometimes, the mother leopard abandons its cubs due to stress and such cubs are left to fend for themselves.

**Habitat restoration**

In 2007, Wildlife SOS began reforestation efforts on 50 acres of barren land in Ramdurga district, Karnataka. This successful effort led to an increase in the water table and also provides a safe haven for wildlife including sloth bears and leopards. As a matter of fact, this project has led to resurgence in agriculture in nearby areas due to the increase in water table.

**Black bears**

In Kashmir, Asiatic black bears often stray onto fields and raid the crops causing serious loss to the farmers. These acts cause the farmers to act violently and have even burnt bears alive as retaliation.

Wildlife SOS has setup 2 rescue centres at Dachigam and Pehalgam to care for rescued bears and simultaneously conducts awareness programs that are aimed at the local communities. Wildlife helpline – Delhi – NCR, Agra, Bangalore

Wildlife SOS has setup a 24x7 helpline for the rescue of injured or stray "urban" wildlife. Injured wildlife are treated by our veterinarians and released if possible or housed at our rescued centres. Some of the animals that we have treated include elephants, tigers, leopards, civet cats, monitor lizards, snakes, kites etc.

**Acknowledgements**

I would like to thank the entire Wildlife SOS team especially our co-founders Kartick Satyanarayan and Geeta Seshamani and the various state forest departments for their dedicated efforts to protect our wildlife as well.

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**WILDLIFE SOS ELEPHANT HOSPITAL:** On November 16th 2018, Wildlife SOS opened India's very first Elephant Hospital. It's a historic milestone not only for the organisation, but more importantly for the injured and ailing elephants throughout India, who can now get the care and love they need in a state-of-the-art facility.


**FIELD OF DREAMS:** Field of Dreams was officially opened for the elephants in November, 2018. The land includes a scenic river, abundant green native foliage, and soft soil that's perfect for strolling.


# Art – 132. INCIDENCE OF ENTERIC SALMONELLOSIS AND ITS SUCCESSFUL MANAGEMENT IN CAPTIVE ASIATIC ELEPHANT *(Elephas maximus)*

**Ilayaraja S., Yaduraj Khadpekar, Gochalan Elango, A. Sha. Arun and Niharika Sharma**

## Abstract

An adult bull elephant aged around 55 years was rescued with severe dehydration, debility, hip abscess and foot lesions was housed at Elephant Conservation and Care Centre, Mathura which is managed by an NGO Wildlife SOS. The elephant started passing mucous coated foul-smelling faeces. However, the food intake and vital parameter were normal. Faecal examination did not reveal any parasitic load. So, the condition was treated with Sulphatrimethoprim and Bolus Ecotas orally for 7 days. However, the foul smell markedly reduced but the mucous still persisted. Later, the faecal consistency gradually changed from semisolid to diarrhoea leading to severe dehydration, debility and later; recumbency. The toenail abscess also got aggravated. Twenty litres of intravenous fluids were administered, which included amino acids, dextrose and multiple electrolytes through ear vein and oral administration of light kaolin and tab lopramide – 10 nos and the elephant was lifted with help of a crane. We avoided rectal rehydration, and the animal was assisted by the crane as the animal had repeated episodes of the same, 5 times in two weeks thus cause lacerate wound on all bony projection while it's falling down and trying to get up. Rest of the time within those two weeks, the elephant was reluctant to lie down for sleeping. Rectal swab and swab from toenail abscess

were taken for bacterial culture examination and antibiogram, which revealed abundant colonies of salmonella which were sensitive to amikacin. Salmonella infections have been associated with severe diarrhoea and stiffness in elephants. Enteric pathogens usually treated with amikacin, ceftiofur, or trimethoprim/ sulfamethoxazole. We administered 10 days course of inj. Amikacin @ 6 mg/kg body weight intravenously and provided coconut, vitamin D3, E and omega 3 fatty acid supplement orally. From the seventh day of treatment the condition of the elephant was improved. The animal started lying down on its own and getting up without any difficulties.

# Art – 133. SPATIAL VARIATION IN LEPTOSPIRAL SEROVARS IN SLOTH BEAR SERA SAMPLES IN INDIA

T. Sabarinath, Karikalan M, **Ilayaraja S., Arun A. Sha,** Surnj Nair, Chandra Mohan S., R.K. Agarwal and A.K.Sharma

## Abstract

Leptospirosis is an important disease of the tropics caused by Spirochaetes belonging to pathogenic species L interrogans, which has been a bane to wildlife in India. A total of 76 sera samples were screened for leptospirosis by Microscopic Agglutination Test (MAT). 56 sera samples were received from Bear Rescue Centre (BRC), Agra and 20 sera samples have been received from BRC, Bannerghatta, Karnataka. Out of these 32 sera samples tested positive for various serovars of leptospira, 8 sera were seropositive for multiple serovars. Pyrogenes was found to be the predominant serovar present in BRC, Agra with 17 seropositive cases. However, none of the sloth bear sera tested in Bannerghatta, Karnataka were positive for serovar Pyrogenes. Similarly, a total of 3 sera samples from BRC, Agra showed agglutinins against serovar Canicola whereas none of the sloth bear sera tested in BRC, Bannerghatta were positive for serovar Canicola. In contrast, MAT results of sloth bear sera tested in Bannerghatta revealed Icterohaemorrhagiae to be the predominant serovar with 11 MAT positive cases followed by Grippotyphosa with 3 MAT positive cases as against 3 and 1 sera samples tested MAT positive for Icterohaemorrhagiae and Grippotyphosa respectively in BRC, Agra. Thus, our study clearly suggests differences in geographic distribution of serovars between agro-climatic zones (Land locked Northern India and peninsular Southern India) which indicate spatial variation in presence of

leptospiral serovars in sloth bear sera samples in India. The application of the present study includes obtaining information on apposite leptospiral serovars pertinent to different agro-climatic zones for developing future vaccination strategies against leptospirosis in sloth bear.

## Art–134. CHOLANGIOCELLULAR CARCINOMA CONCURRENT WITH TUBERCULOSIS IN INDIAN SLOTH BEAR

M. Karikalan, **S. Ilayaraja, Arun A. Sha,** S. Chandra Mohan, R. Singh, Rishendra Verma and A.K. Sharma

### Abstract

The present case report describes an important case of cholangiocellular carcinoma associated with tuberculosis in Sloth bear, which was rescued and housed at Bear Rescue Centre, Agra. Fifteen years old adult male Sloth bear *(Melursus ursinus)* suddenly fell sick and clinically showed yellowish mucous membranes, high fever, poor appetite, enlarged peripheral lymph nodes, respiratory distress and increased size of the abdomen. Serum biochemistry results showed compromised liver and kidney functions. For few days, the animal exhibited decreased body weight, anorexia, emaciation and it died after a week of the onset of clinical symptoms. On systemic post-mortem examination approximately litres of sero-sanguinous fluid could be observed in the abdominal cavity. The liver was moderately enlarged with variable sized pale areas on the sub capsular surface extending deep into the parenchyma. The mesenteric lymph nodes were enlarged and some of them were hard to cut. Similarly, the lungs and the mediastinal lymph nodes revealed nodular lesions. Representative tissue samples were collected for the laboratory examinations. The impression smears taken from liver, lung and lymph nodes were found positive for the acid fast bacilli by ZN staining. On histopathological examination,

lungs sections showed diffuse granulomas, which contained macrophages and fibrous tissue proliferation. The granulomatous lesions revealed only proliferation of macrophages with lymphocytes. The bronchi and bronchioles had increased mucous secreting cells and the lumen contained mucocellular material. The sections of spleen, liver, kidneys, adrenals and mediastinal lymph nodes showed multiple tiny granulomas consisting of macrophages. The duplicate sections of the lungs, liver, kidney, spleen, mediastinal lymph nodes and adrenal glands revealed the presence of acid fast bacilli by staining. The lungs and the mediastinal lymph nodes were found positive for Mycobacterium tuberculosis by multiplex PCR using primers for 12.7-kb fragment of ORF region. The mycobacterial antigen was shown in lung, liver, adrenal and lymph nodes by immunohistochemistry using MPT-64 polyclonal antibodies. Along with the tuberculous lesions, the liver parenchyma also revealed both lobular and interlobular variably dilated irregular acinar structures exhibiting atypia, karyomegaly, nuclear hyperchromatism and presence of numerous mitotic figures. The neoplastic cells were exfoliated and clumping of the lumen was observed. The metastatic lesions were also observed in the mesentric lymph nodes. The neoplastic cells showed intense PCNA nuclear staining by immunohistochemistry. Based on these observations, the case as diagnosed as cholangiocellular carcinoma concurrent with tuberculosis.

# Art – 135. ACOUSTIC NEUROMA IN CAPTIVE LEOPARD *(Panthera pardus)*: A CASE STUDY

Nirupama Jaisingh, Ansar Kamran, Vaseem Mirza, **Arun A Sha** and Manjunath V

## Abstract

Acoustic neuroma is non-cancerous growths that develop on the 8th cranial nerve which connects the internal with brain. Cranial nerve help in transmitting the sound and equilibrium information from the internal ear to brain. It is also called as vestibular schwannomas/neurolemmomas, which grow slowly. One such case was noticed in a four year old female leopard weighing 45 kg. Animal had a history of fall from the tree and showed clinical signs like dullness, incoordination and an area of depression in the temporal region due to the atrophy of the right massator and temporal muscles of right face. Animal was subjected to radiography, which couldn’t reveal any abnormalities. After a week animal showed weight loss, sunken right eyeball, drooping of right eyelid with prolapse of third eyelid, sagging of right lip and anorexia. Blood examination revealed increase total leukocyte count. Animal was treated with inj. dicrysticin 5 ml i/m for seven days, Inj. Beplex 4 ml I/m, inj. neurobion 4 ml i/m for two weeks. Animal showed improvement in condition however, relapse of same clinical signs were observed after two months. Animal was subjected to MRI, which revealed a well marginated mass measuring 3.7x2.9x3.4 cm in right C.P. angle continuing with thickened right 7th and 8th nerve

complex suggestive of acoustic neuroma. The animal exhibited signs of circling, bending of head towards right and hearing impairment. The case was diagnosed as a case of acoustic neuroma.

## Art – 136. SURGICAL MANAGEMENT OF WOUND IN RESCUED WILD LEOPARDS.

Nirupama Jaisingh, Vassem Mirza, P.A. Kalignan, Sanath Krishna, L. Ranganath, and **Arun A. Sha**

### Abstract

Leopard *(Panthera pardus)* is one of the five big cats in the genus *Panthera*. It is a member of the family *Felidae* with wide range in Sahara Africa and parts of Asia. Leopards have the ability to adapt to various habitats ranging from the rainforest to steppe. Due to increase in the urbanization, the habitat of wild animals is getting fragmented and destroyed, which is leading to increase in the frequency of man- leopard conflicts. During the conflict situations either the man or the leopard gets injured. There were three leopards which were rescued in different year with wound on the body.

Case 1: an adult female leopard rescued from Mysore was presented with the deep wound of the neck region. Animal was immobilized, wound was cleaned thoroughly with normal saline and povidine iodine muscles and skin was opposed. Animal was kept under antibiotic and analgesia for a week. Animal recovered uneventfully after 20 days.

Case 2: an adult male leopard rescued from Hassan was presented with lacerated wound around the thoracic cavity measuring 7 cm wide dorsally exposing the thoracic vertebrae and 2 cm wide ventrally. Animal was immobilized using xylazine and ketamine, radiography was taken which

couldn’t reveal any abnormalities. Wound was thoroughly cleaned with povidone iodine and normal saline. Wound was freshened using BP blade ventral area was sutured using monofilament polyamide 1 and treated as open wound. Animal was kept under antibiotic and analgesia for a week. Wound was cleaned and dressed daily. Wound healed in a month.

Case 3: An adult male rescued from Kaglipura was presented with a wound on the left paw. Radiography of paw revealed fracture of the left phalange. After a week animal showed self-mutilation and necrosis of same limb. Animal was subjected to amputation of limb as per standard surgical procedure. Animal was kept under antibiotic and analgesic for a week. Animal recovered uneventfully after a month.

# Art – 137. IMPORTANCE OF RADIOGRAPHY IN DIAGNOSIS OF FOOT ISSUES FOR CAPTIVE ASIATIC ELEPHANTS *(Elephas maximus)*

**Ilayaraja S, Akshay Mohan, Gochalan E, Yaduraj Khadpekar** and **Arun A. Sha**

## Abstract

Elephants are the largest land mammals. In captivity the health of elephants is mainly influenced by factors such as proper nutrition, housing management, timely veterinary care and routine exercise. But most of the time in captivity the above said factors were ignored by the elephant owners thus resulted in different kinds of health issues. So, to address this issue, Wildlife SOS, an NGO started Elephant Conservation and Care Centre, in collaboration with Uttar Pradesh Forest department and rehabilitated more than 20 elephants under our care. Many rescued elephants have multiple health issues including, multiple abscesses and blindness etc. Abscesses of feet and nail art common due to poor upkeep. Many also suffer from hygromas and abscesses over the body. Radiography of feet identifies the extent of abscess, damage to the phalanges and surrounding tissues in case of foot abscess. We performed the radiographic examination of 20 elephants' foot in the skyline view and recorded the different stage of P3 and P2 osteolysis due to abscess condition. Hence, radiography acts as an important diagnostic tool in elephant foot health. In this article we have discussed the same in detail and how we conditioned the elephant, radiograph factors, positioning and challenges to implement the radiographic examination.

## Art – 138. VERTICAL EAR CANAL ABLATION IN A ROYAL BENGAL TIGER

Nirupama Jaisingh, Vaseem Mirza, **Arun A Sha** & Manjunath V.

### Abstract

Otitis is an infection or inflammation of ear. Otitis can affect the inner or outer parts of the ear. The condition is classified according to whether it occurs suddenly and for a short time (acute) or repeatedly over a long period of time (chronic). Vertical canal ablation is used to salvage a functional horizontal canal when vertical canal is severely diseased in otitis. A 15-year-old Bengal Tiger *(Panthera tigris)* weighing 150 kg was observed with head tilting, dullness and anorexia. Physical examination of head revealed pus and maggots in right ear with foul smell suggestive of otitis. Animal was treated for the same continuously for three weeks and no improvement was observed. As a definitive treatment for otitis, animal was subjected for surgery. Animal was anesthetized using xylazine @1 mg/kg bwt and ketamine @3mg/kg bwt intravenously and maintenance with ketamine. Surgical site was shaved, cleaned and prepared for vertical ear canal ablation. Skin was incised in T shape and flapped to expose the vertical canal. Vertical canal was excised till the point of horizontal ear canal opening without damaging the major blood vessels. Skin was suture by simple interrupted method using monofilament polyamide 0 leaving one drainage point near the horizontal canal opening.

Post operatively animal was administered with inj. ceftriaxone @ 30 mg/kg bwt. Intravenously, inj meloxicam @ 0.5 mg/kg bwt subcutaneously and fluid therapy for seven days. Wound was dressed with povidone iodine powder and topicure spray topically every day. Wound was completely healed by 40 days.

**Art – 139. CAVERNOUS HAEMANGIOMA IN A RESCUED WILD BENGAL TIGER**

**Arun A Sha,** Nirupama Jaisingh, **Harikirishnan,**
Vaseem Mirza, **Simon** and Manjunath V.

## Abstract

Cavernous haemangioma is a type of blood vessel malformation or hemangioma where a collection of dilated blood vessels forms benign tumours. Because of this malformation blood flow through the cavity is slow. The cells that form the vessels do not form the necessary junction with surrounding cells. A rescued wild Bengal tiger *(Panthera tigris)* age around 6 years, weighing 200kg body weight had swelling in the right elbow. On physical examination, swelling was found to be soft, fluid filled and causing no pain to the animal. On aspiration serosanguinous fluid was found. There was no increase in size or maturity of the swelling when observed for a week. Animal was anesthetized using xylazine @ 1 mg/kg bwt. And ketamine @2 mg/kg bwt. For close examination of the elbow swelling. Internal examination of swelling after 1 inch of incision revealed multiple cavities and papillae like growth filled with blood. Sample was collected for histopathology and antibiotic sensitivity test. Cavity was cleaned thoroughly with povidine iodine, normal saline, metranidazole and packed with gama benzene ointment and dicrysteine powder gauge. Animal was administered with inj meloxicam@ 0.3 mg / kg bwt. for a week. Histopathology sample revealed stratified squamous epithelial lining suggestive of bengin vascular

lesion – cavernous haemangioma. Cavity was cleaned with povidine iodine, normal saline and calendula ointment applied every day for 15 days. Wound healed by 20 days. Swelling reduced by three months. Keeper was advised to leave the animal in the felid and keep less time in concert floor.

# Art – 140. CALCANEAL FRACTURE OF RIGHT HIND LIMB AND ITS SUCCESSFUL MANAGEMENT IN A CAPTIVE ASIATIC BULL ELEPHANT *(Elephas maximus)*

**Ilayaraja S, Yaduraj K, Gochalan E** and **Arun A. Sha**


## Abstract

The cases of the fractures, especially involving the bones of the legs, in the elephants are difficult to treat and need intensive therapy and management to avoid further development of severe rheumatic disease. However, the fractures do occur due to various reasons such as accidents, fights or secondary fractures due to other injuries and the veterinarians need to tend to them. Here, we present a case of a fracture in a calcaneus bone in an adult bull elephant that was caused by hitting a metal barrier during musth. The fracture was treated using an efficient combination of allopathic treatments backed by laboratory investigations and aurvedic medications. The fracture was observed to heal completely, and the elephant started walking normally on 65th day after commencement of the treatment. Keywords: Avulsion fracture, aurvedic medications, calcaneus bone, elephant *(Elephas maximus).*

## Introduction

The elephants are the largest living mammals in the land. The elephant’s limbs and articulation were designed in such a way to bear the heavy body weight and providing better locomotion. Long bones of elephant’s doesn’t have any bone marrow cavity but filled with cancellous bone or red marrow [3]. Any problem to limbs will cause severe impact on health of these mega herbivores.

Trauma is the potential cause of morbidity and mortality in young elephant. However, limb fractures are unusual in elephant as it has very thick layer of muscles and tissue around the bones; heavy automobile accident, train hits and uncontrollable aggression of musth elephants will remains the cause for the same. Radiography is the most important imaging modality used in musculo skeletal evaluation of elephant especially its useful for the detection of chronic changes in bones or joints of elephants [7, 9, 14]. Elephants are semi-digitigrade in the front feet and semi-plantigrade in hind feet. The metacarpal and metatarsal bones of the foot maintain a relative vertical angulation during weight bearing, but the phalanges compress the digital cushion and lie nearly horizontal when supporting the weight of the body [2]. The hind foot is smaller than the forefoot and has an oval shape, the tarsus consists of seven bones arranged in three rows [19]. Muscles, tendons, collateral ligaments, synovial sheaths, vascular supply and innervation are similar to those of other multi digit mammals [12]. There are several reports available about the elephant's bone fractures [4, 5, 8, 13, 17]. And sprain at carpal joint 14 but no such records were available for avulsion fracture of calcaneus bone and its managements. So author took effort to report this unique case in this article.

**Case history**

An adult bull elephant in elephant care and conservation centre of Wildlife SOS at Mathura presented with severe swelling in the right hind limb at ankle region with open wound, serosanguionus discharge and unable to bear the weight on the affected limb. The interaction with mahout revealed that 10 days before animal showed aggression and not cooperate for mahouts command and kicked the barrier made up of iron angles by its right hind legs as the animal was in the musth and mild swelling noticed in the affected limb after two days and oral non-steroidal anti-inflammatory medication was provided for 5 days but no improvement and mild fluid discharge noticed from skin ulcer at ankle region.

The animal was left in the enclosure without any disturbance to calm down. After a week the animal took for routine exercise walk, the animal walked little bit distance with normal gait after that animal was reluctant to walk by using its right hind limb and limping increased followed by swelling and more pus discharge from the wound.

**Diagnosis and treatment**

On close examination of the affected limb revealed hot painful swelling due to septic abscess and more pus coming out while pressing that swelling. Pus sample were collected from the wound for bacterial culture examination and for Anti-Biotic Sensitivity Test (ABST). The radiographic examination with portable x ray unit with computerised radiography revealed oblique avulsion fracture in the calcaneus bone (fig1). As per the ABST result treatment started with antibiotics along with NSAID, Vitamine D3 and calcium supplements. Open wound was irrigated with pipracilin mixed normal saline solution and dressed with fly replant ointment topically. The fracture area covered with aurvedic oils (Murvuanna and Myaxyl) and cloth bandage having the paste of egg yolk with ayurvedic chooranam powder which were used to treat the fracture in humans in Kerala state (fig 2). The cloth bandage was changed in alternate days but the oils (Murvuanna and Myaxyl) applied daily on the bandage. The animal was kept in the soft mud floor without chaining and mud bed provided for comfortable resting to improve the healing 4,5. The treatment efficacy was monitor by periodical radiographic examination (fig3), evaluate the reduction in swelling by manually measuring the circumference of the ankle region (fig 4), observation of the animal’s range of motion, stride and stance while resting.The healing progress was more satisfactory and complete healing of fracture noticed on 56th day and animal started walking normally on 65$^{th}$ day.

**Fig 1: Elephant standing with swollen right hind limb, Unable to bear the weight and performing Radiographic examination through protected contact wall**


**Fig 2: Applying cloth bandage which was soaked in ayurevedic oils and chooranam mix with egg white on the affected limb just above the open wound.**

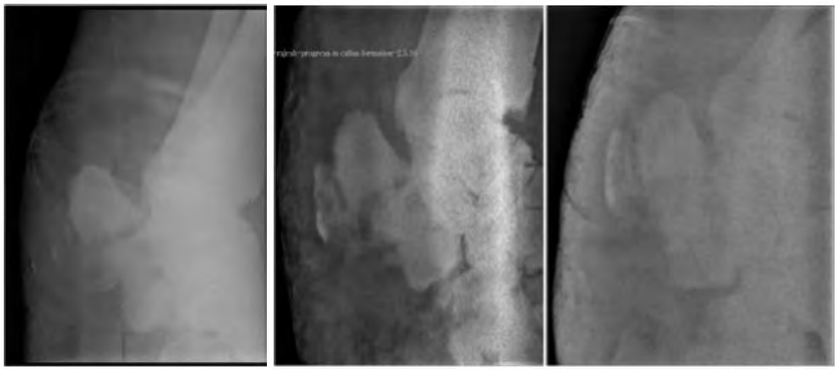

**Fig 3: Radiograph showing gradual healing of the fractured calcaneus bone**

**Fig 4: Gradual improvement in wound healing and reduction in swelling on affected area**

## Discussion

In this case kicking with heavy force on the strong iron barrier is the preliminary cause for this fracture. The secondary bacterial infection, waking and weight bearing caused further aggravation of this condition. An elephant may strike forward with a forelimb or toward the or rearward with a hindlimb [14]. An avulsion fracture is an injury to the bone in a location where a tendon or ligament attaches to the bone. When an avulsion fracture occurs, the tendon or ligament pulls off a piece of the bone. Therapy for traumatic injuries will depend on severity and location of the lesion. Acute inflammation associated with these injuries can be treated with NSAIDS [14] and antibiotic therapy should be based on culture results or broad- spectrum antibiotics should be used [1, 10, 11, 16, 18]. Generally, fracture were fixed by internal or external fixation, POP casting is commonly used for external fixation as it cheaper than the fibre glass casting. Though POP casting is commonly suggested for fracture below the knee and hock [6, 15], it is most suitable in case of large animal; we didn't use such casting because of the open infected wound above the fracture and considering daily dressing. The author experienced the combination of routine veterinary treatment along with the Ayurvedic medication was well accepted by the animal and revealed good and quick healing and no untoward effects were recorded.

**Conclusion**

Aggressive behaviour of this Bull elephant at the time of musth and kicking the barrier is the preliminary cause for the hairline crack on the calcaneus bone. The further weight bearing, movements and secondary bacterial infection on the affected area caused hindrance for the healing which lead to this complete avulsion fracture of this calcaneus bone.

**Acknowledgement**

We greatly appreciate the support and effort of Kartick Satyanarayan and Geeta Sheshamani, co-founders of the Wildlife S.O.S. We thank the elephant care staff and rescue team at Wildlife and Uttar Pradesh Forest Department for their kind co-operation. Our special thanks to Mr. Baijuraj MV, Director Conservation project, Wildlife SOS for organizing the ayurvedic medicines from Kerala.

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**ADVANCING BEAR CARE WORKSHOP:** An International Conference on Bears was hosted in Agra by Wildlife SOS with various International wildlife experts, veterinarians and delegates from over eleven countries. Through this conference the Bear Care Group and Wildlife SOS created and enhanced communication, cooperation and education among bear care professionals by sharing information about bear behavior, husbandry, enrichment, training and veterinary care to further the cause of global bear welfare and conservation.


**REFUSE TO RIDE CAMPAIGN:** Wildlife SOS launched an outdoor campaign to promote the message of Refuse To Ride an Elephant. For a period of one month, the Gatimaan Express train became an educational ride as the headrests on the seats were adorned with the message- "Joyrides on elephants is elephant abuse." The aim is to utilise the advertising space in Gatimaan Express to refrain tourists from riding elephants for their entertainment.

## Art – 141. ECHOCARDIOGRAPHY IN SLOTH *BEAR* *(Melursus ursinus)* – A PRELIMINARY APPROACH

**Ilayaraja Selvaraj, Arun A Sha** and **Puspendra K.Singh**

### Abstract

Echocardiography is the accepted term for the study of cardiac ultrasound. It is a technique by which we can do the imaging of the heart with the help of ultrasonography machine with a suitable probe. These techniques provide valuable diagnostic information without risk to the patient as it is a non-invasive procedure. The M-mode, two- dimensional (2D mode), and Doppler mode are maximum practiced techniques in echocardiography. Since there is no need of anaesthesia and special restraining procedure, the echocardiography is well developed and has become an integral part of cardiac evaluation of humans, small animal practices and reptile medicine especially in ophidian as well. But due to the huge body size and need of special restraining procedure with anaesthesia this technique is not established enough in wildlife practice particularly in sloth bear that belongs to *ursidae* family. The objective of this study is to develop the procedure for assessment of optimal approaches of echocardiography in sloth bears such as instrument selection, patient preparation and positioning etc and establish a cardiac image catalogue. This will help to identify the normal echo-anatomy of the heart and possible measurements B mode and M mode for further evaluation of the normal echocardial anatomy and colour flow Doppler to understand the normal blood inflow and outflow of heart.

**Keywords:** Echocardiography, Heart, Sloth bear, B-mode, M-mode, Doppler mode, Ultrasonography

## Introduction

The use of ultrasound in veterinary medicine is widespread as a diagnostic supplement in the clinical routine of small animals, but there are few reports in wild animals [6, 12]. It is a non-invasive approach for exploration of morphology, topographic anatomy, and biological process in Non-domestic animals [13]. This imaging technique is still underutilised in wildlife medicine due to the limited knowledge of the topography and ultrasound anatomy of their organs and also the need for anaesthesia with special restraining procedure [12]. According to individual anatomical structure in different vertebrate taxa; use of ultrasonography is combined with the characteristic feature for applying this imaging technique [13]. Advanced progress of imaging modality in veterinary sciences is being improved in ultrasound technology through the development of new types of probes for different applications such as cardiovascular, abdominal and intra cavity ultrasonography scanning.

Echocardiography is the accepted term for the study of cardiac ultrasound. Echocardiography uses sound waves in the order of frequency of greater than 20000 Hz. Piezoelectric crystals, in the form of a transducer, emit ultrasonic waves at high frequency when subjected to an alternating current. This same transducer receives the reflected waves and forwards them to be electronically processed and displayed for interpretation via one of three modes such as B-mode, M-mode and Doppler mode [3]. Echocardiography is a safe [1, 8], non-invasive method that provides quantitative information of cardiac wall thicknesses, internal cavity dimensions, valve motion, ventricular function and the presence or absence of intra cardiac structures [4, 5, 7]. The objective of this study is to develop the procedure for assessment of optimal approaches of echocardiography in sloth bears and establish a cardiac image catalogue for further feature study reference. Since the Agra bear rescue facility is a lifetime care and rehabilitation centre for the rescued dancing sloth bears which have come from poor health background [10], the initial and periodic health screening by adopting recent technique is more necessary to keep them in a comfortable healthy status and to establish preventive health care protocols.

## Materials and Method

Since ultrasound propagates poorly through gaseous or bony media, an area free of lung interface must therefore be determined to achieve a proper cardiac imaging. Proper patient preparation and positioning, examination table selection, selection of suitable ultrasonography machine with required probe/ transducer and knowledge of the sonographer regarding the different modes of ultrasonography along with basic organ anatomy and its topography within the body cavity (Fig.1) of the animal patient are major factors which influence the efficient echocardiographic examination in the desired animals.

## Patient preparation and positioning

For getting good cardiac image the patient needs to be stationary in position and the transducer needs to be in contact with the animal's skin as close as it can be without air pockets in between the transducer and skin. This can be achieved by chemical immobilization of the bear and clip or shave the hair on the intercostal region where we can recognise the apical beat then clean thoroughly eliminate the skin debris and dirt if any otherwise it may cause poor image quality by causing hindrance to the ultrasound beam penetration. The bear was tranquilized as per the standard protocol with Injection Xylazine @ 2 mg/kg and Injection Ketamine @ 5 mg/kg intramuscularly using blow pipe [11]. Both left and right side hemithorax region clipped and cleaned thoroughly and the coupling gel is applied on the scanning area 5 minutes prior to start the examination to allow the gel to soak the skin tissue and avoid air pockets. This will enhance the penetration of ultrasound beam and produce good quality image. The bear was kept in right lateral recumbency and the right forelimb gently pulled and kept cranially (Fig.2). The same was followed for left forelimb while positioning the bear in left lateral recumbency.

**Equipment selection**

Sloth bears have body weights ranging between of 60 – 120kg. The patient’s examination table needs to be selected accordingly to bear’s weight without compromising and also it shoulad possess the facility to reach the animal’s hemithorax region with the scanning probe without any difficulties to the operator and bending the probe cable as well. We specially designed the examination table for this procedure in such a way to full fill the above said requirements (Fig.3). Any ultrasound machine with cardiac package and transducer with small foot print can be used for Echochocardiographic studies as like in medical and small animal veterinary practices. We used LOGIQ e manufactured by GE healthcare company and Phased array transducer (model 3S-RS).

**Organ anatomy**

As like other mammalian species sloth bears also possess the well-developed 4 chambered heart with valves in the mediastinal cavity. The cardiovascular system has resemblance as close as to humans and canines, so we followed the echo cardiographic studies as described by the previous authors in mammalian species especially in dogs. We performed right parasternal long (Fig. 4) and short axis (Fig. 5), left apical parasternal location (Fig 6) views along with M mode (Fig.7) and Doppler mode (Fig.8 & 9) recorded the images [14].

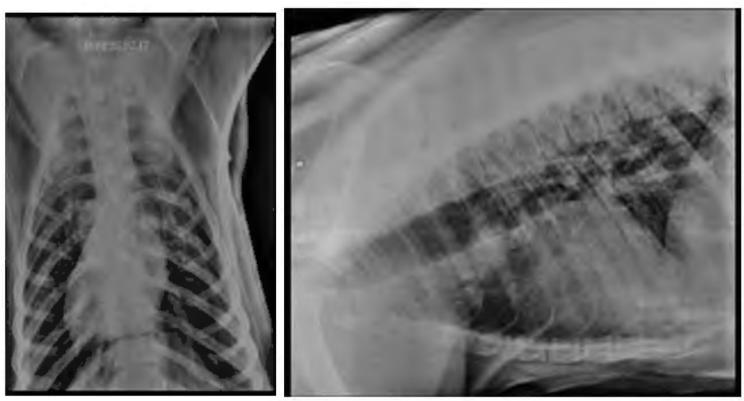

**Fig 1: Radiography showing location of heart inside the thoracic cavity of a normal Sloth bear.**

**Fig 2: The bear kept in right lateral recumbency and the right forelimb gently pulled and kept cranially on the examination table after clipped the hairy**

**Fig 3: Echocardiography examination table model.**

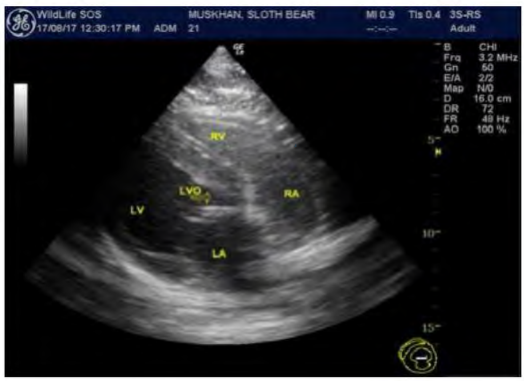


**Fig 4: Right parasternal long axis view**

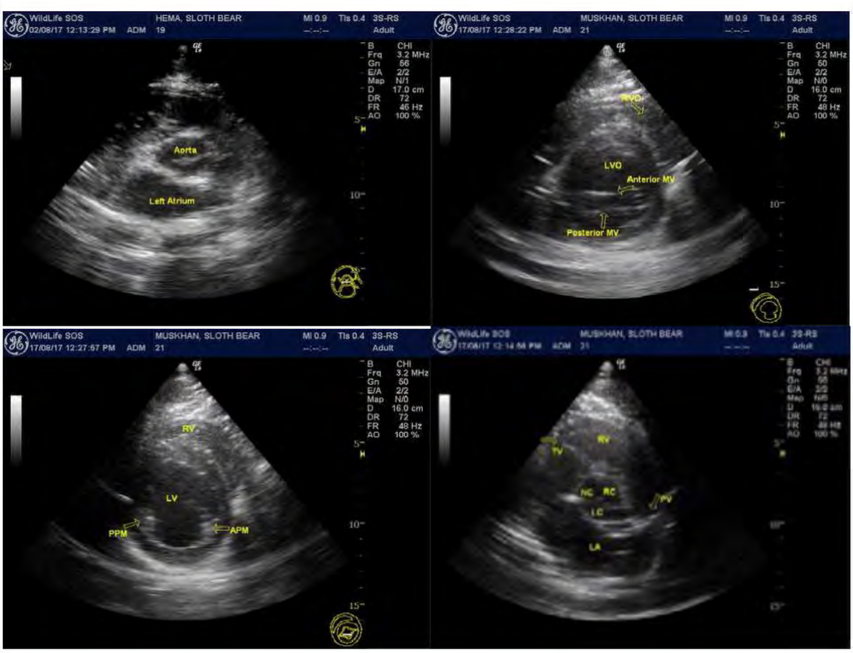


**Fig 5: Right parasternal short axis views**

**Fig 6: Left parasternal apical four & two chamber view**

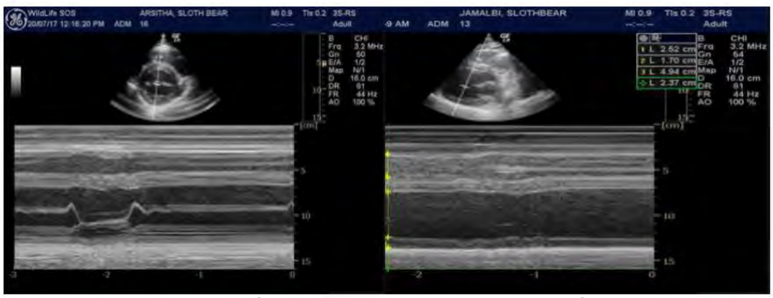

**Fig 7: M mode view for mitral valve activity & left ventricle**

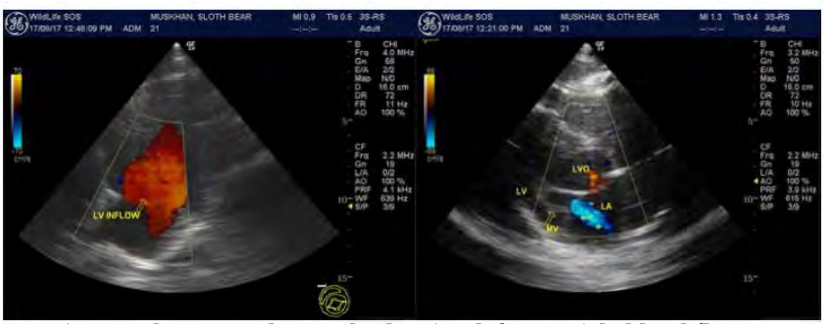

**Fig 8: Colour Doppler mode showing left ventricle blood flow**

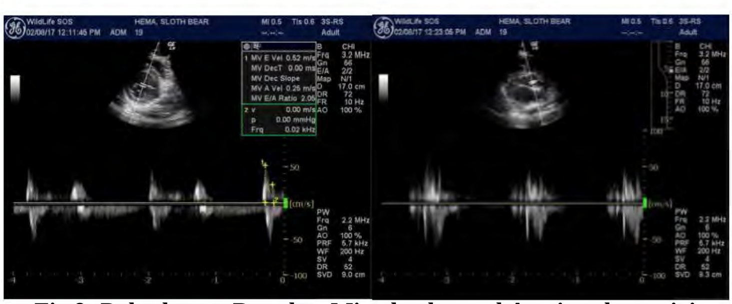

**Fig 9: Pulsed wave Doppler- Mitral valve and Aortic valve activity**

## Types of Echo Display Modes

There are three basic modes to consider in echocardiography for complete evaluation of heart anatomy and its functions effectively. B-mode or brightness is the electronic conversion of the A-mode and A-line information into brightness-modulated dots on a display screen. The brightness of the dot is proportional to the echo signal amplitude [9]. The B-mode display is used for M-mode and 2D gray-scale imaging. This gives good cross-sectional images of the heart and is useful to identify conditions such as pericardial effusion, chamber enlargement and valvular disease.

M-mode (M for motion) is a technique that uses B-mode information to display the echoes from a moving organ, such as the myocardium and valve leaflets, from a fixed transducer position and beam direction on the patient. The echo data from a single ultrasound beam passing through moving anatomy is acquired and displayed as a function of time, represented by reflector depth on the vertical axis (beam path direction) and time on the horizontal axis. Since M-mode can provide excellent temporal resolution of motion patterns, it is used to evaluate chamber size and contractility, as well as indices of cardiac function.

Doppler mode consists of four types of Doppler ultrasound, which rely on the change in frequency of returning echoes depending on whether flow is towards or away from the transducer. Pulsed wave Doppler (PWD) – 'gates' identifies the specific sampling area e.g. across the mitral valve. Unfortunately, PW cannot calculate high velocities due to a phenomenon called 'aliasing'. Continuous wave Doppler (CWD) – can identify high velocity but the exact location of the flow cannot be identified.

Colour flow Doppler (CFD) –provides an easy visual identification of flow direction and velocity by producing red and blue coloration [9]. It can be remembered as BART= blue away red towards. Colour Doppler is angle dependent so that no Doppler shift is recorded when blood flow is 90 degrees to the transducer. The combination of all three types of Doppler is used in practice as per the need or as a routine examination procedure. Power Doppler (PD) detects very-low-velocity blood flow and small blood vessels. It is not prone to 'aliasing' artifact as it is essentially angle independent [2,9].

## Result and Discussion

The right parasternal long axis is the best view for visualising left ventricle (LV) apex, mitral valve (MV), LV out flow tract (LV O), aortic valve (AV), proximal ascending aorta, left atrium and interventricular septum. The right parasternal short axis is the suitable view for LV apex, high papillary muscle level, chordae tendineae of the LV, mitral valve (MV), aortic valve, pulmonary arteries and pulmonic valve. Left parasternal apical long axis will help us to see the LV, MV, and left atrium (LA), with slight anticlockwise rotation the LV O can be seen. Left cranial parasternal location, short axis view at the level of the aortic root provides the right ventricular inflow and outflow tracts clearly. However, we followed the previous author's recommendation, we did necessary fine adjustments of transducer position and angulation and image plane orientation in sloth bears to obtain optimal cardiac images. Since the transducer movement and angulation is too sensitive, we can't achieve proper echocardiography in sloth bear without sedation. This image modality would be helpful to carryout efficient diagnosis of the cardiac problems such as pericardial effusion, dilated cardiomyopathy and degenerative valve disease efficiently in sloth bears.

## Conclusion

Since echocardiography is a non-invasive and risk-free technique and provides a wealth of data concerning cardiac morphology and function, it has to be an integral part of health examination in sloth bears and other captive wild animals as well. Adaptation of this procedure and images should facilitate consistent performance and provide basic information for further studies in future. It should always be borne in mind that echocardiography is a specialist area, requiring a thorough knowledge of cardiac anatomy and pathophysiology. Referral to a cardiologist should be considered if it is an option and is a useful way of learning.

## Acknowledgment

We are sincerely thanking to BCF Technology and Dr. Nicolette Hayward for the generous donation of cardiac probe. We appreciate the great active effort of Ms. Tracianna Morrell Myers., Australia for the donation of Ultrasonography machine. We are grateful to Mr. Kartick Satyanarayan & Geeta Sheshmani Co-Founders, Wildlife SOS for their tire less support and all our animal care staff of wildlife SOS, India.

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**CHHATTISGARH RADIO COLLARING PROJECT:** On September 22nd, 2018, a team of experts from Wildlife SOS and the Chhattisgarh Forest Department successfully radio collared the matriarch of a herd of ten elephants. Wildlife SOS is actively working with Chhattisgarh Forest Department and the local community to develop an early warning system (EWS) to mitigate human-elephant conflict. Putting satellite collars on the elephants will allow us to track the elephants' movements more effectively which in turn will help in predicting potential conflicts before they occur.

**MULTIPLE LEOPARD CUB RESCUES:** Ever expanding population and receding forest cover has forced wild animals to live in close proximity to the human habitations, leading to increased man-animal conflicts. Maintaining a 100% success rate, Wildlife SOS has carried out 10 rescue and reunions within the past year itself. Rescues like these are immensely important for us to know that these cubs will continue to be raised in the wild.


# Art – 142. METAGENOMIC INSIGHTS INTO THE RUMEN MICROBIAL FIBROLYTIC ENZYMES IN INDIAN CROSSBRED CATTLE FED FINGER MILLET STRAW

V. Lyju Jose, Thulasi Appoothy, Ravi P. More and **A Sha Arun**

## Abstract

The rumen is a unique natural habitat, exhibiting an unparalleled genetic resource of fibrolytic enzymes of microbial origin that degrade plant polysaccharides. The objectives of this study were to identify the principal plant cell wall- degrading enzymes and the taxonomic profile of rumen microbial communities that are associated with it. The cattle rumen microflora and the carbohydrate-active enzymes were functionally classified through a whole metagenomic sequencing approach. Analysis of the assembled sequences by the Carbohydrate-active enzyme analysis Toolkit identified the candidate genes encoding fibrolytic enzymes belonging to different classes of glycoside hydrolases (11,010 contigs), glycosyltransferases (6366 contigs), carbohydrate esterases (4945 contigs), carbohydrate- binding modules (1975 contigs), polysaccharide lyases (480 contigs), and auxiliary activities (115 contigs). Phylogenetic analysis of CAZyme encoding contigs revealed that a significant proportion of CAZymes were contributed by bacteria belonging to genera Prevotella, Bacteroides, Fibrobacter, Clostridium, and Ruminococcus. The results indicated that the cattle rumen microbiome and the CAZymes are highly complex, structurally similar but compositionally distinct from other ruminants. The unique characteristics of rumen microbiota and the enzymes produced by resident microbes provide opportunities to improve the feed conversion efficiency in ruminants and serve as a reservoir for cellulosic biofuel production.

**Keywords:**Rumen, Fibrolytic enzymes, Plant polysaccharides, Metagenomics, Microbiome

## Introduction

Ruminants represent a substantial proportion of domesticated animal species worldwide and are the main source of milk, meat, and other dairy products. Ruminants have the ability to digest large amounts of plant polysaccharides by virtue of the composite microflora present in the rumen. The rumen has evolved into an efficient and effective fermentation vat for fiber degradation and the rumen is inhabited by a consortium of microorganisms consisting of bacteria, archaea, fungi, protozoa, and viruses (Miron et al. 2001; Pope et al. 2012), which interact and contribute significantly towards the health of ruminants. Among the various domains of micro-organisms resident in the rumen, bacteria are predominant representing about 95% of the total microbes (Mackie et al. 2000; Lin et al. 1997). The rumen fermentation process mediated by microbial communities affects the quality and composition of milk and meat and the productive performance of the host (Welkie et al. 2010; Stevenson and Weimer 2007; Sundset et al. 2009).

In tropical countries like India, ruminants are primarily fed on lignocellulose based agricultural crop residues. The extensive rumen microbiota are endowed with the potential to hydrolyze the plant polymers into simpler forms that provides nutrients to the host, predominantly in the form of volatile fatty acids and microbial proteins. The rumen essentially functioning as an anaerobic fermenter, has the ability to absorb the digested plant polysaccharides by the resident microflora (Jami and Mizrahi 2012). Studies on the symbiotic rapport between the rumen microbial communities and the mammalian host have posed a challenging area of research for the scientific community in the past due to the lack of adequate techniques to investigate and analyse such complex ecosystem.

Rumen microbes produce an array of fibrolytic enzymes called Carbohydrate-Active Enzymes (CAZymes), including exoglucanases, endoglucanases, glucosidases, and hemicellulases to deal with the complex plant polysaccharides High Throughput Sequencing (HTS) technologies are extensively used to address the intricate process of lignocellulose degradation in ruminants. An improved understanding of the rumen microbial ecosystem could address the challenges in ruminant nutrition and environmental concerns in the Livestock sector.

Numerous metagenomic studies have reported on the diversity of fibrolytic enzymes from the rumen of yak (Dai et al. 2012), reindeer (Pope et al. 2012), Jersey cow (Wang et al. 2013), Angus cattle (Brule et al. 2009), and buffalo (Singh et al. 2014). However, there are no comprehensive scientific reports available on metagenomic studies on the rumen CAZymes profile of Holstein- Friesian crossbred cattle, fed only finger millet straw. Due to the paucity of information on the CAZymes profile in HF cross fed only finger millet straw (a common crop residue fed to ruminants in Karnataka, India), this study was undertaken with the key objectives of deciphering CAZymes diversity in HF cross cattle and to enumerate the composition of metabolically active, CAZyme-contributing microbiota that is involved in the hydrolysis of plant polysaccharides. A comparative analysis of the data obtained in our study and other published herbivore metagenomes was also performed to identify whether any unique CAZyme families exist in the HF cross rumen ecosystem.

## Materials and Methods

### Experimental design and rumen sampling

Three fistulated Holstein-Friesian crossbred steers with an average body weight of 380 ± 15 kg were selected and maintained in individual stands for the feeding experiment at the Experimental Livestock Unit (ELU) National Institute of Animal Nutrition and Physiology, Bangalore, India. The animals were fed with finger millet straw, offered twice daily for a period of 21 days, at maintenance ration (ICAR 2013). The rumen contents were collected from all three animals prior to morning feeding on the last day of the experiment. Approximately 50 ml of rumen digesta samples were collected through the rumen fistula and immediately transported to the laboratory for further processing. Rumen digesta samples were mixed and strained through two layers of muslin cloth and immediately flash frozen in liquid nitrogen. Both liquid and solid portions of rumen digesta samples were then stored at -86 ·c until further processing.

### Total DNA extraction from rumen digesta and quantification

The frozen rumen samples were thawed at room temperature and the solid rumen digesta samples were resuspended in phosphate buffered saline (Amresco, Solon, USA), for 2 h with vortexing to liberate the microbes adhering to feed particles and mixed with the rumen fluid sample. The rumen fluid samples were then centrifuged at 4000 rpm for 5 min and the supernatant obtained was used further for the DNA extraction. In brief, the rumen fluid was centrifuged at 14,000 rpm for 10 min and the pelleted cells were resuspended in a mix of 800 µ1 of CTAB lysis buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA and 100 mM Tris-HCl), (Amresco, Solon, USA) and 0.2 g of glass beads (0.l mm}, (Biospec products Inc, Bartlesville, USA) and kept in a Mini bead beater (Biospec products Inc, Bartlesville, USA) for 3 min. 10 µl of 20 mg/ml proteinase K (Amresco, Solon, USA) and 10 mg/ml lysozyme (Amresco, Solon, USA) were added to the above mixture and incubated at 37°C for 1 h. The tubes were then incubated at 70°C for 30 min with intermittent mixing. An equal volume of Phenol:Chloroform:Isoamyl alcohol (25:24:1) (Amresco, Solon, USA) was added to the above lysate and mixed by inverting until a thick milky white emulsion was formed. After centrifugation at 14,000 rpm for 10 min, the supernatant was transferred to a fresh tube and total DNA was precipitated using 0.3 volumes of chilled ethanol (Merck, Kirkland, Canada). The precipitated DNA was then washed twice with 70% ethanol and the pellet was finally dried using a vacuum concentrator (Concentrator 5301) (Eppendorf, Hamburg, Germany). The quality of extract edgenomic DNA was assessed by running it in 0.8% agarose gel electrophoresis for a single intact band, and A260/280 ratio was determined by Nanodrop 8000 (Thermo Fisher Scientific, Waltham, USA). Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, USA) was used to measure the quantity of DNA.

**Metagenome library preparation and sequencing**

The paired-end sequencing library was prepared using Illumina, Truseq Nano DNA LT Library Preparation Kit (Illumina, California, United States). Subsequently, 200 ng of genomic DNA was fragmented by Covaris (Covaris Inc, Massachusetts, USA) to generate a mean fragment distribution of 550 bp. The fragments were then subjected to end repair using end repair mix and indexing adapters were ligated to the ends of the DNA fragments. The ligated products were purified using SP beads supplied in the kit. The size-selected product was PCR amplified as described in the kit protocol. The amplified library was analyzed in Bioanalyzer 2100 (Agilent Technologies, California, USA) using a High Sensitivity DNA chip (Agilent Technologies, California, USA) as per the manufacturer's instructions. The library was then loaded onto the Illumina MiSeq platform for cluster generation and subjected to paired-end sequencing.

### Metagenome assembly and bioinformatic analysis

De novo assembly of high quality data was accomplished using the CLC Genomics workbench 6.0 (Qiagen, USA) at default parameters (minimum contig length: 200, automatic word size: yes, perform scaffolding: yes, mismatch cost: 2, insertion cost: 3, deletion cost: 3, length fraction: 0.5, similarity fraction: 0.8). Bioinformatic analysis of the metadata was performed with Metagenome Rapid Annotation using Subsystem Technology (MG-RAST) (Meyer et al. 2008) server. The quality of the uploaded sequences was checked using MG-RAST quality filters and the sequences, which failed QC, were removed from further analysis. The metadata were functionally categorized via an RPS-BLAST comparison with the Subsystem data- base, (Overbeek et al. 2014), and KEGG databases (Kane- hisa and Goto 2000).

### Carbohydrate-active enzyme annotation and taxonomic profiling

The fibrolytic gene encoding contigs from the metadata were identified and classified based on the carbohydrate- active enzymes database (Cantarel et al. 2009) (http:// www.cazy.org) by the carbohydrate-active enzyme analysis toolkit (CAT ) (Park et al. 2010) at an E value of 1 x 10-5. Putative plant cell wall polysaccharide-degrading enzymes belonging to different CAZy families were identified and classified based on sequence-based annotation. The CAZyme encoding contigs were analyzed manually for different classes of CAZymes: glycoside hydrolases (GHs), glycosyltransferases (GTs), carbohydrate esterases (Ces), carbohydrate-binding modules (CBMs), polysaccharide lyases (PLs), and auxiliary activities (Aas). The phylogenetic analysis of putative contigs encoding different CAZyme classes (24891 contigs) was performed in parallel to identify their microbial origin. The CAZyme encoding contigs from HF cross metagenome were uploaded on the MG-RAST server v 3.2 (Meyer et al. 2008) for phylogenetic analysis by the MSNR database using the BLASTX algorithm (Wilke et al. 2012) with a minimum identity of 60% and an E-value cut off of 1 x 10-5. The CAZymes obtained in the present study were compared with other accessible metagenomic datasets, cow rumen (Hess et al. 2011), jersey cow (Wang et al. 2013), reindeer (Pope et al. 2012), macropod (Pope et al. 2010), and termite gut (Warnecke et al. 2007).

## Results

### Metagenome sequence data statistics and phylogenetic abundance

The ultimate objective of our study was to elucidate the fibrolytic potential of the rumen microbial community in Indian crossbred (HF) cattle fed finger millet straw. The whole metagenome sequencing of the total DNA from cattle rumen digesta generated about 1.8 gigabases of raw sequences. De novo assembly of the raw sequencing reads after quality check (CLC Genomics Workbench 6.0) (Qia- gen, USA) resulted in 171,594 contigs with an average length of 838 bp. The statistical elements of the assemblies were calculated by in-house perl scripts and the metagenomic data analysis statistics are given in Table 1. Contig-7574 was the largest contig with a length of 25,731 bps. In order to validate the contig assembly, 16 contigs (≥600 hp) from the glycoside hydrolase family were randomly selected and primers were designed to amplify the target gene fragment (Additional file 1: Table S1). Fifteen of the sixteen contigs were successfully amplified using at least one set of the primers and the sequences showed >99% identity to the assembled contigs. The metagenomic data- set was uploaded on the MGRAST server for further bioinformatic analysis. Phylogenetic analysis of metagenomic data at the domain level revealed that 97.5% of sequences binned to bacteria, 1.3% to archaea, and 0.9% to eukaryota (Fig. 1). At the genus level, the most predominant genera were Prevotella, Bacteroides, Clostridium, Ruminococcus, and Parabacteroides, representing more than 47% of the total sequences (Additional file 1: Table S2). The functional annotation using the SEED subsystem (Overbeek et al. 2014) has identified 73,886 predicted functions, out of which 17.6% corresponded to clustering-based subsystems, 9.7% with protein metabolism, and 9.9% to carbohydrates (Additional file 1: Figure SI). The key metabolic pathways and abundance of enzymes in HF cross rumen metadata were predicted using KEGG mapper (http://www.genome. jp/138ab/) and KEGG database (Kanehisa and Goto 2000) (Additional file 1: Figure S2; Table S3).

**Table 1: Rumen metagenome data assembly analysis statistics by using in house Perl scripts**

| Parameters | Number of Sequences |
|---|---|
| Total number of bases uploaded | 14,77,49,531 |
| Total number of sequences uploaded | 1,71,594 |
| Mean sequence length bp uploaded | 838±481 |
| Mean GC count uploaded | 46±10% |
| Artificial duplicate reads | 14 |
| Number of sequences failed QC | 9310 |
| Total number of bases post QC | 12,58,33,189 |
| Total number of sequences post QC | 1,62,284 |
| Mean sequence length post QC | 775±234 |
| Mean GC count post QC | 45±10% |
| Predicted protein features | 2,01,967 |
| Predicted rRNA features | 244 |
| Identified protein features | 97,723 |
| Identified functional categories | 58,691 |

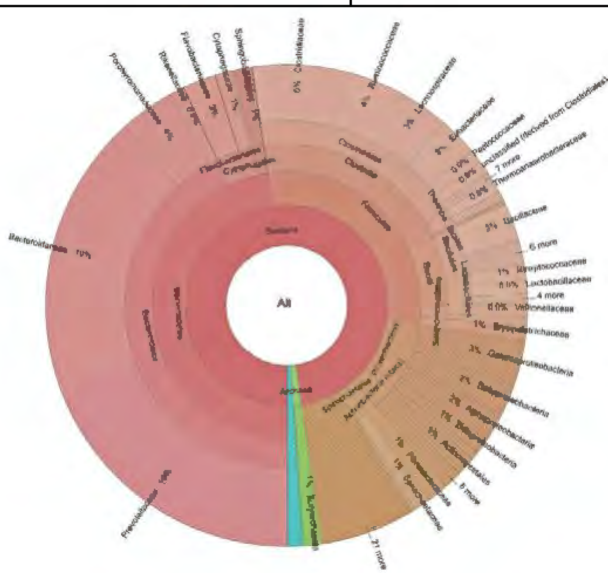

**Fig: 1 Krona chart illustrating the distribution of taxonomic domains indicating the percentages of reads with predicted proteins and ribosomal RNA genes annotated**

**Mining of rumen metagenome for identification of fibrolytic enzymes**

Nucleotide sequence homology based CAZymes annotation was performed for rumen metadata against the CAZy database (Cantarel et al. 2009) (http://www.cazy.org), using the CAZymes analysis Toolkit (CAT) (Park et al. 2010). CAT analysis using the assembled sequences (171,594 contigs) identified a total of 24,891 contigs (14.51% of total contigs) (Fig. 2) that had significant similarity with at least one of the reported CAZyme modules, spanning about 205 different CAZyme families (Additional file 1: Table S4). Approximately 13% of the contigs matching with different CAZyme classes could not be assigned to any of the protein families available in the CAZy database. CAT analysis revealed the number of contigs and respective Pfam domains of the members of different CAZyme classes that are mainly involved in the catalytic hydrolysis of plant cell wall polysaccharides inside cattle rumen.

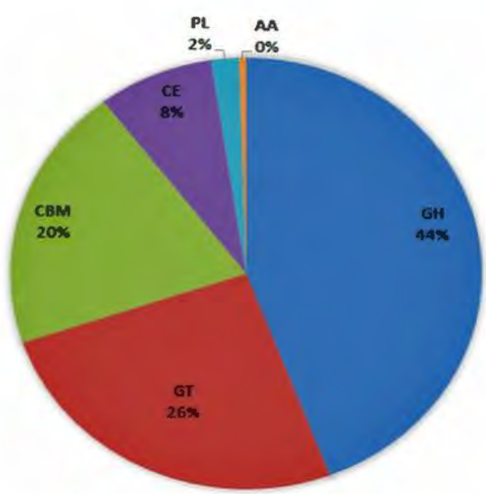


**Fig: 2 CAT analysis showing the distribution of different CAZyme classes across HF cross rumen metagenome. The chart shows the**

**predominance CAZymes encoding putative glycoside hydrolases in the HF cross metagenome followed by glycosyltransferases and carbohydrate binding modules. GH glycoside hydrolase; GT glycosyltransferase; CBM carbohydrate- binding module; PL polysaccharide lyase; and AA auxiliary activities**

The GH catalytic modules corresponding to 11,010 sequences were the most predominant and represented 96 different families altogether. The contigs encoding enzymes belonging to functional class GTs were the second most prominent (25.58% of CAZymes) group in HF cattle rumen and contained 6366 sequences from 40 different families. The enzymes from GT2, GT4, GT51, and GT28 families were present in larger proportion, representing 72.93% of the total GTs. CBM modules included 4945 sequences from 35 families. The CE1 family, encoding feruloyl esterases, which are essential for the solubilization of plant polymer lignin, was the principal CE family with 390 contigs in cattle rumen. There were 1975 contigs associated with thirteen CE families in the CAZy database, representing 7.93% of CAZymes. CAT analysis also detected the presence of fifteen families of PLs (1.93%) and six families of Aas (0.46%) in HF cross rumen metagenome (Additional file 1: Table S4). Out of 24,891 CAZyme encoding contigs, there were 2517 contigs (10.11%) that had two or more distinct CAZy domains. CBM domains were predominant among the multi-domain enzyme families accounting for about 1275 contigs (50.66%}. Class glycoside hydrolases had 807 (32.06%) multi-domain contigs, largely associated with different carbohydrate- binding modules. Ces, GTs, and PLs represented 8.03, 5.44 and 3.77% of multi-domain enzymes, respectively, most of which were linked to CBMs.

A majority of the cattle rumen microbiome cellulases identified were classified as families GH5, GH6, GH9, GH44, GH45, and GH48. Gene fragments coding for putative cellulases belonging to families GHS and GH9 accounted for 4% of the total GHs and were found to be the major families of cellulases present in cattle rumen. Likewise, 11 contigs encoding endoglucanases (GH45) and cellobiohydrolases (GH48) were also documented. Comparatively lesser number of cellulases degrading the main chains of plant cell wall components (10.67% of total GHs), were prevalent in HF rumen, while the enzymes involved in hydrolysis of side chains of plant polymers and oligosaccharides (GHl, GH2, GH3, GH29, GH35, GH38, GH39, GH42, GH43, and GH52), were in abundance and represented a majority of (60.11%) GHs. Putative exo-β-1, 4- glucanases (GH1 and GH3) endo-β-1, 4-glucanases (GH5, and GH12) Involved in the hydrolysis of β-1, 4-linked glucose residues from cellulose were represented by ~20 and ~4% of the total GHs, respectively. A large number of endo-hemicellulase degrading enzymes from GH8, GH10, GH11, GH12, GH26, GH28, and GH53 families were also identified and classified. CAT analysis also identified the presence of debranching enzymes belonging to family GH51 (α-L-arabino furanosidases), GH67 (α-glucuronidases), and GH 78 (α-L-rhamnosidases) that play a key role in depolymerization of hemicellulose. The enzymes effecting the hydrolysis of xylan main chains such as endo-1, 4-β-xylanase and endo-1, 3-β-xylanase were represented by GH10 and GH11 families. These accounted for about 2.09 and 0.3% of the total GHs, respectively (Table 2). Among the 96 GH families reported in this study, families GH3, GH2, and GH43 encoding oligosaccharide- degrading enzymes were most abundant and represented by 993, 927, and 739 contigs, respectively (Additional file 1: Table S4).

## Microbial community analysis of CAZyme encoding contigs

To determine the phylogenetic origin of core microbial populations that significantly contribute CAZymes, all 24,891 CAZyme encoding regions, representing different classes of GHs, GTs, CBMs, Ces, PLs, and Aas, were analyzed separately. Phylogenetic analysis of CAZyme contigs on the MG-RAST server using the MSNR database revealed that the bacterial species belonging to genera Prevotella, Bacteroides, Clostridium, Fibrobacter, and Ruminococcus contributed a greater part of CAZyme encoding gene fragments in the HF cross rumen metagenome. Genera Bacteroides and Prevotella were the most dominant, producing a substantial amount of four major classes of CAZymes (GHs, GTs, Ces, and CBMs) accounting for about 44.63 and 36.1%, respectively. A significant proportion of GTs (23.7%), GHs (13.34%), and Ces (5.07%) were assigned to genus Bacteroides alone, whereas genus Prevotella was found to be the dominant contributor of GHs (19.22%), Ces (10.13%), and CBMs (3.73%). Genera Fibrobacter and Clostridium were found to encode three classes of CAZymes each: CBM, CE, GH and CBM, CE, GT, respectively. A major portion of CBMs were corresponding to members of genera Ruminococcus (25.74%) and Clostridium (12.94%) (Fig. 3).

**Table 2 Overview of the comparative analysis of putative carbohydrate-active enzymes belong to members of various GH families targeting plant structural polysaccharides identified in HF cross rumen with other herbivore metagenomes**

| Ghfamily | Major activity | HF cross | Jerseycow b | Cow< | Reindeer d | Termite 9 | Macropod[1] |
|---|---|---|---|---|---|---|---|
| Cellulases | | | | | | | |
| GHS | Cellulases | 6.95 | 10.53 | 7.88 | 5.56 | 14.62 | 3.72 |
| GH6 | Endoglucanase | 0.10 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 |
| GH7 | Endoglucanase | 0.00 | 0.00 | 0.Ql | 0.00 | 0.00 | 0.00 |
| GH9 | Endoglucanase | 3.16 | 3.51 | | 2.11 | 2.35 | 0.00 |
| GH44 | Endoglucanase | 0.09 | 0.00 | 0.54 | 0.10 | 1.57 | 0.00 |
| GH45 | Endoglucanase | 0.19 | 0.00 | 0.62 | 0.00 | 1.04 | 0.00 |
| GH48 | Cellobiohydrolases | 0.19 | 0.00 | 0.02 | 0.10 | 0.00 | 0.00 |
| Subtotal | | 10.67 | 14.47 | 13.39 | 7.87 | 19.58 | |
| Endo-hemicellulases | | | | | | | |
| GH8 | Endoxylanase | 1.14 | 0.00 | 1.79 | 0.68 | 1.31 | 0.37 |
| GHl0 | Endo-1,4-!3-xylanases | 2.03 | 15.35 | | 3.68 | 12.01 | 4.09 |
| GHll | Xylanases | 0.29 | 0.00 | 0.90 | 0.16 | 3.66 | 0.00 |
| GH12 | Xyloglucanases | 0.53 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 |
| GH26 | ß-Mannase and xylanases | 1.16 | 0.44 | 2.00 | 2.97 | 3.92 | 1.86 |
| GH28 | Galacturonases | | 0 00 | 2.56 | 2.33 | 1.57 | 0.74 |
| GH53 | Endo-1.4-13-galactanases | 10.19 | 7.89 | 2.62 | 2.42 | 3.13 | |
| Subtotal | | 17.92 | 24.12 | 15.45 | 12.23 | 25.59 | 10.41 |
| Xylanoglucanases | | | | | | | |
| GH16 | Xyloglucanases | 2.53 | 0.00 | 2.62 | 2.25 | 0.26 | 1.49 |
| GH74 | Xyloglucanases | 0.45 | 0.00 | 2.09 | 0.85 | 1.83 | 0.37 |
| Subtotal | | 2.98 | 0.00 | 4.72 | 3.10 | 2.09 | 1.86 |

| Debranching enzymes | | | | | | | |
|---|---|---|---|---|---|---|---|
| GHSl | a-L-Arabinofuranosidases | 0.48 | 0.44 | 6.79 | 9.46 | 4.70 | 4.46 |
| GH54 | a-L-Arabinofuranosidases | 3.47 | 0.00 | 0.41 | 0.45 | 0.00 | 0.00 |
| GH62 | a-L Arabinofuranosidases | 0.00 | 0.00 | 0.Ql | 0.00 | 0.00 | 0.00 |
| GH67 | a-Glucuronidases | 0.98 | 0.00 | 0.65 | 1.43 | 2.61 | 1.86 |
| GH78 | a-L-Rhamnosidases | 3.38 | 5.70 | 6.85 | 6.07 | 0.00 | 9.29 |
| Subtotal | | 8.31 | 6.14 | 14.70 | 17.40 | 7.31 | 15.61 |

**Table 2 continued**

| **GHfamily"** | **Major activity** | **HF cross Jersey cow [b]** | **Cow [c]** | **Reindeer [d]** | **Termite[e]** | **Macropod [f]** |
|---|---|---|---|---|---|---|
| Subtotal | 60.11 | 55.26 | 51.75 | 59.40 | 45.43 | 68.40 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Metagenome size | 1.8Gb | 0.28Gb | 268Gb | 030Gb | 0.062Gb | 0.054Gb |

Table lists the percentages of different glycoside hydrolase families targeting plant cell wall polysaccharides and their major activities. GHs are divided into different groups based on their activity and hydrolysis of plant polysaccharides. Enzymes belonging to oligosaccharide degrading cluster were observed as the predominant class representing a major proportion of GHs

- Based on CAZy data base (http://www.cazy.org)
- Wang et al. (2013) fed on Timothy grass hey
- Hess et al. (2011) fed on switch grass
- Pope et al. (2012) natural grazing grass on winter pastures, Norway
- Warnecke et al. (2007) study on wood feeding termite
- Pope et al. (2010) fed onTimothy cannary grass and commercial pellet mix

## Discussion

### Rumen microbiota and CAZymes

Agricultural crop residues represent a significant proportion of renewable carbohydrate resources of energy for ruminants. The host as such cannot produce any enzymes that degrade the plant material, so they take advantage of the symbiotic association with rumen microflora to release the energy in form of carbohydrates and sugars from the recalcitrant plant polysaccharides. Rumen microbial populations are proven to play a vital role in plant fiber degradation in different ruminant species. A considerable variation in rumen microbiome has been observed across individual animals (Jami and Mizrahi 2012) and rumen microbial community structure also tends to vary significantly depending on the animal's age and diet (Kittelmann and Janssen 2011; Li et al. 2012). The rumen microbial profile is also moderately influenced by breed, gender, and other ecological factors like grazing location (Jami and Mizrahi 2012). Nevertheless, most of the published studies on rumen microbial communities have identified the predominance of bacteria belonging to phyla Bacteroidetes and Firmicutes, irrespective of the change in diet, gender, breed, and other ecological factors

(Kittelmann and Janssen 2011; Li et al. 2012; Petri et al. 2013; Stevenson and Weimer 2007; Thoetkiattikul et al. 2013). The results obtained in our studies confirm the earlier reports where the bacteria belonging to phyla Bacteroidetes and Firmicutes are the major contributors of different classes of CAZymes in the cattle rumen ecosystem. This result may indicate that the bacterial populations inhabiting the cattle rumen are largely similar to those of other ruminants in terms of Bacteroidetes and Firmicutes populations. The enzymes produced by these microbial communities are reported to have the potential to digest plant polymers like xylan, pectin, and starch (Stevenson and Weimer 2007). Genus Prevotella which has been established to account for a significant part of genetic and metabolic diversity of microbial communities in ruminants (Purushe et al. 2010). This genera was found to contribute a substantial proportion of CAZymes (>36%) in our study, when the ani- mals were fed with only finger millet straw, that is rich in fiber. Prevotella has already been reported to play a vital part when there was a shift from a high- calorie diet to high-fiber diet (Jami et al. 2013). Microbes belonging to both genera Bacteroides and Prevotella were found to possess a large repertoire of CAZymes; Bacteroides were the chief manufacturers of CAZyme class GTs, whereas Prevotella were the principal contributors of GHs in cattle rumen. The natural capability of rumen microbiota to produce an array of potential enzymes that hydrolyze the rigid lignocellulose biomass has been successfully employed to treat different systems like agricultural residues and straw waste (Hu et al. 2007; Barnes and Keller 2004). The application of different artificial rumen systems for organic waste conversion {Yue et al. 2013) was also being extensively studied. Plant biomass containing cellulose and hemicellulose on earth is considered to be one of thelargest sources of fermentable sugars and energy that could be utilized for various industrial applications, like ethanol production (Jorgensen et al. 2007). Despite the large-scale availability of these plant polysaccharides, the major challenge for accessing these fermentable sugars is the presence of a highly resilient

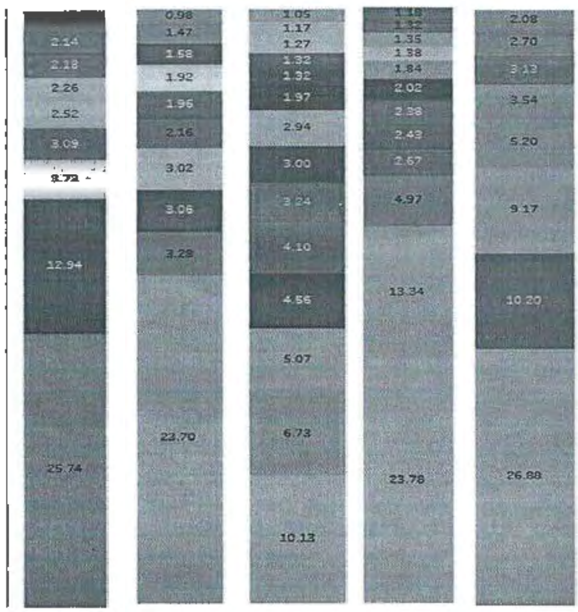


**Fig. 3 Taxonomic distribution of major CAZymes encoding contigs in HF cross rumen metagenome using MSNR database. The barchart shows the percentages of contributions of CAZymes from the major microbial communities in cattle rumen. Bacteria belong to genus Prevotella, Bacteroides, Fibrobacter, Clostridium, and Ruminococcus were identified as the chief producers of CAZymes in cattle rumen ecosystem. GH glycoside hydrolase; GT glycosyltransferase; CBM carbohydrate- binding module; PL polysaccharide lyase; and M auxiliary activities.**

aromatic compound called lignin (Alvira et al. 2010). However, the ruminants with their highly complex efficient microbial communities can produce a broad range of fibrolytic enzymes that can facilitate the hydrolysis of lignocellulose biomass (Hess et al.

2011). However, it should be noted that the NGS-based Illumina-MiSeq sequencing platform employed in this study cannot be used for the quantitative interpretation of the rumen microbial community structure and size due to the bias inherent with the PCR amplification and sequencing of 16s rRNA gene, as it may result in misinterpretation of active community members (Schloss et al. 2011). Cattle rumen has highly diverse and complex CAZymes The CAZy database (http:// www.cazy.org) exclusively deals with the diverse group of enzymes that actively contribute to synthesis and degradation of complex carbohydrates and glycoconjugate. The CAZy database provides manually curated information for all CAZyme families: glycoside hydrolases, glycosyltransferases, polysaccharide lyases, carbohydrate esterases, carbohydrate-binding modules, and auxiliary activities; it now allows one to examine all known families and enzymes involved in cellulolysis, hemicellulolysis, and pectienolysis (Cantarel et al. 2009). Enzymes coding for GH families are highly abundant in most of the genomes and they account for about 50% of the enzymes classified in the CAZy database. Among all major classes of CAZymes that have been reported till date, glycoside hydrolases were observed to be the most predominant and diverse group of catalytic enzymes involved in the hydrolysis of plant polymers in Indian crossbred cattle rumen metagenome. GHs representing glycosidases and transglycosidases are responsible for the hydrolysis of the glycosidic bonds linking two or more carbohydrates or a carbohydrate and a non-carbohydrate moiety accounting for about 6.5% of the total sequences. The total number of contigs obtained in each CAZyme family gives an indication of the abundance of that particular group of enzymes in the HF cross cattle rumen. Out of 135 CAZy families that are reported in the CAZy database till date, 96 families were represented by cattle rumen metagenome. Patel et al. (2014) reported 72 families of GHs from the buffalo rumen ecosystem. The substantially large number of enzymes from the GH family (96 families) in our study could be either due to the difference in NGS sequencing platform or the modifications in the analysis pipeline. Considerably higher number of GH families obtained in our study also indicates that HF cross rumen might have a more intricate process of lignocellulose breakdown than other reported rumen metagenomes.

In parallel to previous reports (Wang et al. 2013; Stevenson and Weimer 2007; Pope et al. 2012), genes encoding cellulases belonging to families GHS and GH9 were present in higher proportion than other cellulase coding families. Dai et al. (2012) reported the exoglucanase activity of enzymes corresponding to the GHS family which is known for endoglucanase, mannanase and endo-xylanase activities. The large number of putative cellulase genes identified in the GHS family (403 contigs) in our study suggests that cattle rumen microbes may have novel strategies for degradation of plant polysaccharides present in the feed. Previous studies on rumen metagenomes have not reported any contigs assigned to the GH6 family, other than one contig from the fosmid library of jersey cow rumen metagenome (Wang et al. 2013). Conversely, Indian crossbred cattle rumen microbiome was found to exhibit a marginally higher amount of endoglucanases corresponding to the GH6 family (6 contigs). Consistent with earlier herbivore-related metagenome and metatranscriptome studies (Brule et al. 2009; Hess et al. 2011; Pope et al. 2012; Warnecke et al. 2007; Qi et al. 2011) no contigs associated with the GH7 cellulase family were found.

Consequently, the scanty representation (11 contigs) of enzymes belonging to the GH48 family displays the possibilities of the non-cellulosomal mode of plant cell wall hydrolysis in cattle rumen. Additionally, the contigs which were affiliated to diverse CAZyme modules (~13%), but could not be classified under any of the known or reported Pfam domains, indicated that the cattle rumen microbiome produces a large number of unique CAZymes which are 149abrador characterized. Complete depolymerization of plant polysaccharides requires collective activities of different groups of enzymes other than GHs and these include GTs, CBMs, Ces, PLs, and Aas. GTs, reported to be involved in the catalysis of glycosidic bonds to form disaccharides, oli- gosaccharides, and polysaccharides from phospho-activated sugar donors (Coutinho et al. 2003; Lairson et al. 2008), were the second most abundant CAZy family HF rumen (-4% of total contigs). Out of the forty GT families identified in cattle rumen metagenome, enzymes belonging to families GT2 and GT4 (cellulose synthase, chitin synthase, α-glucosyltransferase, etc.) represented a significant proportion (>62%) of the total GTs. CBMs which have no reported enzymatic activity on their own, but can potentiate the activities of all other CAZymes (GHs, Ces, and auxiliary enzymes) or act as an appendix module of CAZymes (Tomme et al. 1995; Boraston et al. 2004), accounting for 2.9% of contigs.

In conclusion, high throughput sequencing-based whole metagenomic approach was used to investigate the natural biomass-converting microbial communities in the cattle rumen ecosystem. Comprehensive analysis of metagenome data strongly indicates that the complex digestive system of Indian crossbred cattle possesses a very high degree of a deeply branched and extremely diverse group of enzymes of microbial origin which have the ability to degrade the recalcitrant lignocellulose plant biomass. The CAZymes corresponding to class glycoside hydrolases were found to be the most abundant and diverse group of CAZymes in cattle rumen. Bacteria belonging to genera Prevotella, Bacteroides, Clostridium, Fibrobacter, and Ruminococcus were identified as the key contributors of CAZymes inhabiting the cattle rumen. This study provides a substantially expanded catalogue of enzymes that participate in the deconstruction of plant cellulosic biomass in Indian cattle rumen, which provides an opportunity to improve ruminant nutrition and also to develop proficient fermentation systems for bioconversion of plant biomass into biofuels.

**Additional file**

**1. Additional tables and figures**

**Abbreviations**

CAZymes: carbohydrate active enzymes; HTS: high through put sequencing; HF: Holstein Friesian; ELU: experimental livestock unit; CTAB: cetyl trimeth-ylammonium bromide; MG-RAST:Metagenome Rapid Annotation using Subsystem Technology; QC: quality control; RPS BLAST: reversed position specific basic local alignment search tool; KEGG: Kyoto Encyclopedia of Genes and Genomes; CAT: carbohydrate-active enzyme analysis toolkit; GHs: glycoside hydrolases; GTs: glycosyl transferases; Ces: carbohydrate esterases; CBMs: carbohydrate-binding modules; Pls: polysaccharide lyases; Aas: auxiliary activities.

## Authors' contributions

UV and TA designed and performed the experiment and wrote the manuscript. RPM and ASA analyzed the data. All Author details 1 Rumen Microbiology Laboratory, Animal Nutrition Division, National Institute of Animal Nutrition and Physiology, Bangalore, India. 2 Department of Biotechnology, Jain University, Bangalore, India. 3 Bioinformatics Laboratory, Division of Molecular Entomology, National Bureau of Agricultural Insect Resources, Bangalore, India. 4 Wildlife SOS, Bannerghatta Bear Rescue Centre, Bangalore, India.

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## Competing interests

The authors declare that they have no competing interests.

## Availability of data and materials

The raw data obtained had been deposited in the NCBI Sequence Read Archive (SRA) with the Bio Project ID- PRJNA291680, Accession: SAMN03963232, ID: 3963232.

## Ethical approval

All applicable institutional guidelines for the care and use of animals were followed and ethical committee at National Institute of Animal Nutrition and Physiology, Bangalore, India, has approved the feeding experiment and sample collection.

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# Art – 143. SLOTH BEAR *(Melursus ursinus)* DISEASES AND THEIR MANAGEMENT

**Ilayaraja. S** and **Arun A. Sha**

## Introduction

Sloth bears are coming under the order Carnivora and family Ursidae. The origins of the bear family or *Ursidae* can be traced back to the racoon-sized, dog-like Cephalogale from the middle Oligocene and early Miocene (approximately 20-30million years ago) of Europe. Unlike the bears, Cephalogale had functional carnassial teeth for slicing through meat.

The *Ursidae* is a young family consisting of 5 sub families; Nine bear species are recognised today. In the past, these have been assigned to different genera, but recently it has been suggested that all species except for the spectacled bear and the pandas should be placed within the genus *Ursus* on the basis of the results of various molecular studies, which show that they have evolved recently. The sloth bear *(Melursus ursinus)* is also usually divided into two subspecies. The nominate race, *ursinus*, is found in India, but the subspecies inornatus from Sri Lanka was described by Pucheran in 1855. Sri Lankan Sloth bear also have white markings on their chests and may be significantly smaller than Indian ones (Corbet, GB. & Hill, J.E. (1991).

Sloth bears have shaggy, dusty-black coats, pale, short- haired muzzles, and long, curved claws that they use to excavate ants and termites. A cream-colored “V” or “Y” usually marks their chests. A gap between their upper teeth enables them to suck ants, termites and other insects. Sloth bears’ nostrils can close, protecting the animals from dust or termite nests or beehives. Sloth bears are the only bears to carry young ones on their backs. Sloth bears grow five to six feet long, stand two to three feet long, stand two to three feet high at the shoulder and weigh from 80 kg (in lighter females) to 120kg (in heavy males).

The sloth bear is listed as vulnerable on the World Conservation Union's Red List of Threatened Animals. Sloth bears are completely protected under schedule I of the Indian Wildlife (Production) Act 1972 (as amended in 1986). Sloth bears are listed under Appendix I of CITES.

Sloth bears live in a variety of dry and wet forests, and also in some grasslands, where boulders, scattered shrubs and trees provide shelter. Sloth bears are well known to climb trees to heights of 18 m to forage for fruit and honey (Peyton, B.,1980). Sloth bears dine on mango, fig, ebony, and other fruits (frugivory), also on some flowers. However, ants and termites (myrmecophagy), dug out of their cement-hard nest mounds, are a year-round staple. Also, sloth bears climb trees and knock down honeycombs, beetles, grubs, ants, and other insects round out their diet. During food shortages, sloth bears will eat carrion.

Sloth bears mate during the hot season—May, June, and July. Females usually have delayed implantation/embryonic diaphase as a reproductive strategy and give birth to two cubs after six to seven months of gestation. Cubs are born in an underground den, and stay there for several months. After emerging from the den, cubs stay at their mother's side for two to three years before heading off on their own. Active mostly at night, the sloth bear is a noisy, busy bear. It grunts and snorts as it pulls down branches to get fruit, digs for termites, or snuffles under debris for grubs and beetles. A sloth bear uses its lips like a vacuum, making rapid, loud "kerfump" noises as it sucks insects from their nests. Sloth bears lead solitary lives, and most are nocturnal. (In protected areas, they may be active during the day.) If threatened, these smallish bears will stand on two legs, brandishing their clawed forepaws as weapons. In captivity they are more active in the early morning and late evening and like to be with the socialized group. Infighting among the males is observed at the time of mating season. The impact of human activity and local climatic conditions may also have important effects on the diurnal activity patterns of bears (Yudin, V.G.,1993). The presence of conspecifics, particularly adult male, may influence the activity patterns of individuals of other age/sex classes.

In areas of low human impact, bears may be diurnal, but increased human activity in an area may force the animals to adopt crepuscular or even nocturnal habits.

## Common Diseases Affecting Sloth Bears

Agra bear rescue facility is world largest rescue facility and rescued over 386 sloth bears for the past 15 years and rehabilitated in semi captive conditions and maintained on an enriched native diet of local seasonal fruits and grains. We recorded many health issues such as, viral diseases, bacterial diseases, dental infection, enteritis, infighting injuries, skin infection, neoplasams, idiopathic epilepsy, cerebral infract, obstipation induced urinary incontinence, vulval abscess, paraphymosis, balanitis, orchitis, aural haematoma, inguinal hernia, trichobezoars etc.

## Treatment of Diseases and Its Control

"Medicine is not only a science, but also the art of letting our own individuality interact with the individuality of the patient" – ALBERT SCHWEITZER.

**Rabies**

**Causative Organism: Lyssa virus**

**Incubation period: 7 days to several months**

Symptoms & Lesions: Rabies is an acute viral disease. Rabies in sloth bears is characterized by laboured respiration, distress, pale mucous membrane and inability to ingest food, vomiting of undigested food materials and off fed indicating the dumb form of the disease in most cases and the affected animal will die soon, in 3 to 7 days after exhibiting the symptoms. The animal does not respond to any treatment. The post-mortem examination revealed severe brain congestion. The histology examination performed at IVRI, NICD, Delhi revealed presence of 'Negri bodies'.

**Treatment & Control:**

Symptomatic treatment. Morbidity and mortality controlled by immunisation procedure. Initially we practiced post exposure immunisation schedule and followed by annual revaccination.

**Infectious Canine Hepatitis (ICH).**

Causative organism: Infectious canine hepatitis (ICH) is a highly contagious viral disease-causing acute liver infection in dogs (Carlson et al, 1980), caused by canine adenovirus type 1(CAV-1). CAV-1 is related to but distinct from CAV-2 that causes infectious tracheo bronchitis (kennel cough) in dogs (Stephen JB, et al, 2005). CAV2 is potentially pathogenic for raccoons (Procyon lotor) (Hamir, A. N. 1992 & Jamison et al,1973). Incubation period is 4-7 days (Ettinger et al, 1995). Prevalence of ICH in grizzly bears *(Ursus arctos)* from Alaska were recorded during the period of 1973-1987 (Zarnke, R.L, et al, 1989). Antibodies to canine adenovirus were detected in 8% of 38 *Ursus americanus* -American black bears 0% of 36 grizzly bear (*Ursus arctos*–Brown bear) and 17% of 60 *Ursus maritimus* - Polar bear in Canada, in samples collected 1994-2001 (Philippa, et al., 2004).

**Symptoms & Lesions**

In sloth bears this disease is manifested in per acute form, hence the death occurs suddenly preluded by convulsions and respiratory distress in 10 to 20 minutes. In few acute cases we recorded mild shivering to staggering gait, congested eyes, mild swelling of nasal flaps, pinpoint reddish lesion (petechial haemorrhages) in lips and gums, vomiting, passing brownish black mucoid faeces, increased body temperature 40-42$^{0}$C, intermittent convulsion and hyper excitability, increased clotting time, grunting noise due to abdominal pain leads to death within 6-8 hours. In rare chronic cases with facial swelling, which is manifested after the initial convulsion, leading to comatose condition, paralysis of limbs, intermittent fever with convulsions, hyper excitability and brownish black diarrhoea leading to death within 10 – 14 days in spite of all necessary palliative treatment being given. In post-mortem examination invariably all animal showed the same kind of lesion such as pin point haemorrhagic lesion in buccal mucosa, pale to mild yellowish corneal mucous membrane, severe congestion in serosal and mucosal layer of the intestines and stomach with diffused pin point haemorrhage and hyperaemic mucosa, 10 to 20 ml of sanguineous fluid found in the abdominal cavity, liver is discoloured and mottled with sharp edges, urinary bladder and kidneys were severely congested, lymph nodes showed various degree of congestion and swelling, petechial haemorrhages were noticed at base of the heart and meningitis with cerebral haemorrhage was noticed.

The representative tissue samples (liver, spleen, heart and lymph nodes) of all dead bears with the above-mentioned symptoms and lesions were sent to IVRI. They revealed intranuclear inclusion bodies. ICH is a contagious and lethal disease in bears. The disease occurrence is more in summer when compared to winter and high levels of mortalities were recorded during the months from March to May. Sloth bears show similar symptoms like American black bears *(Ursus americanus)*. The disease is characterized clinically by its rapidly fatal progression (Whetstone, C.A, et al, 1988). CAV-1 is both endotheliotropic and hepatotropic. In addition to acute hepatocellular necrosis; severe acute haemorrhages are observed on the serosal surfaces, within the lymph nodes and liver (Kelly, WR, 1993).

Ataxia, excessive salivation, vomiting, paddling of the legs, nystagmus (Pursell, A.R., et al, 1983) diarrhoea, signs of abdominal pain, such adopting the foetal position (Collins, J.E, etal1984.) anorexia, lethargy, hindlimb ataxia, seizures and paralysis (Fowler, M.E. et al 2003 & Kritsepi, M, et al 1996) are recorded in American black bears *(Ursus americanus)*. Death occurs within 12 hours of the first clinical signs. The surviving bears from an epizootic, showed neurological signs and lethargy for 60 to 90 days. (Fowler, M.E. et al 2003 & Collins, et al, 1984). In two of the surviving bears from the epizootic, unilateral corneal opacity was detected 10 to 14 days after the onset of the clinical signs. The corneal opacity regressed over the next weeks without treatment. (Collins, et al 1984).

## Treatment & Control

Infection spreads through urine, faeces, saliva, probably via the oropharynx. The virus may be shed in urine for six months or more following recovery (John, W.D, et al. 1981 & Fraser, C.M, 1986). CAV-1 is destroyed in the environment by steam cleaning and quaternary ammonium compounds. Otherwise, the virus can survive in the environment for months in the right conditions (Ettinger, et al, 1995). So, immediate isolation of the individual animal and provision of stress free environment, strict and frequent disinfection of enclosures, cages and dens with broad spectrum disinfectants is more important along with palliative treatment with immunomodulators, haematinic and vitamin –C is recommended to control the morbidity and reduce the mortality rate in captive sloth bears based on our experience.

## Rotaviral enterities

Faecal samples of 18 sloth bears were tested for enteric viral infection such as Rota virus, picobirnavirus and Parvovirus as they were showing diarrhoea. All the samples tested negative except for Rotavirus.

The affected bears were isolated and provided symptomatic treatment along with supportive therapy and the animals recovered well after 10 to 14 days of treatment.

## Bacterial diseases

Tuberculosis

Causative organism: Micobacterium uberculosis.

It's a longstanding and challenging issue till date. It is very difficult to diagnose in initial stage and treat in bears. No proper test kit/ procedure available globally to diagnose TB in bears. Regular observation /health monitoring (weight, food intake, activity and G.I & respiratory disorder) will help diagnose TB by correlation with other clinical findings.

**Symptoms & Lesions**

Inappetance, anorexia, gradual weight loss, purulent discharge from eyes, diarrhoea and deep thoraco-abdominal respiration, purulent discharge from nose in advanced cases, lymphadenitis. The post- mortem examination reveals different kinds of lung lesions from small diffused nodular lesion to large pus-filled cavities, congested trachea, enlarged lymph nodes and necrotic lesions in liver and kidney.

**Diagnosis:**

- ➢ Chest radiograph
- ➢ Lung-wash culture
- ➢ Blood & serum analysis.
- ➢ Wild alert TB kit
- ➢ Based on the symptoms Treatment and control
- ➢ Intensive monitoring and immediate isolation of suspected / infected bear and providing supportive therapy/palliative treatment with vitamin C and D.
- ➢ Anti-tuberculosis drugs
- ➢ Expose the animal to sunlight

**Leptospirosis**

**Symptoms and lesions**

Dullness, inappetence, congested mucous membrane and passing brownish black mucoid faeces and showing severe respiratory distress followed by convulsions. On post-mortem examination, we found cerebral haemorrhage, internal petechial haemorrhage, severely congested urinary bladder and discoloration of liver in some cases.

**Diagnosis:**

A detailed serological analysis of healthy and suspected bears (total 32 samples) with MAT (microscopic agglutination test) test in 1 :50 screening dilution against 12 serovars. Out of 32 samples only few bears were found negative the remaining bears were found positive for different serovars. The majority of positive cases were infected with *L.icterohaemorrhagiae*, *L.canicola* and *L.pyrogenes* and *L.pomona*.

However few animal even had MAT titier 1:400 ,1:800 and 1:100 for *L.pyrogenes, L.icterohaemorrhagiae and L.canicola* respectively; no clinical symptoms has been recorded. Thus, it can be assumed that the sloth bears also serve as the potential carrier for leptospirosis.

Treatment and control

Annual vaccination. Treating with Doxycycline along with other supportive therapy. Improve the hygiene and immediate isolation of infected or suspected bear from the enclosure.

## Fungal disease

### Candidiosis

**Causative organism: Candida albicans**

It was reported in one young bear cub. The cub showing sudden dullness, reduced milk intake and crying frequently and passed turbid urine. The urine culture revealed more number of Candida albicans colonies. Treatment provided with fluconazole b.i.d mixed with milk for 10 days. The animal recovered well and started passing transparent urine without crying and food intake and activities also improved without any further complications.

## Neoplasms

In sloth bears are prone to both malignant (lymphoid leukemia, cholangiocellular carcinoma, adenocarcinoma, seminoma, Adenocarcinoma and intra alvolar carcinoma) and benign neoplasoms (capillary haemangioma, cementoma, fibro epithelial polyp and growth inoropharynx).

**Cholangiocarcinoma**

Bile duct cancers can start in the bile ducts within the liver. These are called intrahepatic cholangiocarcinomas. Or they can start in the bile ducts outside of the liver. These are called extrahepatic cholangiocarcinomas. The affected animal starts showing the symptoms of inappetance, bulged abdomen, diarrhoea with foul smelling and muciod blackish brown in colour. Symptomatic treatment doesn't give much improvement. Diagnostic ultrasonography of abdominal cavity will help confirm the hyper- echoic hard mass in hepatic system and ascites in abdominal cavity. All visible mucous membrane are icteric and dehydration will further lead to clay-coloured stools, and dark urine. The animal may die 10 to 15 days after exhibiting the symptoms. The detailed post-mortem examination revealed, severely icteric body, abdominal cavity filled with amber colour fluid, intestines shrunken and adhered tightly to the stomach. In the stomach, dark blakish brown mucoid material was noticed. Gallbladder associated with tumour mass and the liver lobes with multiple nodules. The histopathological examination of the tumor mass suggested cholangio carcinoma.

**Leukemia**

Leukemia is characterized by proliferation of lymphoblasts, it is described in a rescued dancing sloth bear *(Melursus ursinus)*. A 12- year-old male sloth bear in Agra Bear Rescue Facility showed symptoms of anorexia, lethargy, dehydration and limping of hind leg. The animal was isolated in the retiring den and treated symptomatically with antibiotic, vitamin-B complex, methyl prednisolone acetate and 7 litres of isotonic fluid (DNS 0.9 & 5%) was infused into the bear to improve the hydration status but no improvement could be noticed. The animal was tranquilized for detailed clinical examination and treatment. The initial clinical examination revealed leucocytosis and moderate blast like cells were found in blood smear. After a few days (10th day), the second clinical examination of animal showed high number of lymphoblast cells in blood smear. Then, chemotherapy was initiated with Imatinib Mesylate but the condition of the animal deteriorated which later succumbed to the disease. The post-mortem examination revealed enlargement of lymph nodes and spleen and liver. Histopathologically the liver section revealed severe degeneration of hepatocytes and swollen hepatocytes with pale cytoplasm and finely granular nature. The portal areas were heavily infiltrated by large blast form of lymphocytes. The lymphoblasts also infiltrated the parenchyma focally. The nuclei in good number of infiltrated cells were enlarged and hyperchromatic. Mitotic figures and pleomorphism was not noticed. The lymph node section showed lymphoid hyperplasia with blast like lymphocytes having hyperchromatic nuclei. The normal lymphoid follicular structures were replaced by diffused sheet of lymphoblasts. The femur bone marrow section was severely infiltrated with lymphoblast cells. Finally, this case was concluded as lymphoid leukemia.

**Seminoma**

One adult male bear died suddenly after showing respiratory distress. The detail Postmortem examination revealed unidentified mass in the mediastinal region. Liver showing generalized congestion and no major abnormality was noticed.

Histopathological examination of liver section revealed sinusoidal congestion. Lung tissue section revealed normal appearing alveoli and bronchioles with vascular congestion. Thoracic mass-section revealed large areas of necrosis with small part of preserved tumour showing lobules of tumour cells separated by fibrous septa. The tumour cells have vacuolated fragile cytoplasm, vesicular nucleus and nucleolus. Features are suggestive of germ cell tumour “Primary mediastinal seminoma”.

**Adenocarcinoma**

An adult rescued male sloth bear approximately 15 yrs old was exhibited limping on its right forelimb and not responding to any treatment Later the bear showed symptoms of vomition and frothy secretion from mouth. However, no respiratory distress was observed. Subsequently the bear became recumbent and died on 27th day .A complete necropsy was performed and tumour masses of varying sizes were found in all the vital organs. Histopathology of lung, liver, spleen and kidney revealed multiple foci of well differentiated adenocarcinomas.

**Intra Alvolar Carcinoma**

A hand reared adult male sloth bear aged 10 years started showing symptom of gradual limping on its right for limb, inappitance and passing brownish diarrheic faces with mucous. The radiographic examination of the affected limb no revealed any abnormality. The symptomatic treatment not given any cure. Later the animal become anorectic, developed ascites and died due to respiratory distress. Post- mortem examination revealed abnormality in lung, liver, kidney and spleen. Histopathology of lung revealed tumour cells proliferation occupying the alveolar lumen, increased mucus and goblet cell hyperplasia were observed in bronchi and bronchioles and bronchiolar epithelium was also hyperplastic and desquamated.

Kidney section showed completely necrosed and degenerated proximal convoluted tubules. some tubules showed tall columnar epithelium and filled with mucus and severe interstitial fibrosis with haemorrhages and congestion in the kidney vessels. Spleen section showed smooth muscle proliferation which reduced the lymphoid element. Tumor metastatic emboli were found in blood vessels of lung, kidney and spleen. The condition was confirmed as intra alveolar carcinoma with metastasis in liver, spleen and kidney.

**Benign growth in Oropharynx region**

An abnormal mass growth was observed in the oropharynx region of a rescued female sloth bear during intubation for gas anaesthesia. The growth was removed surgically and subjected to histological examination. The mass was found to be composed of respiratory lining epithelium and squamous epithelium. There was marked oedema and fibrin deposition in subepithelium. There was mild lymphocytic infiltrate. Neoplastic lesions were not seen thus suggesting non-cancerous/benign growth. The animal recovered well, no abnormality or recurrence of growth was noticed. The bear remains active and is having food normally.

**Benign Fibro Epithelial Polyp**

Fibro epithelial polyps or hypertrophied anal papillae are essentially skin tags that project up from the dentate line and the junction between the skin and the epithelial lining of the anus. They are usually small in size, but sometimes they become enlarged, causing unexpected medical conditions. Fibro epithelial polyps of the anus, also known as hypertrophied anal papillae, are lesions that have attracted little attention both in the medical and veterinary literature. A fibro epithelial polyp, which was present in the perianal region of a rescued dancing sloth *bear (Melursus ursinus),* was successfully excised surgically which relieved the bear from irritation from the same.

**Ocular Capillary Haemangioma**

Haemangioma is a benign, abnormally dense collection of dilated small blood vessels that may occur in the skin or internal organs. In humans, hemangiomas may be present at the time of birth or may develop shortly after. They mostly resolve on their own by 5 to 10 years of age but some may take several years to disappear. In animals, sporadic cases have been reported in cattle, horse, sheep, dog, swine and fowl. However, it is very rarely reported in wild animals and especially bears except for a case of Cutaneous Haemangioma in a Giant Panda *(Ailuropoda melanoleuca)* (S. Rourke NL et al,2006). Here we describe a case of Ocular Capillary Haemangioma in an adult rescued male Sloth Bear *(Melursus ursinus)*.

A rescued adult male dancing bear on admission, showed severe lacrimation from the left eye and was scratching the eye frequently. On close observation, the conjunctiva of the affected eye was found to be congested with a swelling on the upper eyelid, a red coloured mass was observed to be emerging from the affected eye from behind the upper eyelid hindering the bear's vision from that eye. The animal was tranquilized for detailed inspection of the eye, using a ketamine-xylazine combination (Page, 1986. The mass was found to be oval shaped with 1.5 cm length and 1 cm width, erythematous and emerging from the cornea. A surgery was conducted excising the mass from the eyeball under general anaesthesia with isoflurane. Capillary bleeding was controlled using an electric cauterizer. The bear was treated with a course of oral antibiotics, anti- inflammatory and Vitamin A supplements for next 5 days. Antibiotic eye drops were applied for 5 days. On subjecting the mass to histopathological examination using H & E stain, it was found to be a Capillary Haemangioma. It was suspected to I caused by the constant irritation of cornea by the rope passing over the eye and thus rubbing on it frequently.

## Parasitic Infestation

Ascarids infestation, Taenia infestation and Hepatozan ursi were recorded in sloth bears. Hepatozoon species are parasites that infect a wide variety of domestic and wild animals. Hepatozoon infection could be detected in 38 (70%) out of 54 blood samples of Indian sloth bears. (captive and wild), suggestive of high prevalence of Hepatozoon infection in Indian sloth bears. Sequencing of partial 18S rRNA gene of the positive samples and BLAST analysis indicated that the nearest phylogenetic neighbour was H. ursi with which they exhibited 99–100% similarity. Additionally, Hepatozoon sp. Isolated from wild sloth bears of India were identical to those in captive sloth bears and phylogenetically related to H. *ursi* reported from Japanese black bears from Japan. To our knowledge, this is the first report on the molecular characterization of H. ursi infection in Indian sloth bears. Frequent faecal analysis and periodical rotational deworming will prevent this parasitic infestation in the captive population.

## Dental Diesease

Very few cases of Gigantiform cementoma, a rare lesion found in the jaw, have been encountered in bears. Cementoma occurs as a benign fibro-cemento-osseous lesion around the apices of vital teeth and causes severe disfigurement of the jaws. A cementoma was found in a rescued dancing sloth bear *(Melursus ursinus)* in Agra Bear Rescue facility, Agra, India. The upper right canine was extracted using a surgical technique. A mucoperiosteal flap was raised from the medial aspect of right upper canine to distal second premolar. Then the Buccal bone was removed. The histopathological examination of the incised mass revealed the hyperplastic squamous lining of the epithelium had chronic inflammation.

**Periapical Abssces**

An abscess is an abnormal cavity containing pus. The cavity is formed in tissues, due to local suppurative inflammation. Infection around the apex of a tooth leads to perepical abscess; usually it is secondary to periodontal or endodontic disease and it is characterized by acute, severe painful swelling in the area of the affected tooth and also has heavy bacterial load. We treated many clinical case of preiepical abscess in rescued dancing sloth bears. Treatment was done by surgical intervention and extracting the affected tooth was the permanent remedy for the animal.

# Art – 144. THREATS TO WILDLIFE IN AND AROUND AGRA CITY, UTTAR PRADESH AND ITS MANAGEMENT

**Ilayaraja S., M.V. Sharma** and **Arun A. Sha**

## Abstract

Due to the change in land use pattern, unsustainable use of natural resources, climate change, and increasing pollution, the global biodiversity is losing much faster than the natural extinction. Conversion of land by human beings, which results in habitat loss is seen in the tropical forests, temperate, boreal, and arctic region. Increasing human population, increase in the waste production, and pollutants in the urban development are taking a toll in the animal habitat, where the animals coexist with the human. It is very important to educate the person about the conservation ethics, who lives in close proximity with wild animals. There is also a need to teach appropriate: measures to avoid brutal conflicts with the animals. Wildlife SOS is a NGO carrying out round the clock rescue operations in and around Agra city to prevent the conflict situations and also is trying to implement the wildlife protection act, 1972. In this study, the authors have encountered the type of wildlife cases since last one year, which are of potential threat to the natural existence of the wild animals such as skull fracture in python, burning injuries of python, other traumatic injuries due to barbed wire in the fence, severe electrocution injuries in peafowl, dog bite injuries, fractures and mixed bacterial infections. Human made traumatic injuries to hyena such as skull fracture and paraplegia, protruding of the eye ball, etc. Injury to Macaque, include dog bitten wound, paraplegia,

electrocuted wound, bullet wound etc. Monitor lizard and civet cat with traumatic injuries caused by poachers were also observed. So, the authors took effort to report the wildlife cases encountered to further create the awareness and share the experience with other wildlife veterinarians and wildlife activists.

# Art – 145. SLOTH BEAR PEDE-MARKING CAUGHT ON VIDEO

**Shanmugavelu Swaminathan, Attur Shanmugam Arun, Thomas Sharp, Kartick Satyanarayan** and **Geeta Seshamani**

Chemical communication through scent marking plays a significant role in the behavioural lives of solitary predators, including bears. Different forms of scent marking have been documented among the bear species. Grizzly bears *(Ursus arctos)* and American black bears *(U. americanus)* have been observed scratching trees, rubbing against trees or other objects, and pede-marking twisting their feet into the ground to apply scent (Taylor et al. 2015, Sergiel et al. 2016). Andean bears (*Tremarctos ornatus)* rub against trees and other objects (Filipczyková et al. 2017). Polar bears *(U. maritimus)* leave a scent in their tracks (Owen et al. 2014). Giant pandas *(Ailuropoda melanoleuca)* apply a waxy-sticky secretion from their anal gland on a tree of other substrate, sometimes using an elaborate handstand to do so (Kleiman 1983, Nie et al. 2012). Sloth bear *(Melursus ursinus)* scent marking has not been widely discussed, though Laurie and Seidensticker (1977) reported sloth bears rubbing and scratching trees.

The role of pede-marking in bears is not fully understood, and likely varies by species. Pedal glands, both apocrine sweat glands and holocrine sebaceous glands, are well developed in the polar bear (Owen et al. 2014). Brown bears also have relatively prominent apocrine sweat glands (Sergiel et al. 2016). Sloth bears have few prominent apocrine sweat glands on their interdigital skin or around hair follicles (Patil et al. 2016) and lack the rows of prominent apocrine sweat glands present in polar bears (Owen et al. 2014, Patil et al. 2016). It has been suggested, therefore, that sloth bear sexual beahvior is likely communicated by other sources, including urine (Patil et al. 2016).

We captured 8 wild sloth bear pede-marking events on video over a period of 1.5 years (January 2014–June 2016, which may represent the first documented cases of this behaviour in this species. These events were recorded by 4 camera traps, which we set to monitor sloth bear dens in eastern Karnataka, India. All of the subjects were identified as adult males. Additionally, though it is difficult to tell sloth bear individuals apart via camera-trap photograph, we could identify some individuals in multiple videos. In 1 location where we observed 3 pede-marking incidents, 2 were of the same bear and 1

*Camera trap photos (extracted from videos) of wild male sloth bears sniffing and pede-marking in Karnataka, India. The pede-marking involved twisting the foot on the ground in an apparent attempt to apply scent. Left and center photos are of apparently the same bear (based on hair pattern) in the same cave doing the same 179abrador179, a month apart. These 3 photos correspond with events 2, 4, and 8 in the accompanying table. Photos: Shanmugavelu Swaminathan.*

A different bear. The videos showed bears grinding and twisting their back feet into the ground. Some of the videos also captured the bear sniffing the ground before or after the pede-marking event. During some of the pede-marking events the bear was walking forward, while in others it was standing still while twisting and grinding the back paws. One video shows the bear intermittently pede-marking, defecating and rubbing its back side against the entrance of a cave. Pede-marking events typically only lasted a few seconds, though several of the videos captured multiple pede-marking events.

We also observed that one male captive bear at our Bannerghatta Bear Rescue Facility (an 18-year-old male that was rescued from the “dancing bear” trade) displayed the same behaviour. Possibly of relevance, whereas the vast majority of male bears at our facility are castrated, this individual had been vasectomized instead.

**Sloth bear pede-marking events, eastern Karnataka, India.**

| Event | Date | Time | Area | Sex | Comments |
|---|---|---|---|---|---|
| 1 | 13 Jan 2014 | 22:59 | Ramnagara | Male | Same den entrance as event #3. |
| 2 | 8 Feb 2015 | 04:51 | Gudeokotte | Male | Same den as event #4 (see photo). |
| 3 | 22 Feb 2015 | 19:58 | Ramnagara | Male | Possibly same bear as event #1 (1 year later) |
| 4 | 11 Mar 2015 | 19:42 | Gudeokotte | Male | Possibly same bear as event #2, based on fur pattern on back and strikingly similar behavior (see photo). |
| 5 | 5 Jan 2016 | 21:21 | Sulikeri | Male | Pede-marking while facing the camera, not by a den entrance. |
| 6 | 14 Jan 2016 | 04:59 | Sulikeri | Male | Sniffing and pede marking not by a den. |
| 7 | 31 Jan 2016 | 07:29 | Sulikeri | Male | Same location as #5 and #6; appears to be the same individual as in #6. |
| 8 | 7 June 2016 | 07:52 | Ariskere | Male | Defecated twice while pede-marking, and also rubbed hind quarters on a rock near the den entrance (see photo). |

The 8 videos clearly document the use of pede-marking by wild sloth bears. However, the frequency and purpose of this Pede-marking remains uncertain. The largely nocturnal Pede-marking of this species may be a reason why this Pede-marking has eluded observation in the past. Our sampling was biased towards because that is where we set cameras, but we wondered if the behaviour was intended to keep intruders away from valued den sites.

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# Art – 146. MIXED BACTERIAL INFECTION AND ITS SUCCESSFUL TREATMENT INTERVENTION IN PEA FOWL *(Pavo cristatus)*

**Ilayaraja S, Puspendra K Singh, Arun A Sha** and **Baijuraj MV**

## Abstract

In Kosi at Mathura district, Uttar Pradesh few pea fowls died with the history of dullness, anorexia, whitish diarrhea and with facial swelling, so the forest department requested Wildlife SOS to rescue the sick birds from that area. On close examination of the bird revealed nasal blocking and swelling below both the eyes, mucoid discharge from the nostril, impaired vision due to occluded eye because of mucous. Swab collected from oral, cloacal and ocular region sent for detail microbiological examination and antibiogram along with blood sample. The bacteria isolated from ocular swab were pseudomonas spp, E. coli, aeromonas, streptococcus and the bacteria isolated from cloacal, oral swab were E. coli, aeromonas, streptococcus which were sensitive to enrofloxacin, ciprofloxacin, tigenium, imipenem, meropenem, piperacillin + tazobactam, chloramphenicol, gentamycin. Treatment provided as per the result. 10 days after the treatment the hard caceous pus material excavated after from the swelling by the surgical incision and wound opposed with 4.0 absorbable suture materials under local anaesthesia by using lignocaine gel. After 2 weeks wound healed and bird recovered, started showing normal appetite. Bird released back in to the wild in a suitable habitat after complete recovery.

**Keywords:** Antibiogram, Bacterial infection, Local anesthesia, Pea fowl

## Introduction

Pea fowl *(Pavo cristatus)* is national bird of India protected under schedule I of 1972 of Indian wildlife protection act and commonly found in tropical subtropical/ tropical moist lowland, deciduous forests, cultivated lands [1].

An abscess is a collection of pus locally within closed cavity in an organ, tissue. Pyogenic organism cause death of cell which is liquefied by the proteolytic enzymes mainly from the Neutrophils, resulting in the pus in the cavity [17]. Organism that mostly affected with formation of abscess is streptococci and staphylococci [2]. Streptococci are gram positive, non- motile, non – spore forming cocci occurring singly, in pairs, or in chains [3]. Streptococcal also found to be an important cause of corneal ulcer, endophthalmitis, conjunctivitis [7]. Pseudomonas aeruginosa is a chronic infection than acute and causes blindness of eye by penetrating the cornea [19]. Aeromonas species have been associated with health effects like wound infections, septicemia and diarrhoeal illness [11]. Aeromonas also reported to cause ophthalmic infection [5, 10, 14]. E. coli is a gram-negative bacterium in the family enterobacteriaceae. These bacteria are normal inhabitants of the intestines of humane, mammals, reptiles and even some birds [6]. Shammas HF also presented a case of endogenous E. coli endophthalmitis [16]. In low potential avian influenza virus infection symptoms like mild to moderate and occasionally severe respiratory lesions in gallinaceous species including edema of the eyelids, conjunctivitis, catarrhal to fibrinopurulent rhinitis, sinusitis, and tracheitis can be found [12].

## Material and Method

After few birds death at Kosi, in ‘Mathura district’ with a history of whitish diarrhea, anorectic with severe swelling on both side of eyes with mucoid discharge from the nostril and eyes, Wildlife SOS team rescued the sick bird from the affected area as requested by the forest department which was having the symptoms of whitish diarrhea and severe swelling on face with nasal discharge. Detail physical examination revealed hard swelling just below the eyes (figure 1) which is affecting the eye vision thus cause difficulty to consume its food. Radiographic examination also done to ruled out no fracture on the skull bone.

The thermography examination revealed irregular thermal patches suggesting hot inflammatory swelling. Blood sample and swab collected from oral, cloacal and ocular region sent for microbiological examination and antibiogram to Indian Veterinary Research Institute.

## Result and Treatment

Before obtaining result report first aid treatment provided with broad-spectrum antibiotics in order to stabilize the health condition and control the infection. The ocular lesions and swelling were rinsed and cleaned with metronidazole which is generally covers the causative organisms most commonly found in the infection. The occluded purulent discharge from the eye lids gently removed from the both the side and all the discharge from the nose was washed and cleaned sterile gauze. Ointment Silver sulfadiazine mixed with dimethyl sulphoxide applied on swelling on both side. A single dose of long acting of anti- biotic injection Enrofloxacin and antiinflammatory injection meloxicam for 3 days given to the bird.

Result of microbiological examination revealed ocular swab was positive for pseudomonas spp, E. coli, aeromonas, streptococcus and oral and cloacal swab was positive for E. coli, aeromonas, streptococcus. Blood plasma antibody titter found

found > 500 against aeromonas, streptococcus isolates. The isolates were sensitive to enrofloxacin, ciprofloxacin, tigenium, imipenem, meropenem, piperacillin + tazobactam, chloramphenicol, gentamycin as per the result obtained from antibiogram. The isolates were resistance to beta- lactam drugs and extended spectrum beta lactamase (ESBL) types. Microbiological examination negative for infectious coriza and no symptoms found of the same disease. Injection chloramphenicol given for 5 days and topical treatment done with dimethyl sulphoxide (DMSO) and eye drop containing chloramphenicol for 10 days. Condition of the bird improved gradually and started having grains with normal appetite. After the antibiotic course and topical treatment, the swelling on the left side reduced comparatively. Nasal discharged greatly reduced but no improvement on the swelling on the right side of the eye. So, the decision made to incise the swelling to evacuate the pus material. Fallowed by lignocaine gel application; Swelling incised and hard caceous pus removed surgically and incised area sutured on opposition by 4.0 absorbable suture materials [18] (figure 2). Two weeks after surgery the wound healed, and bird recovered uneventfully.

**Discussion**

Differential diagnosis is more important to relevant the cause of infection. In present case study the bird was showing symptoms of dullness, anorexia, whitish diarrhea. Swab culture from cloaca found coli and Aeromonas species, streptococcus species. Similar symptoms were found in aeromonas species infection [11] (Marisa Di Bari 2007). E. coli infection cause also cause abdominal pain, rectal bleeding and diarrhoeal symptoms [15]. On close examination revealed bird having nasal blocking and swelling below the both the eyes, mucoid discharge from the nostril. Ocular swab on culture examination found pseudomonas species isolates. Microbiological examination negative for infectious coriza and avian influenza virus and no symptoms found of the same disease. Pseudomonas species infection found to be cause complete blindness when directly or indirectly introduced into the cornea [19] and Streptococci infection cause formation of abscess [2]. Aeromonas also reported to cause ophthalmic infection [5, 10, 14]. We found chloramphenicol has sensitivity over pseudomonas, streptomycin and Aeromonas in antibiotic sensitivity test. So, treatment given with parental administration of chloramphenicol. After 10 days pus become hard which was removed surgically.

## Conclusion

In current case; Location of abscess was just below the eye ball of both the side which is affecting the vision and causing more pain so the bird couldn't approach and consume the feed. Such a bird with a hindered vision is also easy prey for stray dogs and other predators. Any swelling in the body especially in the head and neck region need to be differentiates immediately and treatment needs to provide accordingly to save the animal.

## Acknowledgement

We are sincerely thanking to Mathura forest department, Uttar Pradesh for their effort to save the bird, All animal care staff of wildlife SOS, and Mr. Kartik Satyanarayan & Geeta Sheshmani Co- Founders, Wildlife SOS for their kind support

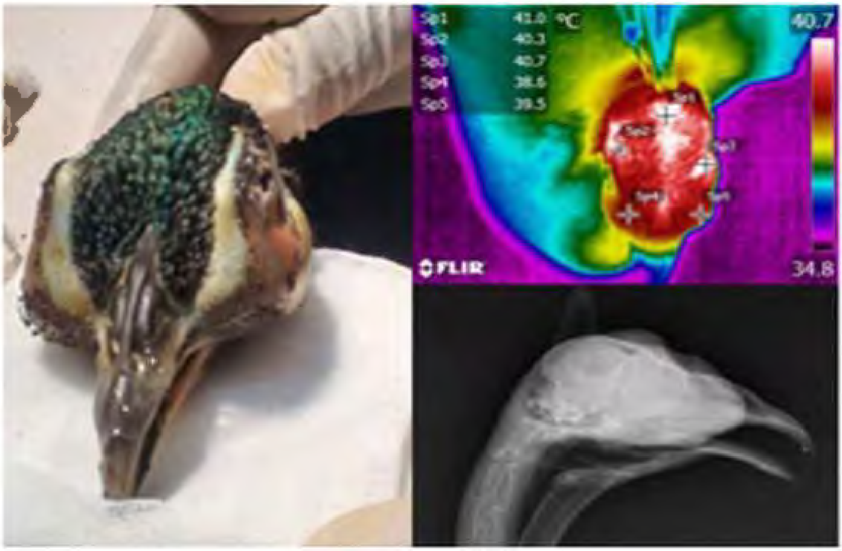


**Fig 1: Hard swelling below the eyes. Radiographic examination not revealed any fracture Thermography revealed sever inflammatory hot sports on the head.**

**Fig 2: Making incision on the swelling, evacuating the calcified pus and Incision opposed with simple interrupted suture.**

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## Art – 147. COXO-FEMORAL OSTEOARTHRITIS IN A RESCUED DANCING SLOTH BEAR *(Melursus ursinus)*

**llayaraja Selvaraj, Arun A. Sha, Puspendra K Singh** and **Yaduraj Khadpekar**

**Abstract**

Arthritis is an inflammatory condition of the joint. Types of arthritis are classified based on the condition and involvement of tissue in the articulation. Osteoarthritis is characterized by degeneration and hypertrophy of bone and cartilage. The rheumatic diseases are well documented in small animals but nothing much for any wild animals. In Agra Bear Rescue Facility one of the rescued adult female dancing bears showed the symptoms of lameness on its left hind limb, due to pain its activity and food intake was reduced. However, we had provided symptomatic treatment but did not get good response. As a routine protocol tranquilization procedure was carried out to do a closer examination, radiography of the joints and confirmed the chronic osteoarthritis at coxo-femoral joint damage due to prolonged malnourishment and forced to perform in an unusual standing position on the roads by the kalander (animal performer); we made the radiographic evaluation of hip joints as a mandatory procedure in our routine health check-up. Thus, we diagnosed varied degrees of osteoarthritis at coxo-femoral joint in different animals and started providing the above said palliative treatment to enhance their life quality and minimize the stress and sufferings.

**Keywords:** Etodolac, Meloxicam, Osteoarthritis, Coxo-femoral joint, Sloth bear, Pain management.

## Introduction

A1thritis is an inflammatory condition of the joint. Based on the condition structure classified and involvement of tissue in the articulation, arthritis is into different types. Osteoarthritis is an inflammatory and Degenerative Joint Disease (DID) characterized by degeneration and hypertrophy of bone and cartilage (Venugopalan, 2002) due to phenomenon of aging or a mecha nic a lly derived process (Hunter et al., 2002; Radin et al., 1982; Resnick D, 2002.). Bears with erosion of the particular cartilage would have severe pain and would have loss of movement, stiffness and difficulties in walking, sitting and risinag (Kitchener et al., 2000 & 2004). DJD is reported in different bear specie s such a *Ursus americanus* American black bear (Storms et al. 2002 & 2004), *Ursus maritimus* Polar bear (Harper et al., 1988), *Tremarctosornatus* – Spectacled bears (Dierenfeld, 1988), but such report for the sloth bear is rarely available except the Paleopathologic examination study of dry-bone skeletons· of captive bears from three different museums in the USA (Greer et al., 1997). Anima ls caught in t he wild seldom (about 1%) have any evidence of OA, in contrast with captive wild animals (Rothschild & Martin., 1993; Rothschild & woods., 1992; Rothschild et al.,1999). Sloth bears in India has been used by the street performers (Kalandar) as dancing bear after the brutal training. The daily stress owing to malnourishment, indiscriminate beating, making them to stand frequently in hind limbs (unusual posture) making them more prone to many kinds of diseases. In this article we have reported a case of osteoarthritis at coxo-femoral joint in a living sloth bears and its management.

## Materials and Methods

In Agra Bear Rescue Facility one of the rescued adult female dancing bears, age around 14 years showed the symptoms of lameness on its left hind limb but no visible injury or swelling was noticed either on hind limbs or hip region. However we had provided symptomatic treatment but with less response, the decision was made to immobilise the bear with lnj Xylazine hydrochloride @ 2mg/kg and lnj.Ketamine hydrochloride @ 5mg/kg for close clinical and radiographic examination of the hip and knee joint. The animal was positioned on dorsal recumbency for taking true ventro-dorsal hip joint articulation viewing image, right lateral recumbency position for taking left knee joint image and blood sample were collected for detail edhemato -biochemical analysis.

## Result and Treatment

The detail radiographic examination of the hip joint revealed osteoarthritis of left coxo-femoral joint (fig 1) and the haemato- biochemical values were within range. The animal was shifted to a small enclosure for better observation and provides suitable enrichments to improve the bear’s activity along with treatment and feeding regulated to maintain its

**Fig. 1: Radiograph showing osteoarthritis of left coxo-femoral joint and normal knee joint of sloth bear**

body weight Meloxicam @ 0.2 mg /kg and Etodoloc 400 mg sustain release tablet administered orally per day for 7 days along with multi vitamin, minerals and glucosamine supplementation. However, the animal was unable to bear its weight fully on left hind limb and its activity pattern and food intake became normal and it started walking without much difficulty.

## Discussion

Degenerative Joint Disease (DJD is a common disorder of umans and animals. It is generally regarded as a non-inflammatory condition of particular cartilage resulting from natural aging, trauma, or disease. DJD is a seque l to cartilage insult, but because cartilage lacks intrinsic vascularity ‘it does not become in flamed, thus the designation of DJD as a non-inflammatory condition (Charles et al., 1985. Animals and some microbes lack the capacity to synthesize pantothenate (vitamin BS and are totally dependent on the uptake of exogenous pantothenic acid (Antonio Sampedro et al., 2015. The deficiency of vitamin BS leads to arthritis and rheumatism (Barton- wright & Elliott., 1963. Removal of an animal from its natural habitat is associated with a 10-fold increase in the prevalence of osteoarthritis, whether the animals were zoo or colony raised (Rothschild & Martin., 1993; Rothschild & woods., 1992; Rothschild et al., 1999. Whiteside et al., (2006 stated that Meloxicam has shown good clinical efficacy for the treatment of osteoarthritis and other painful conditions in large felids. Ball et al., (2001) reported the use of Etodolac as an adjunct to managing osteoarthritis in captive Bengal tigers *(Panthera tigris bengalis)* (Ball et al., 2001) and Budsberg et al., (1999) also described the efficacy of Etodolac in improving hind limb function in dogs with osteoarthritis of the hip joint. Kitchener (2004) suggested that ensuring an appropriate level of activity in zoo bears may be important to minimize the development of skeletal diseases.

**Conclusion**

The authors strongly suspect that the predisposing cause for osteoarthritis in this rescued dancing sloth bear might be due to its previous history of kalandars making the bears stand on its hind limbs a lot which is unusual in nature thus the constant pressure and overload on hip joints and hind limbs. After we learned that these rescued sloth bears were more prone to Coxo-femoral osteoarthritis, we made the radiographic examination and evaluation of hip joins as a mandatory procedure to find normal (Fig. 2) and affected hip joints (Fig. 3).

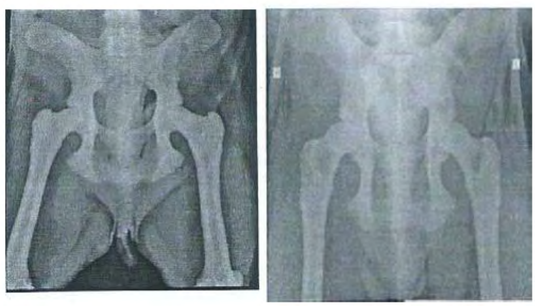

**Fig 2: Normal hip joint of Sloth bear (Female & Male)**

Thus, we diagnosed various degrees of osteoarthritis at coxofemoral joint and initiated the treatment as mentioned. Providing isolated enclosure, suitable enrichment activities along with periodical medication (meloxicam, etodolac, vitamins, mineral and glucosamine supplement) and radiographic evaluation to further improve the treatment and care will minimize the pain and suffering thus will help to provide better quality of life to the bears in captivity.

**Acknowledgement**

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**Fig. 3: Various digress of hip joint osteoarthritis is sloth bears**

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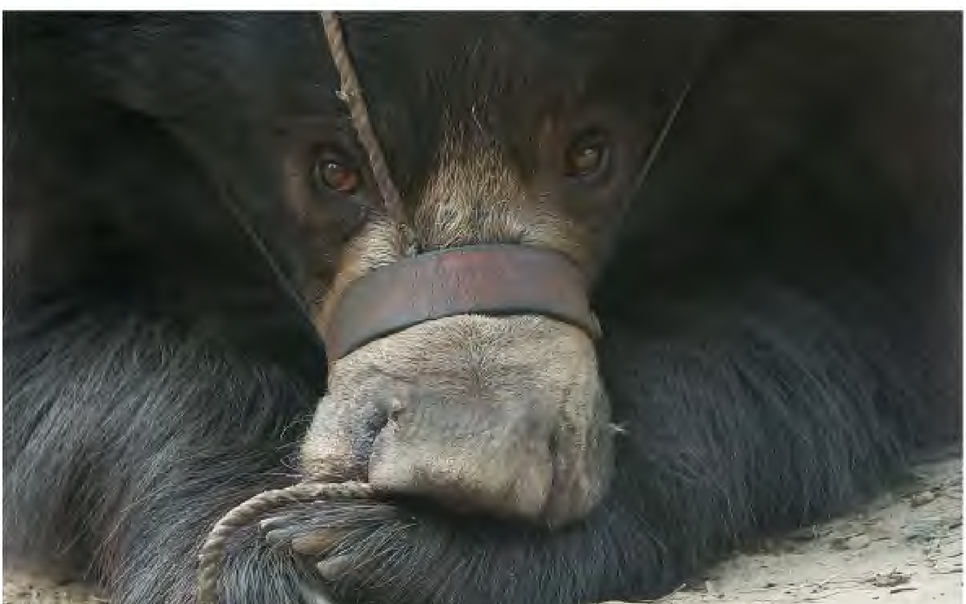
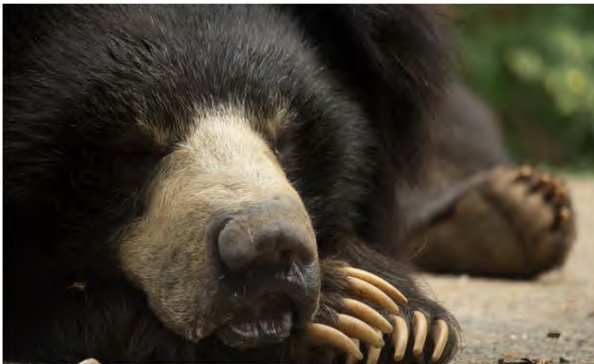
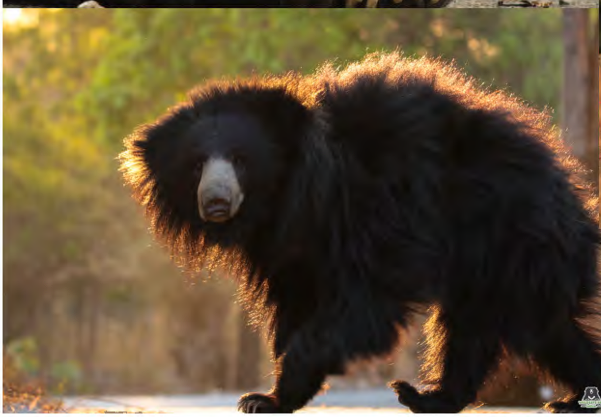
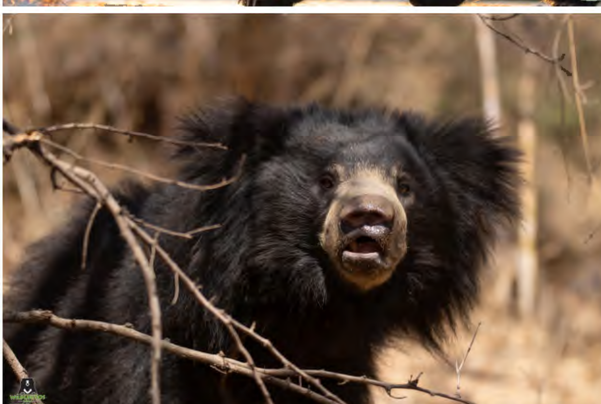
**INDIAN STAR TORTOISE REPATRIATION:** Wildlife SOS, along with the Singapore based rescue facility ACRES, brought back 50+ Indian Star Tortoises to their native habitat in Karnataka. After a three month quarantine period, the tortoises were tagged with radio transmitters for the study of their survival and movement. Intensive monitoring measures have been put in place to track these animals upon reintroduction in the wild to evaluate the success of this unique project.



**RANGILA BEAR RESCUE:** On 11th July, 2018, Wildlife SOS welcomed its newest member, Rangila. The dancing bear was confiscated at the Indo Nepal border and repatriated to India with the help of Indian and Nepalese government. After months of struggle and a 1000 km journey, Rangila has been successfully rehabilitated at the Agra Bear Rescue Facility.







# Art – 148. BASELINE HEMATOLOGY AND SERUM BIOCHEMISTRY RESULTS FOR INDIAN LEOPARDS
*(Panthera pardus fusca)*

**Arun Attur Shanmugam,** Sanath Krishna Muliya, **Ajay Deshmukh,** Sujay Suresh, Anukul Nath, Pa Kalaignan, Manjunath Venkataravanappa and Lyju Jose

## Abstract

Aim: The aim of the study was to establish the baseline hematology and serum biochemistry values for Indian leopards *(Panthera pardus fusca)*, and to assess the possible variations in these parameters based on age and gender.

## Materials and Methods

Hemato-biochemical test reports from a total of 83 healthy leopards, carried out as part of routine health evaluation in Bannerghatta Biological Park and Manikdoh Leopard Rescue Center, were used to establish baseline hematology and serum biochemistry parameters for the subspecies. The hematological parameters considered for the analysis included Hemoglobin (Hb), packed cell volume, total erythrocyte count (TEC), total leukocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and MCH concentration. The serum biochemistry parameters considered included total protein (TP), albumin, globulin, aspartate aminotransferase, alanine aminotransferase (ALT), blood urea nitrogen, creatinine, triglycerides, calcium, and phosphorus.

## Results

Even though few differences were observed in hematologic and biochemistry values between male and female Indian leopards, the differences were statistically not significant. Effects of age, however, were evident in relation to many hematologic and biochemical parameters. Sub-adults had significantly greater values for Hb, TEC, and TLC compared to adults and geriatric group, whereas they had significantly lower MCV and MCH compared to adults and geriatric group. Among, serum biochemistry parameters the sub-adult age group was observed to have significantly lower values for TP and ALT than adult and geriatric leopards.

## Conclusion

The study provides a comprehensive analysis of hematologic and biochemical parameters for Indian leopards. Baselines established here will permit better captive management of the subspecies, serve as a guide to assess the health and physiological status of the free ranging leopards, and may contribute valuable information for making effective management decisions during translocation or rehabilitation process.

**Keywords:** hematology, Indian leopard, Panthera pardus fusca, serum biochemistry

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## Introduction

Leopards are one of the widely distributed wild felids in the world, with nine subspecies found in varied habitats across their extent range [1]. The species is currently categorized as "vulnerable" on the IUCN Red List, with some subspecies identified as "endangered" or "critically endangered" [2]. India hosts substantial numbers of the subspecies Panthera pardus fusca, the Indian leopard which survives in protected areas and multiple use forests all across India, except for the arid deserts and regions above the timberline in the Himalayas [3]. The subspecies is even known to persist close to human populations by feeding on live- stock and domestic dogs [4]. Ensuing this, incidences of human-leopard conflicts have also seen a sharp rise in recent past in India, leading to casualties on both sides. This has, in turn, led to increased veterinary interventions in the species. Further, a large population of Indian leopards exist in captivity maintained in almost all the Indian zoos [5].

Given the difficulty in detecting signs of disease and distress in wild animals, comprehensive health assessments, including hematology and serum biochemistry studies, have been crucial to assess the effects of many health-related problems [6]. Such information has also been proven useful to assess the subclinical effects of pathogens [7] and physiological [8,9], ecological [10], or nutritional [7] status, among other issues.

To interpret a laboratory report to be normal or abnormal, the values expected to be obtained from healthy animals (reference intervals) must be known first [11]. However, unlike other large felids [12-15], a reliable baseline hematological and serum biochemistry references, that can be used to assess the health status of Indian leopards is still lacking. The reference intervals available are usually based on limited population testing and do not account for variation within the subspecies or within subpopulations defined by age, gender, and other factors [16,17]. Further, these parameters can also be influenced by several additional extrinsic factors such as the presence of pathological condition, phlebotomy techniques used, and chemical immobilization before sampling [18]. Thus, this study was undertaken to establish the baseline reference interval of 7 hematology and 11 serum biochemistry variables from Indian leopards and to assess the influence of age and gender on these variables, while keeping the extrinsic factors constant.

## Materials and Methods

### Ethical approval

Not applicable: None of the animals were sampled solely for the purpose of this study. The study was carried out by compiling and analysing available hemato-biochemical test reports, carried out as part of routine health evaluations in both the study sites.

### Study area and animals

Hemato-biochemical test reports from a total of 83 healthy leopards, carried out as part of routine health evaluation in Bannerghatta Biological Park, Karnataka (BBP, n=41) and Manikdoh Leopard Rescue Center, Maharashtra (MLRC, n=42) between January 2014 and January 2016 were used to establish baseline hematology and serum biochemistry parameters for the subspecies. The leopards from BBP included both captive born leopards from zoo section and rescued free ranging leopards from rescue center, whereas leopards from MLRC were mainly rescued free ranging leopards. While the reports from animals which appeared to be healthy and clinically stable during sampling were considered, reports from the ones with visible injuries and known history of illness were excluded from the analysis.

## Blood collection and processing

Since it is crucial that samples are obtained under consistent conditions to arrive at baseline value, the restraint and phlebotomy techniques were also considered. Based on available information, uniform protocols were followed in both the institutions. All the leopards considered for this study were physically restrained by securing them in squeeze cages, and phlebotomy was done from lateral coccygeal vein, without any chemical immobilization. Hematological parameters were evaluated using commercially available automated analyzers (BBP: HumaCount 30TS, HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany; MLRC: BC-1800, Vector Biotek Pvt. Ltd. Gujarat, India), whereas the serum biochemistry assays were performed on semi- automated clinical chemistry analyzers (BBP: ERBA Chem Pro, Transasia House, Mumbai, India; MLRC: Vchem+, Vector Biotek Pvt. Ltd. Gujarat, India) using commercially available biochemical kits, calibrated with control reagents before sample analysis. Further, all the samples were confirmed to have undergone laboratory analyses within 2-3 hours of collection.

## Hemato-biochemical parameters

The hematological parameters considered for the analysis included Hemoglobin (Hb in g/dl), packed cell volume (in %), total erythrocyte count (TEC in 106/µl), total leukocyte count (TLC in 103/µl), mean corpuscular volume (MCV in fl), mean corpuscular Hb (MCH in pg), and MCH concentration (in g/dL). The serum biochemistry parameters considered included total protein (TP in g/ dl), albumin (in g/dl), globulin (in g/dl), aspartate aminotransferase (AST in IU/L), alanine aminotransferase (ALT in IU/L), blood urea nitrogen (in mg/dl), creatinine (in mg/dl), triglycerides (in mg/dl), calcium (in mg/dl), and phosphorus (in mg/dl).

## Statistical analysis

For the purpose of statistical analysis, leopards were grouped according to their gender (male, n=34 and female, n=49) and further into three age groups: Sub-adults (1-3 years old; n=19), adults (3-10 years old, n=38) and geriatric animals (10 years and above, n=26), based on available zoo/rescue center records. The mean and standard deviation for each hemato-biochemical value obtained were calculated as per the standard procedure [19]. If any value was less than or equal to the first quartile −3 times the interquartile range or greater than the third quartile +3 times the interquartile range, they were regarded as extreme outliers and were removed before further analyses and then the middle 95% of test results were considered as the reference interval. Data were further tested for normality using the Shapiro– Wilk test for normality using R version 3.2.5. The dataset which followed normal distribution was tested using independent t-test to compare the mean of the hematological parameters, whereas the parameters which did not meet the criteria of normality were tested using Mann–Whitney U-tests (two factors) to evaluate significant differences. The variation between different age classes irrespective of gender was tested using one-way ANOVA followed by Tukey test for the data following the normal distribution pattern and Kruskal– Wallis test for the data which did not meet the criteria. Significance level was $p<0.05$ for all tests.

## Results

The results and descriptive statistics for hemato-biochemistry parameters analyzed in this study are listed in Tables-1-4. Even though few differences were observed in hematologic and biochemistry values between male and female Indian leopards, the differences were statistically not significant.

**Table-1:** Overall haematology and serum biochemistry results for Indian leopards *(Panthera pardus fusca*) from BBP and MLRC.

| Parameters | N | Minimum | Maximum | Mean± SD | SEM | 95% CI of the mean | |
|---|---|---|---|---|---|---|---|
| | | | | | | LCI | UCI |
| TLC ($10^3$/µl) | 83 | 8.0 | 36.9 | 16.2±6.1 | 0.7 | 14.9 | 17.5 |
| TEC ($10^6$/µl) | 83 | 5.4 | 12.7 | 8.7±2.0 | 0.2 | 8.3 | 9.1 |
| Hb (g/dl) | 83 | 10.5 | 20.2 | 14.7±2.5 | 0.3 | 14.2 | 15.3 |
| PCV (%) | 83 | 32.8 | 65.4 | 48.5±6.8 | 0.8 | 47.0 | 50.0 |
| MCV (fl) | 83 | 45.9 | 93.9 | 58.6±11.3 | 1.2 | 56.2 | 61.1 |
| MCH (pg) | 83 | 13.6 | 28.0 | 18.1±3.0 | 0.3 | 17.4 | 18.7 |
| MCHC (%) | 83 | 21.7 | 36.0 | 30.7±2.8 | 0.3 | 30.1 | 31.3 |
| TP (g/dl) | 83 | 4.3 | 11.1 | 7.0±1.3 | 0.1 | 6.7 | 7.3 |
| Albumin (g/dl) | 83 | 1.6 | 7.0 | 3.9±1.1 | 0.1 | 3.7 | 4.1 |
| Globulin (g/dl) | 83 | 0.3 | 6.6 | 3.0±1.2 | 0.1 | 2.8 | 3.3 |
| AST (IU/L) | 41 | 9.9 | 90.8 | 41.3±19.2 | 3.0 | 35.5 | 47.2 |
| ALT (IU/L) | 83 | 2.0 | 146.7 | 44.0±28.2 | 3.1 | 37.9 | 50.0 |
| CRT (mg/dl) | 83 | 0.4 | 4.2 | 1.6±0.7 | 0.1 | 1.4 | 1.7 |
| BUN (mg/dl) | 83 | 3.1 | 56.3 | 27.6±11.4 | 1.3 | 25.1 | 30.0 |
| Glucose (mg/dl) | 41 | 14.1 | 159.0 | 58.6±26.9 | 4.2 | 50.4 | 66.9 |
| Triglyceride (mg/dl) | 41 | 4.0 | 59.9 | 29.5±12.5 | 2.0 | 25.6 | 33.3 |
| Ca (mg/dl) | 83 | 1.5 | 27.0 | 9.9±3.2 | 0.3 | 9.2 | 10.6 |
| P (mg/dl) | 83 | 2.2 | 12.4 | 5.5±1.8 | 0.2 | 5.1 | 5.9 |

TLC=Total leukocyte count, TEC=Total erythrocyte count, Hb=Hemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, TP=Total protein, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, BUN=Blood urea nitrogen, Ca=Serum calcium, P=Serum phosphorus, CI=Confidence interval, LCI=Lower confidence interval, UCI=Upper confidence interval, BBP=Bannerghatta Biological Park, MLRC=Manikdoh Leopard Rescue Center, SEM=Standard error of mean, SD=Standard deviation

**Table-2:** Comparison of hematology and serum biochemistry results for male and female Indian leopards (*Panthera pardus fusca*) from BBP and MLRC.

| Parameters | Male | | | | | | | Female | | | | | | | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | Min | Max | Mean±SD | SEM | 95% CI of the mean | | n | Min | Max | Mean±SD | SEM | 95% CI of the mean | | |
| | | | | | | LCI | UCI | | | | | | LCI | UCI | |
| TLC ($10^3$/μl) | 34 | 8.7 | 36.9 | 17.2±6.5 | 1.1 | 15.0 | 19.4 | 49 | 8.0 | 28.2 | 15.5±5.9 | 0.8 | 13.9 | 17.0 | 0.37 |
| TEC ($10^6$/μl) | 34 | 5.8 | 12.7 | 8.9±2.2 | 0.4 | 8.2 | 9.7 | 49 | 5.4 | 11.8 | 8.6±1.8 | 0.3 | 8.0 | 9.1 | 0.99 |
| Hb (g/dl) | 34 | 10.5 | 20.2 | 15.0±2.7 | 0.5 | 14.1 | 15.9 | 49 | 11.0 | 19.9 | 14.6±2.3 | 0.3 | 14.0 | 15.1 | 0.57 |
| PCV (%) | 34 | 32.8 | 65.4 | 49.2±7.2 | 1.2 | 46.8 | 51.6 | 49 | 34.0 | 60.7 | 48.0±6.6 | 0.9 | 46.2 | 49.7 | 0.83 |
| MCV (fl) | 34 | 47.0 | 88.1 | 57.5±11.1 | 1.9 | 53.8 | 61.2 | 49 | 45.9 | 93.9 | 59.4±11.6 | 1.7 | 56.0 | 62.7 | 0.45 |
| MCH (pg) | 34 | 14.2 | 28.0 | 18.3±3.3 | 0.6 | 17.2 | 19.4 | 49 | 13.6 | 25.0 | 17.9±2.8 | 0.4 | 17.1 | 18.6 | 0.59 |
| MCHC (%) | 34 | 24.4 | 35.1 | 31.1±2.3 | 0.4 | 30.3 | 31.9 | 49 | 21.7 | 36.0 | 30.4±3.0 | 0.4 | 29.6 | 31.1 | 0.46 |
| TP (g/dl) | 34 | 4.6 | 10.4 | 6.8±1.2 | 0.2 | 6.4 | 7.2 | 49 | 4.3 | 11.1 | 7.1±1.4 | 0.2 | 6.7 | 7.4 | 0.31 |
| Albumin (g/dl) | 34 | 1.9 | 7.0 | 4.0±1.1 | 0.2 | 3.6 | 4.4 | 49 | 1.6 | 6.6 | 3.9±1.1 | 0.2 | 3.5 | 4.2 | 0.46 |
| Globulin (g/dl) | 34 | 0.3 | 5.6 | 2.9±1.2 | 0.2 | 2.4 | 3.3 | 49 | 0.3 | 6.6 | 3.2±1.3 | 0.2 | 2.8 | 3.5 | 0.66 |
| AST (IU/L) | 22 | 10.6 | 90.8 | 44.7±21.0 | 4.5 | 35.9 | 53.4 | 19 | 9.9 | 77.5 | 37.5±16.8 | 3.9 | 29.8 | 45.1 | 0.24 |
| ALT (IU/L) | 34 | 2.0 | 120.2 | 37.0±22.9 | 3.9 | 29.3 | 44.7 | 49 | 3.7 | 146.7 | 48.8±30.6 | 4.4 | 40.1 | 57.4 | 0.91 |
| Creatinine (mg/dl) | 34 | 0.5 | 3.0 | 1.5±0.6 | 0.1 | 1.3 | 1.7 | 49 | 0.4 | 4.2 | 1.6±0.7 | 0.1 | 1.4 | 1.7 | 0.32 |
| BUN (mg/dl) | 34 | 3.9 | 56.3 | 27.5±13.6 | 2.3 | 22.9 | 32.1 | 49 | 3.1 | 49.9 | 27.6±9.7 | 1.4 | 24.8 | 30.3 | 0.63 |
| Glucose (mg/dl) | 22 | 14.1 | 159.0 | 59.4±30.7 | 6.6 | 46.6 | 72.2 | 19 | 14.1 | 95.1 | 57.7±22.4 | 5.1 | 47.7 | 67.6 | 0.81 |
| Triglycerides (mg/dl) | 22 | 4.7 | 59.9 | 31.1±12.0 | 2.6 | 26.1 | 36.1 | 19 | 4.0 | 52.9 | 27.6±13.2 | 3.0 | 21.7 | 33.4 | 0.39 |
| Ca (mg/dl) | 34 | 3.5 | 14.7 | 9.6±2.7 | 0.5 | 8.7 | 10.5 | 49 | 1.5 | 27.0 | 10.1±3.5 | 0.5 | 9.1 | 11.0 | 0.67 |
| P (mg/dl) | 34 | 2.4 | 12.4 | 5.6±2.3 | 0.4 | 4.8 | 6.3 | 49 | 2.2 | 8.9 | 5.5±1.5 | 0.2 | 5.1 | 5.8 | 0.70 |

TLC=Total leukocyte count, TEC=Total erythrocyte count, Hb=Hemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, TP=Total protein, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, BUN=Blood urea nitrogen, Ca=Serum calcium, P=Serum phosphorus, CI=Confidence interval, LCI=Lower confidence interval, UCI=Upper confidence interval, BBP=Bannerghatta Biological Park, MLRC=Manikdoh Leopard Rescue Center, SEM=Standard error of mean, SD=Standard deviation

Effects of age, however, were evident in relation to many hematologic and biochemical parameters. Sub-adults had significantly greater values (mean ± standard deviation) for Hb (16.0±2.9 g/dL), TEC (10.1±1.7 × 106/μl), and TLC (19.1±4.1 × 106/μl) compared to adults (Hb:14.4±2.1g/dL; TEC:8.4±1.9× 106/μl and TLC: 15.8±6.8 × 103/μl) and geriatric group (Hb: 14.3 ±2.5 g/ dL; TEC: 8.1±1.9 × 106/μl and TLC: 14.7±5.8 × 103/ μl), whereas they had significantly lower MCV (50.8±2.1 fl) and MCH (16.3±1.1 pg) compared to adults (MCV: 60.8±12.8 fl and MCH: 18.8±3.4 pg) and geriatric group (MCV: 61.3±10.7 fl and MCH: 18.2±2.8 pg). Among, serum bio-chemistry parameters the sub-adult age group were observed to have significantly lower values for TP (6.5±1.4 g/ dl) and ALT (25.8±14.5 IU/L) than adults (TP: 7.0±1.4 g/dl and ALT: 46.8±26.8 IU/ L) and geriatric leopards (TP: 7.3±1.4 g/dl and 53.1±32.1 IU/L) age groups.

**Table-3: Comparison of hematology results for sub-adult, adult and geriatric Indian leopards** *(Panthera pardus fusca)* **from BBP and MLRC.**

| Parameters | n | Min | Max | Mean | SEM | SD | 95% CI of the mean | | p value |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | LCI | UCI | |
| Sub-adults | | | | | | | | | |
| TLC ($10^3$/µl) | 19 | 10.3 | 26.4 | 19.1 | 0.9 | 4.1 | 17.2 | 20.9 | 0.027 |
| TEC ($10^6$/µl) | 19 | 6.8 | 12.7 | 10.1 | 0.4 | 1.7 | 9.4 | 10.8 | 0.001 |
| Hb (g/dl) | 19 | 10.5 | 20.2 | 16.0 | 0.7 | 2.9 | 14.7 | 17.2 | 0.05 |
| PCV (%) | 19 | 32.8 | 65.4 | 49.9 | 1.9 | 8.4 | 46.1 | 53.6 | 0.537 |
| MCV (fl) | 19 | 47.0 | 54.2 | 50.8 | 0.5 | 2.1 | 49.8 | 51.7 | 0.002 |
| MCH (pg) | 19 | 14.2 | 18.4 | 16.3 | 0.2 | 1.1 | 15.8 | 16.8 | 0.009 |
| MCHC (%) | 19 | 29.7 | 33.6 | 31.9 | 0.1 | 1.2 | 33.7 | 34.0 | 0.112 |
| Adults | | | | | | | | | |
| TLC ($10^3$/µl) | 38 | 8.7 | 27.9 | 15.8 | 1.1 | 6.8 | 13.6 | 17.9 | -a |
| TEC ($10^6$/µl) | 38 | 5.4 | 11.8 | 8.4 | 0.3 | 1.9 | 7.8 | 9.0 | - |
| Hb (g/dl) | 38 | 11.0 | 20.2 | 14.4 | 0.4 | 2.1 | 13.7 | 15.1 | - |
| PCV (%) | 38 | 34.0 | 60.9 | 48.5 | 1.0 | 6.4 | 46.4 | 50.5 | - |
| MCV (fl) | 38 | 46.2 | 88.6 | 60.8 | 2.1 | 12.8 | 56.7 | 64.8 | - |
| MCH (pg) | 38 | 13.6 | 23.9 | 18.8 | 0.6 | 3.4 | 17.8 | 19.9 | - |
| MCHC (%) | 38 | 25.6 | 34.2 | 30.4 | 0.6 | 3.4 | 29.4 | 31.5 | - |
| Geriatric | | | | | | | | | |
| TLC ($10^3$/µl) | 26 | 8.7 | 27.9 | 14.7 | 1.1 | 5.8 | 12.5 | 16.9 | -a |
| TEC ($10^6$/µl) | 26 | 5.4 | 11.8 | 8.1 | 0.4 | 1.9 | 7.4 | 8.9 | - |
| Hb (g/dl) | 26 | 11.0 | 20.2 | 14.3 | 0.5 | 2.5 | 13.4 | 15.3 | - |
| PCV (%) | 26 | 34.0 | 60.9 | 47.6 | 1.2 | 6.3 | 45.1 | 50.0 | - |
| MCV (fl) | 26 | 46.2 | 88.55 | 61.3 | 2.1 | 10.7 | 57.1 | 65.4 | - |
| MCH (pg) | 26 | 13.6 | 23.88 | 18.2 | 0.5 | 2.8 | 17.1 | 19.3 | - |
| MCHC (%) | 26 | 25.6 | 34.2 | 30.3 | 0.5 | 2.4 | 29.3 | 31.2 | - |

*[a]p values for sub-adult, adult and geriatric group comparison are listed in the top (sub-adult) half of the table. TLC=Total leukocyte count, TEC=Total erythrocyte count, Hb=Hemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, CI=Confidence interval, LCI=Lower confidence interval, UCI=Upper confidence interval, BBP=Bannerghatta Biological Park, MLRC=Manikdoh Leopard Rescue Center, SEM=Standard error of mean, SD=Standard deviation*

## Discussion

Flagging haematology and biochemistry values obtained from an animal as either normal or abnormal is the first interpretive step in interpreting hemato-biochemical reports. However, this is not as simple as it may seem in wild animals as reference intervals are usually based on limited population testing and do not account for variation within subpopulations defined by age, sex, subspecies, or extrinsic factors such as instrumentation and phlebotomy techniques. Further, adequate numbers of normal animals must be sampled to arrive at intervals that are valid for healthy animals from the defined population. Thus, unlike published studies [16], sampling large numbers of animals to make the results most reflective of the healthy population is highly desirable. A reference interval is typically defined as values encompassing the median 95% of a tested population of apparently healthy animals [11]. Inherent in this definition is that 2.5% of the healthy population will have values outside either side of the median 95%, suggesting they are abnormal [11]. Thus, the nonparametric method used to arrive at reference intervals in this study, wherein the test values were rank ordered, and any outliers were removed and then the middle 95% of test results define the reference interval are less biased, compared to other methods [9,11,20].

In felids, the number, size and Hb concentration of circulating erythrocytes is known to increase gradually after 1 month of age and, at 3-4 months reaches values similar to those of adults depending on the amount of iron in the diet [21]. However, this study showed significantly greater TEC and Hb counts in sub-adults compared to adult and geriatric animals. This might be subsequent to transient polycythemia from splenic contraction [22], a momentary response to epinephrine due to stress, anger, and fear as observed in sub-adults, most of them with a history of being and the RBC counts are known to revert to normal in a short period [22].

Higher TLC observed in sub-adults may also be indicative of low-level stressor factors inherent with the animals newly introduced to captive environment. The significantly lesser MCV and MCH values observed in sub-adults, in comparison to geriatric and adult age groups leopards can be attributed to the age and dietary differences that permit older age classes to produce relatively larger erythrocytes than younger age classes; similar to the patterns observed in other carnivores [20,23,24].

Among serum biochemistry parameters, sub- adults had significantly lower TP compared to adults and geriatric leopards. The increased serum concentrations of TPs in the older age groups could be explained as an age-related phenomenon, having been observed in other carnivores [25,26] and also probably due, in part, to the increase in gammaglobulins induced by either vaccinations or increased contact with environmental microorganisms [27]. Further, the TP levels for be normally low, consistent with the expected higher physiological demand for proteins during their growth phase [28].

ALT and AST are used as general indicators of liver function, with ALT being more liver-specific [29]. In this study, older age classes were observed to have higher ALT activity compared to young animals and in particular, sub-adults had significantly lower ALT levels than geriatric animals. This observation in older age classes can be attributed to physiological variations related with age, hormone action, reproductive phases as well as exposure of liver to a wide array of toxins, infectious, agents, drug metabolites and endo- toxins over time [30].

## Conclusion

This study provides a comprehensive analysis of hematologic and biochemical parameters for Indian leopards, including the possible variations in these parameters based on age and sex. To the best of authors' knowledge, there are no similar published reference intervals available for the subspecies and thus the baselines provided here will permit better captive management of the subspecies, serve as a guide to assess the health and physiological status of the free ranging leopards, and may contribute valuable information for making effective management decisions during translocation or rehabilitation process.

**Table-4:** Comparison of serum biochemistry results for sub-adult, adult and geriatric Indian leopards (*Panthera pardus fusca*) from BBP and MLRC.

| Parameter mean | n | Minimum | Maximum | Mean | SEM | SD | 95% CI of the LCI | UCI | p value |
|---|---|---|---|---|---|---|---|---|---|
| Sub adult | | | | | | | | | |
| TP (g/dl) | 19 | 4.3 | 11.1 | 6.5 | 0.3 | 1.4 | 5.9 | 7.2 | 0.01 |
| Albumin (g/dl) | 19 | 1.9 | 5.0 | 4.0 | 0.2 | 0.8 | 3.6 | 4.3 | 0.91 |
| Globulin (g/dl) | 19 | 0.3 | 6.6 | 2.7 | 0.3 | 1.4 | 2.0 | 3.3 | 0.168 |
| AST (IU/L) | 19 | 9.9 | 77.7 | 39.6 | 4.5 | 19.8 | 30.7 | 48.5 | 0.561 |
| ALT (IU/L) | 19 | 2.0 | 50.2 | 25.8 | 3.3 | 14.5 | 19.2 | 32.3 | 0.001 |
| Creatinine (mg/dl) | 19 | 0.4 | 4.2 | 1.7 | 0.2 | 1.0 | 1.2 | 2.1 | 0.213 |
| BUN (mg/dl) | 19 | 7.5 | 56.1 | 25.7 | 2.7 | 11.9 | 20.4 | 31.1 | 0.645 |
| Glucose (mg/dl) | 19 | 14.1 | 159.0 | 66.1 | 7.3 | 31.8 | 51.8 | 80.3 | 0.249 |
| Triglycerides (mg/dl) | 19 | 4.0 | 52.9 | 27.4 | 3.0 | 13.0 | 21.5 | 33.2 | 0.62 |
| Ca (mg/dl) | 19 | 3.5 | 27.0 | 9.3 | 1.1 | 5.0 | 7.1 | 11.6 | 0.193 |
| P(mg/dl) | 19 | 2.6 | 12.4 | 6.2 | 0.5 | 2.1 | 5.2 | 7.2 | 0.181 |
| Adult | | | | | | | | | |
| TP (g/dl) | 38 | 4.9 | 10.1 | 7.0 | 0.2 | 1.4 | 6.6 | 7.4 | -[a] |
| Albumin (g/dl) | 38 | 1.8 | 6.7 | 3.9 | 0.2 | 1.2 | 3.6 | 4.3 | - |
| Globulin (g/dl) | 38 | 0.3 | 5.2 | 3.0 | 0.2 | 1.2 | 2.6 | 3.4 | - |
| AST (IU/L) | 16 | 13.7 | 49.0 | 45.3 | 5.2 | 20.7 | 35.1 | 55.4 | - |
| ALT (IU/L) | 38 | 21.3 | 147.0 | 46.8 | 4.4 | 26.8 | 38.3 | 55.3 | - |
| Creatinine (mg/dl) | 38 | 0.5 | 2.5 | 1.7 | 0.1 | 0.6 | 1.5 | 1.8 | - |
| BUN (mg/dl) | 38 | 3.1 | 56.3 | 27.5 | 1.8 | 11.4 | 23.9 | 31.1 | - |
| Glucose (mg/dl) | 16 | 24.1 | 66.2 | 53.5 | 5.6 | 22.5 | 42.4 | 64.5 | - |
| Triglycerides (mg/dl) | 16 | 22.9 | 43.9 | 31.1 | 3.4 | 13.6 | 24.4 | 37.8 | - |
| Ca (mg/dl) | 38 | 7.3 | 14.2 | 10.0 | 0.4 | 2.5 | 9.2 | 10.8 | - |
| P(mg/dl) | 38 | 2.22 | 12.4 | 5.4 | 0.3 | 1.6 | 4.9 | 5.9 | - |
| Geriatric | | | | | | | | | |
| TP (g/dl) | 26 | 4.9 | 10.08 | 7.3 | 0.2 | 1.2 | 6.9 | 7.8 | -[a] |
| Albumin (g/dl) | 26 | 1.8 | 6.7 | 3.8 | 0.2 | 1.0 | 3.5 | 4.2 | - |
| Glo (g/dl) | 26 | 0.3 | 5.23 | 3.4 | 0.2 | 1.1 | 2.9 | 3.8 | - |
| AST (IU/L) | 6 | 13.7 | 49 | 36.5 | 5.5 | 13.6 | 25.7 | 47.4 | - |
| ALT (IU/L) | 26 | 21.3 | 146.7 | 53.1 | 6.3 | 32.1 | 40.8 | 65.5 | - |
| Creatinine (mg/dl) | 26 | 0.5 | 2.54 | 1.4 | 0.1 | 0.5 | 1.2 | 1.6 | - |
| BUN (mg/dl) | 26 | 3.1 | 56.3 | 29.0 | 2.2 | 11.3 | 24.6 | 33.3 | - |
| Glucose (mg/dl) | 6 | 24.1 | 66.2 | 48.9 | 6.0 | 14.8 | 37.1 | 60.8 | - |
| Triglycerides (mg/dl) | 6 | 22.9 | 43.9 | 31.7 | 3.0 | 7.3 | 25.8 | 37.5 | - |
| Ca (mg/dl) | 26 | 7.3 | 14.21 | 10.1 | 0.4 | 2.2 | 9.3 | 11.0 | - |
| P(mg/dl) | 26 | 2.2 | 12.4 | 5.2 | 0.4 | 1.9 | 4.5 | 5.9 | - |

[a]p values for sub-adult, a dult an d geriatric gro p comparison re listed i n the top (sub-adult) half of the tabl . TP=Total protein, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, BUN=Blood urea nitrogen, Ca=Serum calcium, P=Serum phosphorus, CI=Confidence interval, LCI=Lower confidence interval, UCI=Upper confidence interval, BBP=Bannerghatta Biological Park, MLRC=Manikdoh Leopard Rescue Center, SEM=Standard error of mean, SD=Standard deviation

## Authors' Contributions

AAS and SKM conceived and designed the study. AAS, SKM, AD, SS and PK were involved in collection and compilation of data from BBP and MLRC. MV and LJ performed the hematology serum biochemistry analysis. AN carried out all the statistical analysis required for the manuscript. AAS, SKM, AD, SS, PK, MV, AN, and LJ contributed to the manuscript writing and the reviewing of the literature. All authors read and approved the final manuscript.

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## Competing Interests

The authors declare that they have no competing interest.

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# Art – 149. BIRDS SPECIES DIVERSITY OF THE GUDEKOTE SLOTH BEAR SANCTUARY, BELLARY DISTRICT, NORTH KARNATAKA, SOUTHERN INDIA

**Reegan P, Swaminathan S, Arun A Sha, Yogaraj P** and **Balasubramaniyan G**

## Abstract

Ecological investigation of Bird species diversity of the avifauna recorded during a survey of the Gudekote sloth bear Sanctuary, Bellary District, North Karnataka. The survey was carried out between March 2015 and April 2016. Transect count and point count methods were used to investigate the abundance of birds. Observation was conducted by periodically walking along the study area early in the morning and late in the afternoon. Different diversity indices and statistical methods were used to analyze data collected during the field survey. A total of 132 bird species and 50 families were recorded. Terrestrial habitat contributed much in terms of species composition (87%) than aquatic habitat (13%). Shannon's diversity index indicate that terrestrial habitat had higher species diversity (H' = 3.9996) than aquatic habitat (H' = 3.0717). The overall bird diversity in both terrestrial and aquatic was (H'=4.2669). Appropriate management of bird attractant sites is very important to discourage birds from the Gudekote sloth bear Sanctuary. A complete checklist of species recorded from the area is also given along with respective relative abundance levels recorded during the survey.

**Keywords:** Species, diversity, birds, Gudekote sloth bear Sanctuary.

## Introduction

Bird community evaluation has become an important tool in biodiversity conservation and for identifying conservation actions in areas of high human pressure. The Indian subcontinent is known for diverse and rich bird species whose taxonomy, distribution and their general habitat characteristics are well documented in India.

Bird communities have been studied fairly well both in temperate and tropical forests. However, only a very little information is known about the bird community structure and their dynamics in India. Understanding the diversity and structure of bird communities is essential to delineate the importance of regional or local landscapes for avian conservation. Determinations of bird populations in different habitats are central to understanding the community structure and niche relationships, as well as for intelligent management of populations. Moreover, seasonal monitoring is equally important to trace the dynamic movement of birds in such habitats.

Birds are among the most easily defined and readily recognized categories of animals, due to the presence of the feather, which is unique to them. In addition to feathers, the development of forelimbs as wings, mostly in flight; feathered tail that serves for balancing, steering and lifting; toothless horny beak and skeleton exhibiting unique adaptations, mainly for flight and bipedal locomotion are characteristics of birds (Wallace et al. 1975; Padian et al. 1998).

Birds are both visually and acoustically conspicuous organisms of most ecosystems. Because they are comparatively easy to identify, birds have received considerable attention of humans (Mclay 1974; Whelan et al. 2008). Although they occupy most of the earth's surface, most species are found only in particular regions and habitats, whereas others are cosmopolitan (Van Tyne et al. 1959). Patterns of abundance and distribution of birds are strongly related to environmental factors, which determine their presence and activity. The power of flight allows them to move easily through the air and yet they are perfectly adapted to every environment that fit their requirements for successful reproduction and survival (Welty 1985; Estrella 2007). India harbors 1200 species of birds among 13% of the 9600 bird species of the world (Ali et al. 1987). However, with the new classification coming into force, the number of species may well be 1300 (Javed et al. 2000).

Urban biodiversity has received very little attention from conservation biologist as compared to natural and protected ecosystem (Jules et al. 1997; Vandermeer 1997). The main aim of this paper is to make comprehensive based line information about the bird species for the future as well as to create awareness for their conservation.

## Study Area

The Gudekote Sloth bear Sanctuary is located in Kudligi and Sandur Talukas of Bellary District, Karnataka lies 14° 55' to 14° 47'N latitude and 76° 35' to 76° 43' E longitude covered a total geographic area of 47.54 Sq. km. Sanctuary spreads in rocky hillocks and plain forest which in surviving of the drier plains of North Karnataka. (Fig. 1).

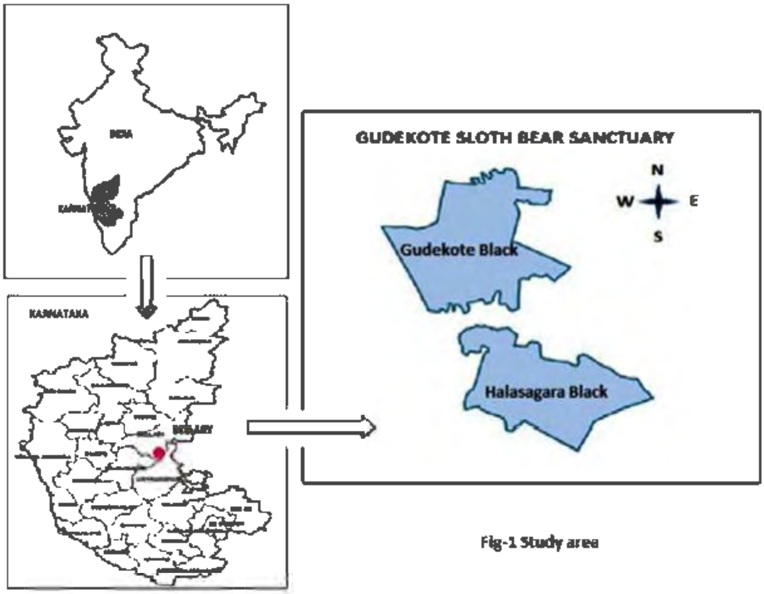

Fig-1 Study area

**Materials & Methods**

Data were collected using three methods: transect walk, point transects and direct observations during March 2015 to April 2016. Line Transect method was used for the bird survey. This method proved most efficient in termsof data collection per unit effort (Yallop et al. 2003). This survey involves an observer moving slowly along the routes and recording all birds detected on either side of the route. The length of transects depends on the type of survey but is usually constrained by accessibility and thus fixed. Line transects are often used to collect data in large, open areas and is more efficient than point count as one tends to record more birds per unit time.

The most of the surveys of the aquatic birds were conducted between November 2015 and February 2016 using a transect line approach (Bibby et al.1992). The line transect method proved most efficient in terms of data collection per unit effort (Yallop et al. 2003). A total of three transects was established along the various flat terrains available within the field areas. Each transect had a total length of 2000 m, with 100 m of transects. For each transect, an observer recorded any bird species and numbers in the area with the aid of binoculars. At each site, birds' observations were carried out twice monthly; morning between 0630 to 9000 h and evening, between 1600 and 1800 h by walking slowly along transects. Birds were counted as the bird seen and heard and birds in flight were also recorded. The birds were identified using Bushnell binoculars (10x42) and field guides (Richard Grimmett 2015; Kottur 2014)

**Data Analysis Bird:**

**Species diversity:**

The relative abundance of a species was obtained by dividing the abundance of a species by the total abundance of all species combined based on the assumption that the frequently seen

$$H = -\sum_{i=1}^{R} pi \ln pi$$

the species the more abundant it is (Welty 1975).

Where, Pi = Proportion of individual species and R = total number of species of the community (number seen and heard).

**Results and Discussion**

A total of 5176 individual birds representing 132 species, 50 families were observed in Gudekote Sloth bear Sanctuary (Table 1). The maximum (8.19%) of species were recorded Columbidae family, followed by Muscicapidae (7.65%), Charadriidae (6.91%), Ardeidae (6.04%), Nectariniidae (5.96%), Phasianidae (5.58%), Corvidae

(5.19%), Sturnidae (4.52%), Pycnonotidae (4.38%), Cisticolidae (4.09), Leiothrichidae (4.01), Ploceidae(3.65%), Cuculidae (3.63%), Apodidae (3.18%), Meropidae (3.05%), Coraciidae (2.68%), Alcedinidae (1.89%), Upupidae (1.50%), Campephagidae (1.15%), Megalaimidae (1.13%), Podicipedidae (1.13%), Accipitridae, Rallidae(1.00%), Aegithinidae (0.92%), Motacillidae (0.85%), Dicaeidae, Dicruridae, Phalacrocoracidae(0.81%), Psittacidae (0.75%), Alaudidae (0.73%), Caprimulgidae (0.71%), Laridae (0.71%), Anatidae (0.57%), Zosteropidae (0.50%), Bucerotidae (0.48%), Strigidae (0.48%), Scolopacidae (0.38%), Turdidae (0.28%), Ciconiidae (0.25%), Acrocephalidae (0.21%), Threskiornithidae (0.19%), Laniidae (0.15%), Picidae (0.13%), Tephrodornithidae (0.11%), Falconidae (0.09%), Anhingidae (0.057%), Oriolidae (0.05%), Monarchidae (0.03%) and Pteroclididae (0.01%), families were recorded during the study periods (Fig.2). Terrestrial habitat contributed much in terms of family composition (78%) than aquatic habitat (22%). (Fig.3).

Terrestrial habitat contributed much in terms of species composition (73%) than aquatic habitat (27%). We observed that terrestrial habitat had a higher number of birds (4495 individuals, 87%) than water birds (681 individuals, 13%) (Fig.4). However, in overall abundance, Red-vented Bulbul *(Pycnonotusgoiavier)* had the highest relative frequency followed by Red wattled lapwing, Purple Sunbird, Yellow-wattled lapwing, Green Bee-eater, and Jungle Babbler. Higher relative frequency of birds could be contributed by the high frequency of occurrences to some of the birds.

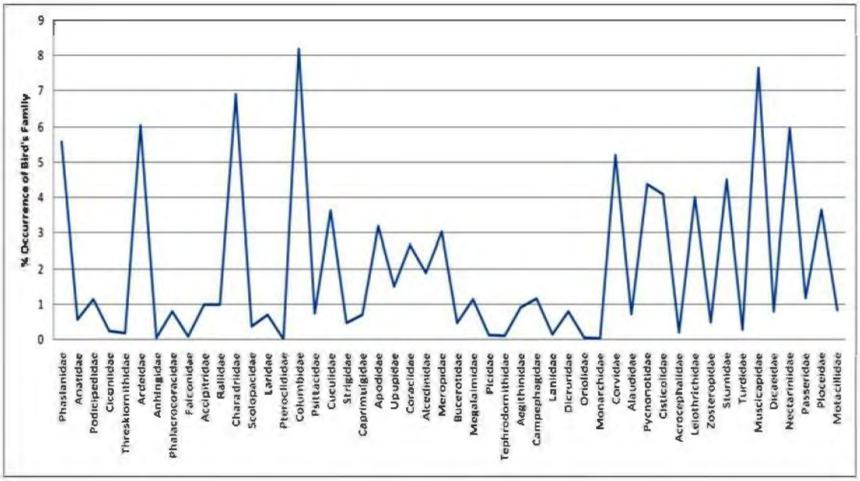


**Fig 2: Percentage representation of bird species in Gudekote Sloth bear Sanctuary.**

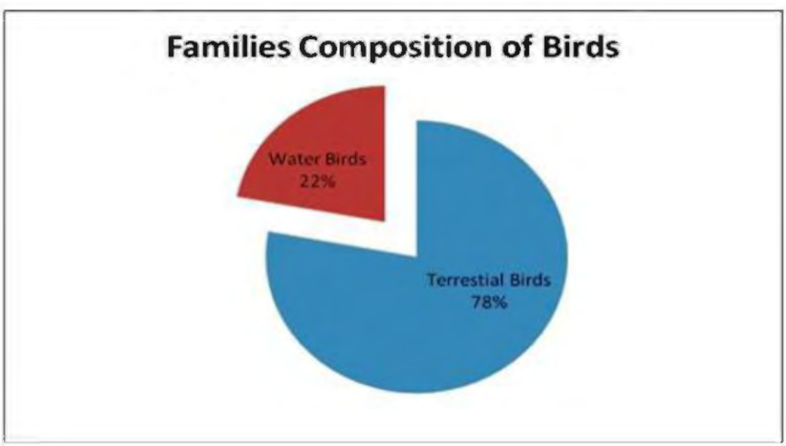


**Fig 3: Families Composition of Birds in Gudekote Sloth bear Sanctuary.**

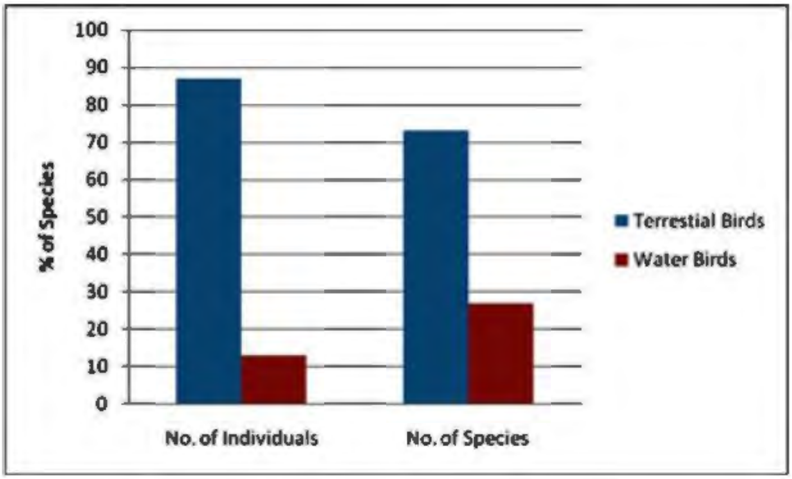


**Fig 4: Species Composition of Birds in Gudekote Sloth bear Sanctuary.**

Shannon's diversity index indicate that Gudekote black had higher species diversity (H' = 4.3709) than Halasagara black(H' = 3.7855). The overall birds' diversity for both Gudekote black and Halasagara black was (H'=4.2667) (Fig.5). Gudekote black habitat contributed much in terms of family composition (58%) than Halasagara black habitat (42%). Gudekote black habitat contributed much in terms of species composition (62%) than Halasagara black habitat (38%). We observed that Gudekote habitat had higher number of birds (3522 individuals, 68%) than Halasagara (1654 individuals, 32%) (Fig.6).

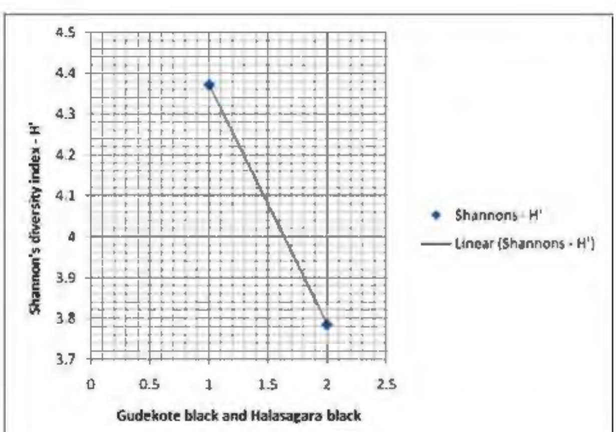


**Fig 5: diversity indices in Gudekote black and Halasagara Black at**

**Gudekote Sloth bear Sanctuary**

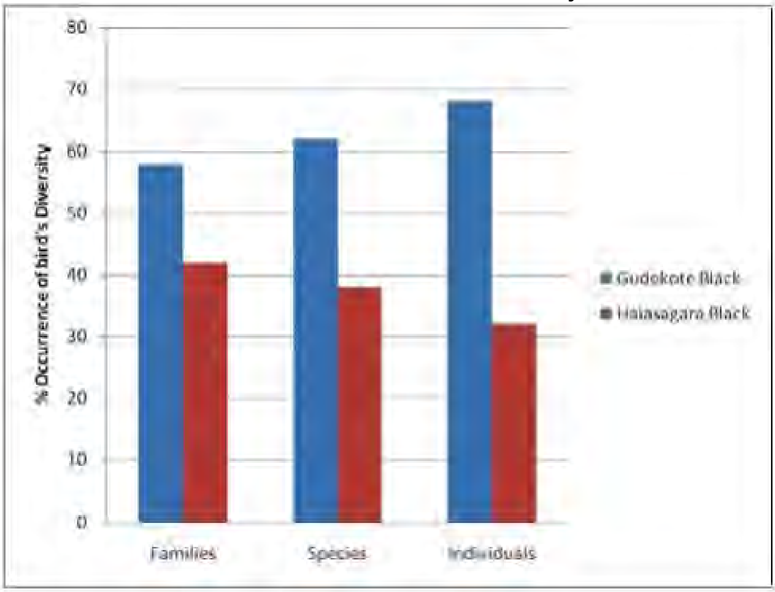


**Fig 6: Percentage of bird diversity in Gudekote black and Halasagara black for Gudekote Sloth bear Sanctuary.**

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## Table 1: Check list of birds in Gudekote Sloth bear Sanctuary

| S.No | Common Name | Scientific Name | Family |
|---|---|---|---|
| 1 | Grey Francoline | Francolinus pondicerianus | Phasianidae |
| 2 | Common Quail | Coturnix coturnix | Phasianidae |
| 3 | Rain Quail | Coturnix coromandelica | Phasianidae |
| 4 | Jungle Bush Quail | Perdicula asiatica | Phasianidae |
| 5 | Rock Bush Quail | Perdiculaargoondah | Phasianidae |
| 6 | Painted Spurfowl | Galloperdix lunulata | Phasianidae |
| 7 | Indian Peafowl | Pavo cristatus | Phasianidae |
| 8 | Lesser Whistling-duck | Dendrocygna javanica | Anatidae |
| 9 | Bar-headed Goose | Anser indicus | Anatidae |
| 10 | Cotton Pygmy-goose | Nettapus coromandelinus | Anatidae |
| 11 | Indian Spot-billed Duck | Anas poecilorhyncha | Anatidae |
| 12 | Northern Pintail | Anas acuta | Anatidae |
| 13 | Common Teal | Anas crecca | Anatidae |
| 14 | Little Grebe | Tachybaptus ruficollis | Podicipedidae |
| 15 | Painted Stork | Mycteria leucocephala | Ciconiidae |
| 16 | Asian Openbill Stork | Anastomus oscitans | Ciconiidae |
| 17 | Woolly-necked Stork | Ciconia episcopus | Ciconiidae |
| 18 | Black-headed Ibis | Threskiornis melanocephalus | Threskiornithidae |
| 19 | Eurasian Spoonbill | Platalea leucorodia | Threskiornithidae |
| 20 | Striated Heron | Butorides striata | Ardeidae |
| 21 | Black-crowned Night Heron | Nycticorax nycticorax | Ardeidae |
| 22 | Indian Pond Heron | Ardeola grayii | Ardeidae |
| 23 | Grey Heron | Ardea cinerea | Ardeidae |
| 24 | Purple Heron | Ardea purpurea | Ardeidae |
| 25 | Cattle Egret | Bubulcus albus | Ardeidae |
| 26 | Great Egret | Casmerodius albus | Ardeidae |
| 27 | Intermediate Egret | Mesophoyx intermedia | Ardeidae |
| 28 | Little Egret | Egretta garzetta | Ardeidae |
| 29 | Darter | Anhinga melanogaster | Anhingidae |
| 30 | Little Cormorant | Phalacrocorax niger | Phalacrocoracidae |
| 31 | Indian Cormorant | Phalacrocorax fuscicollis | Phalacrocoracidae |
| 32 | Common Kestrel | Falco tinnunculus | Falconidae |
| 33 | Peregrine Falcon | Falco peregrines | Falconidae |

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|---|---|---|---|
| 34 | Black-winged Kite | Elanus caeruleus | Accipitridae |
| 35 | Black Kite | Milvus migrans | Accipitridae |
| 36 | Brahminy Kite | Haliastur indis | Accipitridae |
| 37 | Pallid Harrier | Circus macrourus | Accipitridae |
| 38 | Shikra | Accipiter badius | Accipitridae |
| 39 | White-breasted Waterhen | Amaurornis phoenicurus | Rallidae |
| 40 | Common Moorhen | Gallinula chloropus | Rallidae |
| 41 | Yellow-wattled Lapwing | Vanellus malabaricus | Charadriidae |
| 42 | Red-wattled Lapwing | Vanellus indicus | Charadriidae |
| 43 | Little Ringed Plover | Charadrius dubius | Charadriidae |
| 44 | Common Snipe | Gallinago gallinago | Scolopacidae |
| 45 | Common Greenshank | Tringa nebularia | Scolopacidae |
| 46 | Green Sandpiper | Tringa ochropus | Scolopacidae |
| 47 | Little Stint | Calidris minuta | Scolopacidae |
| 48 | River Tern | Sterna aurantia | Laridae |
| 49 | Whiskered Tern | Childonias hybrid | Laridae |
| 50 | Painted Sandgrouse | Pterocles indicus | Pteroclididae |
| 51 | Common Pigeon | Columba livia | Columbidae |
| 52 | Eurasian Collared Dove | Streptopelia decaocto | Columbidae |
| 53 | Red Collared Dove | Streptopelia tranquebarica | Columbidae |
| 54 | Spotted Dove | Stigmatopelia chinensis | Columbidae |
| 55 | Laughing Dove | Stigmatopelia senegalensis | Columbidae |
| 56 | Rose-ringed Parakeet | Psittacula krameri | Psittacidae |
| 57 | Jacobin Cuckoo | Clamator jacobinus | Cuculidae |
| 58 | Common Hawk Cuckoo | Hierococcyx varius | Cuculidae |
| 59 | Grey-bellied Cuckoo | Cacomantis passerines | Cuculidae |
| 60 | Asian Koel | Eudynamys scolopaceus | Cuculidae |
| 61 | Blue-faced Malkoha | Rhopodytes viridirostris | Cuculidae |
| 62 | Southern Coucal | Centropus (sinensis) parroti | Cuculidae |
| 63 | Barn Owl | Tyto alba | Strigidae |
| 64 | Spotted Owlet | Athene brama | Strigidae |
| 65 | Eurasian Eagle Owl | Bubo bubo | Strigidae |
| 66 | Indian Nightjar | Caprimulgus asiaticus | Caprimulgidae |
| 67 | Asian Palm Swift | Cypsiurus balasiensis | Apodidae |
| 68 | Little Swift | Apus affinis | Apodidae |
| 69 | Coomon Hoopoe | Upupa epops | Upupidae |
| 70 | Indian Roller | Coracias benghalensis | Coraciidae |

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|---|---|---|---|
| 71 | White-throated Kingfisher | Halcyon smyrnensis | Alcedinidae |
| 72 | Common Kingfisher | Alcedo atthis | Alcedinidae |
| 73 | Pied Kingfisher | Ceryle rudis | Alcedinidae |
| 74 | Green Bee-eater | Merops orientalis | Meropidae |
| 75 | Blue-tailed Bee-eater | Merops philippinus | Meropidae |
| 76 | Indian Grey Hornbill | Ocyceros birostris | Bucerotidae |
| 77 | Coppersmith Barbet | Megalaima haemacephala | Megalaimidae |
| 78 | Yellow-crowned Woodpecker | Dendrocopos mahrattensis | Picidae |
| 79 | Lesser Goldenback | Dinopium benghalense | Picidae |
| 80 | Common Woodshrike | Tephrodornis pondicerianus | Tephrodornithidae |
| 81 | Common lora | Aegithina tiphia | Aegithinidae |
| 82 | Small Minivet | Pericrocotus cinnamomeus | Campephagidae |
| 83 | Bay-backed Shrike | Lanius vittatus | Laniidae |
| 84 | Long-tailed Shrike | Lanius schach | Laniidae |
| 85 | Black Drongo | Dicrurus macrocercus | Dicruridae |
| 86 | Indian Golden Oriolus | Oriolus (oriolus) kundoo | Oriolidae |
| 87 | Asian Paradise-flycatcher | Terpsiphone papadisi | Monarchidae |
| 88 | Rufous Treepie | Dendrocitta vagabunda | Corvidae |
| 89 | Indian Jungle Crow | Corvus (macrorhynchos) culminates | Corvidae |
| 90 | House Crow | Corvus splendens | Corvidae |
| 91 | Rufous-tailed Lark | Ammomanes phoenicura | Alaudidae |
| 92 | Oriental Skylark | Alauda gulgula | Alaudidae |
| 93 | Red-vented Bulbul | Pycnonotus cafer | Pycnonotidae |
| 94 | White-browed Bulbul | Pycnonotus luteolus | Pycnonotidae |
| 95 | Yellow-browed Bulbul | Acritillas indica | Pycnonotidae |
| 96 | Grey-breasted Prinia | Prinia hodgsonii | Cisticolidae |
| 97 | Jungle Prinia | Prinia sylvatica | Cisticolidae |
| 98 | Plain Prinia | Prinia inornata | Cisticolidae |
| 99 | Zitting Cisticola | Cisticola juncidis | Cisticolidae |
| 100 | Common Tailorbird | Orthotomus sutorius | Cisticolidae |
| 101 | Clamorous Reed Warbler | Acrocephalus stentoreus | Acrocephalidae |
| 102 | Blyth's Reed Warbler | Acrocephalus dumetorum | Acrocephalidae |
| 103 | Greenish Warbler | Phalloscopus trochiloides | Acrocephalidae |
| 104 | Common Babbler | Turdoides caudate | Leiothrichidae |
| 105 | Jungle Babbler | Turdoides striata | Leiothrichidae |
| 106 | Large Grey Babbler | Turdoides malcolmi | Leiothrichidae |

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|---|---|---|---|
| 107 | Oriental White-eye | Zosterops palpebrosus | Zosteropidae |
| 108 | Common Myna | Acridotheres tristis | Sturnidae |
| 109 | Brahminy Starling | Sturnia pagodarum | Sturnidae |
| 110 | Orange-headed Thrush | Zoothera citrine | Turdidae |
| 111 | Oriental Magpie Robin | Copsychus saularis | Muscicapidae |
| 112 | Pied Bushchat | Saxicola caprata | Muscicapidae |
| 113 | Indian Robin | Saxicoloides fulicatus | Muscicapidae |
| 114 | Black Redstart | Phoenicurus ochruros | Muscicapidae |
| 115 | Asian Brown Flycatcher | Muscicapa dauurica | Muscicapidae |
| 116 | Tickell's Blue Flycatcher | Cyornis tickelliae | Muscicapidae |
| 117 | Pale-billed Flowerpecker | Dicaeum erythrorhynchos | Dicaeidae |
| 118 | Purple-rumped Sunbird | Leptocoma zeylonica | Nectariniidae |
| 119 | Purple Sunbird | Cinnyris asiaticus | Nectariniidae |
| 120 | House Sparrow | Passer domesticus | Passeridae |
| 121 | Chestnut-shouldered Petronia | Gymnoris xanthocollis | Passeridae |
| 122 | Streaked Weaver | Ploceus manyar | Ploceidae |
| 123 | Baya Weaver | Ploceus philippinus | Ploceidae |
| 124 | Indian Silverbill | Euodice malabarica | Ploceidae |
| 125 | Red Avadavat | Amandava amandava | Ploceidae |
| 126 | Scaly-breasted Munia | Lonchura punctulata | Ploceidae |
| 127 | Black-headed Munia | Lonchura Malacca | Ploceidae |
| 128 | Yellow Wagtail | Motacilla flava | Motacillidae |
| 129 | Grey Wagtail | Motacilla cinerea | Motacillidae |
| 130 | White Wagtail | Montacilla alba | Motacillidae |
| 131 | White-browed Wagtail | Motacilla maderaspatensis | Motacillidae |
| 132 | Paddyfield Pipit | Anthus rufulus | Motacillidae |

# Art – 150. ARCHITECTURE OF SHOULDER MUSCLES OF SLOTH BEAR (*Melursus ursinus)*

Annie V. R., K.V. Jamuna, **Arun A. Sha,** R. V. Prasad, V. Girish Kumar, Shruti, Rajani Chunkkath, Sunilkumar Patil, Manjunath K., Bharath Kumar

## Abstract

Sloth bear is one of the youngest carnivores occupying a wide range of habitats in the Indian subcontinent. Carcass of sloth bears were studied from Bannerghatta Bear Rescue Centre, Bangalore. The study was undertaken to systematically establish the myological characteristics of shoulder region muscle which might help to carry out morphological assessments. In this study it was observed that well developed supraspinous fossa was filled by M. supraspinatus and infraspinous fossa was filled by M. infraspinatus. The powerful deltoideus muscle consisted of two bellies, arose from acromion process and from the fascia overlying M. infraspinatus. Subscapularis muscle was divided into five fascicular tracts and additional subscapularis minor muscle which was positioned medial to M. subscapularis proper. M. coracobrachialis consisted of two heads, short head inserted beneath the lesser tubercle and longus inserted on supracondylar crest. In sloth bear attachment of muscle was more proximally compared with other carnivore which may account for slow gait of bears and the muscle mass has reflected the quadrupedal walking of sloth bear.

## Introduction

Sloth bears *(Melursus ursinus)* are medium-sized nocturnal, insectivorous species currently seen only to southern Asia They occupy a wide range of habitats on the

Indian mainland including wet or dry tropical forests, savannas, scrublands and grasslands.

They evolved from ancestral brown bears and have been classified as an Ursid. Weighing 70-90kgs these adapt climbers subsist primarily on leaves and fruits. Two separate subspecies are recognized, the Sri Lankan sloth bear *(Melursus ursinus inornatus)* and the Indian sloth bear *(Melursus ursinus ursinus)*. Sloth bear is classified as "Vulnerable" in the 1996 IUCN Red List of Threatened Animals and is listed on Appendix I of CITES. (IUCN Red List, Version 2014.2). A number of investigators studied the muscles of bears generally for phylogenetic analyses, but the myology of Sloth Bear remains largely undocumented. This study augments the soft tissue data for sloth bear, including detailed description of the shoulder muscles.

Skeletal musculature constitutes the active part of the locomotor system. Skeletal muscles always attaching to bone or cartilage, provides force for locomotion and posture of individual parts of the body or the body as a whole. It also plays an important role in supporting the body weight and formation of abdominal wall (Konig and Liebich, 2004). Each movement of a body part is produced by the involvement of several muscles either simultaneously or one after another. The action of a muscle depends on its origin, course, insertion and point of rotation (Williams and Warwick, 2008). Locomotion and animal posture greatly influence the anatomy of a muscle due to the high frequency and high loads of forces involved.

## Materials and methods

Dissections were conducted on the left and right forelimbs of three succumbed captive sloth bears. The bears lived at the Wildlife SOS, Bannerghatta Bear Rescue Centre of Bannerghatta Biological Park, Bangalore, Karnataka and included two adult males of 15- and 13-years age, an adult female of 11 years. Following necropsy, the specimens were stored in 10% formalin and dissections were carried out. Digital photographs were taken at each level of the dissection.

Muscle origins and insertions were recorded. Data from the forelimb dissections were compared with previous accounts of this species and other carnivores. The terminology in this report conforms to the standards of the Nomina Anatomica Veterinaria (Waibl et al. 2005).

## Results

### Muscles on Lateral aspect of Shoulder

M. Supraspinatus

M. supraspinatus took its origin from supraspinous fossa and from cranial aspects of scapular spine. It filled supraspinous fossa (Fig-1) and was covered by cervical part of M. trapezius. Distally its strong muscular belly curved far around neck of scapula (Fig-2) so that it also appeared on medial surface of shoulder joint. As the muscle crossed shoulder joint, M. supraspinatus inserted via a stout tendon onto the dorsal aspect of greater tubercle. M. supraspinatus extends and stabilizes the glenohumeral joint.

M.Infraspinatus

M. Infraspinatus was a triangular shaped muscle which occupied infraspinous fossa and extended caudally beyond the fossa. M. infraspinatus arose from infraspinous fossa, scapular neck, caudal aspect of scapular spine and from the circular area on lateral aspect of lesser tubercle of humerus. M. infraspinatus was largely covered by the scapular part of M. Deltoideus (Fig-1). The muscle inserted via a stout tendon on lateral aspect of greater tubercle (Fig-2). M. infraspinatus stabilized gleno-humeral joint and laterally rotate the humerus.

It may also assist in extension or flexion of gleno-humeral joint, depending on position of humeral head relative to glenoid cavity.

## M. Deltoideus

deltoideus was a powerful bipennate muscle, consisting of two portions M. deltoideus pars acromialis and M. deltoideus pars scapularis (Fig-1). M. deltoideus par acromialis arose by fleshy fibres from posterior edge of acromion. M. deltoideus pars scapularis originated from fascia covering M. Infraspinatus. It was completely separable from M. deltoideus pars acromialis. It was inserted onto deltoid tuberosity by aponeurosis. Insertion of deltoideus muscle on humerus was via direct attachment of muscle fibres to humeral shaft through fibrous attachment. Deltoid tuberosity was found on lateral surface below the middle of shaft obliquely in the form of a thick ridge.

## Teres minor

M. teres minor was a small but well-defined muscle observed distocaudally on the scapula (Fig-3). This muscle was wedged below M. infraspinatus. Some fibers of M. infraspinatus took origin from the surface of that tendon. It was inserted by a short, stout tendon onto small circular area present distal to the greater tubercle of humerus, just below the insertion of M. infraspinatus. M. teres minor will act as a flexor for shoulder joint.

**Muscles on medial aspect of shoulder**

M. Subscapularis

Subscapularis, a rectangular muscle, originated from subscapular fossa and caudal border of scapula. M. subscapularis wrapped around the cranial edge of scapula and also took origin from the surface of M. supraspinatus. M. subscapularis was divided into five tracts by fascial septa (Fig-4).

In addition, a tract of parallel fibers which was separated from main mass of M. subscapularis by a fascial septum was noticed along the posterior border of scapula and formed M. subscapularis minor (Fig-5). Posterior half of subscapularis minor was completely covered by M. teres major, which took origin from this surface. Fibers of M. subscapularis minor maintained their identity from M. subscapularis proper down to their insertion, ventral part of lesser tubercle of humerus. M. subscapularis proper fibers passed over M. coracobrachialis tendon and inserted onto remaining part of lesser tubercle of humerus.

M. Teres major

M. teres major was a sheet like muscle which originated from the surface of M. subscapularis minor and from proximal half of caudal border of scapula (Fig-4). It had been displaced completely onto medial surface of the scapula by M. subscapularis minor. M. teres major terminated in a flat tendon, that united with the tendon of M. latissimus dorsi and inserted into roughened scar on the crest of humerus (Fig-6).

M. Coraco-brachialis

L. coracobrachialis was a strap like muscle arose from a rudimentary coracoid process. Tendon of origin of M. coraco-brachialis coursed deep to tendon of M. subscapularis and it passed over the head of humerus and divided into two bellies. First belly was coraco-brachialis brevis (Fig-3) which was a short muscular slip and was inserted onto inner side immediately below the lesser tuberosity of humerus. Second belly or main portion of the muscle was M. coraco-brachialis longus (Fig-3) which continued down on the inner side of the limb. M. coracobrachialis inserted via fleshy and tendinous fibers onto supra condylar crest.

**Concluding-remarks**

**Intraspecific variation with other bears and with other species**

The origin of M. Supraspinatus is variable in red panda wherein muscle possessed an additional origin from the hamate process (Fischer et al., 2009). Hunt (1991) mentioned that deloideus muscle was larger in chimpanzee and may be an adaptation for vertical climbing. M. Teres minor was indistinguishable from M. infraspinatus in American black bear (Shepherd (1883). In present study, short head of biceps was absent in contrast to study made by Miller (1952), who suggested that in gibbons, short head was originating from the lesser tubercle of humerus whereas in monkey, it was from the coracoid process of scapula by common tendon with coracobrachial muscle (Ferreira et al., 2007). So, in sloth bear this muscle might have less flexion capacity in shoulder level. Davis (1949) found that in carnivore muscle which were attached far away from joints are responsible for fast movement compared with the sloth bear.

## Traits shared with family ursidae and carnivora

In bears infraspinatus muscle extended caudally beyond the fossa and similar findings were demonstrated by Evans and de Lahunta (2013) in dog. In sloth bear M. deltideus par acromialis originated from acromion process of scapula. In other bears also similar findings were recorded by Shepherd (1883) and Fujino (1994). In present study M. teres minor was quite distinct running distocaudally on scapula and inserted to outer side of greater tubercle as in polar bear (Kelley, 1888), in red panda (Fischer et al., 2009) and in dogs (Evans and de Lahuta, 2013). Teres major muscle had been displaced completely on medial surface of scapula by M. subscapularis minor. This arrangement was well supported by distinct post scapular fossa in bears.

## Primitive retentions

Data from present study was matched with reports made in orangutans (Oishi et al., 29). He proposed that size difference might reflect the functional specialization for their different positional and locomotor behaviour. Sloth bears possess large post scapular fossa. Davis (1949) revealed that degree of development of post scapular fossa had direct co-relation with the size of animal.

## Conclusion

This study provides additional soft tissue features that can be incorporated in future phylogenetic studies of Ursidae family. In addition, the shoulder muscles provide a unique resource for those analyzing the functional anatomy of fossil. The shoulder muscles of sloth bear are characterized by a number of primitive retentions of other bears. Features that are most likely to be derived in the forelimb of sloth bear include M. subscapularis was divided into five tracts by fascial septa and an addition M. subscapularis minor was there on posterior aspect. M. teres major had been displaced completely onto medial surface of scapula and terminated on the crest of lesser tubercle of humerus. So, the animal exhibits more walking habit compared with that of other primates.

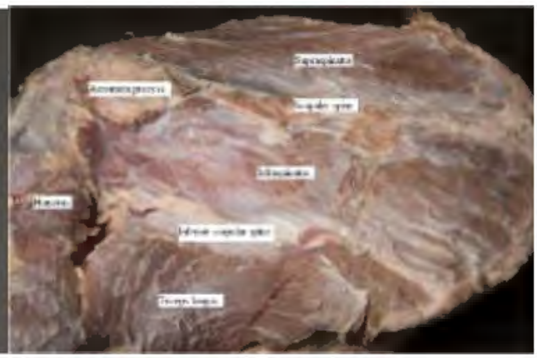

**Fig 1- Figure showing Muscles of shoulder lateral**

**Fig 2- Figure showing M. Supraspinatus and M. Infraspinatus**

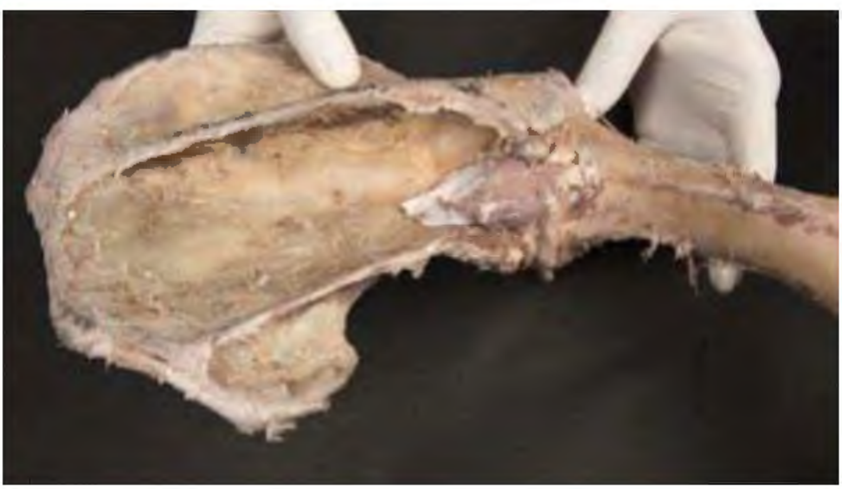

**Fig 3- Figure showing M. Teres Minor**



**Fig 4 Figure showing Muscles of shoulder medial aspect**

**Fig 5 -Figure showing M. Subscapularis**

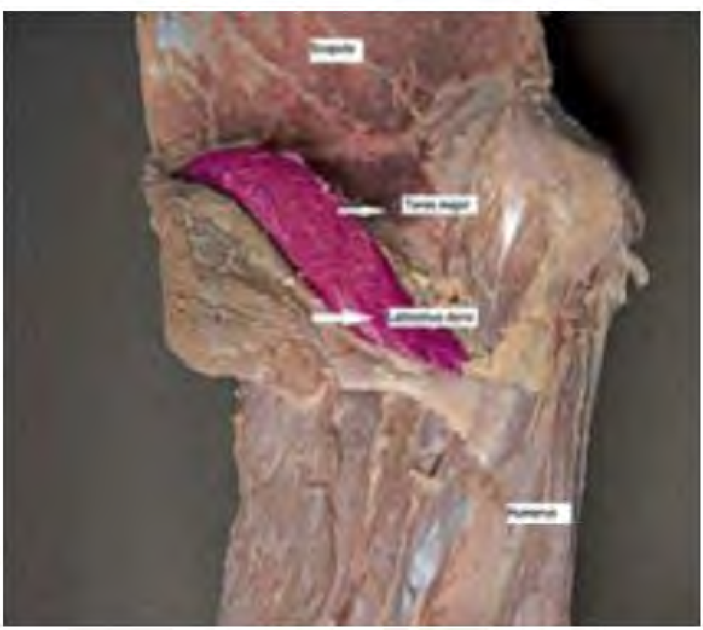

**Fig 6- Figure showing M. Teres Major**

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# Art – 151. SLOTH BEAR ATTACK BEHAVIOR AND A BEHAVIORAL APPROACH TO SAFETY

**Thomas R. Sharp, Shanmugavelu Swaminathan, Attur Shanmugam Arun,** Tom Smith, **Kartick Satyanarayan,** and **Geeta Seshamani**

They [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. So, the natives give bears a wide berth; together with the elephant, they command the greatest respect from jungle dwelling folk.

—Kenneth Anderson, Man-Eaters and Jungle Killers

[The sloth bear] is also more inclined to attack man unprovoked than almost any other animal, and casualties inflicted by it are unfortunately very common, the victim being often terribly disfigured even if not killed, as the bear strikes at the head and face. Blanford (author of The Fauna of British India, Including Ceylon and Burma) was inclined to consider bears more dangerous than tigers.

—Robert A. Sterndale, Natural History of the Mammalia of India.

**Summary**

Sloth bears *(Melursus ursinus)* are known to behave aggressively toward humans and are believed to be one of the most dangerous wild animals in India. Although several papers have documented sloth bear attacks, no attention has been

given on how to behave in sloth bear country to avoid encounters, or how to react to a sloth bear attack to minimize injuries and the likelihood of death. Wildlife SOS field research teams interviewed a total of 342 people, including 180 that had either been attacked or that had witnessed an attack, and 162 people that have had encounters with wild sloth bears that did not result in an attack. Our research and investigation confirmed that all attacks were defensive-aggressive in nature; we found no evidence for predatorial motivations. Our findings also show that people who had been making noise while moving through sloth bear country were less likely to be attacked. Our data also reveal that 9% of individuals who fought back during an attack were killed, and 11% of people who attempted to run were killed, whereas there were no deaths among people who merely fell to the ground and did not fight back. However, the data also revealed that those who fell to the ground and did not fight back were more likely to sustain serious injuries than those who did fight back.

## Introduction

Sloth bears are known for their propensity to behave aggressively toward humans and are believed to be one of the most dangerous wild animals in India (Sterndale 1884, Pillarisett 1993). It is not known exactly how many people are seriously injured or killed by sloth bears in India during a given year. Nonetheless, in the state of Madhya Pradesh, there were 48 sloth bear–related human deaths and 687 maulings between 1989 and 1994 (Rajpurohit and Krausman 2000) for an average of 6 deaths and 115 maulings per year. Compared to American black bears *(Ursus americanus)* in the United States and Canada during the last 110 years (1900–2009), there have been 63 documented human deaths due to predatory attacks (Herrero et.al. 2011).

Additionally, Herrero (1985) estimates that during the past 100 years in the United States and Canada, roughly 100 people have been killed by brown bears *(Ursus arctos)*. Most people working and living in sloth bear habitat do not possess firearms or have access to bear (pepper) spray or other commonly used bear deterrents (e.g., flares, screamers, shotgun deterrent rounds).

Additionally, there is currently no messaging that emphasizes the importance of bear avoidance, how to behave when encountering a sloth bear, or how to react to a charging sloth bear. Sloth bears are considered very unpredictable and often aggressive. Given the number of attacks and the associated human casualties, coupled with the lack of firearms and bear spray, a behavioral-based approach to reducing bear encounters and associated attacks in the wild could be useful for saving human lives. Such an approach has been very successful in Canada and the United States, and has helped people better understand these mammals. This study is the first comprehensive effort to identify a behavioral approach to reducing risk from sloth bears in India.

**Objectives**

Determine a sloth bear's motivation(s) for attack: defensive or predatorial. Determine the circumstances under which defensive attacks occur. Determine defensive behaviors that sloth bears typically exhibit. Determine the most effective ways for humans to avoid sloth bears in the wild. Determine the best way to react when observing a wild sloth bear, based on its behavior. Determine the best way to respond if attacked by a wild sloth bear, based on its attack motivation.

## Study Area

Sloth bears occupy mainly lowland habitats throughout India, extending south to Sri Lanka and north to Nepal. However, Wildlife SOS works extensively in the southern Indian state of Karnataka, and so we largely interviewed people from that region, namely in the districts of Ramnagaram, Arasikere, Tumkur, Koppal and Ballary in Karnataka.

Wildlife SOS currently operates four sloth bear rescue centers across India. The Bannerghatta Bear Rescue Center (BBRC) in the state of Karnataka houses roughly 80 bears. We collected video footage of bear behavior at this facility to analyze sloth bear behavior.

## Methods

We employed the following four methods to determine the motivations behind sloth bear attacks, and the best ways to avoid encounters and attacks. Literature review – We conducted a thorough literature review of past sloth bear attacks and other aspects of their ecology that could help predict wild sloth bear behavior when encountering humans. This included sloth bear diet, behavioral details of attacks, and known behavioral elements of inter- and intra-specific sloth bear interactions, including with tigers *(Panthera tigris)*. Interviews – We interviewed 342 people in their native language who have had a variety of encounters or observations of sloth bear behavior,including the following: People who had been attacked by sloth bears (n=180). People who have had an encounter with a sloth bear in the wild that did not result in an attack (n=162). Veterinarians and biologists who have more than 10 years of experience working with sloth bears at the Wildlife SOS sloth bear rehabilitation centers, and who have observed sloth bear behavior toward humans as well as between Sloth bears Video documentation of sloth bear behavior – We recorded videos of intra-specific sloth bear interactions and behavior at the Wildlife SOS bear facilities in Agra and Bangalore as well as bear charge videos. Comparison of bear behavior – We compared the behavior of sloth bears to that of bears with better understood and documented human attack behavior, namely American black *(Ursus americanus)* and grizzly *(Ursus arctos)* bears.

## Results

### Sloth Bear Attack Motivation

We studied documented sloth bear attacks to assess motivation for attack. We initially posited that attacks would be either predatorial or defensive in nature, as is the case with North American bears. If an attack was deemed defensive, we attempted to assess whether the animal was protecting cubs, a food cache, or was surprised and therefore defensive-aggressive. The analysis of the data gathered indicates that sloth bear attacks appear to be wholly defensive. We did not find a single case that was clearly predatorial in nature when conducting our intensive literature review or in the attacks we documented. However, we did find accounts, both historical and contemporary, of sloth bears partially consuming human corpses— occasionally including those they had killed themselves. Our reasons for not labeling these attack predatory is illustrated in the examples that follow.

We studied two historical accounts of sloth bear maulings that included consumption of the victim. The most famous is the "sloth bear of Mysore," which was reported to not only have mauled 24 people and killed another 12, but also partially consumed three of its victims (Anderson 1957). The second account involves the "sloth bear and cubs of Chandra," which threatened small villages for a six-week span, reportedly consuming more than one victim. More recently, Bargali et al. (2005) reported two incidents in which a bear that had killed a person remained in the area feeding on body parts. However, because we do not know how this event unfolded, we cannot reasonably conclude what the bear's initial motivation was.

During our interviews, 4% (n=7) of victims claimed their attack to have been predatorial. It is important to recognize that a non- predatorial attack could easily appear predatorial from the victim's perspective, especially if there seemed to be no other apparent motivation for the confrontation. Additionally, sloth bear attacks tend to focus on the victim's head region, leading some to believe the attack was predatorial; however, focusing on the head and face does not appear to be linked to predatorial attacks in bears in general (Smith et al. in review) but is indicative of the attack strategy. Herrero (1985) surmised that bears attack the head and neck region largely because they perceive our teeth as a weapon threat, the same as they would with conspecifics.

The lack of evidence for sloth bear predatorial attacks is consistent with their diet (Joshi et al. 1997, Garshelis et al. 2008). These studies indicate that red meat is only rarely a component in the normal diet of a sloth bear. Although the occasional small rodent or reptile has been found in sloth bear scat, even these food types are likely ingested incidentally while foraging for insects and grubs. Similarly, T. Smith (personal communication) has found bees (Bombus spp.) in brown bear scat, incidentally ingested while foraging on the inflorescences of cow parsnip *(Heracleum lanatum)*. There is no evidence to suggest that sloth bears purposely forage for even small mammals. However, sloth bears are known to occasionally scavenge on larger mammals, including humans. In 1995, Kartick Satyanarayan found a human index finger in a sloth bear scat during a tiger-scat collection drive on a research project in Central India. It was later confirmed that a tribal burial site was nearby, and the bear scavenged on a human corpse that had been excavated by other scavengers.

Perhaps Kenneth Anderson said it best roughly 60 years ago (Anderson 1957) when he wrote the following about the famous Sloth bear of Mysore case:

Local rumors had it that the bear had taken to eating its victims, the last three of whom had been partly devoured. I had no opportunity to verify the truth of these rumors but felt that they might be true to some extent as the Indian sloth bear is a known devourer of carrion at times, although generally he is entirely vegetarian, restricting himself to roots, fruit, honey, white ants [termites] and similar delicacies. So fresh meat, even human meat, might not be unwelcome. It is possible that scavenging on the remains of humans, especially those that the bears themselves killed, has led to the belief that sloth bears prey on people. Consuming a victim initially attacked for defensive reasons is not unique to sloth bears; it has also been described in grizzly bear literature (Herrero 1985). It is likely that the bear is simply being an opportunistic omnivore by feeding on human flesh.

Defensive Attacks: Circumstances

Grizzly bear defensive attacks can be subdivided into four categories 1) a mother protecting her young, 2) a bear protecting its food cache, 3) a surprise encounter, and 4) a harassed bear. Our data and the literature suggest that defensive sloth bear attacks are motivated by three of these four categories—the protection of young, surprise encounters, and harassed bears. We have found no cases of a sloth bear attacking to protect a food cache.

The "harassed bear" category refers to attacks provoked by human harassment (often chronic) that leads to a bear charge and physical contact. For sloth bears, this category varies a bit from how it is used for grizzly bears. This type of harassment in the case of sloth bears often includes people throwing objects and yelling at the bear. This situation can also escalate into what is termed an "attack spree." These are cases in which a harassed bear kills multiple people in what appears to be self-defense, usually because the bear appears unable to escape, or is motivated to become aggressive by the overall threat of the situation. It therefore feels forced to confront one person after another. Attack sprees have been documented between brown bears and humans in Alaska (T. Smith, personal observation), though sloth bear attack sprees last longer and appear, at least superficially, to put the animal in more of a frenzied state.

During our interviews with 181 sloth bear attack victims, almost half of the attacks (n=84, 46%) involved a female with dependent young. These cases fall under the "mother protecting her young" category. Of the 161 encounters that were reported and did not end in an attack, 40% (n=65) involved a female with dependent young. Of the 181 attacks, 52% (n=94) involved single bears. The motivation for these attacks was most likely due to surprise encounters. Single bears accounted for 60% (n=96) of the encounters that did not result in an attack. The remaining 2% (n=3) of attacks involved a pair of bears.

## Defensive Behaviors

Grizzly bear defensive behavior such as laying their ears back, slapping the ground, jaw popping, and huffing are well documented. Sloth bear defensive behaviors have not been explicitly documented. The ears being drawn or pinned back is a common defensive reaction among bears and many other species of wildlife (e.g., felids and canids). However, this behavior is rarely, if ever, exhibited by sloth bears. Sloth bear charge videos taken by Wildlife SOS and other wildlife videographers show that sloth bear ears are not laid back during a charge. Additionally, sloth bear ears do not appear pulled back during tiger encounters (http://www.arkive.org/tiger/panthera-tigris/video-ti11b.html). Aditya Dicky Singh's 10-photograph series of a tiger/sloth bear interaction (http://www.dickysingh.com/2011/04/10/ bear-tiger-confrontation-10-pics-that-tell-a-story/) provides several interesting details. One photograph (Figure 1) clearly shows the heads of each animal during the most intense moments of the confrontation. The contrast is remarkable; while the tiger clearly has its ears pulled back, the sloth bear does not; in fact, the bear's head appears larger than usual. The sloth bear's shaggy head potentially conceals drawn-back ears, rendering them useless as a means for communicating stress. Additionally, it seems possible that the fur on the head makes the bear appear larger and thus more intimidating. Pulling back the ears would potentially make the head look smaller. Another tiger/sloth bear confrontation photographed by Julien Boulé shows a sloth bear aggressively squaring off with a tiger and holding its ground (Figure 2). Once again, the tiger's ears are pulled back while the sloth bear's are not.

**Figure 1. Sloth bear/tiger encounter demonstrating the appearance of a very large sloth bear head (photograph by Aditya Dicky Singh).**

**Figure 2. Sloth bear/tiger interaction demonstrating a sloth bear's aggressive stance toward a tiger; the bear's ears are not pulled back in a defensive posture (photograph by Julien Boulé).**

Sloth bears may also attempt to look larger by getting up on their two hind legs during attacks on humans or in encounters with tigers. A bear on two hind legs will appear larger and more intimidating (Figure 3). A bipedal bear also brings all three weapons, two paws and its teeth, into play simultaneously, presenting a formidable threat to would-be attackers.

**Figure 3. Sloth bear/tiger interaction demonstrating the sloth bear's predilection to rise onto two hind legs during a dangerous encounter (photograph by Aditya Dicky Singh).**

Sloth bears, unlike most other bear species, are very vocal, and will actively voice their uncertainty and discomfort with a situation. However, only 4% of the attack victims (n= 7) noted that the bear vocalized before it charged and made physical contact. Yet it is clear in video of sloth bear charges and sloth bear/tiger interactions that bears are markedly vocal during such encounters. A Wildlife SOS video from the BBRC captured a vocalization that sounds more reminiscent of a gorilla's charge than a bear's. It seems clear that these vocalizations add a startling and intimidating element to the charge.

Overall, sloth bears appear to forego the subtle defensive/stress displays that grizzly bears and American black bears make when warning people or other animals that their stress level is rising. However, sloth bears do use several methods to intimidate a potential threat, after which an attack may or may not occur.

## Avoiding Encounters in the Wild

We asked the interviewees involved in 181 bear attacks if he had been making noise before the encounter. Roughly two-thirds (n=111, 67%) stated that they had not been. We also analyzed sloth bear encounters that did not result in an attack or physical contact. Of the 126 interviewees who experienced a sloth bear encounter without an attack, and could recall if they were making noise, 78% (n=98) claimed to have been making noise, whereas 22% (n=28) had not been.

## Injuries and Responses to Attacks

To identify potential patterns, we assessed the severity of bear- attack injuries based on how the victim reacted to the confrontation. Our intent was to identify responses that resulted in the least amount of bodily injury. We paid particular attention to the three most common responses to an attack: 1) fighting back, 2) running, and 3) falling to the ground and not fighting back.

## Fighting Back

We interviewed 69 individuals involved in separate incidents who fought back when attacked (or who had witnessed it). Approximately 9% of these people were killed, 12% were severely injured, and 50% suffered minor injuries (Figures 4 and 5).

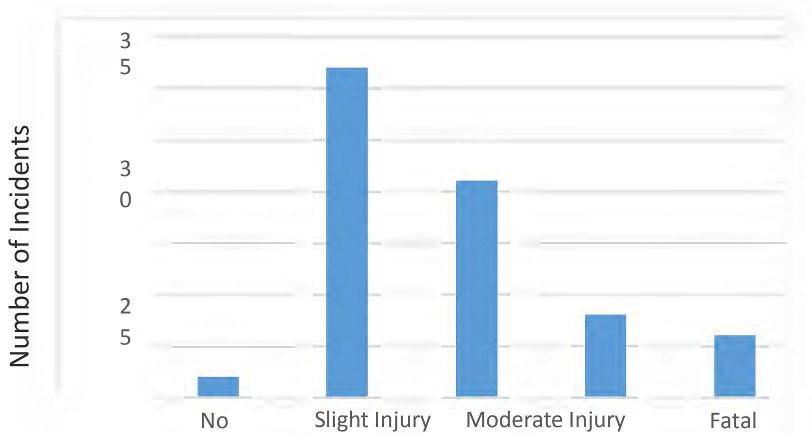


**Figure 4. Number of incidents (n=69) by injury type that resulted from fighting back with an attacking sloth bear.**

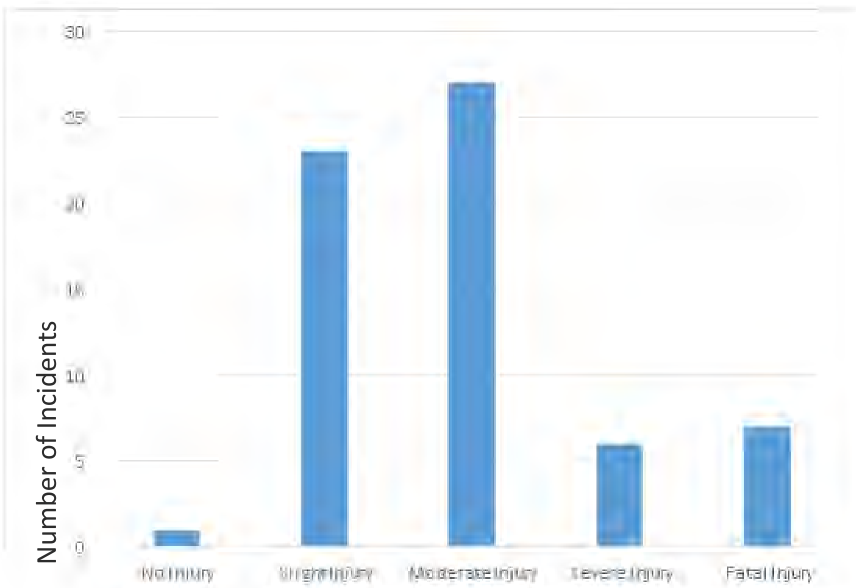


**Figure 5: Number of incidents (n=64) by injury type that resulted from running from an attacking sloth bear.**

## Running

We interviewed 64 individuals who were attacked by a sloth bear (or witnessed an attack) when attempting to run away. In all, 11% that ran were ultimately attacked and killed. Another 9% were severely injured, and 42% suffered moderate injuries (Figures 6 and 7).

## Playing Dead (Falling to the Ground and Not Fighting Back)

We interviewed 23 individuals who played dead (i.e., fell to the ground and did not fight back) when attacked. No individuals who played dead suffered fatal injuries. However, 18% of those playing dead suffered severe injuries, while 65% incurred moderate injuries (Figures 8 and 9).

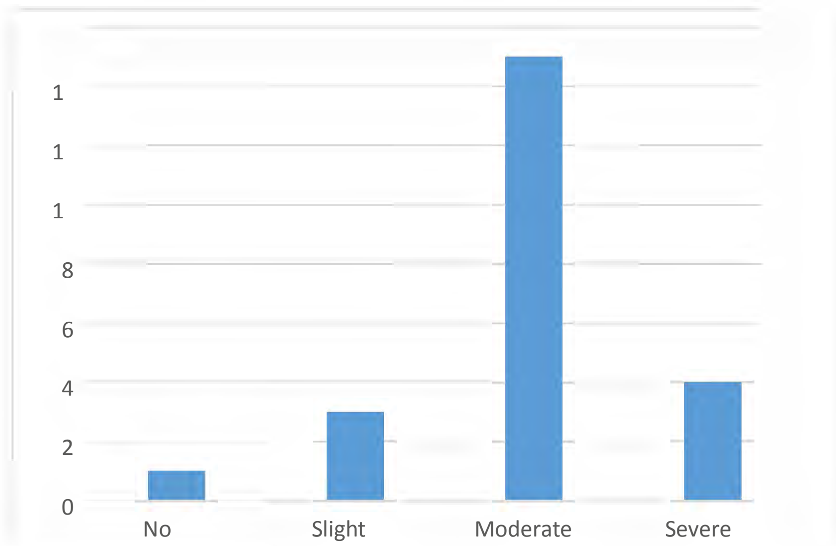


**Figure 6. Number of incidents (n=23) by injury type that resulted from playing dead with an attacking sloth bear.**

## Comparison to other Bear species

Although anecdotal reports of bear attacks on humans exist for the polar bear *(Ursus maritimus)*, Asiatic black bear *(Ursus thibetanus)*, Andean bear *(Tremarctos ornatus)*, panda bear *(Ailuropoda melanoleuca)*, and sun bear *(Ursus malayanus)*, American black bear and grizzly/brown bear attacks have been the most studied and best understood. Importantly, the grizzly bear accounts for more than 80% of all bear attacks in North America (Herrero 1985, Smith et al. in review), even though they are far outnumbered by black bears. Smith et al. (in review) report that grizzly bears are 26 times more likely to engage in conflict with humans than are black bears, and 6 times more likely than polar bears. Nonetheless, bear attacks in North America have averaged 7.6 attacks/ year in the last decade (Smith et al. in review). This level of human-bear conflict pales in comparison to that of human–sloth bear conflict in India. Although data are lacking for the entire country of India, human-bear conflict statistics from a single Indian state prove this point. Rajpurohit and Krausman (2000) documented sloth bear attacks in the state of Madhya Pradesh from 1989 to 1994. They found that sloth bears inflicted 48 fatalities and 687 maulings during a 6-year period, for an average of 123 attacks/year, 16 times more than the entire state of Alaska for the same time. From this perspective, the sloth bear appears to be a far greater threat to human safety than any bear species on the North American continent. However, differential contact rates with humans clearly play a role in these statistics. Whereas the human density in North America is roughly 22.9/km2, it is reported to be 389.9/ km2 for India, or approximately 17 times greater. Just as this differential population density correlates highly with the difference in bear attack frequency between North America and India, Smith et al. (in review) demonstrated that population growth in Alaska over 130 years accounts for 87% of the variation in bear attacks during the same period. Indeed, the more people enter bear habitat and commingle with them, the more likely are human-bear encounters and risk of subsequent injury and/or death, whether grizzly or sloth bear. We do not conclude, therefore, that sloth bears are more dangerous than grizzlies, but rather that they are quite similar in their intolerance of human incursions into their habitat.

## Discussion

### Attack Motivation

Historical data and recent interviews with attack victims and witnesses all support the premise that the motivations of sloth bear attacks are exclusively defensive in nature, not predatorial. In fact, we were unable to document or find documentation for a single sloth bear predatorial attack. Occasionally a victim reported an attack as predatorial, and though these attacks do not appear predatorial when independently analyzed, we understand how they could appear as such. Attack victims have reported feeling ambushed by an animal lying in wait because the bear appeared to be hidden and therefore could have likely left the area without ever being detected. It then follows that people might conclude that the motivation for attack was predatory. Other victims reported seeing a sloth bear hiding behind a tree, only to attack after detection. Again, this appeared to the victim as predatorial. However, video footage of a stressed bear at the BBRC shows it running behind a tree, only to charge again at people outside its fenced area. The attack might look predatorial because it seems logical that a non-predatorial bear would either stay hidden or run off rather than attack.

Another contributor to the belief that sloth bears may be predatorial is the existence of documented cases, both recent and historical, of sloth bears partially consuming their victims' corpses. However, upon closer inspection, it appears that the initial bear attacks in these cases were defensive in nature (or at the very least the motivation is unknown), and that the bears began consuming the corpse(s) opportunistically. As we mentioned, sloth bears do occasionally scavenge larger mammals (Schaller 1984). Additionally, other bears (specifically, grizzlies [Herrero 1985]) have been documented feeding on victims killed during defensive attacks.

The known ecology and diet of the sloth bear seems to corroborate the lack of predatorial attacks. It is clear from studies (e.g., Laurie and Seidensticker 1977, Gopal 1991, etc.) that red meat plays nearly no role in the diet of this species. Red meat is generally limited to the occasional rodent, which was likely consumed inadvertently while the bear foraged for insects. Even scavenging on wildlife carcasses appears rare for this species.

The sloth bear's defensive nature appears to be the result of co- evolving with large predators, namely tigers, which are known to occasionally kill and eat sloth bears. However, it is instructive to compare the sloth bear's survival strategy to that of the American black bear. Although the sloth bear behaves defensively aggressive, the American black bear almost never attacks defensively. Even a female with cubs will run in the face of danger, while the cubs climb trees. Sloth bear cubs do not climb trees when threatened, but rather cling to their mother's back for the first 9 months of their lives. Mother sloth bears with cubs have been documented fighting off tigers by charging them with cubs clinging to their backs. The reasons for this difference in strategies between black and sloth bears may be explained by differences in their respective habitats and the other species of wildlife occupying them. American black bears are largely restricted to forested habitats, whereas sloth bears are often found in grasslands and scrub jungle, where the opportunity to climb a tree is not always present. Additionally, though sloth bears occasionally climb trees for honey and other foods, they are not nearly the climbers that American black bears are. This may be partly due to the sloth bear's very long claws, which are adapted to digging rather than climbing trees. The tendency of sloth bears to attack without much provocation could have evolved as a way to mitigate threats in their environment. Bouskila and Blumstein (1992) state that "animals rarely have perfect information, and generally are expected to maximize fitness by overestimating rather than underestimating risk. Overestimation costs, such as lost feeding opportunities, have milder fitness consequences than the cost of underestimating danger, which might be immediate death." Frid and Dill (2002) concur with this assessment, stating that underestimating a potential risk has much harsher consequences than overestimating a perceived threat. Sloth bears are mid- to small-sized bears that coexist with many predatorial species such as tigers and leopards *(Panthera pardus)* as well as megafauna such as the Asian elephant *(Elephas maximus)* and Indian rhinoceros *(Rhinoceros unicornis).* Although sloth bears flee potential danger when given the chance, they often use the strategy of "the best defense is a good offense," and charge the putative threat.

North American bear species (black, brown/grizzly, and polar have been known to see humans as potential prey. We did not find predation to be a motivation for sloth bear attacks in India; most are the result of surprise encounters. This is important because it suggests the solution to sloth bear-human conflict lies in human behavior modification rather than arming people with expensive deterrents (e.g., firearms, bear spray, shotgun deterrent rounds, an economic impossibility for most. If a person acts appropriately in sloth bear habitat, making noise and telegraphing their presence as they move about, our research suggests that most bear encounters will be avoided. When avoidance measures (i.e., making noise appropriately, hiking in groups, etc. are the focus of bear safety messaging, the nuances of how to best defend oneself during an attack (e.g., fight or flight become much less important.

**Defensive Behaviors**

Sloth bears do not appear to display signs of stress before charging. This may be related to the species' predilection to charge with less provocation than bear species such as grizzlies or American black bears. Sloth bears do appear to incorporate several actions meant to intimidate, including vocalizing and raising up on their hind legs. Sloth bears may also use their shaggy fur to appear larger to potential predators.

Notably sloth bears do not pull their ears back during a tiger encounter. Since tiger attack sites are typically the back of the neck, pulling one's ears back is not particularly useful. Work by Walther (1969) and Ghalambor and Martin (2000) suggests that prey have evolved predator-specific antipredator behaviors. This may be the case here: no need to pull ears back for tigers whereas they may do so with other sloth bears. Further observation of sloth bear-sloth bear aggression is required to determine if sloth bears behave differently to different types of threats. Bear-bear attacks in North America are generally frontal attacks of the head and face and as such laying the ears flat makes more sense.

**Avoiding Encounters in the Wild**

Our data strongly suggest that making noise while moving through sloth bear habitat helps to avoid sloth bear encounters and attacks, and that encounters are less likely to turn into attacks. This suggests that if the bear is not startled in close quarters, its preference is to leave the area without incident. This is not surprising, and is similar to findings with other bear species, namely the grizzly and American black bear.

**How to react to a Sloth bear attack**

The results of our interviews aimed at determining a behavioral approach to sloth bear attacks yielded some mixed messages. Fighting an attacking sloth bear resulted in approximately 9% fatalities, while running from an attacking sloth bear resulted in 11% fatalities. Playing dead when confronted by an attacking sloth bear resulted in no fatalities. Therefore, if the goal is simply to survive the encounter, falling to the ground and covering up in some fashion seems advisable. However, if we combine fatalities with serious injuries, all three courses of action result in approximately 20% of individuals being killed or severely injured. Further, a higher percentage of people who played dead and covered up in some fashion suffered a higher rate of moderate injuries (65%) than did those who fought back (30%) or those who ran (42%). The reasons for this are difficult to interpret; however, a partial explanation may be that those who played dead did not cover up in the most protective manner. In fact, what many of the victims reported doing while playing dead does not constitute effective protection. Therefore, it is at least possible that if people were taught how to properly protect their head and neck regions from injury, the severity of the injuries would lessen.

Those who fought back and did not die faired relatively well, as only 46% suffered minor injuries. However, 9% were fatally injured and another 12% were severely injured. We are unable to ascertain why nearly half of the victims escaped with minimal injuries while 21% were killed or severely injured. The differences in injury may have to do with some unreported action on the victim's part that resulted in. It may also merely reflect the odds of an injury being severe or fatal. When a bear's jaws encompass the head, injuries are most often fatal, whereas when canines slip off the curvature of the skull, severe injuries result but the brain remains protected. With this in mind, one can see how under the same attack scenario, one individual may die while another might survive.

Finally, those who attempted to run from the attacking bear fared worst, with a higher percentage dying than in the other response scenarios (11%). There is little doubt that running triggers a chase response in sloth bears, just as it does in grizzly bears (Herrero 1985). There have been many cases of sloth bears chasing, catching, and mauling human victims (Sharp and Sonone 2011).

## Future Directions

Further follow-up studies on sloth bear attacks and responses to victim behavior will help confirm if reacting to a sloth bear attack in the same manner as to a defensive grizzly bear attack would be most beneficial for the victim. Additionally, outreach to communities in sloth bear habitat can educate them on them how to avoid encounters with sloth bears, or what to do if they are charged and attacked.

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## Abbreviations:

## FLA: Full Length Article

## ABT: Abstract

## IV c) PUBLICATION BASED INDEX

| ARTICLE NUMBER | YEAR PUBLISHED | PUBLISHED IN | Published as Poster/ Report/ FLA/ABT | PAGE No. |
|---|---|---|---|---|
| 102 | 2011 | National Conference on Environment and Biodiversity of India held on 30th-31st December 2011, New Delhi, pp 125 | ABT | 01 |
| 103 | 2011 | National Congress on Wildlife Health and Foresnsics 15-16 September 2011, MPCVV, Jabalpur (M.P.) | ABT | 03 |
| 104 | 2012 | International Conference on Reptile and mphibian Medicine, Cremona, Italy | ABT | 04 |
| 105 | 2014 | American Association of Zoo Veterinarians Journal 2014 | ABT | 06 |
| 106 | 2015 | 7th Kerala Veterinary science congress 2015, Pookode, Wayanad. | ABT | 08 |
| 107 | 2015 | International Bear News, Fall 2015, Volume 24, No. 3, pp 10-13 | FLA | 10 |
| 108 | 2015 | International Symposium on Ecology and Health Management of Asiatic Elephant (*Elephas maximus*) held on 19-20 November organized by Assiciation of Indian Zoo & Wildlife Veterinarians (AIZWV), pp 318. | ABT | 20 |
| 109 | 2015 | International Symposium on Biodiversity, Agriculture, Environment and Forestry organized by Association for the Advancement of Biodiversity Science, pp 152 | ABT | 21 |
| 110 | 2015 | International Symposium on Biodiversity, Agriculture, Environment and Forestry organized by Association for the Advancement of Biodiversity Science, pp 106 | ABT | 23 |
| 111 | 2015 | International Symposium on Ecology and Health Management of Asiatic Elephant (*Elephas maximus*) held on 19-20 November organized by Association of Indian Zoo & Wildlife Veterinarians (AIZWV), pp 279. | ABT | 25 |
| 112 | 2015 | International Journal of Applied Research, Volume1, No.10, pp 672-675 | FLA | 27 |

| | | | | |
|---|---|---|---|---|
| 113 | 2015 | 7th Kerala Veterinary science congress 2015, Pookode, Wayanad. | ABT | 39 |
| 114 | 2015 | Advancing Bear Care Vietnam Workshop, Vietnam | ABT | 41 |
| 115 | 2016 | CATnews 63, Spring 2016, pp 19-20 | FLA | 43 |
| 116 | 2016 | Journal of Veterinary Medicine and Science, Volume 2, pp 221-225 | FLA | 45 |
| 117 | 2016 | XXV National Congress of Veterinary Parasitology and National Symposium on One Health Approach – Plausible Solution For Sustainable Parasite Control Organized by Department of Veterinary Parasitology, Madras Veterinary College, TANUVAS, Chennai, Feb – 600 007. Pp 138 | ABT | 53 |
| 118 | 2016 | 19th ADNAT Convention International Symposium on Microbiome in Health and Disease Organized by Association of the Promotion of DNA Fingerprinting and other DNA technologies, Hyderabad & ICAR – National Institute of Animal Nutrition and Physiology, Bangalore, Feb 2016 pp 56. | ABT | 63 |
| 119 | 2016 | 19th ADNAT Convention International Symposium on Microbiome in Health and Disease Organized by Association of the Promotion of DNA Fingerprinting and other DNA technologies, Hyderabad & ICAR – National Institute of Animal Nutrition and Physiology, Bangalore, Feb 2016, pp 68 | ABT | 64 |
| 120 | 2016 | Journal of Wildlife Diseases, Volume 52, No.2, pp 400-402 | FLA | 66 |
| 121 | 2016 | Indian Journal of Veterinary Anatomy May 2016, Volume 8, No. 1, pp 70-71 | FLA | 68 |
| 122 | 2016 | APCRICON 2016 18th National Conference of Association for Prevention and Control of Rabies in India held on 9-10 July, 2016 organized by Kempegowda Institute of Medical Sciences, pp28. | ABT | 75 |
| 123 | 2016 | 40th Annual Congress of Indian Society for Veterinary Surgery & National Symposium Biomaterials & Stem Cells for Tissue Repair and Regeneration in Veterinary Surgery held on 2-4, December, 2016, pp 231. | ABT | 79 |

| | | | | |
|---|---|---|---|---|
| 124 | 2016 | 40th Annual Congress of Indian Society for Veterinary Surgery & National Symposium Biomaterials & Stem Cells For Tissue Repair and Regeneration in Veterinary Surgery held on 2-4 December, 2016, pp 238. | ABT | 81 |
| 125 | 2016 | 40th Annual Congress of Indian Society for Veterinary Surgery & National Symposium Biomaterials & Stem Cells For Tissue Repair and Regeneration in Veterinary Surgery held on 2-4 December, 2016, pp 240. | ABT | 83 |
| 126 | 2016 | International Bear News, (December) Fall 2016, Volume 25, No. 3, pp 10-12 | FLA | 84 |
| 127 | 2016 | Elephant Managers Association conference at Oklahoma, USA in October 2016. | ABT | 85 |
| 128 | 2016 | Elephant Managers Association (EMA) conference, Oklahoma, USA | ABT | 90 |
| 129 | 2017 | National Congress on Wildlife Health & Annual Convention of Association of Indian Zoo and Wildlife Veterinarians (AIZWV) jointly Organized by Association of Indian Zoo and Wildlife Veterinarians (AIZWV) & ICAR & Indian Veterinary Research Institute, Jan (2017) pp 121 | ABT | 91 |
| 130 | 2017 | National Congress on Wildlife Health & Annual Convention of Association of Indian Zoo and Wildlife Veterinarians (AIZWV) jointly Organized by Association of Indian Zoo and Wildlife Veterinarians (AIZWV) & ICAR & Indian Veterinary Research Institute, Jan 2017, pp 180-185. | FLA | 92 |
| 131 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar, pp. 52. | ABT | 93 |
| 132 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar, pp 55. | ABT | 105 |
| 133 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar, pp 6 | ABT | 107 |
| 134 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar, pp 107 | ABT | 109 |
| 135 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar, pp. 108, | ABT | 111 |

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|---|---|---|---|---|
| 136 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar. Pp 118 | ABT | 113 |
| 137 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar. Pp 120. | ABT | 115 |
| 138 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar. Pp 120. | ABT | 116 |
| 139 | 2017 | International Journal of Applied Research, Volume 3, No. 8, pp 448-451. | FLA | 118 |
| 140 | 2017 | International Journal of Veterinary Sciences and Animal Husbandry, Volume 2, Issue 4, pp 04-09. | FLA | 120 |
| 141 | 2017 | AMB Express, Volume 13, No. 13, pp | FLA | 127 |
| 142 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar, pp 33 | FLA | 139 |
| 143 | 2017 | National Congress on Wildlife Health held on 06-07 January, organized by ICAR-Indian Veterinary Research Institute, Izatnagar, pp 54 | ABT | 168 |
| 144 | 2017 | International bear news, Summer 2017, Volume 26, No. 2 | FLA | 184 |
| 145 | 2017 | International Journal of Applied Research, Volume 3, Issue 8, pp 112-114 | FLA | 186 |
| 146 | 2017 | Shanlax International Journal of Veterinary Science, Volume 4, April 2017, No.4, pp | FLA | 190 |
| 147 | 2017 | Veterinary World, Volume 10, July 2017, pp 818-824 | FLA | 198 |
| 148 | 2017 | International Journal of Life Sciences Research, Volume 5, Issue 3, Sept 2017, pp 128-135 | FLA | 207 |
| 149 | 2017 | Indian Journal of Applied Research, Volume 7, Nov 2017, Issue 11, pp 295-297 | FLA | 224 |
| 150 | 2017 | International Association for Bear Research & Management, March 2017 | ABT | 238 |
| 151 | 2017 | International Bear News, Spring 2017, Volume 26, No. 1, pp 14-15 | FLA | 248 |

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