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A COMPENDIUM OF SCIENTIFIC PUBLICATIONS OF WILDLIFE SOS

Volume - IV

An Official Publication of Wildlife SOS(R)

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NOTE FROM THE CO-FOUNDERS

Dear Friends and Supporters of Wildlife SOS,

We write to you today with immense pride and gratitude as we share the remarkable journey and accomplishments of Wildlife SOS over the past two decades. Our mission, deeply rooted in the preservation of India's incredible wildlife, started in 1995, with the inception of the 'Dancing Bear Project.' This endeavour signified an end of a centuries-old tradition that subjected hundreds of highly endangered sloth bears to unspeakable cruelty.

These bears, poached from the wild as cubs, endured excruciating procedures to prepare them for the trade. At the tender age of six months their canines were possibly knocked-out, and a thick rope pierced through their muzzles, controlling their every movement. Over the years, Wildlife SOS has successfully rescued and rehabilitated over 600 bears, liberating them from a life of agony while also empowering the Kalandar community involved in this practice through alternative livelihoods and education initiatives. This project exemplified our commitment to both wildlife welfare and the well-being of the communities connected to it.

While the 'Dancing Bear Project' is a significant milestone, our conservation efforts extend far beyond its scope. We have actively engaged in various initiatives, including running helplines for the rescue of reptiles and other urban-wildlife, elephant conservation and care, leopard conservation, Asiatic black bear and Himalayan brown bear conservation through human-wildlife conflict mitigation, anti-poaching efforts, and habitat conservation.

Our success is deeply rooted in collaboration, working closely with local communities and government agencies to combat illegal wildlife trade and trafficking. Each rescue operation exemplifies our commitment to timely intervention, ensuring the success of our missions. While the challenges we face are formidable, our determination remains unshaken. Together with our dedicated team, supporters, partners, and individuals like you, we have achieved extraordinary milestones. And together, we will continue to script stories of hope and conservation.

Geeta Seshamani

Geeta Seshamani
Co-founder & Secretary
Wildlife SOS

Kartick Satyanarayan
Co-founder & CEO
Wildlife SOS

भारतीय वन्यजीव संस्थान
Wildlife Institute of India
(An Autonomous Institute under Ministry of Environment, Forest & Climate Change, Govt. of India)
पत्रपेटी सं०/Post Box No. 18, चंद्रबनी, देहरादून/Chandrabani, Dehradun - 248001, उत्तराखण्ड, भारत /Uttarakhand, INDIA

Azadi Ka Amrit Mahotsav

Foreword

In the intricate drapery of our world, there exists a profound and symbiotic relationship between humans and the magnificent array of wildlife. This delicate equilibrium is constantly challenged by the myriad of obstacles from natural disasters to human-induced threats. It is in these moments of adversity that the tireless efforts of scientists, veterinarians, and dedicated individuals shine brightly, illuminating the path toward understanding, compassion, and sustainable coexistence.

This compendium is a comprehensive compilation of publications addressing various aspects of veterinary interventions in wild animals including case studies, ex-situ care and management and efforts towards rehabilitation of wild sentient. The chapters within these covers are not merely scientific publications; they are chronicles of resilience, compassion, and the unwavering commitment of the Wildlife SOS team who have devoted their lives to safeguarding the wellbeing of the diverse array of wondrous inhabitants. Each case study has been meticulously documented, analyzed and presented. From pioneering veterinary interventions to ground-breaking conservation strategies, this compendium serves as a testament to the power of interdisciplinary collaboration and the unyielding spirit of those who dedicate their lives to the welfare of animals.

It is my sincere hope that this compendium will enlighten the minds of its readers and serve as a ready reckoner for Wildlife health practitioner's including Zoo veterinarians, researchers, students, and managers.

I extend my deepest gratitude to the authors, researchers, and practitioners whose unwavering dedication and ceaseless pursuit of knowledge have made this compendium possible. May their stories serve as a source of inspiration, reminding of our shared responsibility to be stewards of managing wild animal.

Dr. Parag Nigam
Scientist-G &
Head, Department of Wildlife Health Management

ई.पी.ए.बी.एक्स./EPABX : +91-135-2640114, 2640115, 2646100; फ़ैक्स/Fax : 0135-2640117 ई-मेल/Email: wii@wii.gov.in वेब/Website www.wii.gov.in Twitter: @wiiofficial1

Dr. A Sha Arun

B.V.Sc., M.V.Sc (Wildlife Medicine), M.S. (Psychotherapy)., Ph.D

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

ACKNOWLEDGEMENTS

Dear Team,

It gives me great pleasure to introduce the volume four of **"A Compendium of Scientific Publications of Wildlife SOS."** This compendium is a testament to the dedication and passion for research that defines the spirit of Wildlife SOS.

Our deepest gratitude goes out to our co-founders, whose enduring support has been the bedrock of our organization's success. We trust in their ongoing encouragement as we continue on this intellectual journey.

This volume is a collective triumph, a result of the unyielding commitment of our team. I extend my heartfelt appreciation to Ms Vasavi and team for their invaluable contributions. Their tireless work has been instrumental in shaping this compendium.

I also extend my thanks to our diligent animal keepers, whose unwavering cooperation laid the groundwork for the research presented in these pages. Your dedication has been the cornerstone of this endeavor.

To my peers and colleagues, I extend my gratitude for your constructive feedback and unwavering support. Your insights have played a pivotal role in refining our work, and I am truly grateful.

Lastly, I cannot overstate the vital role played by the various state forest departments across the country. Their unwavering support has been instrumental to our research endeavors, and we look forward to continued collaboration in the pursuit of knowledge and conservation.

Thank you all for your tireless dedication and contributions.

Warm regards,

(Attur Shanmugam Arun)

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

IV) CONTENTS

iv a) ARTICLE TITLE INDEX

Art – 220. MUCINOUS CHOLANGIOCARCINOMA IN CAPTIVE SLOTH BEAR

(*Melursus ursinus*)

Karikalan M., **Ilayaraja S., Arun A. Sha**, C. Mohan S. and A.K. Sharma

Abstract

A 28 years old male sick sloth bear in captivity died with the history of poor appetite, distended abdomen with liver disorder at Agra Bear Rescue Centre. Post-mortem examination revealed pale mucous membranes, weak and debilitated body condition and presence of about two litres of serosanguinous fluid in the peritoneal cavity. Multiple mulberry sized semi-solid nodular growths were observed on the liver. The surface of the liver had adhesions with most of the abdominal organs. Serosal surfaces of most of the abdominal organs were also covered with thick mucus material. Histopathologically, the liver section showed variable sized areas where architecture of normal hepatic parenchyma was replaced by fibroadenomatous structures. The irregular variably dilated acinar structures resembled neoplastic bile ductules infiltrating the fibrous tissue stroma. The lining epithelial cells exhibited nuclear and cellular atypia with presence of many mitotic figures. The neoplastic cells, at times, exfoliated into the lumina. The lumina in majority of ductules contained mucin which stained pink with PAS stain. The neoplastic cells of proliferating bile ductules were intensely positive for cytokeratin immunostaining. Based on the characteristic histopathological features, the present case was diagnosed as mucinous cholangiocellular carcinoma, a rare variant of cholangiocellular carcinoma.

Keywords: Cholangiocarcinoma, cytokeratin, mucin, PAS, sloth bear

Sloth bear (*Melursus ursinus*), a bear species commonly found in the Indian subcontinents, is listed under Schedule I of the Wildlife (Protection) Act 1972, and it is classified as vulnerable in the IUCN Red List of Threatened Species[1]. Cholangiocarcinoma (bile duct carcinoma) is a malignant tumour arising from bile duct epithelial cells. Cholangiocellular carcinoma has been reported commonly in domestic animals[2]. Most of the cholangiocarcinomas are characterized by acinar or tubulo-papillary structures lined with cuboidal or columnar epithelial cells, but sero-mucinous abdominal effusions are highly unusual in animals, however, some isolated cases of mucinous form in humans and cats have been documented[3,4]. In captive bears, neoplasms involving the hepatobiliary and gastrointestinal systems have been frequently reported[6]. Several case studies showed occurrence of hepatic neoplasms (cholangiocarcinoma, intrahepatic biliary neoplasm and hepatocellular carcinoma) in polar bear, sun bear, Himalayan black bear and sloth bears[5]. The exact cause of hepatic cancer in bears is unknown. The present paper describes a case of mucinous cholangiocellular carcinoma in captive sloth bear.

A 28 year old male captive sloth bear which fell sick for more than 2 months with the history of poor appetite, slowly bulging abdomen and compromised liver functions, died at Agra Bear Rescue Centre, Keetham, Agra, Uttar Pradesh. Postmortem examination was conducted immediately and the gross lesions were recorded. Therepresentative tissues pieces of visceral organs like liver, lung, kidney, spleen, heart, intestines, stomach, and mesenteric lymph nodes were collected and fixed in 10% neutral buffered formalin (NBF) for routine histopathological examination. Duplicate sections of liver were also stained with Periodic Acid Schiff (PAS) to confirm the mucous material. Immunohistochemistry was performed on liver tissue sections using Mouse anti-pancytokeratin antibodies (Sigma, USA).

Post-mortem examination revealed pale mucous membranes, poor body condition with debilitation, rough hair coat and presence of 1.5-2.0 liters of serosanguinous fluid in the abdominal cavity. Multiple mulberries sized nodular growths were seen bulging from the liver surface and had adhesions with serosal surfaces of other the abdominal organs (Fig. 1). Serosal surfaces of most of the abdominal organs were also covered with mucus material. Histopathologically, the liver section showed disruption of normal architecture of hepatic parenchyma which in some areas was replaced by connective tissue stroma with numerous infiltrating acinar structures resembling bile ductules (Fig. 2). The cells were cuboidal or columnar, with a moderate amount of clear or slightly granular cytoplasm, most of the neoplastic cells showed nuclear and cellular atypia with presence of abnormal mitotic figures (Fig. 3,4). At some places, the neoplastic cells were exfoliated into the lumen. Intra luminal mucin confirmed by PAS staining was also observed as pink material (Fig. 5). On immuno- histochemistry with pancytokeratin antibodies, the neoplastic epithelial cells of proliferating bile ductules were found intensely positive for cytokeratin immunolabelling (Fig. 6).

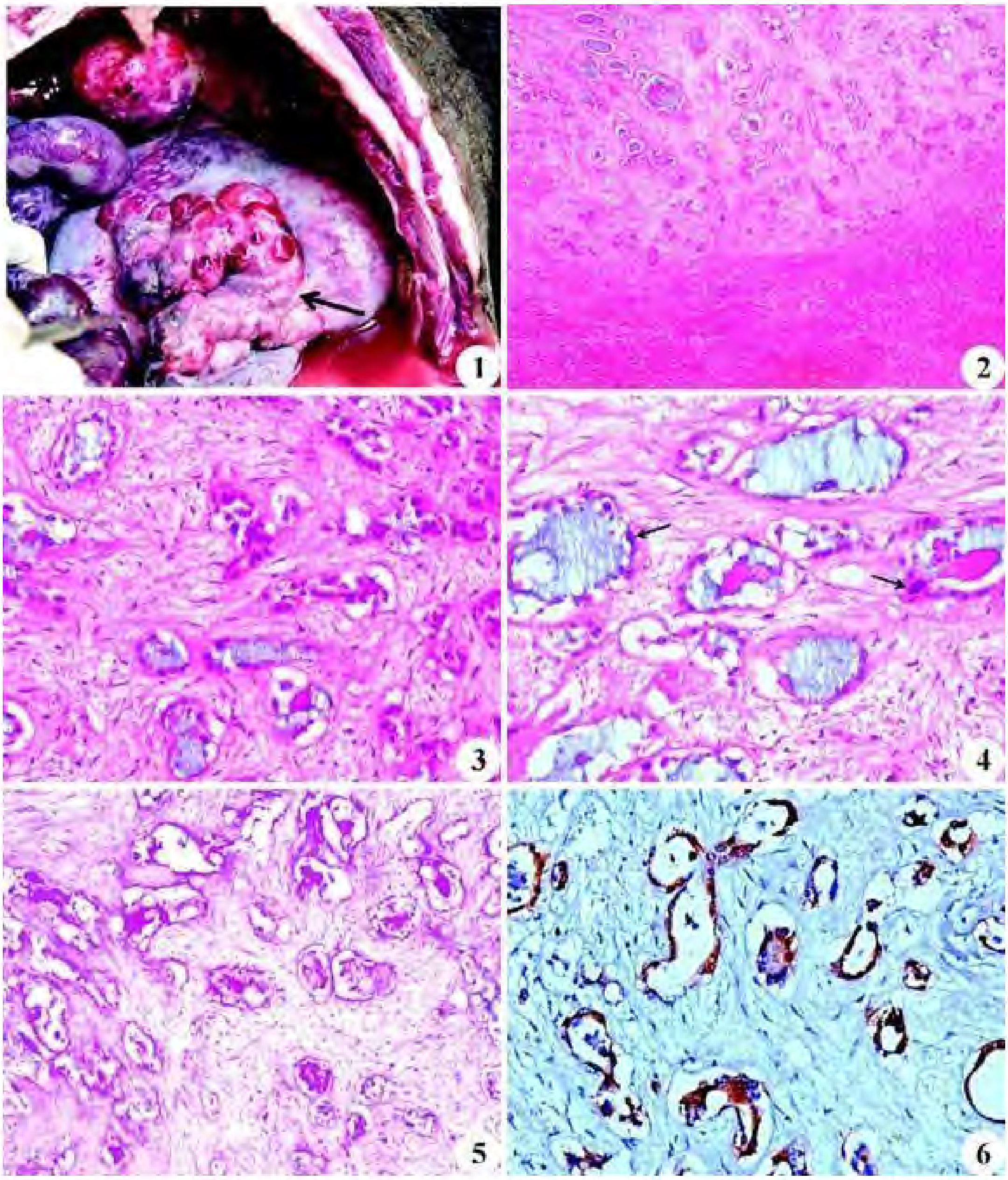

Fig.1. Abdominal cavity showing multiple mulberries sized coalescing nodules elevated from the liver surface (arrow) with serosanguinous translucent peritoneal effusion; Fig.2. Section of liver showing irregular acinar structures containing mucus in connective tissue stroma. H&E x40; Fig.3. Irregular bile ductules lined by neoplastic epithelial cells containing intra luminal mucus. H&E x200; Fig.4. Mucin containing dilated acini lined by neoplastic cells with cellular atypia, hyperchromatic nuclei and mitotic figures (arrow). H&E x400; Fig.5. Liver section showing pinkish mucus material in lumina of neoplastic cholangioles. Periodic Acid Schiff x100; Fig.6. Neoplastic bile ductules showing cytokeratin positive lining epithelial cells. IPO-DAB x200.

Mucin-producing cholangiocarcinoma is an uncommon biliary tract neoplasm of animals which is commonly reported in humans and only few cases were reported in animals, mostly in cats[3,4]. Several studies in humans showed that incidence of mucinous cholangio- carcinoma ranged from 4% to 90% of hepatic neoplasms[6]. The exact pathogenesis of mucinous cholangiocarcinoma is not understood properly. The present case in sloth bear appeared to be unusual because of the presence of mucinous secretory component of the cholangiocarcinoma. Most of the cholangiocarcinomas reported in animals are non-mucinous type characterized by acinar or tubulo-papillary structures, lined with cuboidal or columnar neoplastic epithelial cells[2]. It was reported that the epithelial cells lining some of the intrahepatic biliary ducts apparently underwent malignant transformation to a phenotype that secreted a mucinous substance[7], and accumulated within the acinar structures leading to peritoneal effusion. However, one case of sloth bear at Ohio zoo, USA which died of extrahepatic biliary carcinoma had numerous mucin-producing neoplastic glands in fibrous stroma. Although the exact cause of biliary and hepatocellular neoplasms is not known, it has been reported that in bears, high levels of dietary fat and low levels of vitamin A and selenium may predispose to the development of hepatic neoplasia[9]. In present case, deficiency of vitamin A cannot be ruled out.

Though hepato-biliary neoplasms are not uncommon in sloth bears but the present case has unusual pathology of mucin producing neoplastic cells and is of rare occurrence.

Acknowledgements

The authors are thankful to the Director, ICAR-IVRI and Wildlife SOS for providing necessary facilities.

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Art – 221. ARCHITECTURE OF ARM MUSCLES OF SLOTH BEAR

(*Melursus ursinus*)

VR Annie, KV Jamuna, **A Sha Arun**, RV Prasad, VK Girish and Shruti

Abstract

The present study was undertaken to systematically establish the myological characteristics of the arm region of sloth bear which might help to carryout morphological assessments. Carcasses of sloth bears were obtained from the Bannerghatta Bear Rescue Centre of Bannerghatta Biological Park, Bangalore. In this study it was observed that M. Biceps brachi was principally made up of the long head and its tendon coursed within bicipital groove. M. brachialis lodged in the musculo spiral groove and coursed in a spiral like fashion. M. triceps comprised of five heads with M. triceps longus and medialis again subdivided two parts, got inserted on the olecranon process. M. triceps lateralis was smaller than M. triceps longus which run diagonally across the outer surface of arm. M. anconeus lied deep to the distal part of the M. triceps. In sloth bear attachment of muscle was more proximally compared with other carnivore which may account for the slow gait of bears.

Keywords: arm region, mycological characteristic, sloth bear

1. Introduction

Sloth bears are nocturnal, insectivorous species. They belong to ursidae family which includes eight species, viz., Giant Panda, Spectacled Bear, Sun Bear, Sloth Bear, Asiatic Black Bear, American Black Bear, Brown Bear and Polar Bear. Each species shows remarkable variations in their physical features and habits. They exhibit morphological variation within and between species and the differences in behaviour, as well as habitual and specific activities are due to their anatomical peculiarities [1]. The scientific name for the sloth bear is *Melursus ursinus*. Sloth bear is classified as "Vulnerable" in the 1996 IUCN Red List of Threatened Animals and is listed on Appendix I of CITES. In India, trade and export of sloth bear is illegal and the bear is completely protected under Schedule I of the Indian Wildlife Protection Act of 1972 that is amended in 1986 [2].

Locomotion and animal posture greatly influence the anatomy of a muscle due to the high frequency and high loads of forces involved. Skeletal musculature constitutes the active part of the locomotor system [3]. 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. Based on the action muscles are classified as extensors, flexors, adductors, abductors, supinator, pronator, rotator etc [4].

The gross myological similarities, differences and functional adaptations of muscle may serve as a basis for study of the functional morphology of locomotion as well as phylogenetics and systematics at various taxonomic levels [5]. However, the information concerning sloth bear locomotor system is scanty and no detailed information is available on the muscular system. Hence, the present study was to study the gross morphological features of muscles of the arm region in sloth bear.

2. Materials and Methods

Dissections were conducted on the left and right forelimbs of three captive adult sloth bears lived at the Wildlife SOS, Bannerghatta Bear Rescue Centre of Bannerghatta Biological Park, Bangalore, Karnataka. Following necropsy, the forelimbs were stored in 10% formalin and dissections were carried out. Then skin and superficial fascia overlying the forelimb muscles were removed. Prior to the removal of muscles, observations were made on origin, insertion, position and relationship of each muscle. Since connective tissue function in binding all other structures together, major work in dissection was the removal of connective tissues. Deep muscles were exposed by transecting the more superficial ones and reflecting them.

A transection was made only after careful study of superficial characters of muscles under consideration. The transection was carried out at about midway between origin and insertion in most cases. By following this procedure neither the origin nor insertion was cut into and it was possible to replace the cut edges for later observations of transected muscle. Each muscle had to be reflected to its points of attachments. The terminology used conforms to standards of Nomina Anatomica Veterinaria [6].

3. Results and Discussion

3.1.1 Craniolateral muscles of Arm

3.1.2 M. Biceps brachi

M. Biceps was a large fusiform muscle in flexor compartment of the upper arm region (Fig. 1-j). Biceps brachi was principally made up of a long head designated as M. biceps brachi caput longus (Fig.4-c). M. Biceps brachi arose from top of glenoid cavity (Fig.3-a) and its tendon coursed within bicipital groove (Fig.3-b). Later, muscle soon expanded into a large fleshy belly which ended in a strong tendon near elbow which was inserted on the bicipital tubercle of radius (Fig.4- d). Bicipital tubercle was observed just below the neck of the radius on medial border which had the form of a rough ridge. It's function was to extend the shoulder joint and flex the elbow joint. In addition, it stabilized the elbow joint when standing.

A remarkable difference between sloth bear and other primates was with a short head. Our study revealed that in sloth bear, it was absent which was contrary to the records in primates where in it took origin from the coracoid process of scapula. According to some authors both head of M. biceps brachi of primates ran over shoulder giving a biarticular function (crossing shoulder and elbow joint) [7]. So, in sloth bear this muscle might have less flexion capacity in shoulder level.

3.1.3 M. Brachialis

M. brachialis was lodged in the musculo-spiral groove and it coursed in a spiral like fashion towards the cranial aspect of humerus (Fig.5-a). Our findings were supported by some of the authors who stated that muscle originated from the caudolateal aspect of humerus and coursed towards cranial aspect of brachium [8]. Tendinous origin of M. brachialis anticus was positioned external to insertion of M. deltoideus. During its course, it was closely connected with lateral head of M. Triceps (Fig.2-e), covered cranial part of humerus and was inserted onto proximal part of ulna.

There was no significant difference in origin of brachialis with any other species. This muscle was significantly larger in orangutans and interpreted as a morphological specialization to arboreal locomotion [9].

3.2 Caudomedial muscles of Arm

3.2.1 M. Triceps brachi

M. triceps comprised of five heads viz., M. triceps longus anterius (Plate 6-e), M.

triceps longus posterius (Fig.1-e), M. triceps lateralis (Fig.7-a), M. triceps medialis longus (Fig.8- b), M. triceps medialis brevis (Fig.8-c). M. triceps longus was a large triangular mass lying along posterior side of arm. It's origin extended along whole axillary border of scapula, from infraglenoid tubercle to vertebral angle. At it's origin M. triceps longus was subdivided into anterior and posterior parts, of which anterior part was considerably larger. Anterior head arose by fleshy fibers, from proximal 3/4th of axillary border of scapula and from prominent crest separating infraspinous fossa from post-scapular fossa (Fig.6-d). Posterior head continued line of origin of anterior head along crest separating fossa, but did not attach to bone. It arose from fascia covering M. subscapularis minor lying directly deep into it and from fascia covering M. infraspinatus and M. teres major (Fig.1-e). Origin extended posteriorly to the vertebral border of scapula. At about middle of the arm, M. triceps longus fused with M. triceps lateralis and remained distinct from M. triceps medialis.

M. triceps lateralis (Fig.6-f) was smaller than M. triceps longus which run diagonally across the outer surface of the arm. On its deep surface throughout it's length it was intimately united with M. triceps medialis. M. triceps lateralis arose almost exclusively from surface of M. brachialis lying immediately beneath (Fig.6-i). Distal half of M. triceps lateralis was fused with adjacent surface of M. triceps longus.

M. triceps medialis was composed of a long head and a small intermediate head, which were separated by M. coracobrachialis brevis at their origins but fused below at middle of the arm. Long head (Fig.1-g) originated from a triangular area on posterior surface of shaft of humerus. Long head was separated from M. triceps lateralis only for a very short distance beyond their origin. Intermediate head (Fig.1-f) took a tendinous origin from a short line on postero-medial edge of shaft of humerus, immediately beneath and behind the tendinous insertion of M. latissimus dorsi. M. triceps medialis was inserted by fleshy fibers, into medial surface of the olecranon.

Giant panda was the only other carnivore where in subdivision into anterior and posterior was recorded. In contrast, American black bear, possessed only four heads [10] and in other bears three heads [11]. Such a recorded notable difference in size of triceps indicating that enlargement of this muscle group was associated with locomotion.

In giant ant eater triceps was very extensive and predicted that wider origin could be an indication of muscle strength required for habits of animals [11]. In sloth bear, M. triceps longus posterius was extending along whole axillary border of scapula. However, in other carnivores, origin of this muscle was restricted to proximal half or less of axillary border, except in giant panda, in which it extended nearly as far as in bears. As these muscles were inserted on olecranon process, they will assist in extension of the elbow joint and act against flexor rotational forces.

3.2.2 M. Anconeus

We observed anconeus as a single, flat triangular muscle which arose from posterior side of distal end of the humerus and was fused with elbow joint capsule and fanned out to insert extensively on entire width of caudal part of olecranon and proximal ulnar shaft (Fig.9-b). Anconeus muscle was insignificant muscular portion derived from triceps muscle[7]. In sloth bear, it was a single muscle, but this muscle was splited into two layers and united at about axial line of humerus in other bears [12]. It was inserted on entire width of posterior of olecranon in red panda [8]. Its function was to extend elbow joint and it's contraction may also tense joint capsule thereby preventing its entrapment between humerus and ulna.

Fig 1: Medial surface of shoulder and arm

Fig 2: Lateral surface of shoulder and arm

a – Point of origin
b – Tendon of M. Biceps
c – Muscle belly
d – Supraspinous fossa
e – Infraspinous fossa
f – Glenoid cavity
g – Acromion process
h – Humerus

Fig 3: Origin of M. Biceps brachi

a – Point of origin
b – Tendon of M. Biceps
c – Muscle belly
d – Bicipital tubercle

Fig 4: Origin and insertion of M. Biceps brachi

a – M. Brachialis
b – Humerus
c – M. Ancon

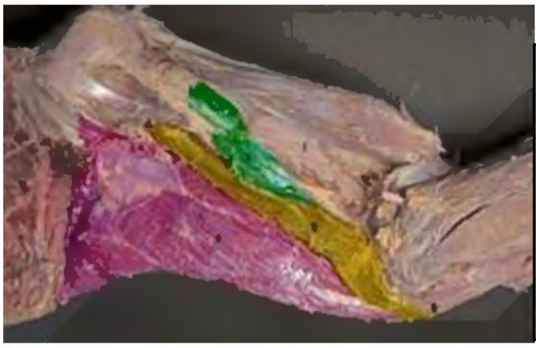
Fig 5: Caudal aspect of arm region

a – M. Supraspinatus
b – Infraspinous fossa
c – Scapular spine
d – Inferior scapular spine
e – M. Tricpes longus anterius
f – M. Triceps lateralis
g – Humerus
h – olecranon process
i – M. Brachialis

Fig 6: Caudo-lateral aspect of shoulder and arm region

a – M. Triceps lateralis
b – M. Triceps longus anterius

Fig 7: Origin and insertion of M. Triceps brachi



a – M. Triceps longus anterius
b – M. Tricpes medialis longus
c – M. Triceps medialis brevis
d – Origin of M. Triceps longus
e – Origin of M. Medialis longus
f – Origin of M. Medialis brevis
g – Olecranon process
h – Scapula

Fig 8: Origin and insertion of M. Triceps brachi

a – M. Anconeus
b – Olecranon process
c – Humerus
d – Lateral condyle
e – Medial condyle

Fig 9: Origin and insertion of M. Anconeus

Conclusion

The present study was conducted with the objective of generating more data on morphology of arm muscles in sloth bear. The sloth bear's front legs are longer than its hind legs. They are capable of galloping faster than running humans. They are capable of climbing on smooth surfaces. They are good swimmers, and primarily enter water to play. Because of their locomotory behaviour the muscles have some similarities with primates and with brachitors. M. brachialis lodged in the musculospiral groove and coursed in a spiral like fashion. M. triceps was having five heads. Heavy mass and long fascicle length of fibres produced more force which moving. So, the animal exhibit more walking habit compared with that of other primates.

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Art – 222. PREVALENCE, MOLECULAR DIAGNOSIS AND MANAGEMENT OF *KLEBSIELLA* SPECIES IN CAPTIVE SLOTH BEARS (*Melursus ursinus*)

Muthusami Palanivelrajan, **Attur Shanmugam Arun***, Madurai Ganesan Jayathangaraj, Kumanan Vijayarani, Bhaskaran Ravi Latha and Purushothaman Sridevi

Abstract

Genus *Klebsiella* from faeces of sloth bears was screened by using culture morphology, Gram's staining, biochemical tests and polymerase chain reaction. Our results showed that out of 60 samples collected, 22 samples (36.67%) were cultured on *Klebsiella* Selective Agar Base with *Klebsiella* Selective Supplement and Gram's stain revealed rod-shaped Gram-negative organism with purple-magenta colony-like colonies. The biochemical tests of cultured samples revealed negative to indole production and methyl red test, positive to Voges-Proskauer test, positive to Simmon citrate utilization test, negative to H_2S production and that produced acid over acid reaction in TSI agar and positive to urea production in cultured samples. All *Klebsiella* species isolates were sensitive to azithromycin followed by enrofloxacin and resistant to clindamycin and methicillin.The *gyr*A gene was amplified by PCR for the genus *Klebsiella* and found to be positive of 36.67%. This study may provide information for developing strategies in the future in the control of *Klebsiella* species infections in sloth bears.

Keywords: Antibiogram, *Klebsiella* spp., *gyr*A gene, Sloth Bear, Ursidae

Genus *Klebsiella* is Gram-negative, non-motile, usually encapsulated rod-shaped bacteria, belonging to the family Enterobacteriaceae and the family members are generally facultative anaerobic. The Genus *Klebsiella* includes 6 species with 3 subspecies and it consists of 77 capsular antigens (K antigens), leading to different serogroups (Janda *et al*., 2006). Genus *Klebsiella* are increasingly important opportunistic pathogens associated with severe hospital-acquired infections (nosocomial bacterial infections) such as septicaemia, pneumonia and urinary tract infections (Brisse and Verhoef, 2001). Enterobacteriaare considered part of the intestinal tract of mammals and some bird species, and they are capable of spreading in the environment and becoming ubiquitous under the appropriate conditions (Gerlach, 1994). Information on *Klebsiella* bacteria of sloth bear (*Melursus ursinus*) is generally lacking and no information could be accessed on *Klebsiella* specifically from the Indian subcontinent. The aim of this study was to investigate *Klebsiella* spp. infection in sloth bears.

Materials and Methods

During this study in 2017, the faecal samples (n=60) of captive sloth bears (*Melursus ursinus*) from 15 juveniles, 15 sub-adults, 15 adults and 15 geriatric animal groups and these animals are housed in different enclosures (n=5) at Bannerghatta Bear Rescue Centre (BBRC), Wildlife SOS, Bangalore, Karnataka were collected in nutrient broth using sterile swabs and kept at 4 ^{o}C until further processing. The swabs were incubated at37^{o}C for 24 hours under aerobic conditions. A loop full of cultured broth was streaked aseptically onto Hichrome™ *Klebsiella* Selective Agar Base with *Klebsiella* Selective Supplement under laminar floor hood and incubated at 37°C for 24hrs under aerobic conditions. The isolated colony was stained with Gram's stain and biochemical tests (HiIMViC™) Biochemical Test Kit, TSI agar and urea agar) and was confirmed by Polymerase Chain Reaction (PCR).

Antimicrobial resistance pattern of *Klebsiella* species isolates were studied by Modified Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2006) for the following antibiotics: amoxicillin/clavulanic acid (30mcg), azithromycin (15mcg), cefotaxime (30 mcg), clindamycin (2 mcg), gentamicin (10 mcg), enrofloxacin (10 mcg), methicillin (5 mcg), streptomycin (300 mcg) and tetracyline (30 mcg).

For the PCR reaction, the DNA was extracted from the isolates by using boiling method (Medici *et al*., 2003). One ml of the pre-enriched culture was transferred to a 1.5ml micro-centrifuge tube. The cell suspension was centrifuged for 10 min at 10,000rpm. The supernatant was discarded carefully. The pellet was resuspended in 300 µlof DNase-RNase- free water by vortexing. The tube was centrifuged at 10,000 rpm for 5 min, and the supernatant was discarded carefully. The pellet was resuspended in 200 µl of DNase-RNase-free water by vortexing. The microcentrifuge tube was incubated for 15 min at 100°C and immediately chilled on ice. The tube was centrifuged for 5 min at 10,000 rpm at 4°C. The supernatant was transferred to a new microcentrifuge tube and incubated again for 10 min at 100°C and chilled immediately on ice. Further, it was stored at -20°C. An aliquot of 5 µl of the supernatant was used as template in the PCR.

The molecular weight of the *gyr*A gene corresponding to the *Klebsiella* species was 441bp (Brisse and Verhoef, 2001). The following primers were used to amplify the *gyr*A gene (Forward Primer: *gyr*A-A 5'- CG CGT ACT AT ACG CC ATGAACGT A –3' and Reverse Primer: *gyr*A-C 5'-ACCGTTGATC ACTTCGGTCAGG–3'). The PCR was performed in a 25 µl reaction mixture consisting of 12.5 µl of 2X PCR master mix, 1 µl of each primer, 2 µl of extracted DNA and finally volume was adjusted with nuclease free water. Amplification was carried out in thermocycler with initial denaturation 94°C for 4 min followed by 30 cycles each of denaturation at 94°C for 45 seconds, annealing at 56°Cfor 45 seconds, extension at 72°C for 50 seconds with a final extension period of 10 minutes at 72°C. 10 µl of each PCR products was electrophoresed on 1.5% agrose gel containing ethidium bromide in the presence of 100bp ladder. The presence specific amplicon of 441 bp was viewed under UV transilluminator.

The PCR amplifies the *gyr*A gene of the one of the isolate was sequenced using commercial sequencing service.

Results and Discussion

Out of 60 samples collected, 22 samples (36.67%) were cultured on Hichrome™ *Klebsiella* Selective Agar Base with *Klebsiella* Selective Supplement and purple- magenta colony - like colonies (Fig. 1) were picked for Gram's staining and revealed Gram-negative rod-shaped organism.

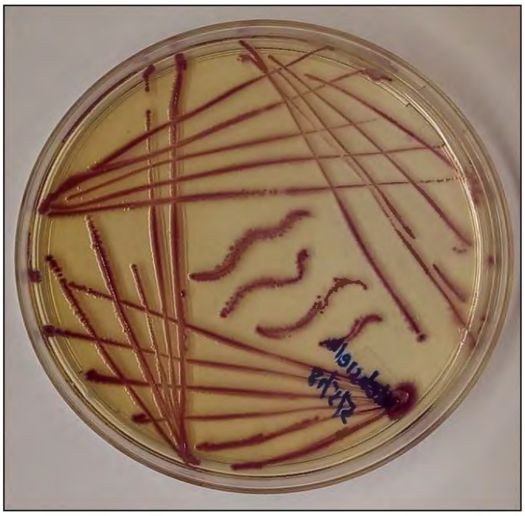

Fig. 1: Klebsiella species on Hichrome™ Klebsiella Selective Agar Base with Klebsiella Selective

Usage of biochemical tests in HiIMViC™ Biochemical Test Kit, TSI agar and urea agar revealed that were negative to indole production and methyl red test, positive to Voges-Proskauer test, positive to Simmon citrate utilization test, negative to H_2S production and that produced acid over acid reaction in TSI agar and positive to urea production in cultured samples. All *Klebsiella* species isolates which were studied have been found sensitive to azithromycin followed by enrofloxacin. They were found resistant to clindamycin and methicillin.

Supplement

Further, based on the PCR assay (Fig. 2) among the enclosures, a higher prevalence of *Klebsiella* species infection was noticed in Chitrakuta Block (50.00%) followed by Jambhava Block (42.86%) and Dr GKV Block(38.46%) (Table 1). Statistically, there was no significant difference among enclosures (P>0.05). Similarly, the highest infection (40.00%) was found in both sub-adult group and adult group followed by 33.33% of infection in juvenile group and geriatric group. There was no significant difference among animal groups (P>0.05) (Table 2). In male sloth bears, 40.74% of *Klebsiella* species infection was found during this study. The prevalence of *Klebsiella* species in male and female did not differ significantly (Table 3).

Fig. 2: Amplification of gyrA gene of Klebsiella species by PCR in 1.5% Agarsoe; Lane L: DNA ladder (500bp), Lane PC: Positive control, Lane NC: Negative control, Lane 1-5: gyrA gene amplification (441bp)

Table 1: Prevalence of *Klebsiella* species among enclosures

Enclosure	n	*Klebsiella* species
Panchavati Block	12	2 (16.67%)
Chitrakuta Block	10	5 (50.00%)
Kishkinda Block	11	4 (36.36%)
Dr. GKV Block	13	5 (38.46%)
Jambhava Block	14	6 (42.86%)
Overall	60	22 (36.67%)
$\chi 2$		3.08^{NS}

n – Number of samples collected; NS - Not Significant (P>0.05).

Table 2: Prevalence of *Klebsiella* species among animal groups

Animal Groups	n	*Klebsiella* species
Juvenile	15	5 (33.33%)
Sub-adult	15	6 (40.00%)
Adult	15	6 (40.00%)
Geriatric	15	5 (33.33%)
Overall	60	22 (36.67%)
$\chi 2$		0.29^{NS}

n – Number of samples collected; NS - Not Significant (P>0.05).

Table 3: Prevalence of *Klebsiella* species among animal genders

Animal genders	N	*Klebsiella* species
Male	27	11 (40.74%)
Female	33	11 (33.33%)
Overall	60	22 (36.67%)
$\chi 2$		0.35^{NS}

n – Number of samples collected; NS - Not Significant (P>0.05).

The *gyr*A gene was chosen for amplification since presence of this gene indicates the *Klebsiella* species. All the isolates from the sloth bears were positive for the presence of *gyr*A gene. The sequence results were compared with that of *Klebsiella* species isolate sequences using nBLAST (Nucleotide local alignment service tool) available at https://blast.ncbi.nlm.nih.gov/Blast. cgi?PAGE_TYPE=BlastSearch. The sequence data is provided as supplement.

The *Klebsiella* organisms revealed during the culture related studies carried out with the fecal samples obtained from captive sloth bears reared at BBRC had purple-magenta colonies and were positive for catalase test and citrate test, in addition to Voges-Proskauer test. This biochemical identification made during the study was in accordance to the reports furnished by Davies *et al*. (2016) who opined that *Klebsiella pneumonia* was consider as one of the most important gram-negative opportunistic pathogens and isolates were revealed to have the ability to catabolise citrate promote hydrolysis of urea and decarboxylation of lysine and were also associated with negative indole production. Encountering the rod shape organisms of *Klebsiella* sp. in this study was alsoinaccordance with the reports presented by Abdel-Aziz *et al*. (2017) who opined about this rod-shaped bacterium and were the gram-negative and facultative anaerobic organisms. The colonies identified as *Klebsiella* sp. using standard biochemical procedures were also subjected to molecular confirmation by polymerase chain reaction, in this study.

The present study revealed increased sensitivity of the *Klebsiella* sp. organisms to azithromycin followed by enrofloxacin. The findings of comparatively a high sensitivity towards enrofloxacin by *Klebsiella* sp. organisms in this study was in agreement with the reports furnished by Du *et al*. (2014) who additionally detailed that in case of Chinese hares, the antibiotic agents like ampicillin, penicillin, polymyxin B, imipenem, meropenem and vancomycin were resistant. However, the zoonotic bacteria including the *K. pneumonia* were quoted to be resistant to ceftiofur in a most prevalent manner, followed by ampicillin and ceftriaxone. Similar to the finding in this study related to enrofloxacin, sensitivity of the *Klebsiella* sp. organisms were reported by Seliskar *et al*. (2007) towards enrofloxacin, in addition to ciprofloxacin but at the same time, it was revealed that these organisms were resistant to azithromycin in dogs; however, in the current study with bears, these were found to be more sensitive to azithromycin. The variations in sensitivity might be due to different species, environment, and virulence as well as nature of microbes present in the species.

The overall positivity of *Klebsiella* sp. organisms was 36.67 percent among the captive sloth bears in the study encountering of genus *Klebsiella* during the study with captive sloth bears was an agreement with the findings reported by Du *et al*. (2014) who however revealed drug-resistance *Klebsiella pneumonia* in Chinese hares (*Lepus* *sinensis*) the findings of genus *Klebsiella* among the captive sloth bears in the study might be due to the opportunistic nature of these pathogens.

It becomes significant to mention the report of Brissee and Verhoef (2001) who specifically quoted that bacteria of the genus *Klebsiella* are increasingly important opportunistic pathogens associated with severe hospital-acquired infections such as septicaemia, pneumonia and urinary tract infections. Bacteria belonging to the genus *Klebsiella* had been reported in multiple species of wild fauna like red deer (Dias *et al*., 2018), hares (Du *et al*., 2014), wild birds (Matias *et al*., 2016) and other wild animals (Eze *et al*., 2018).

Similar to the case with detection of other bacterial organisms in this study, clinically affected captive sloth bears with bacteria of genus *Klebsiella* were however not encountered during this study at Bannerghatta Bear Rescue Centre, Bangalore. In this regard, the incidences of *Klebsiella pneumonia* were documented by different authors in different species. Species specific study with regard to genus *Klebsiella* might be more useful in revealing heterogeneity as well as the complex epidemiology of the *Klebsiella* sp. and however, studies on the phylogenetic relationships between *Klebsiella* sp. and subspecies among the captive sloth bears are highly lacking, in this regard.

Interestingly, the presence of genus *Klebsiella* was encountered among the captive sloth bears, regardless of the type of enclosures, sex of the animal and age groupof the animal. However, the variation with regard to the presence of *Klebsiella* sp. revealed in this study failed to reveal any significance among age group or sex or enclosures.

Acknowledgements

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Supplement

Partial nucleotide sequence of *gyr*A gene from *Klebsiella* species isolated from sloth bear (*Melursus ursinus*)

>Consensus A A A T C A G C C C G T G T C G T T G G T G A C G T A A T C G G T A A A T A C C A C
C C K C A T G G T G A C T C T G C G G T A T A C G A C A C C A T T G T C C G T A T G G C T C A G
C C G T T C T C G C T G CG AT A Y A T G C T G G T G G A T G G Y C A G G G K A A C T T C G G TT
C C A T C G A T G G C G A Y T C C G C G G C G G C G A T G C G T T AT A C G G A A A T C C G TC
T G G C G A A R A T T G C C C A T G A G Y T G A T G G C C G A T C T G G A A A A A G A G ACGG
T T G A T T T Y G T C G A C A A C T A T G A C G G C A C G G A A A A A A T Y C CW G A C G T YA
T G C C R A C C AAA A T Y C C T A A C C T G Y T G G T A G A A C G G T T C G T T C C G G T AT
C G C M G T A G G K A T G G C

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

Art – 223. SLOTH BEAR ATTACKS ON THE DECCAN PLATEAU OF KARNATAKA, INDIA

Robert Sharp, Shanmugavelu Swaminathan, Attur Shanmugam Arun, Tom Smith**, Kartick S,** and **Geeta Seshamani**

Abstract

The sloth bear (*Melursus ursinus*) is endemic to India, Nepal, Bhutan, and Sri Lanka, and is among the least studied of all bear species. Sloth bears behave aggressively toward humans when they feel threatened and are considered among the most dangerous wild animals in India. Our research objective was to interview those that had close encounters with sloth bears and attack survivors so that we could learn from these unfortunate events and prepare persons to be safe in sloth bear country. Consequently, we interviewed 342 people—162 that had close encounters that did not result in attacks and 180 that were involved in attacks—in the southern Indian state of Karnataka between the years 1985 and 2016. Our surveys revealed that all attacks were defensive in nature, that people that had been making noise while moving through sloth bear country were less likely to be attacked, and that persons in groups of ≥2 were very rarely attacked. Nine percent of people that fought back during an attack were killed and 11% of people that attempted to run from the bear were killed. There were no deaths among people that fell to the ground and did not fight back. Drawing from this work and that of others, we provide suggestions for safe conduct in sloth bear country.

Keywords: bear attack, bear behavior, Deccan Plateau, human–bear conflict, India, Karnataka, *Melursus ursinus,* sloth bears

India is among the most biodiverse countries in the world and is recognized internationally as a global treasure (Myers et al. 2000). With only 2.4% of the earth's land area, India accounts for approximately 8% of the world's animal species. India is also home to 1.3 billion people (United Nations 2017), and this burgeoning population places tremendous pressure on the nation's natural capital. As a result, India's rich wildlife heritage suffers from habitat loss, fragmentation, air and water pollution, and human–wildlife conflicts.

The sloth bear (*Melursus ursinus*) is endemic to India, Nepal, Bhutan, and Sri Lanka (Garshelis et al. 1999). Of the 4 species of bears in India, the sloth bear is the most widespread; but details of its occupied range are still being refined and there are only a few scientifically sound population studies; therefore, the worldwide population estimate of this animal is still rough and ranges anywhere from 10,000 to 25,000 individuals. However, the International Union for Conservation of Nature lists the sloth bear as vulnerable (Dharaiya et al. 2016) based on the fact that most of the species' habitat is not protected and is being degraded, and the human population in India is growing steadily. Sloth bear populations are currently threatened by poaching; capture of live bears; killing of nuisance bears; loss of habitat by destruction, fragmentation, and degradation; and conflict with humans (Garshelis et al. 1999).

Maintaining a low level of human–carnivore conflict is a critical component if carnivore conservation is to be effective (Loe and Roskaft 2004). Unfortunately, sloth bears behave aggressively toward humans and are considered one of the most dangerous wild animals in India (Sterndale 1884, Pillarisett 1993). Consequently, human– sloth bear conflict leads to retaliatory killings, removal of nuisance bears, and undermines efforts to conserve them. It is not known how many people are seriously injured or killedby sloth bears in India annually. However, a number of research publications on the subject depict adesperate situation for both people and bears through-out their range (Rajpurohit and Krausman 2000, Bargaliet al. 2005, Debata et al. 2012, Mardaraj 2014, Patel and Dharaiya 2014, Ratnayeke et al. 2014, Garcia et al. 2016, Dhamorikar et al. 2017,Lamichhane et al. 2018). For example, in the state of Madhya Pradesh, 48 sloth bear– inflicted fatalities and 687 maulings were reported be- tween 1989 and 1994 (Rajpurohitand Krausman 2000), for an average of 8 deaths and 115 maulings/year. By comparison,only 63 bear-inflicted fatalities caused by American black bears (*Ursus americanus*) occurred in North America over a 109-year span (1900–2009; Herrero et al. 2011). Additionally, Herrero (1985) estimated that in a 100-year span in the United States and Canada, approximately 100 persons were killed by brown bears (*U. arctos*).

Chronicling human–sloth bear conflict is the first step to resolving conflict. Once we understand the nature of these events, we can determine the best ways to avoid attacks, as well as how to behave during an attack. This paper reports on human–sloth bear conflict in the Deccan Plateau in the southern state of Karnataka, India, in an effort to understand why these events occur and how to avoid them.

Study area

Our study of human–sloth bear conflict took place in the southern Indian state of Karnataka (Fig. 1). Karnataka has 3 predominant geographic zones: a coastal region bordered by the Arabian Sea to the west, a hilly region consisting of the Western Ghats, and the plains and rocky scrub jungle of the Deccan Plateau. Sloth bear habitats in Karnataka are considered some of the highest-quality habitats for the species (Puri et al. 2015). Our study was conducted wholly in the Deccan Plateau of Karnataka. The study area has a semi-arid climate characterized by hot summers (24.2°–45°C) during April–June and low rainfall (571.92–802.00 mm) from June to November.

Methods

We interviewed persons in Karnataka that had (1) been attacked by sloth bears, (2) witnessed an attack (i.e., sometimes the victim did not survive), or (3) had an encounter with a sloth bear in the wild that did not result in an attack. The survey form we used for interviews is included in Appendix 1 (Supplemental Material).

For clarity, we defined a 'bear attack' as a human–bear interaction during which the bear came in physical contact with the person (Smith et al. 2005). A 'human–bear incident' (or simply 'bear incident') did not result in the bear making physical contact with the person, yet the person was at significant risk of injury. We term 'human– bear conflicts' as the sum of bear attacks and bear incidents. We collected data on bear incidents for analysis comparison with bear attacks because we believed that by studying noncontact incidents we could learn important aspects of human–sloth bear conflict. Hence, in this paper, we report on both bear attacks (injury events) and bear incidents (non-injury events).

A database, containing information provided by attack victims, contained up to 59 variables for each conflict, including date and time of conflict, location, number of persons, number of bears involved in the attack, human injury, etc. (Appendix 1). We restricted analyses and conclusions to those aspects of data we felt confident were accurate and unbiased.

We assigned human activity to categories describing what the person(s) was doing at the time of the bear encounter. Categories included unknown (unreported), walking, farming, cattle or goat grazing, gathering forest materials, defecating, in the vicinity of their home, or other. Consistent with Smith and Herrero (2018), we ascribed probable cause of the sloth bear encounter using the following categories:

1. Surprise encounter—the human–bear encounter was abrupt and the human and bear were surprised. Such encounters generally occurred at close range (<50 m). A person collecting leaves in the forest and suddenly encountering a mother and cubs wouldbe an example of a surprise encounter.
2. Predation—was identified by a series of behaviors: searching, following and testing, attacking (capturing), killing, sometimes dragging a per- son, sometimes burying, and often feeding upona person. Vocalizing and stress behaviors by thebear were usually absent (Herrero and Higgins 2003).

We qualitatively ranked visibility of the land cover in which conflicts occurred. Those rated 'poor' were either heavily forested, dense shrub lands, or rough terrain with short sight distances. We also rated low light levels (as in dawn, dusk, and nighttime) as poor. Land cover rated 'fair' had shrub and tree cover, but also open areas providing increased visibility, as compared with those rated 'poor.' Land cover rated 'good' was typically very open, such as agricultural fields (those with low-profile crops), meadows, and poorly vegetated barren areas.

We used the chi-square goodness-of-fit test to determine whether observed and expected data were independent of each other, such as whether party group size and bear encounter group size were different (Dytham 2003).

Fig. 1. Location of sloth bear attack surveys, Deccan Plateau (cross-hatched) in the Indian State of Karnataka, India (in gray), 1985–2016.

Table 1. Sloth bear–inflicted injuries caused by known bear group size in Karnataka, India, 1985– 2016.

Injury level	Females with cubs	Single bears	2 similar-size bears	Row total
Unknown	6	5	0	11
Injury unknown	1	1	0	2
No injury	1	0	0	1
Slight injury	23	40	0	63
Moderate injury	31	36	0	67
Severe injury	14	7	2	23
Fatality	7	5	1	13
Grand total	83	94	3	180

Results

Our review of the Web of Science and Zoological Abstracts databases yielded 20 research papers that ad- dressed human–sloth bear conflict. We downloaded and reviewed each paper and have included them as references in this report.

We conducted interviews between June 2014 and March 2016 in the Karnataka districts (no. of interviews conducted are in parentheses) of Ramnagara (*n*=49), Arasikere (*n*=67), Tumakuru (*n*=104), and Ballari (*n*=122). Interviews yielded information regarding 342 conflicts comprising 15,268 data entries. These incidents occurred over a 33-year period from 1985 to 2016. Of the 342 incidents reported, 53% (*n*=180) consisted of physical contact and injury, whereas the remaining 47% (*n*=162) did not result in contact. These conflicts involved 149 female sloth bears with dependent young, 190 single sloth bears of unknown gender, and 3 pairs of adult sloth bears.

Injuries

One hundred sixty-eight people reported being injured in 180 sloth bear attacks, including 13 fatalities, 23 severe injuries, 67 moderate injuries, 63 slight injuries, and2 persons injured without the specific degree reported (Table 1). In 1 case where the bear made physical contact with the person, the person was not injured; and in 11 cases it was unknown whether the person was injured or the extent of the injury. Of those that reported, <1% suffered no injuries, 37% slight injury, 40% moderate injury, 14% severe injury, and 9% fatality. Of the reported 168 sloth bear–inflicted injuries, females with dependent young accounted for 45% (*n* = 76), single bears 53% (*n* = 89), and a pair of similar-sized bears 2% (*n* = 3).

Temporal nature of sloth bear conflicts

Bear attacks (*n*=159) and bear incidents (*n*=157) occurred during every month of the year on the Deccan Plateau (Fig. 2). Most attacks (*n*=76, 48%) occurred during the winter months (Nov–Feb), followed by summer (Mar–Jun; *n*=49; 31%), and monsoon (Jul–Oct;*n*=34, 21%). Likewise, most incidents (*n*=77, 49%) occurred during the winter months (Nov–Feb), followed by summer (Mar–Jun; *n*=63, 40%), and the monsoon (Jul–Oct; *n*=17, 11%).

Time of day was reported for 342 human–sloth bear conflicts (Fig. 3), including 180 bear attacks and 162 bear encounters. The most frequent time of day humans were attacked by sloth bears was during dark hours (morning and night when there was no light), accounting for 46% (n= 82) of all attacks. The dark hours, both before and after midnight, also accounted for the most bear incidents at 59% (n = 95). Dusk accounted for the second-most attacks (n = 35; 19%) and the second-most incidents (n =26, 16%). Dawn accounted for the third-most attacks (n =23, 13%) and the third-most incidents (n = 16, 10%). The remaining 22% of attacks (n = 40) and 15% of incidents (n = 25) occurred during daylight hours.

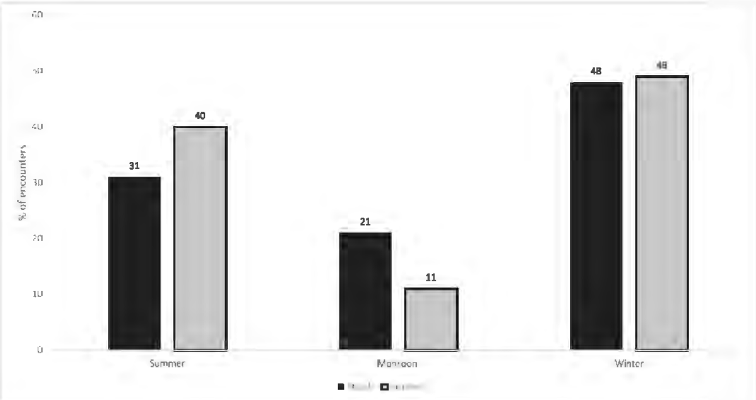

Fig. 2. The occurrence of all sloth bear–human conflicts by season, Karnataka, India, 1985–2016

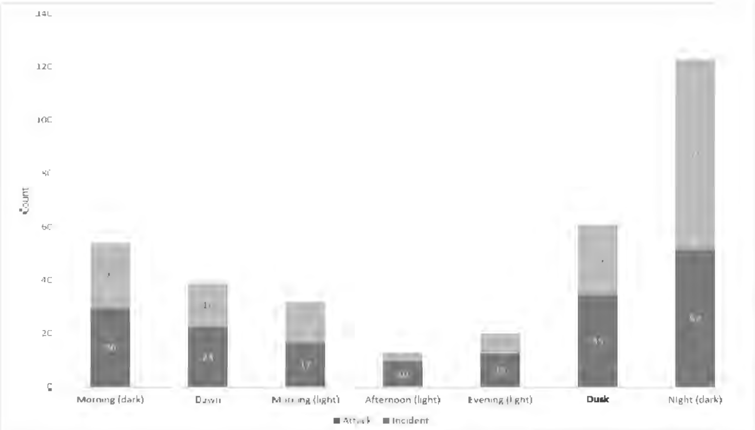

Fig. 3. The occurrence of all sloth bear–human conflicts by time of day, Karnataka, India, 1985–2016.

Profile of bears involved

The slight majority of attacks involved single bears (52%, *n*=93), although females with dependent young made up 47% (*n*=84); and roughly 2% (*n*=3) were pairs of bears, which could have been mother bears with large young or a different grouping. Incidents with sloth bears that did not result in an attack were primarily single bears 60% (*n*=96) and females with young 40%(*n*=65).

In 340 conflicts (179 attacks and 161 incidents), persons reported the bear's activity when a person first became aware of the bears (Fig. 4). In a majority of attack instances, bears were either moving away from the direction from which people had been approaching (32%, *n*=58) or foraging (30%, *n*=54). Roughly 12% (*n*=22)of the bears were already charging at the person when they first became aware of the bear. A small number of bears were walking in the direction of the people (8%,*n*=15), apparently unaware that people were present. In incidents that did not did not result in attacks, the bears were most often moving away from the direction from which people were approaching (49%, *n*=79) or bears were foraging (25%, *n*=41). Roughly 7% (*n*=12)of the bears were already charging at the person when the person first became aware of the bear, but the bear then ran off without making physical contact. A small number of bears were walking in the general direction of the people (8%, *n*=13), unaware that people were present in the area. Bears involved in conflicts with people (*n*=324) had the following outcomes: bears suffering no injury (*n*=321, 99%) and bears wounded to some degree (*n*=3, 1%).

In conflicts that resulted in an attack, the sloth bears' most common response when confronted by a person(*n*=176) was to charge the person (89%, *n*=156). The second-most common response was to make loud vocalizations before charging (5%, *n*=9). Sloth bears were also documented walking away first (4%, *n*=7), and ignoring the person (2%, *n*=4). In conflicts that did not result in an attack (*n*=162), the sloth bears' most common response was to charge (47%, *n*=76). In these instances, no contact was made, and bears left the area immediately. The second-most common response was to immediately run from the site of confrontation (36%, *n*=59). Other initial reactions by bears to sudden human encounters included standing on their hind legs (4%, *n*=6), holding their ground and making noise (5%, *n*=8), and walking away slowly (2%, *n*=3).

When sloth bears charged persons, 94% charged once, and the remainder twice (6%). Sixty-one percent of the time sloth bears charged victim, they vocalized. In 97% of all cases, sloth bears did not return after the initial encounter. The remaining 3% of victims said that the bear did return, but never more than twice, to the site of the encounter.

Profile of humans involved

The majority of persons involved in bear conflicts(n=371), both in attacks and incidents, were adult men (94%). Data for 180 groups that were attacked show that158 were alone (88%) and the remainder were with one other person (n= 22, 12%). Data for 162 groups that were not attacked show that 155 were alone (96%) and 7 (4%) were with another person. If bear encounters were independent of group size, we would expect human– sloth bear conflicts to reflect the proportion of group sizes reported. However, observed distribution varied from these expectations, with single persons involved more than expected and groups ≥2 involved less than expected (χ^2=786.46, 1=df, P< 0.001). The activity (n= 342) persons were most often engaged in when encountering bears was farming (n=156, 46%), followed by grazing cattle or goats (n=69, 20%; Fig. 5).

Just prior to encountering a sloth bear, 68% of persons stated that they were moving quietly, not making any noise to alert a sloth bear of their approach. The remainder (32%) said that they were making noise. At the moment of encounter, the most common response was to run from the bear (39%). The next most common responses reported were to (1) climb a tree (22%), (2) lie prone on the ground (14%), (3) stand their ground (9%), (4) attempt to defend themselves with a stick or tool (5%), (5) attempt to climb a tree by running to it (2%), and finally, some few (6) fainted or passed out when suddenly confronted with a bear (1%). The resulting injuries from running from a sloth bear were highly significant with 2% uninjured, 36% suffering slight injuries, 42% moderate injuries, 9% severe injuries, and 11% fatalities. Although all injury categories were significantly different from expected categories, persons suffering no injuries, severe injuries, and fatalities occurred less than expected, whereas slight and moderate injuries occurred more than expected (χ^2= 41.00, 4 df, P < 0.001).

Victims of attacks reported fighting back 24% of the time. When victims that chose to fight back ceased fighting (69%; 27 of 39), they reported that the bear stopped attacking. Fighting an attacking sloth bear resulted in 9% fatalities (n=6), whereas running from an attacking sloth bear resulted in 11% (n=7) fatalities. Falling to the ground and not fighting back when confronted by an attacking sloth bear resulted in no fatalities.

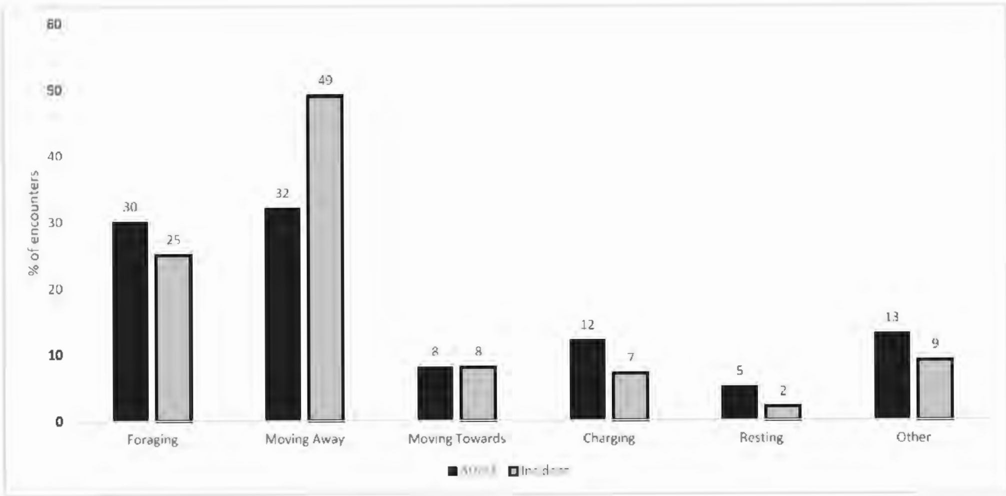

Fig. 4. Sloth bear activity at the time of human–bear conflict, as reported by victims, Karnataka, India, 1985–2016.

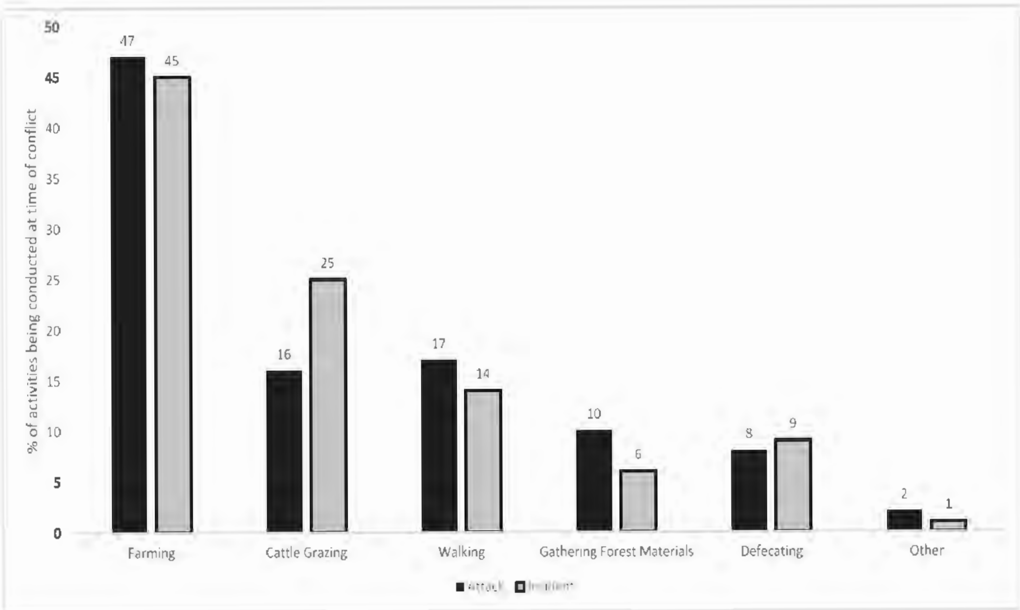

Fig. 5. The activity of persons involved in human–sloth bear encounters, Karnataka, India, 1985–2016.

Profile of encounters

When attack victims first became aware of the sloth bear, 70% were ≤3 m and 88% were ≤5 m from the bear. In incidents that did not result in an attack, 50% were ≤3 m and 73% were ≤5 m from the bear. Only 3 attacks occurred when a person was ?10 m from a bear. The farthest a bear was from the person before it attacked was 12 m. When contact was made, most victims (38%) were running, standing (34%), laying on the ground (15%), or climbing a tree (2%). For attacks for which an estimate of duration was provided (*n* 52), 94% of respondents reported that their encounter was very brief in duration (<3 min), whereas the remaining 6% said their encounter lasted <10 minutes.

Most encounters occurred in agricultural areas (227 of 339, 67%), followed by forested areas (76 of 339, 22%) on roadways (30 of 339, 9%), in riparian areas (4 of 339, 1%), and in scrub vegetation (2 of 339, <1%). Attack locations took place in agricultural areas (131 or 179, 73%), forested areas (29 of 179, 16%), and roadways (16 of 179,9%). Visibility of habitats where sloth bears were encountered was poor (76% of respondents). An additional 20%were confronted by sloth bears in medium-visibility areas, and only 5% were in open areas.

Discussion

There have been a number of research efforts to document human–sloth bear conflict in India and Sri Lanka (Rajpurohit and Krausman 2000, Bargali et al. 2005, De-bata et al. 2012, Mardaraj 2014, Patel and Dharaiya 2014, Ratnayeke et al. 2014, Garcia et al. 2016, Dhamorikaret al. 2017, Lamichhane et al. 2018). Those studies, along with this one, shed light on the nature of human–sloth bear interactions and provide guidance for minimizing sloth bear conflicts. For example, the vast majority of bear victims in Karnataka were alone. A small number were in groups of 2 and no persons in a group of >2 were attacked or even had a close encounter. This observation is generally consistent with findings by Ratnayeke et al.(2014) and Garcia et al. (2016), who both reported that the larger the group, the less chance there was of an attack. Garcia et al. (2016) speculated that larger groups likely deter bears because they are noisier and more intimidating. Traveling and working in groups of ≥2 persons is the simplest thing that can be done to provide greater safety in sloth bear habitat. However, this strategy only works if people remain together rather than dispersed. If people can hold a conversation while hiking, they are likely close enough to have the benefits of being in a group. We surmise that when walking singly, people make less noise and that has led to surprise encounters. Our data also clearly show that if a person bolts and runs when suddenly confronted by a sloth bear, the bear will chase and take them down (41% of incidents).

One attack was considered predatory by locals because the bear ate a portion of the victim. However, we believe that sloth bear attacks are wholly defensive in nature; they appear to attack humans only because humans are perceived as a threat, not because they are considered food. Consumption of human flesh may simply be opportunistic, not a behavior that reflects the initial motivation for the attack. Sloth bears do not hunt large mammals; it is not part of their foraging ecology (Garshelis et al. 1999). However, they have been documented scavenging on large mammals (Laurie and Seidensticker 1977). Additionally, it must be noted that other bears, namely the brown bear, have been documented eating people that they had killed in a defensive, rather than predatorial, attack (Herrero 1985).

Although a few reports of predatory sloth bears exist, we suspect that this notion has arisen not only because sloth bears have on occasion eaten human flesh, but because a non-predatory attack could easily appear predatory from the victim's perspective, particularly if there seemed to be no other apparent motivation for the confrontation. Sloth bear attacks tend to focus on the victim's head and neck region, leading some to believe the attacks are predatory. However, focusing on the head and neck does not appear to be linked to predation in bears in general (Smith and Herrero 2018), but is instead indicative of bears' evolved attack strategy. Herrero (1985) speculated 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 question of whether all sloth bear attacks are defensive, or if occasionally they attack in a predatory fashion, is far more than just an intellectual curiosity about sloth bear behavior. If our conclusion is correct (that sloth bears only attack in a defensive capacity), it greatly simplifies the message of how to react if one finds oneself attacked by a sloth bear. Instead of attempting to discern whether the attack is predatorial or defensive, as one must do when faced with a brown bear attack, when attacked by a sloth bear there would be one course of action that clearly could be recommended. Thus, the message is simpler and easier to remember at the critical moment.

Numerous interdependent findings merit discussion, including the roles played by the victim's gender, group size, time of day, activity at the time of the attack, distance to the sloth bear when first encountered, whether or not the victim was making noise, and visibility of the attack area. The majority of person attacked were men (90%), alone at the time of attack (88%), who encountered bears in darkness (77%), first became aware of the bear presence at very close range (1–5 m; 88%), were moving silently and not making noise to alert bears (49%), and were in agricultural field (67%). These variables can independently or in combination influence the probability of a human–sloth bear confrontation (Rajpurohit and Krausman 2000, Bargali et al. 2005, Debata et al. 2012, Mardaraj 2014, Patel and Dharaiya 2014, Ratnayeke et al. 2014, Garcia et al. 2016, Dhamorikar et al. 2017, Lamichhane et al. 2018). When a person violates best practices in all of these areas, the odds of an interaction and attack greatly increase.

In rural India, it is primarily the role of men to water and protect crops at night from being consumed and destroyed by wild herbivores. For example, in one village in northern India, farmers estimated that as much as 40% of their crops were lost to crop-raiding wildlife (Parvaiz 2017). This dangerous situation, of sloth bears being drawn to highly nutritious, high-density crops, while farmers await with sticks expecting to drive off much less dangerous wildlife during hours of darkness, is the most common human–sloth bear encounter scenario on the Deccan Plateau. Possible solutions to crop-raiding include natural fencing consisting of dense saplings, and awareness campaigns that inform farmers about a variety of techniques for safeguarding their farms (Parvaiz 2017). In North America, persons are cautioned not to enter bear habitat without a deterrent option, such as a fire armor bear spray (Smith and Herrero 2018). People in India generally do not have access to deterrents common in the West, such as guns and bear spray. Singh et al. (2018) recommended persons working in sloth bear habitat construct a 2–3-m-long wooden stick, festooned with bells (noise-makers) and threatening dowel rods (defensive), to form a nonlethal, defensive tool for warding off attacking sloth bears. Although this low-cost solution may provide a deterrent option for some situations, it appears cumbersome and is likely difficult to deploy rapidly, a drawback shared by firearms (Smith et al. 2012). Adding this to the fact that the majority of persons involved in sloth bear encounters in Karnataka were first made aware of the bear's presence at 1–3 m distance (this study), their being armed only with a stick would have resulted in serious limitations. Nonetheless, sticks have been useful for defending oneself or one's acquaintances during sloth bear attacks (Bargali et al. 2005, Dhamorikar et al. 2017), and represent a first step toward a defensive approach to sloth bear safety.

If the goal is simply to survive an encounter, falling to ground and not fightingback result in a 100% survival rate. However, many of those that fell to the ground anddid not fight back were still seriously or moderately injured. In fact, people that fell to the ground suffered a higher rate of moderate injuries (65%) than did those that fought back (30%), or those that ran away (42%). The reasons for this are difficult to a certain;

however, it appears that those that played dead rarely did so using a protective position: (1) face down, and (2) protecting the head and neck. It is likely that if the victims used a protective position when falling to the ground, they would have suffered fewer injuries. 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 injuries would lessen.

Many of those that fought back suffered only minor injuries (46%); however, 12% were severely injured and 9% died. Likely, these reported differences in injury have to do with unreported actions on the victim's part or the motivation of the bear to press the attack. When a bear's jaws encompass the head, injuries are most often fatal; whereas when its canines slip off the curvature of the skull, severe lacerations occur, including avulsions of the ear(s) and nose, but the brain remains protected. With this in mind, one can see how under the similar attack scenarios, one individual may die while another might survive. Finally, those that attempted to run from the attacking bear fared poorly, 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).

Many victims of sloth bear attacks are only slightly injured. This is likely related to the fact that most attacks occur when the human and bear are very close to one another and the bear reflexively reacts to the threat (the person) and instinctively attacks. However, the bear's objective is to escape the situation unharmed, not to unnecessarily engage in a fight. So, once the bear determines "this is not a tiger, or a serious threat" the bear stops, evaluates, and leaves the area in a hurry. However, if the victim actively engages in fighting the bear, then the bear may be more prone to view the victim as a true threat and thus press the attack further, potentially inflicting serious injuries or even killing the victim. However, even in cases where people fought back, the bear likely will take any apparent opportunity to run away. This is likely the reason that many people that did fight back suffered only slight injuries.

It is important to not lose sight of the fact that avoiding sloth bear encounters is the most important action people can take while in bear habitat. Were people to simply practice bear avoidance techniques (e.g., hiking in groups, making noise appropriately, etc.), then knowing how to survive an attack becomes much less important to understand. Those people that violate this basic principle—avoid bears in the first place—put themselves unnecessarily in harm's way. Therefore, we highly recommend that persons working, traversing, or recreating in sloth bear habitat focus mostly on avoidance behaviors. If that is done correctly, worrying about how to defuse a bear encounter or how to survive a mauling is not nearly as important.

Here we summarize a number of insights regarding safety in sloth bear country that will be beneficial for those people that work and live in sloth bear habitat in India:

1) Sloth bears are wholly defensive, meaning that they only attack if they feel threatened. This means that by telegraphing one's presence to the surrounding terrain (i.e., making noise), bears will be alerted to an oncoming human and move clear to avoid confrontation.

2) If attacked, protection of the head and neck is critical. The defensive positions described by Herrero (1985) are recommended to protect the head and neck.

3) Sloth bear conflicts occurred during every month of the year in Karnataka. One must practice bear safety (e.g., make noise, travel in a group, be alert at all times, etc.) year-round.

4) Sloth bear conflicts occurred largely during the dark hours of the day (77%), and in agricultural areas when sloth bears were coming in to drink water or feed. Stationing single persons to water or protect crops from raiding wildlife puts people at a high risk of injury.

5) Sloth bear family groups were often involved in conflicts (49% of all encounters), but single bears were involved as much as any other cohort (51%).

Encounter group size plays an important role in avoiding bear conflicts: single persons were involved in the vast majority of bear conflicts. Hence, singles were involved significantly more than expected, whereas groups of ≥2 persons were involved significantly less than expected.

6) Sloth bear conflicts were most common in areas of poor visibility, or at times of low light resulting in poor visibility. When poor visibility areas cannot be avoided, or when at low light or no light times of day, people should group together, be alert, and make noise to avoid surprising bears.

7) Persons should carry a 2-m stick. The stick may be used to fight off a bear attacking another person. Singh et al. (2018) suggested carrying a stick modified with bells and multiple dowels extending from it (aka "Ghanti Kathi") to optimize safety in bear country.

8) Running is rarely a successful strategy for dealing with an aggressive sloth bear, unless there is a bear-safe haven (e.g., vehicle, cabin, etc.) very close by.

Playing dead, a strategy of last resort, can save one's life as long as the victim lies prone, face down, and protects their neck with hands clasped over it.

It is our hope that studies such as this will inform bear safety messaging for persons living in sloth bear country. We know that if these best practice guidelines are adopted, both people and bears will enjoy a more peaceful coexistence. India without sloth bears would be a loss for everyone, so it is our hope that this work will contribute positively to both their future and human safety.

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Art – 224. FUNCTIONAL SPECIALIZATION OF FORELIMB MUSCLES OF SLOTH BEAR

(*Melursus ursinus)*

V.R. Annie, Shruti., K.V. Jamuna., **A. Sha. Arun.,** R.V. Prasad and V. Girishkumar

Abstract

The objective of this study was to establish the functional characteristics of forelimb muscle of sloth bear. In sloth bear, scapular muscles had long fascicle followed by forearm and manus muscles. There was a significant reduction in muscle mass distribution from proximal to distal portion. In shoulder and arm region, physiological cross-sectional area (PCSA) was greater in proximal than distal muscle group, particularly in the shoulder extensors and elbow extensors. In forearm and manus region, it was greater in caudomedial aspect of muscles particularly in the pronators of forearms and exors of manus. Hence, it enabled greater grip strength.

Key words: Functional specialization, forelimb, Sloth bear

Bears are the youngest among carnivore families arisen from canine like ancestors. The scientific name for sloth bear is *Melursus ursinus*. Bears are plantigrades that help to support the great body weight of the animal. Locomotion and animal posture greatly influenced the anatomy of a muscle due to the high frequency and high loads of forces involved. They exhibit morphological variation within and between species and the differences in behaviour, as well as habitual and specific activities are due to their anatomical peculiarities (Drapeau 2008). Each movement of the body is produced by the involvement of several muscles either simultaneously or one after another. The general role of the forelimb is to provide the mobility necessary to reach the irregular supports of an arboreal substrate (Aiello and Dean (2002). The morphology of the forelimb is always compromised between the functional demands for mobility (arboreal supports) and to allow effective use of the forelimb as a manipulatory organ (Larson 1993).

Materials and Methods

Dissections were conducted on the left and right forelimbs of three captive adult sloth bears from the Wildlife SOS, Bannerghatta Bear Rescue Centre of Bannerghatta Biological Park, Bangalore Karnataka. Following necropsy, the forelimbs were stored in 10% formalin and dissections were carried out. Muscle origins and insertions were recorded.

The muscle fascicle length was measured by using flexible tape after placing the muscle in 10% sulfuric acid at 70^{o} C. Then the muscles from other limb were incubated at 40^{o} C at 48 hrs before recording weights using an electronic scale to the nearest 0.1g. Muscle volume was estimated by dividing muscle mass by muscle density (1.06g/cm^{2}), (Mendez and Keys (1960). The Physiological Cross-Sectional Area (PCSA) was calculated as muscle volume fascicle length, and Fmax was determined by multiplying the PCSA by the maximal isometric stress of vertebrate skeletal muscle (0.3 MPa; Medler 2002). Pennation angle were not included in the study, because tendons of muscle bellies of most muscles were closely attached to skeletons without forming greater angles, nearly all angles were smaller than 10^{0}.Cosine of 10^{0} angle is very close to one and would thus have little effect on estimations of PCSA.

To examine the distribution of muscle mass within the limbs, muscles were grouped according to their function. Muscle function was determined usingthe anatomical position and previously published work (Williams and Warwick, 2008).

The data was subjected to statistical analysis by using the Graph Pad Prism version 5.01 (2007), a computerized statistical software. One way ANOVA with Tukey's post test was employed to know the differences between muscle and functional group. The values were expressed as Mean ± SE.

Results and Discussion

On average, M. subscapularis had the longest muscle fascicles (mean FL, 17.46 ± 1.38 cm) (Fig. 1). Fascicle length was low in elbow compared with more proximally located forelimb muscles. In forearm and manus region the extensors got the longest muscle fascicles comparing flexors, supinators and pronators. In extensors group the M. common digital extensor had got longest muscle fascicles and in the flexors group, the M. Brachioradialis had got the longest muscle fascicle. In supinators the M. brachioradialis was having longest fascicle length while the abductor I longus was shortest muscle fascicle length (Fig. 2). Oishi *et al*. (2009) observed that their orangutan digital had flexors longer fascicles compared with chimpanzees, that was likely due to the increased need for mobility in the wrist joint during arboreal behaviours in orangutans and a greater emphasis on power in the chimpanzees during quadrupedal locomotion.

Unilateral forelimb muscles accounted on average 2342.5 g. The greatest part of forelimb muscle mass consisted of muscles that crossed shoulder (Fig. 3). Among shoulder muscles, 67.98% of muscle mass contributed to extension and 37.35% to flexion, indicating that a larger proportion shoulder muscles were meant for lowering and lifting arm in sagittal plane (extensors and flexors, respectively). However, most of these muscles also contributed to abduction, endorotation and exorotation. Extensors constituted dominant group in the elbow and they also made up more than one-third of total forelimb musculature. In forearm and manus region flexor had got the highest muscle mass compared to other extensors, supinators and pronators.

From the present study, it was found that M. triceps had on average largest PCSA and hence had highest force-producing capacity of all forelimb muscles ($F_{\max}$ =1240.36N) (Fig.4). In shoulder, extensors group dominated with largest PCSA closely followed by exorotators, endorotators and flexors (Fig. 5). PCSA of functional muscle groups showed that, elbow musculature had a clear dominance for extension.

In the forearm flexors had got highest percent of PCSA compared to supinators, extensors and while the PCSA of 5.9% accounts for pronators (Fig. 7). In flexors the M. deep digital flexors ranked highest in PCSA while the M.Brachioradialis ranked the lowest PCSA (Fig.6). In all non-human ape species also, the digital flexor muscles had the greatest PCSA, among the distal muscles. The muscle group in non-human great apes enabled greater grip strength (Myatt *et al*. 2012).

In extensors the M. ulnaris lateralis got the highest PCSA while the M.extensor digiti Iet II got the lowest PCSA. In supinators the M. abductor I longus got the highest force producing capacity while *M. brachioradialis* got the lowest force producing capacity.

PCSA was proportional to amount of force a muscle can produce (Zajac 1992). Data from present study depicted that overall PCSA was generally greater in proximal muscle group than distal, particularly for shoulder extensors and elbow extensors. Among non-human ape species proximal muscles had the greatest PCSA, in particular shoulder muscle group (Myatt *et al.loc. cit*)

Total force generated from forelimb (7879.59 N) for locomotion, 29.7% was contributed from shoulder extensors and 22% from elbow extensors. Thorpe *et al*. (1999) and Michilsens *et al*. (2009) stated in chimpanzee and in gibbon, elbow flexors had long fascicle length which resulted in a larger PCSA similar to that of extensors which reflected need for large excursions and force production at elbow during climbing.

Muscle PCSA are longer in all sloth bear than in human muscles. This significant difference reflects the use of the forelimb for locomotion in sloth bear but not human.

Summary

The overall PCSA was greater in proximal than the distal muscle group, particularly in the shoulder extensors and elbow extensors. In the forearm and manus region PCSA was greater in the caudomedial aspect of muscles of forearm, particularly in the pronators of forearms and flexors of manus. Overall, the fascicle length of the sloth bear were longer in the extensor group of muscle. There was a proximal to distal distribution of muscle mass in the sloth bear forelimb, with the heaviest muscle groups near the body. Flexor group of muscle had got more muscle mass in the forearm and manus than any other functional group. These significant differences reflected the different locomotor behavior and the use of forelimb as a manipulatory organ for locomotion as well as the manipulatory organ in sloth bear.

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Art – 225. COMPARATIVE ASSESSMENT OF ADAPTIVE CAPABILITIES OF WILD AND CAPTIVE INDIAN SLOTH BEAR (*Melursus ursinus)* BASED ON RHYTHMIC CHANGES IN BIOCHEMICAL RESPONSE

A.Sha. Arun, V. Sejian and R. Bhatta

Abstract

` The study is to establish the adaptive capabilities of wild and captive Indian Sloth Bear based on changes in the biochemical response influenced by the gender, age and habitat. The free-ranging and captive sloth bears that were residing in Bannerghatta Bear Rescue Centre, Bangalore, Karnataka, India were chosen for this study. An analysis of serum biochemical variables like glucose, total cholesterol (TC), triglycerides (TG), albumin, total protein (TP), serum glutamic-pyruvic transaminase/alanine aminotransferase aspartate amino- transferase (SGPT/ALT), serum glutamic-oxaloacetic transaminase/ aspartate aminotransferase (SGOT/AST), urea, Alkaline Phosphatase, calcium (Ca), phosphorus (P) and magnesium (Mg) was determined in Indian sloth bear. The study was conducted during the years 2017 and 2018 in the state of Karnataka, India. Statistically, no difference was found in the biochemical variables between the sexes. Instead, age and habitat-related significant differences were found in certain biochemical variables like cholesterol, triglycerides, calcium, phosphorus, magnesium, urea and AST. The findings of this analysis serve as a reference to assess the health and physiological status of Indian sloth bear.

Keywords: Age; biochemical variables; captive; wild; habitat; Indian sloth bear

Introduction

Biochemical variables or blood profile is an indice for the physiological status, nutrition, and health of animals (Marchal etal. 2012; Shanmugam et al. 2017) and humans (Rietjens et al. 2005). Certain studies have implicated nutritional restriction, lack of natural activity like predation, captive management practices, seasonal variations, age, illness and stress for the observed biochemical differences in animals (Couch et al. 2017). In general, these serum biochemical variables can be used for different purposes such as to test the quality (tenderness) and composition of meat (Abou-Kassem et al. 2019), infection (López-Olveraet al. 2013), stress (Trevisi and Bertoni 2009), infertility (Feng et al. 2015). Sex-associated changes in biochemical variables have also been observed in birds and animals such as chicken (Panigrahy et al. 2017) and red deer (López-Olvera et al. 2013). The biochemical variables are also analyzed for captive animals such as wild ruminants (Peinado et al. 1999), buffalos (Couch et al. 2017) and captive birds like Andean Condor (Doussang et al. 2018). The inter-species biochemical parameter variation has also been reported (Elarabany 2018).

Among the bears, sloth bears (*Melursus ursinus*) are endemic to the Indian subcontinent and they are on the endangered list due to fragmentation and loss of habitat, reduced natural food resources, illegal hunting, etc. (Bargali et al. 2004; Yoganand et al. 2006). With the idea to improve their natural habitat and resume normal patterns of behaviour, the zoosand sanctuaries are taking the responsibility to restrict them in a more extensive and safe area. However, several factors like captive conditions, age progression, restrictive natural and physical activity, dietary pattern and stress affect the normal blood chemical profile (Dontas et al. 2011; Klinhom et al. 2017). The successful conservation and efficient breeding demand that the environmental factors be enriched for the animals in a captive state.

In literature, few studies have reported the evaluation of biochemical variables in different species of *Ursus* (Brannon 1985; Græsli et al. 2014) and their relevance in various scenarios such as pathophysiological conditions like atherosclerosis (Arinell et al. 2012), seasonal variations (Græsli et al. 2015), fasting (Lohuis et al. 2005), hibernation (Chauhan et al. 2002), etc. Further, the method of capture (wild or captive), sex, and age are also related to the difference in variations in blood profiles in different types of bears (Matula et al. 1980; Huber et al. 1997).But in the case of Indian sloth bear, there is a lack of reference values for the biochemical variables. Also, the serum biochemistry values for captive Indian sloth bear are not yet published. Therefore, the first objective of this study was to establish the serum biochemical variables for the Indian sloth bear population. Secondly, the pattern of biochemical variables and their comparison based on sloth bear's age, sex and habitat condition was performed.

Materials and Methods

The free ranging and rehabilitated captive sloth bears from lifetime care facility of Wildlife SOS at Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park, Bannerghatta, Bangalore, Karnataka, India were used in this study during the year 2017 and 2018. Before initiating the study, all sloth bears were clinically tested (as determined by body temperature, hydration, heart/respiration rate and a detailed external physical examination) for normal physical health and normal behaviour responses. The captive sloth bear's diet included local seasonal fruits and grains.

A Ketamine-xylazine drug combination containing ketamine hydrochloride (5 mg/kg body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg/kg body weight; Troy Laboratories Pty Ltd.) was administered using a distance projectile drug delivery system to immobilize the sloth bears. Ten minutes after the immobilisation, blood was collected from the jugular vein using a 20-ga sterile hypodermic needle in vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA) without ethylene diamine tetraacetic acid. Immediately the blood samples were stored on cold packs at 4–8°C and transported to the laboratory for further analysis.

Biochemical variables studied

The following serum biochemistry variables were analysed: glucose (mg/dl), total cholesterol (mg/dl), triglycerides (mg/dl), total protein (g/dl), albumin (g %), aspartate amino-transferase (SGOT/AST in U/L), alanine aminotransferase (SPGT/ALT in U/L), alkaline phosphatase (U/L), urea (mg/dl), calcium (mg/dl), phosphorus (mg/dl) and magnesium (mg/dl). Commercially available automated analyzers (BBP: HumaCount 30TS, HUMAN Gesellschaft fürBiochemica und DiagnosticambH, Wiesbaden, Germany; MLRC: BC-1800, Vector Biotek Pvt. Ltd. Gujarat, India) were equipped for the analysis.

Statistical analysis

The collected data were tabulated and statistically analysed for each of the biochemical variables for the entire population as well as for the population based on sex (male andfemale), habitat (wild and captive population) and age (age group from I-IV). The descriptive analysis included sample size (SS), mean ± SD (standard deviation), 95% confidence level (CL)of mean with upper confidence level (UCL) and lower confidence level (LCL). An independent *t-test* was performed to analyse the statistical significance in the mean value. For all the tests, two significance levels, significant (*$p < 0.05$) and highly significant (**$p < 0.01$) were considered. SPSS version 21 was used for all statistical analyses.

Results

The descriptive statistical analysis was performed on different biochemical variables for the entire sloth bear population. The data are exemplified in Table 1. The whole population was further grouped based on their gender (Table 2), habitat (Table 3) and age (Table 4).

Based on the gender (male or female), the distribution of biochemical parameters is presented in Table 2. It was observed that the mean values of variables such as glucose, total cholesterol, triglycerides, total protein, albumin, AST, ALT, alkaline phosphatase, urea, calcium, phosphorus and magnesium were not statistically different between the male and female sloth bear. However, for certain biochemical variables, minor variations in distribution levels were observed between sexes. Glucose was found to be higher in males (85.50 ± 43.76 mg/dl) as compared to the female (69.08 ± 25.14 mg/dl) sloth bear. Similarly, in male vs. female sloth bear, the triglycerides (308.41 ± 21.29 mg/dl vs. 279.02 ± 27.44 mg/dl) and urea (187.00 ± 193.63 mg/dl vs. 76.83 ± 38.95 mg/dl) were higher in the male sloth bear. The total cholesterol was found to be lower in male sloth bear(192.75 ± 17.53 mg/dl) when compared with the female sloth bear (215.63 ± 18.20 mg/dl). Other biochemical variables such as ALT, phosphorus, AST, calcium, alkaline phosphatase and magnesium were found to be similarly abundant in both the male and female populations.

Based on habitat, the entire sloth bear population was also categorized into wild or captive population. The significance of biochemical parameter was evaluated by comparing the mean values of each parameter between the wild and captive sloth bears (Table 3). The mean value of total cholesterol was significantly higher in captive (223.70 ± 13.60 mg/dl) as compared to the wild sloth bear (143.08 ± 22.74 mg/dl). Similarly, between captive and wild, other variables like triglycerides (324.52 ± 25.53 vs. 222.08 ± 19.07 mg/dl) and calcium (324.52 ± 25.53 vs. 222.08 ± 19.07 mg/dl) were elevated in captive sloth bear. In contrast, other variables like AST (50.92 ± 4.36 U/L vs. 352.50 ± 62.23 U/L), urea (24.10 ± 2.017 mg/dl vs. 188.66 ± 53.85), phosphorus (5.60 ± 0.24 vs. 11.72 ± 0.85) and magnesium (1.87 ± 0.07 mg/dl vs. 2.72 ± 0.31 mg/dl) were significantly lower in captive animals as compared to the wild sloth bear population. The remaining biochemical variables such as glucose, total protein, albumin, alkaline phosphatase and ALT were not significantly different in their distribution between the wild and captive animals.

Table 4 represents the distribution of biochemical parameters based on the age group of the sloth bear. The entire sloth bear population was categorized into four age groups: sloth bear of age 1–6 years were grouped in age group I, 7–12 years were in the age group II, 13–18 years in the age group III and sloth bear of age 19–25 years were in age group IV (Table 4). A significant difference was found in the presence of total cholesterol, albumin, SPGT/ALT and calcium across different age groups ($p < 0.05$). For biochemical variables like

SGOT/AST, urea, phosphorus and magnesium a highly significant difference was observed across the age groups ($p < 0.01$). As the sloth bear progressed in their age, a gradual decrease in the total cholesterol was observed. The total cholesterol in the age group I (1–6 years) corresponded to 245.55 ± 19.36 mg/dl. In animals with age limit of 7–12 years total cholesterol was found to be 229.08 ± 30.21 mg/dl. The reduced cholesterol was found in animal 13–18 years (age group III), the total cholesterol level was reduced to 200.46 ± 21.50 mg/dl and the lowest level of 149.08 ± 21.76 mg/dl was found in animals ranging from 19 to 25 years (age group IV). This reduction was statistically significant ($p < 0.05$). The level of albumin significantly increased ($p < 0.05$) as the animal became old. The albumin level increased from 2.31 ± 0.18 g% in the age group I to 2.74 ± 0.18 g% in age group IV. The sloth bear in the age group II (2.55 ± 0.10 g%) and III (2.64 ± 0.17 g%) had a similar level of albumin. The distribution pattern of AST was different; AST was significantly higher in age group IV (329.84 ± 61.56 U/L), when compared with the age group I (42.72 ± 3.00 U/L), II (54.30 ± 6.58 U/L) and III (53.71 ± 10.32 U/L). Another enzyme ALT was found to be significantly different between age groups I and II or III. In age group I, the ALT level corresponded to 2.64 ± 0.33 U/L, whereas in group II it was found to be 9.75 ± 1.52 U/L and 9.29 ± 2.24 U/ L in group III. Comparatively in age group IV, the level was slightly lower (7.59 ± 1.09 U/L) with respect to group II and III. The distribution level of urea was significantly higher in age group IV (175.69 ± 51.21 mg/dl) when compared to other age groups (I, II and III) which represented an average of 25.00 ± 3.33 mg/dl. The mineral levels also varied across the age group. Calcium level gradually and significantly dropped as the age progressed, whereas a significant and elevated phosphorus and magnesium level were recorded with the progress in age.

Table 1. Overall statistical description of biochemical variables for entire sloth bear population.

				Range		95% CL of mean	
Variables	Sample size	Mean ± SD	SEM	Minimum	Maximum	LCL	UCL
Glucose (mg/dl)	25	75.65 ± 33.76	7.54	30	178	62.0	91.09
TC (mg/dl)	49	170.85 ± 89.12	19.92	50	346	134.80	209.84
TG (mg/dl)	38	253.95 ± 95.47	20.34	81	457	212.55	295.04
TP (g/dl)	51	6.87 ± 0.56	0.12	5.7	7.8	6.63	7.11
Albumin (g %)	51	2.69 ± 0.59	0.12	1.9	3.6	2.44	2.94
SGOT/AST (U/L)	51	121.88 ± 23.21	51.20	8	700	78.81	170.89

SPGT/ALT (U/L)	50	8.72 ± 4.53	1.01	3	20	6.92	10.84
Alkaline Phosphatase (U/L)	51	19.85 ± 16.06	3.59	3	62	13.50	27.58
Urea (mg/dl)	51	120.90 ± 165.59	37.02	10	471	54.35	189.99
Ca (mg/dl)	42	7.89 ± 1.19	0.26	5	10	7.34	8.36
P (mg/dl)	51	9.22 ± 3.91	0.87	3.85	15.18	7.64	10.92
Mg (mg/dl)	50	2.46 ± 0.90	0.20	1	4	2.09	2.89

SD: Standard deviation; SEM: standard error of mean; CL: confidence level; LCL: lower confidence level; UCL: upper confidence level; TC: total cholesterol; TG: triglycerides; TP: total protein; Ca: calcium; P: phosphorus; Mg: magnesium: SGPT/ALT: serum glutamic-pyruvic transaminase/alanine aminotransferase; SGOT/AST: serum glutamic-oxaloacetic transaminase/aspartate aminotransferase

Table 2. Comparison of biochemical variables of entire sloth bear population based on gender.

	Male				Female				
	95% CL of Mean				95% CL of Mean				
Parameter	SS	Mean±S.D	LCL	UCL	SS	Mean±S.D	LCL	UCL	*p*-Value
Glucose (mg/dl)	11	85.50 ± 43.76	59.80	118.99	14	69.08 ± 25.14	55.36	83.65	0.175
TC (mg/dl)	25	192.75 ± 17.53	159.41	226.53	24	215.63 ± 18.20	180.19	251.61	0.081
TG (mg/dl)	17	308.41 ± 21.29	256.81	373.47	21	279.02 ± 27.44	227.07	330.51	0.051
TP (g/dl)	26	6.83 ± 0.87	6.49	7.15	25	6.63 ± 1.11	6.14	7.03	0.473
Albumin (g %)	26	2.53 ± 0.56	2.31	2.74	25	2.62 ± 0.55	2.421	2.85	0.125
SGOT/AST (U/L)	26	123.80 ± 34.47	67.08	197.96	25	119.80 ± 31.66	67.69	185.80	0.934
SPGT/ALT (U/L)	25	7.13 ± 1.44	4.73	10.34	25	8.05 ± 4.68	6.43	10.04	0.386
Alkaline Phosphatase (U/L)	26	18.19 ± 3.02	13.17	24.39	25	22.12 ± 22.19	14.33	31.88	0.296
Urea (mg/dl)	26	187.00 ± 193.63	63.50	328.15	25	76.83 ± 38.95	20.00	161.30	0.144
Ca (mg/dl)	20	7.63 ± 1.48	6.55	8.48	22	8.065 ± 0.98	7.49	8.56	0.217
P (mg/dl)	26	8.30 ± 3.72	8.15	13.26	25	10.60 ± 3.92	6.30	10.51	0.957
Mg (mg/dl)	26	2.90 ± 1.00	2.18	3.58	24	2.17 ± 0.730	1.85	2.63	0.056

SS: Sample size; SD: standard deviation; CL: confidence level; LCL: lower confidence level; UCL: upper confidence level; TC: total cholesterol; TG: triglycerides; TP: total protein; Ca: calcium; P: phosphorus; Mg: magnesium; SGPT/ALT: serum glutamic-pyruvic transaminase/alanine aminotransferase; SGOT/AST: serum glutamic-oxaloacetic transaminase/aspartate aminotransferase.

Table 3. Comparison of biochemical variables of entire sloth bear population based on habitat.

		Captive 95% CL of mean			Wild 95% CL of Mean				
Parameter	SS	Mean±S.D	LCL	UCL	SS	Mean±S.D	LCL	UCL	*p*-Value
Glucose (mg/dl)	13	83.15 ± 18.06	50.37	118.50	12	73.83 ± 10.40	57.46	93.29	0.202
TC (mg/dl)	37	223.70 ± 13.60	196.93	249.80	12	143.08 ± 22.74	101.65	192.78	0.005**
TG (mg/dl)	26	324.52 ± 25.53	277.57	369.13	12	222.08 ± 19.07	186.50	255.44	0.015*
TP (g/dl)	39	6.67 ± 0.17	6.30	7.00	12	6.95 ± 0.17	6.58	7.31	0.281
Albumin (g %)	39	2.54 ± 0.08	2.38	2.72	12	2.76 ± 0.18	2.30	3.04	0.071
SGOT/AST (U/L)	39	50.92 ± 4.36	43.05	59.66	12	352.50 ± 62.23	243.70	473.23	0.001**
SPGT/ALT (U/L)	38	7.61 ± 1.07	5.81	9.77	12	7.54 ± 3.41	5.46	9.78	0.975
Alkaline Phosphatase (U/L)	39	20.07 ± 3.24	14.16	27.13	12	20.25 ± 4.19	13.46	29.08	0.978
Urea (mg/dl)	39	24.10 ± 2.017	20.47	28.26	12	188.66 ± 53.85	90.17	297.68	0.001**
Ca (mg/dl)	30	8.92 ± 0.27	8.42	9.50	12	7.33 ± 0.34	6.65	7.94	0.002*
P (mg/dl)	39	5.60 ± 0.24	5.14	6.08	12	11.72 ± 0.85	9.95	13.48	0.001**
Mg (mg/dl)	38	1.87 ± 0.07	1.72	2.01	12	2.72 ± 0.31	2.13	3.33	0.001**

*p < 0.05; **p < 0.01

SS: Sample size; SD: standard deviation; CL: confidence level; LCL: lower confidence level; UCL: upper confidence level; TC: total cholesterol; TG: triglycerides; TP: total protein; Ca: calcium; P: phosphorus; Mg: magnesium; SGPT/ALT: serum glutamic-pyruvic transaminase/alanine aminotransferase; SGOT/AST: serum glutamic-oxaloacetic transaminase/aspartate aminotransferase

Table 4. Comparison of biochemical variables of entire sloth bear population based on age.

	Age Group I				Age group II				Age group III				Age group IV				
95% CL of Mean																	
Variables	SS	Mean±S.D	LCL	UCL	SS	Mean±S.D	UCL	LCL	SS	Mean±S.D	LCL	UCL	SS	Mean±S.D	LCL	UCL	*p*-Value
Glucose (mg/dl)	0	0	0	0	4	54.50 ± 10.34	30.00	74.50	8	97.88 ± 28.22	45.60	154.99	13	74.31 ± 9.58	59.86	97.26	0.305
TC (mg/dl)	11	245.5 ± 19.36	206.03	283.96	12	229.08 ± 30.21	165.90	287.19	13	200.46 ± 21.50	159.82	242.98	13	149.08 ± 21.76	106.96	197.99	0.032*
TG (mg/dl)	11	354.64 ± 34.53	282.26	422.61	8	323.38 ± 50.52	210.21	427.50	6	266.75 ± 62.94	131.50	385.60	13	231.85 ± 20.08	192.38	271.48	0.073

TP (g/dl)	11	6.59 ± 0.32	5.87	7.18	13	6.86 ± 0.21	6.37	7.27	14	6.50 ± 0.36	5.688	7.133	13	6.99 ± 0.16	6.65	7.32	0.576
Albumin (g %)	11	2.31 ± 0.18	2.10	2.53	13	2.55 ± 0.10	2.35	2.74	14	2.64 ± 0.17	2.29	3.00	13	2.74 ± 0.18	2.38	3.11	0.018*
SGOT/AST (U/L)	11	42.72 ± 3.00	37.37	49.22	13	54.30 ± 6.58	41.20	66.99	14	53.71 ± 10.32	34.75	75.79	13	329.84 ± 61.56	206.92	454.66	0.001**
SPGT/ALT (U/L)	11	2.64 ± 0.33	2.00	3.33	12	9.75 ± 1.52	6.69	12.71	14	9.29 ± 2.24	5.86	14.84	13	7.59 ± 1.09	6.05	9.40	0.013*
Alkaline	11	16.27 ± 3.74	9.92	25.54	13	17.769 ± 6.30	9.72	34.15	14	23.21 ± 6.08	12.90	38.20	13	22.38 ± 4.41	14.75	31.33	0.265
Urea (mg/dl)	11	25.00 ± 3.33	18.50	32.12	13	25.53 ± 3.41	19.00	33.14	14	23.28 ± 4.03	16.846	31.876	13	175.69 ± 51.21	82.164	286.581	0.001**
Ca (mg/dl)	11	9.33 ± 0.69	8.07	10.87	9	8.73 ± 0.50	8.4	9.04	9	8.53 ± 0.31	7.85	9.12	13	7.50 ± 0.35	6.81	8.17	0.048*
P (mg/dl)	11	5.18 ± 0.29	0.56	1.23	13	5.60 ± 0.55	4.69	6.77	14	5.94 ± 0.40	5.12	6.77	13	11.25 ± 0.92	9.37	13.15	0.001**
Mg (mg/dl)	11	1.60 ± 0.13	1.28	1.81	13	2.00 ± 0 .07	1.85	2.15	13	1.93 ± 0.13	1.65	2.21	13	2.69 ± 0.29	2.18	3.27	0.001**

*p < 0.05; **p < 0.01

SS: Sample size; SD: standard deviation; CL: confidence level; LCL: lower confidence level; UCL: upper confidence level; TC: total cholesterol; TG: triglycerides; TP: total protein; Ca: calcium; P:phosphorus; Mg: magnesium; SGPT/ALT: serum glutamic-pyruvic transaminase/alanine aminotransferase; SGOT/AST: serum glutamic-oxaloacetic transaminase/aspartate aminotransferase.

Discussion

The biochemical variables of animals are influenced by their different states such as age, habitat and sex. Biochemical differences were found in the captive and wild populations of different species such as gray wolves (Constable et al. 1998) and leopards. In the present study, we have made an attempt to analyse and establish a correlation between the serum biochemical variables and different states of Indian sloth bear population. Biochemical variables like total protein, albumin, total cholesterol, triglycerides, calcium, phosphorus, magnesium, ALT, AST, urea and alkaline phosphatase were estimated in the entire population of sloth bear. The biochemical data were compared to draw a further influence of different states like sex, age, habitat on the representation of biochemical variables in sloth bear.

In the present study, a minor variation was found in the mean values of biochemical variables such as total cholesterol, triglycerides, total protein between the male and female sloth bear. However, this variation was not statistically different, suggesting that gender does not influence the distribution of biochemical variables. The present data were in coherence with data reported by Veeraselvam et al. (2018) where in serum biochemical analysis revealed no significant difference in the level of albumin, total protein, glucose, AST, ALT, calcium, phosphorus, uric acid and cholesterol between male and female captive sloth bears.

The biochemical profile of total cholesterol, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), urea, calcium, phosphorus and magnesium were found to be age-related, whereas, habitat-related significant difference in biochemical variables was observed for total cholesterol, triglycerides, AST, urea, calcium, phosphorus and magnesium. In this study, we observed a significantly elevated level of total cholesterol and triglycerides (TG) in captive sloth bears. In humans (Torii et al. 2003) and animals (Buja et al. 1983) higher lipid concentrations are associated with diseases like atherosclerosis. However, in brown bear (*Ursus arctos*) higher levels of lipids like cholesterol and triglycerides help to sustain the hibernation phase and these were found to be resistant to atherosclerosis. In contrast to our study, Frank et al. (2006) reported a significant variation in the total lipid concentration (TG and cholesterol) within the population of captive American black bears (*Ursus americanus*) (Arinell et al. 2012). It can be inferred that diet or captive management practices may result in different patterns of plasma lipoprotein within a population. Based on the age group, the difference in biochemical variables was clearly evident in the case of total cholesterol, which reduced with an increase in the age of sloth bear.

Alterations in the renal functions have been associated with aging in animal models as well as in humans. The older animals are known to succumb to aging-related diseases including renal disease (Vlassara et al. 2009). The level of urea, globulin and creatinine are considered as biochemical markers for evaluating renal function. In the present study, the elevated level of urea in adult sloth bear (age group IV) as compared to other age groups could possibly be related to renal dysfunction (Marchal et al. 2012). However, considering the distribution based on habitat, it can be inferred that wild animals are consuming diet, which is high in protein content as compared to their captive counterparts (Moen et al.2010). In humans, the reduced serum albumin level is associated with aging and muscle loss (Visser et al. 2005; Gomi et al. 2007). The observed elevated level of albumin in age group IV as compared to other age groups could be related to the existing renal disease in ageing sloth bear. However, further screening is recommended to comment conclusively on this aspect.

The distribution profile of enzymes like SGPT/ALT and SGOT/AST was age- and habitat-related. In literature, there have been contrasting results with respect to ALT and AST. In this study, with respect to ALT, no significant difference was detected between the wild and captive sloth bears. Similarly, even in wild and captive animals like giraffe (Schmidtet al. 2011), the ALT level did not vary significantly. Our result on the level of AST and urea in wild and captive sloth bear was in agreement with the observations in grey wolves(*Canis lupus*) (Constable et al. 1998) and Canada lynx (*Lynx canadensis*) (Moen et al. 2010) where AST and urea level were significantly higher in wild wolves and free-ranging lynx as compared to their captive counterparts. However, in contrast to our studies significantly higher level of ALT was observed in free-ranging wolves and free-ranging lynx. An elevated level of AST in serum was observed in captive emus undergoing the hatching period which was then normalized after the hatching period (Almeida et al. 2018). Similarly, AST and ALT had significantly higher activity in healthy lactating cows. Clinically, the plasma AST and ALT are determined to assess the level of muscular/tissue damage in response to injury or muscle stress (Huang et al. 2006; Harr et al. 2008;Tvarijonaviciute et al. 2017). It could be possible that with increasing age, there is more muscular damage in the sloth bear. However, more test is ascertained.

We also analysed the blood mineral profile. Calcium, phosphorus and magnesium metabolism are closely related and are usually controlled by kidneys (Blaine et al. 2015). In general, mineral abnormalities are related to chronic illness in the animal. Calcium was higher in captive animals as compared to the wild bear which could possibly be due to the additional dietary calcium supplements provided as a part of a formulated diet in the captive animals. However, with an increase in age, there was a clear reduction in the serum calcium levels, which also indicates a lower absorption of calcium as the age progresses. In younger ones higher calciumis related to bone development and lower calcium level in adults is related to lactation and lack of dietary calcium. Further, our data are in agreement with Yang et al. (2017). In the captive and non-hibernation state, the young (<4 years) Asiatic black bear (*Ursus thibetanus*) had higher calcium levels in blood in comparison to adult bear (≥4 years). According to Vestergaard et al. (2011), serum calcium level is not an exact representation of calcium flow in the system, but rather it suggests the functional status of hormones and tissues system, which are actively involved in the maintenance of total calcium level. It can be inferred that with age the functioning of hormone and tissue system may be weakened resulting in less absorption of calcium. In this study, the other two minerals, namely, phosphorus and magnesium had an elevated level in wild animals and adults (age group IV). In ruminants, the loss in homeostasis of the calcium, magnesium and phosphorus is related to age and diet. In captive ruminants, diet change resulted in a positive and significant change in the levels of phosphorus and magnesium (Miller et al.2010). From our data, we can conclude that monitoring the diet composition of sloth bears can aid in normalizing the mineral level in blood.

However, it cannot be neglected that there could be possible limitations in the current study. In this study, besides the different factors like sex, age and habitat, variation in biochemical profile could be due to characteristics of an individual bear. Altogether from our studies and related studies we can draw an inference that level of biochemical molecules in the serum can be case-specific and also factors like diet, physiological processes, metabolic changes, habitat, captive management practices and other environmental factors could influence the biochemical profile in Indian sloth bear. Also, analysis of biochemical variables like vitamin D and other enzymes which are involved in the metabolism pathway can shed light on emerging variations in the biochemical profile of wild and captive sloth bear.

Conclusion

The study attempted to elucidate the influence of age, sex and habitat on the targeted blood biochemical variables. The targeted biochemical variables in this study are distinguished from the point of view to consider the changes in diet, habitat or management practices. Further, the study attempts to deduce the relationship between these variables and different states of sloth bear. The results from the study established that in sloth bear the observed biochemical variable changes were both age and habitat dependent. Thus, the current findings provide a useful reference to evaluate the health of animals based on captive animals' diets. We assume that the housing conditions, feeding pattern and composition, and healthy management practices can be effective in normalizing the biochemical variables in captive animals.

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Art – 226. SCENT-MARKING BEHAVIOR BY FEMALE SLOTH BEARS DURING ESTRUS

Yaduraj Khadpekar, John P. Whiteman, Barbara S. Durrant, Megan A. Owen, and Sant Prakash

Abstract

The sloth bear (*Melursus ursinus*) is one of the least studied bears. Important aspects of sloth bear biology and ecology, such as reproductive physiology and behavior, are largely unknown. Increased scent-marking by anogenital rubbing during breeding season has been recorded in other bear species. We studied the genital rubbing behavior of 37 captive female sloth bears (2–18 yr of age) at the Agra Bear Rescue Facility, India, for 4 breeding seasons over a period of 3.5 years (1 Jun 2015 to 31 Dec 2018). Data on changes in vulva visibility and presence of genital rubbing behavior were collected daily during the breeding period and twice per week for rest of the year, throughout the study period. Vulva visibility was scored as 0 (not visible), 1 (slightly visible), and 2 (fully visible), and a female was considered to be in estrus if the vulva was slightly or fully visible. Presence of genital rubbing was recorded as 1 and its absence as 0. Occurrence of genital rubbing coincided with estrus, as defined by vulva visibility scores. Statistical analysis indicated that female age and the number of males with physical proximity (i.e., in the same enclosure) were significantly correlated with the occurrence of genital rubbing behavior. The number of females in physical proximity and the number of females in the vicinity without physical proximity (i.e., not in the same enclosure but sharing a fence) did not significantly affect this behavior. The results of our study suggest that the genital rubbing behavior by female sloth bears in estrus is likely a form of scent-marking, serving a communicative function, and could be influenced by male presence. This behavior may be a key factor in attracting a mate during the breeding season in the wild.

Keywords: chemical communication, estrus, genital rubbing, keeper check sheets, *Melursus ursinus,* reproductive behavior, sloth bear, vulva scores, zoo research

For animals living under human care, long-term data collection from a relatively large number of individuals may be accomplished using systematically collected keeper feedback. Indeed, feedback from animal keepers has been used effectively in studies of reproduction in black rhinoceros (*Diceros bicornis;* Carlstead et al. 1999), cheetah (*Acinonyx jubatus;* Wielebnowski 1999), Asian elephant (*Elephas maximus;* Duer et al. 2016), and giant panda (*Ailuropoda melanoleuca;* Kleiman et al. 1979). This approach has proven to be advantageous in rescue sanctuaries and conservation centers for bears. For example, Khadpekar et al. (2018) recently demonstrated the research benefits of utilizing the working knowledge of keepers regarding the behavioral dynamics of sloth bears (*Melursus ursinus*) in their charge, for acquiring valid and reliable behavior data on large numbers of bears; particularly when logistical constraints preclude the hiring of dedicated research staff.

The sloth bear is one of the 4 bear species found in India (Menon 2014). It is distributed throughout the Indian subcontinent, including Nepal and Sri Lanka. Recently, it was found to be extirpated from Bangladesh (Islam et al. 2013). Distribution of the remaining population is patchy and corresponds with forest cover (Garshelis et al. 1999, Yoganand et al. 2006). Although there are no reliable population estimates currently, habitat degradation, human– bear conflicts, and poaching are considered to be major threats likely causing population declines (Dharaiya et al. 2016).

Despite being a relatively common bear species in India, as well as in zoos worldwide, the sloth bear is arguably one of the least studied and least understood ursids. Limited information is available on the basic aspects of sloth bear biology, such asdiet, behaviour (Joshiet al. 1995, 1997, 1999), and reproductive physiology (Puschmannet al. 1977). From the scant data available, sloth bears are thought to be seasonal breeders (Spady et al. 2007). Puschmann et al. (1977) described finding a free blastocyst in the uterus of a female sloth bear 4 months after mating, indicating the likelihood that sloth bears experience delayed implantation. However, to our knowledge,there are no detailed studies that describe reproduction and related behavior in this bearspecies.

It is important to understand how sloth bears signal their reproductive status because their patchy distribution and declining numbers in an increasingly human-dominated landscape may hinder the ability of each sexto locate the other for successful mating (Wilmers et al. 2013). A reduction in encounter rates caused by low population density—an "Alleeeffect"—can have population-level consequences (Courchamp et al. 1999, Stephens and Sutherland 1999). Scent marking can be used by mammals for reproductive communication (Eisenberg and Kleiman 1972), and this may be especially important for solitary animals, such as sloth bears, for assessing the location and status of potential mates in advance of face-to-face encounters (Owen et al. 2014). For example, there are a number of studies on different species of primates that describe urogenital scent-marking, such as by rubbing the vulva on a branch by bringing it forward while sitting (Horwich 1983, Lazaro-Perea et al. 1999). Female giant pandas are known to scent-mark by squatting and rubbing the anogenital region on horizontal surfaces, or by rubbing it on vertical surfaces with a leg-lift posture (Kleiman et al. 1979). This method of scent communication is especially prominent during estrus (Bonney et al. 1982, Lindburg et al. 2001, McGeehanet al. 2002). Brown bears (*Ursus arctos*) and American black bears (*U. americanus*) are known to scent-mark by body rubbing on trees and by pede-marking (Green and Mattson 2003, Taylor et al. 2015). Andean bears (*Tremarctos ornatus*) have also been observed to scent-mark trees by body rubbing (Filipczyková et al. 2016). However, there is very limited information available on scent-marking by sloth bears. Laurie and Seidensticker (1977) observed sloth bears scraping tree trunks and rubbing their flanks on those trees. Recently, Swaminathanet al. (2017) reported pede-marking behavior by sloth bears in the wild.

In this study, we hypothesized that genital rubbing in female sloth bears is a form of scent-marking, used to convey information regarding estrous status to male conspecifics. We predicted that observed rubbing behavior would peak during estrus. We tested this expectation using keeper collected behavioral data from 37 captive individuals over a period of 3.5 years.

Study site

The study was carried out at the Agra Bear Rescue Facility (ABRF) at Agra in the state of Uttar Pradesh, India (27°15r14.76rrN 77°51r17.07rrE). The ABRF is located inside the Soor Sarovar Bird Sanctuary and spans 2 sides of the Yamuna River. It houses sloth bears rescued from the 'dancing bear' trade (Seshamani and Satyanarayan 1997) and from human–bear conflicts. When we initiated the study, the facility housed 224 sloth bears. The bears were housed in 14 separate enclosures of different configurations, ranging in size from 2,100 to 11,800 m^2. All but one enclosure held a mixed sex group. Each enclosure in ABRF comprises a den and an open field attached to it with an electric fence boundary. The dens are enclosed cemented rooms used for feeding and other husbandry purposes. In general, the bears have free access to the dens and attached fields.

Methods

Study animals

We selected 37 female sloth bears ranging in age from 2 to 18 years at study initiation. Of these, 1 bear was<5 years, 5 bears were between 5 and 10 years, 12 bears were between 10 and 15 years, and 19 bears were >15 years of age. All these bears were rescued, so their ages were approximate, with the probable error of 6 months. We included ≥1 female from each of the 14 enclosures in the study. With 2 exceptions, all females shared their enclosure with ≥1 male. All males had been previously neutered and thus all females were unmated throughout the study period. All the study females in mixed- sex groups were housed with males that were >5 years old. One female that was housed alone at the start of the study was later moved to another enclosure, where she was housed with 2 or 3-year-old males.

Data collection

At the start of the study, we created a data-collection check sheet for keepers. The check sheet was designed to collect information on 10 physiological and behavioral parameters, including information on vulva visibility and the presence or absence of genital rubbing behavior (Fig. 1). Before beginning data collection, we trained the primary keepers from all enclosures on how to use the check sheets to collect data. Training included example images of bears to standardize observations. On the check sheets, vulva visibility observations were categorized as (a) not visible ('0'), (b) slightly visible ('1'), and (c) Fully visible ('2'; Fig. 2). Genital rubbing was described as rubbing the vulvar region on the den floor, den walls, the ground in the open field, or other substrates (e.g., water trough, wooden logs, trees), and was categorized as present ('1') or absent ('0'). Keepers marked the applicable category for each parameter on the check sheet at the end of their duty time; thus, the data reflected the opportunistic observations during their husbandry duties between 0800 and 1700 hours. Data collection began on 1 June 2015, with the exception of one female that entered the study in May 2017, and continued until 31 December2018. It was carried out either daily (Apr through Jul; the observed breeding season in sloth bears; unpublished observations from ABRF) or twice per week outside the breeding season (Aug through Mar). In the absence of the primary keeper of a study bear, the replacement keeper had been previously trained in data collection and could identify the bear. Females with a vulva visibility score of 1 or 2 were considered to be in estrus (Durrant et al. 2003). Keepers collected 19,433 bear-days of observations. Keepers carried out data collection as approved by the Institutional Animal Care and Use Committee of San Diego Zoo Global (permit 14-036).


Bear Name______________

Date_____ Month__________ Year________

Vulva is:	Vulva colour:	Meals skipped:	Aggressiveness:
Not visible:	Grey:	1:	Less:
Slightly visible:	Pink:	2:	Normal:
Fully visible:	Red:	3:	More:
Mounting others:	**Mounting received:**	**Rubbing vulva:**	**Mating vocals:**
None:	None:	Yes:	Yes:
Once or twice:	Once or twice:	No:	No:
More than twice:	More than twice:		
Water play:	**Activity:**	**Notes:**	
None:	Less:		
Normal:	Normal:		
More:	More:		

Fig. 1. Keeper check sheet used for data collection on female sloth bear (Melursus ursinus) behavior in estrus at the Agra Bear Rescue Facility, India (1 Jun 2015 to 31 Dec 2018). Keepers marked the applicable category for each parameter on the day of data collection.

Fig. 2. Photographs of (a) a sloth bear (Melursus ursinus) exhibiting vulva visibility during estrus. Visibility scoring system used for data analysis included (b) 0—not visible, (c) 1—slightly visible, and (d) 2— fully visible. Images are of the captive female sloth bears at the Agra Bear Rescue Facility, India (1 Jun 2015 to 31 Dec 2018).

Data analysis

We did all statistical analyses using Program R (R Core Team 2018). We visually examined the relationship between vulva visibility and genital rubbing behavior by plotting vulva visibility and rubbing behavior data along the timeline of the entire study period. We carried out 3 different analyses using mixed-effects binomial logistic regressions (Program R—package lme4; Bates et al. 2015) to evaluate the correlation of selected study factors with genital rubbing behavior by female bears. In the first analysis, presence or absence of genital rubbing behavior was the response variable. Predictors included a fixed effect of vulva visibility score on the given day, and a random effect of individual bear ID.

The second analysis addressed estrus duration and influence of proximity to other females and males. We used the number of days during which each bear exhibited genital rubbing out of the entire estrus length in each year as a response variable. Fixed effects predictors included (1) bear age, (2) total duration of estrus that year for that bear (as indicated by vulva visibility), (3) number of males in the same enclosure (i.e., with physical proximity), (4) number of females in the same enclosure, (5) number of males in the adjacent enclosure sharing a fence (i.e., with only auditory, visual, and olfactory proximity), and (6) number of females in the adjacent enclosure sharing a fence. We added individual bear ID and year of data collection as random effect predictors. Based on the results of this model and our expectation that the physical proximity with males would be the best predictor of genital rubbing, we also assessed a model with fewer predictors. The predictors in this model included the individual fixed effect of number of males in the same enclosure, and the random effects of individual bear ID and year of data collection. The response variable was the same as the previous model. We comparedthe model performance between these 2 models with Akaike's Information Criterion (AIC) scores.

The third analysis addressed the consequence of changes in female proximity to males. During the study period, 4 bears were moved to different enclosures for husbandry purposes. For 3 of those bears, this move substantially changed the number of males across the fence. The other bear that was initially housed alone was moved to another enclosure with 2 males. The third analysis was similar to the second analysis, but was carried out using only the data on these 4 bears. However, the data from4 bears were not sufficient to include all the predictors in a single model; therefore, we used 2 different models to assess the individual fixed effects of (1) number of males in the same enclosure and (2) number of males in the adjacent enclosure sharing a fence, respectively. In each of these models, the response variable and the random effects were similar to the second analysis. Some of the bears were already in estrus at the start of the data collection in June 2015, so we excluded data for those bears in that year from all statistical analyses.

Results

During estrus, female bears exhibited genital rubbing on vertical surfaces such as den walls, den metal gates and tree barks in the field, as well as on the horizontal surfaces and substrates such as den floor, grass, wooden logs, and natural floor in the field. Female bears carried out genital rubbing on the vertical surfaces by backing up to them and rubbing the vulvar region on the surface a few times in horizontal pattern while standing. On horizontal surfaces, bears did this by squatting on the hind legs and rubbing the vulvar region by moving it back and forth a few times. In both cases, a wet patch was observed on the surface after the female had moved away. It is not clear whether it was genital secretion or urinary excretion.

The occurrence of vulva visibility (scores 1 and 2) and genital rubbing (score 1) coincided temporally (Fig. 3). Vulva visibility scores of 1 and 2 both had a significant positive effect on genital rubbing behavior (Table 1). These results suggested that the onset of genital rubbing coincided with vulvar swelling. The results of the second statistical analysis are presented in Table 2. The age of the bear was observed to have a significant negative effect on the period for which genital rubbing was exhibited during estrus. Estrus duration did not have significant effect. The number of males in the same enclosure had a marginal positive significant effect. The number of females in the same enclosure and across a fence, and the number of males across a fence, were nonsignificant predictors. When the individual fixed effect of number of males in the same enclosure was evaluated, it was found to have a significant positive effect (z=1.96, P= 0.049). The AIC scores of the 2 models used for this analysis were 670.6 and 667.6, respectively.

When we analyzed only the 4 bears that were moved to different enclosures, the number of males in the same enclosure was found to have a significant positive effect on genital rubbing behavior. The number of males across a fence had a marginal positive significant effect (Table 3).

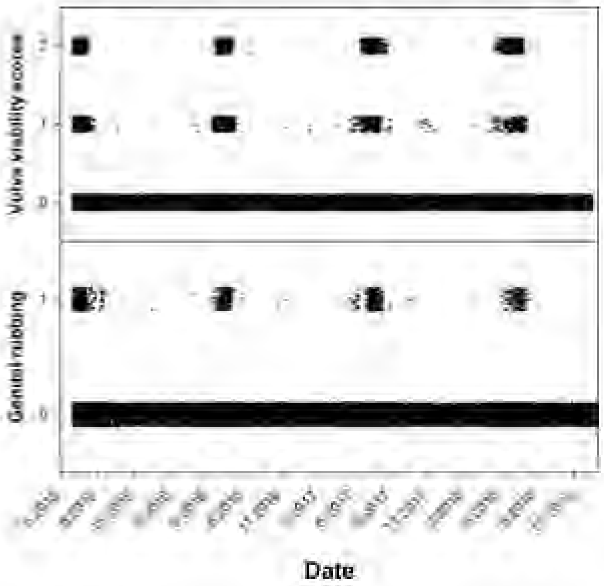

Fig. 3. Occurrence of high vulva visibility and genital rubbing behavior in female sloth bears (Melursus ursinus) during the study period. Vulva visibility was 0 (not visible), 1 (slightly visible), and 2 (fully visible). Presence of genital rubbing behavior was 1 (present) or 0 (absent). Each data point represents one day for one bear. Data points are slightly offset in X and Y space for visual clarity. The occurrence of genital rubbing behavior coincided with the period of high vulva visibility, used as an indicator of estrus. The data were collected over 3.5 years (1 Jun 2015 to31 Dec 2018) from 37 captive female sloth bears at the Agra Bear Rescue Facility, India.

Table 1. Results of mixed-effects binomial logistic regression analysis with binomial response variable of presence (score of 1) and absence (score of 0) of genital rubbing of female sloth bears (*Melursus ursinus*). Fixed effects predictor was the ordered categories for vulva visibility as 0 (not visible), 1 (slightly visible), and 2 (fully visible). Individual bear ID was a random effects predictor. The data were collected over 3.5 years (1 Jun 2015 to 31 Dec 2018) from 37 captive female sloth bears at the Agra Bear Rescue Facility, India.

Predictor	Parameter estimate	SE	*z*	*P*
Vulva visibility score[a] 1 (slightly visible)	3.575	0.137	26.15	<0.001
2 (fully visible)	5.228	0.131	39.81	<0.001

Table 2. Results of mixed-effects binomial logistic regression analysis with response variable of the number of days on which each female sloth bear (*Melursus ursinus*) exhibited genital rubbing out of the entire estrus length in each year. Random effects predictors included individual bear ID and year of data collection. The data were collected over 3.5 years (1 Jun 2015 to 31 Dec 2018) from 37 captive female sloth bears at the Agra Bear Rescue Facility, India.

Predictor	Parameter estimate	SE	*Z*	*P*
Age of the bear	— 0.096	0.047	— 2.03	0.043
Estrus duration	—0.013	0.009	—1.35	0.178
No. of males in same enclosure	0.090	0.050	1.81	0.070
No. of females in same enclosure[a]	—0.024	0.056	—0.42	0.671
No. of males across fence[b]	0.014	0.051	0.28	0.780
No. of females across fence[b]	—0.040	0.041	—0.97	0.333

[a]The numbers of males and females in the same enclosure indicate the no. of bears of different sexes with whom the study female bears had physical proximity during estrus.

[b]The numbers of males and females across fence indicate the no. of bears from different sexes with whom the study female bears had only auditory, visual, and olfactory proximity (i.e., not in the same enclosure but sharing a fence) during estrus.

Table 3. Results of mixed-effects binomial logistic regression analysis on the data from 4 sloth bears (*Melursus ursinus*) that were moved to a different enclosure during the study period. The response vari- able was the number of days on which each bear exhibited genital rubbing out of the entire estrus length in each year. Random effects predictors included in-dividual bear ID and year of data collection. The data were collected over 3.5 years (1 Jun 2015 to 31 Dec 2018) from captive female sloth bears at the Agra Bear Rescue Facility, India.

Parameter Predictor	estimate	SE	*z*	*P*
No. of males in same enclosure[a]	3.238	1.165	2.78	0.005
No. of males across fence[b]	0.124	0.070	1.79	0.073

Discussion

We found that genital rubbing behavior in female sloth bears was positively associated with estrus, as indicated by vulvar swelling (i.e., visibility). The fact that female genital rubbing behavior was more likely to occur in the presence of males than females suggests that it is a form of scent-marking, playing a role in inter-sexual communication.

Scent communication has been documented in many species of ursids; however, the form of scent deposition varies between species, and the specific functional role of chemical communication has not been well-described for bear species other than the giant panda (Swaisgood et al. 2000). Available information on scent communication of the giant panda comes primarily from the long- term studies of captive individuals (Swaisgood et al. 2000, Lindburg et al. 2001, McGeehan et al. 2002). For the rest of the bear species, the few studies that exist come from wild bears and they primarily report scent-marking by males, not females. Green and Mattson (2003) observed that body rubbing (mostly the dorsal region) on trees by brown bears in the Yellowstone region (USA) peaked during the mating season. They did not specify whether it was the case for both males and females. However, Clapham et al. (2012) noticed that free-ranging adult male brown bears at Glendale Cove, British Columbia, Canada, scent-marked with greater frequency during the breeding season, although the adult females did not appear to increase scent-marking to advertise their estrous state. The males also appeared to use scent-marking for communicating dominance to conspecifics. Brown bears in general tended to choose conspicuous trees on frequently visited trails to scent-mark, and the scent-marking behavior and motor patterns changed with increasing age (Claphamet al. 2013, 2014). Taylor et al. (2015) observed that male American black bears scent-marked more than females during the breeding season.

To our knowledge, until now, anogenital scent-marking and its association with the estrous status in female ursids is well-described only in the giant panda (Swaisgoodet al. 2000, Lindburg et al. 2001, McGeehan et al. 2002, Nie et al. 2012). Chemical analysis of the scent marks from the giant pandas has shown that they include a number of compounds that can be used to identify the gender and the individual identity of the depositing animal (Hagey and Macdonald 2003). Of note, the giant panda is the only bear to have a well-developed anogenital scent gland (Zhang et al. 2008), although anal scent sacs have been documented in brown bears (Rosell et al. 2011). However, no information is available on the presence of anal glands or scent sacs in sloth bears. Studies on estrous behavior in captive populations of giant pandas have shown that female giant pandas increase scent-marking through anogenital rubbing and/or urination during estrus (Swaisgood et al.

2000), with rates of marking peaking about 4–8 days prior to ovulation (Lindburg et al. 2001). Nie et al. (2012) found evidence suggesting that free-ranging female giant pandas also scent-marked more during the breeding season. Our data from the female sloth bears indicate that the females scent-mark almost exclusively during breeding season through genital rubbing.

The influence of the proximity to males on the estrous cycle of females has been demonstrated in many species of domestic animals (Rekwot et al. 2001), as well as wild animals, such as the bandicoot rat (*Bandicota bengalensis;* Sahu and Ghosh 1982) and cotton-top tamarins (*Saguinus oedipus;* Widowski et al. 1992). The presence of ale in the same captive enclosure with a female has been shown to increase the probability of cycling in female sun bears (*Helarctos malayanus;* Frederick et al. 2013). There are, however, no data available on the effect of proximity to males and other females on reproductive behavior and scent-marking by female sloth bears. Our results indicate that physical access to a greater number of males during estrus can increase the occurrence of genital rubbing in female sloth bears. However, the presence of other females, both with and without direct physical access, does not affect this behavior. This finding provides evidence to support our hypothesis that genital rubbing in the sloth bear is a form of scent-marking used for intersexual communication. Although the *P*-values for the influence of proximity to males in some of our analyses were slightly above our threshold of 0.05, the overall results indicate the positive influence of male proximity on the genital rubbing by female bears in estrus (Amrhein et al. 2019).

Female brown bears are known to reproductively senesce with advancing age, especially after 15 years of age (Schwartz et al. 2003) and giant pandas show a dampened expression of estrus-associated marking behavior with age (M.A. Owen, unpublished data). The significant negative correlation of age with genital rubbing in female sloth bears might be a subtle indicator of reproductive senescence, similar to brown bears and giant pandas. Another possible explanation is the reduction in the overall activity levels of the sloth bear females with advancing age, as observed in giant pandas (Kleiman 1983).

The odors from the scent-markings of females in estrus are known to strongly influence and arouse sexual behavior in males of mammalian species (Ferkin et al. 2004, Cerda-Molina et al. 2006) and this pattern is well-established in giant pandas (Swaisgood et al. 2000, 2004). Our finding of the high rate of occurrence of genital rubbing by female sloth bears during estrus indicates that this might be one of the key factors in chemical communication via scent-marking for attracting a male partner in wild populations during the breeding season. Studies on polar bears (*Ursus maritimus*) have shown that the sex and reproductive status of conspecifics is conveyed via pedal scent, suggesting that scent is important for finding mates (Owen et al. 2014). Mating success can be a critical factor in population viability and ultimately survival of a species (Molnar et al. 2008), and sloth bear populations in the wild have patchy distributions (Yoganand et al. 2006);therefore, the role of this female scent-marking behavior may be vital in their reproduction dynamics. The evident effects of age, and the proximity to males, are thus of great importance. In addition, further studies are needed on the reproductive behavior and physiology of slothbears, as well as density-dependent effects on their reproductive success in the wild.

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Art – 227. SURGICAL MANAGEMENT OF GUNSHOT WOUND IN A RESCUED WILD SLOTH BEAR *(Melursus ursinus)* USING PLATELET RICH FIBRIN (PRF)

Arun A. Sha, Tista Joseph, **Ilayaraja**, Nithin and **Adhithyan**

Platelet-Rich Fibrin (PRF) is considered a bio-fuel for wound healing. It is an autologous fibrin matrix containing a large number of platelets and leukocyte cytokines. Platelets release numerous growth factors that play a crucial role in wound healing. Platelet rich fibrin provides sustained release of growth factor at the site of healing. The present study was conducted in a rescued sloth bear housed at Bannerghatta bear rescue centre who had suspected for gunshot wound on the right-side shoulder. The wound was two centimeter deep and 3cm wide. After a month of the injury, the wound was partially healed, but the entry wound was infected and there was a frequent discharge of purulent exudate from the wound. The animal was immobilized using Xylazine Ketamine combination, 1Oml blood was collected in a serum separator tube. Blood was centrifuged at 3000 rpm for 10 minutes and the middle portion containing the fibrin clot was collected in the wound edges were debrided and filled with the semisolid PRF, the procedure was repeated for three times with one week interval, and the wound was sutured using catgut during the final session. The animal showed remarkable improvement following the procedure and complete healing was observed by end of the second week. PRF is mainly used in periodontal and soft tissue surgeries. The benefits of PRF in wildlife veterinary practice still remains relatively unexplored and holds potential. The results observed in the current study revealed a promising avenue that can be further studied to a more significant effect.

Art – 228. THE SUBTLE ART OF ANIMAL WELFARE AND ETHICS IN VETERINARY PRACTICE

Arun A. Sha

Homosapiens (wise man' in Latin) have for long assumed a place for themselves atop the evolutionary pyramid. Refined ability to reason and finesse in experiencing and expressing complex emotions are some primary criteria cited as reasons. Exponential technological advancements, increased exposure to comfort and extreme isolation from other species have resulted in us becoming self-centric leading to the risk of losing our subtle yet profound trait of being 'humane. The Subtle Art of Animal Welfare a Ethics in Veterinary Practice rekindles and sensitizes our approach towards animals by including examples and interactive discussions with the intention of reinforcing our awareness, understanding and approach regarding the subject.

The genesis of animal ethics and its current understanding:

Ethics stem from the moral dimensions that back our everyday decisions. There is consistent evidence of 'respect' towards animals in the Indian sub-continent that can be found in the ancient scriptures. Reference to Kamadhenu, the miraculous 'all giving' cow in Hindu mythology and the many animals each attributed as the official mascot of different gods namely the spectacled cobra (*Naja naja*) associated with Lord Shiva, Peacock (*Pavo cristatus*) associated with Lord Subramanya, Garuda the Kite (*Haliastur Indus*) associated with Lord Vishnu or even the much hated rodent species like the Mice (*Mus booduga*) associated with Lord Ganesh all point towards our reverence towards fellow species and an apparent attempt to co-exist.

Modern philosophers and thinkers from the West have debated elaborately on the necessity for being ethical towards animals and thereafter about the degree to which ethics is applicable to animals. While some have discounted the very idea of showing moral consideration to these sentient beings, others have had a more consequentialist or obligatory approach in this regard.

Modern ethical theories namely Contractarian theory, Utilitarian theory, the theory of Animal Rights, Relational views, and Respect for Nature each reflect on the three perspectives mentioned earlier.

Criticism:

Ethical theories have been subjected to their share of criticism, understandably due to the complexity of moral values and variety of ethical theory. Ethical theories are neither infinitely varied collection of subjective, private judgements nor are they mere personal preferences. They are instead general agreements with objective reasons on rights and wrongs pertaining to our actions that facilitate a harmonious existence in the society.

Animal Welfare:

The science of animal welfare concerns how sentient animals experience their life in terms of their physical functioning, mental state/feelings and natural behaviours. A change in one of these aspects could very much result in the welfare of the animal being compromised. Application of the modern ethical theories to areas concerning animal welfare will yield a holistic view and help set a tone to the line of action. Breeding dogs/captive wild animals with heritable defects, farming animals for meat, controlling infectious diseases in animals (zoonotic or otherwise) and use of animals in research are some areas to be covered taking into consideration the socio-economic angle. A discussion on standard veterinary practices in the above mentioned areas of concern shall help in arriving at a common consensus within the veterinary community to better the animals' welfare.

The Wild Twist:

Specific focus on welfare and ethics for wild animals in rehabilitation will help refine our perspective in understanding the subject considering the rarity of these species where requirements are far more stringent and laws far less lenient, taking into consideration the five freedoms of animal welfare.

While most areas of concern superimpose ethical theories on domestic animals, welfare and ethics for wild animals in rehabilitation require special attention for the following reasons:

a. Wild animals inherently belonging to the wild (read away from anthropogenic pressures) are rescued (and rehabilitated if necessary) with minimum interference to relieve it from distress caused by anthropogenic forces. Ex: Animals that can be remotely assisted and empowered to help itself should never be brought into captivity. So, an orphan animal must not be displaced from the wild before ascertaining the whereabouts of the mother and attempt to reunion.

b. Humans not being a natural part of a wild animal's world, an animal in captivity deserves optimum care from its caregivers considering its rescue itself to be a welfare initiative. Ex: Avoiding public display of captive wild animals under rehabilitation as human contact is frightening for them.

c. Exposing wildlife to public might be a requirement to increase awareness and prevent further breach in the animals' welfare and ethics. For this, a responsible roadmap towards creating public empathy towards the animals must be done with the help of animals that are habituated to people. Note: Failure to create empathy might result in stimulating people to possess these wild animals as pets, indirectly encouraging trade in exotic species which would prove detrimental to the animal.

d. Rehabilitation of wild animals is a laborious process with the time taken often varying within species and also between species. Although general guidelines are available for their release, a careful observation of the animal's behaviour /instinct and readiness is a must. Ex: Enrichment (behavioural and feeding) of wild animal before being considered for release.

e. Release of rehabilitated animals is a thorough process of selecting a site (usually closest to the site of rescue) to ensure the site will cater to all the needs of the animal. IUCN's guidelines for re-introduction and translocation will aide in a successful release. A drastic change in environmental factors at the site where the animal was rescued may result in searching for another suitable site for release. A complete health checkup of the animal to ensure it doesn't carry diseases that could spread to other species is a must.

f. To be conservation oriented is extremely important in rehabilitating wild animals. A welfare initiative however significant should not compromise the existence of a wild population. Ex: Releasing non-native to the wild might a. Expose the species to unfamiliar predators and lack of cover from them. b. Invasive species can detrimental effects on native flora and fauna.

g. Empowering communities works both ways. In a country like India where there is a clear divide between ancient and modern, rich and poor, traditions and the lack of respect for it, it is challenging to achieve empowerment by eradicating myths and false beliefs on one side and sensitising those living a fast, self-centric life on the other.

With eighteen plus years of experience in rescuing and rehabilitating wild animals which include Sloth Bears (Melursus ursinus), Leopards (Panthera pardus), Indian Elephant (Elephas maximus indicus), Snakes of different species (Suborder: Surpentes) and Striped Hyenas (Hyaena hyaena), it would be humbling to discuss the subject of animal welfare a ethics with respect to our wild friends that are perhaps equally deserving if not more than their domestic counterparts in leadinga life with dignity, at least in their later years.

Art – 229. CLINICAL MANAGEMENT OF INTESTINAL IMPACTION AND COLIC IN AN ASIAN ELEPHANT

Yaduraj Khadpekar, **Ilayaraja Selvaraj, Arun. A. Sha** and M. Kamalanathan

Abstract

Intestinal impaction and resultant colic is a common non-infectious disease condition reported in captive Asian elephants. If not detected and treated in time, it can prove fatal for the animal. A 16-year-old female Asian elephant presented with severe colic, a distended abdomen, absent food and water intake and inability to pass dung. The case was diagnosed as intestinal impaction on clinical signs and history. It was successfully treated with the administration of NSAIDs, stool softeners, systemic fluids, antibiotics, and encouraging physical activity.

Introduction

Intestinal impaction is not an uncommon condition affecting the Asian elephant (*Elephas maximus*) in captivity (Chandrasekharan *et al*. 2009; Sarma 2011). It is a non-infectious disease condition resulting from partial or complete obstruction of the intestinal tract by undigested food material, foreign bodies or a hard faecal bolus (Dumonceaux 2006). We have examined 15 such cases in captive Asian elephants and in all of them, a hard faecal mass was the cause of impaction. It can be very painful and uncomfortable for the elephant and, if not diagnosed and treated in time, can prove fatal. Compared to other species, surgical management of intestinal impaction in elephants is difficult and challenging, because of the large visceral cavity, large intestinal volume, and higher chances of incision dehiscence. Therefore, therapeutic management is the preferred mode of treatment. Here we describe one such case of intestinal impaction in an Asian elephant and its clinical management.

Case details

A sixteen-year-old female Asian elephant presented with a history of rapid bloating of the abdomen and lack of defaecation for over 7 h. The animal was reported to have fed normally on fodder of sorghum crop till evening. At around 8:30 pm, the keeper had noticed the elephant showing signs of discomfort, groaning, and with a bloated abdomen, and had immediately reported it. Clinical examination was carried out within 2 h of reporting of the case.

On observation, the elephant exhibited symptoms of tenesmus like frequent squatting and straining to expel the faeces, along with restlessness, trunk biting, frequently sitting down and standing, inappetence, an abnormal posture with hind legs spread apart, and groaning. A pear-shaped bloated abdomen was noticed on physical examination (Fig. 1). The elephant continued to exhibit symptoms such as frequent sitting and standing, changing positions, groaning, tenesmus, intermittent open mouth gaping, and restlessness, suggestive of severe colic. Analysis of CCTV footage showed that the elephant had not defecated after 1:00 pm. The rectal temperature was recorded to be normal (37.1ºC). Per-rectal examination was carried out to check for any object or faecal bolus causing obstruction in the rectum. However, the rectum was found to be empty. The condition was tentatively diagnosed as colic resulting from intestinal obstruction, and immediate treatment was initiated.

Figure 1. The pear-shaped bloated abdomen of the elephant.

An attempt was made to administer 00 ml of antacid and anti-bloat syrup (DIGENE®, Abbott India Limited, Goa, India) orally to the elephant. But it was unsuccessful as the elephant was reluctant to take anything orally and refused to swallow the syrup.

The body weight of the elephant was known to be 3300 kg from recent records. The non- steroidal anti-inflammatory drug meloxicam (MELONEX POWER™, Intas pharmaceuticalsLtd., Ahmedabad, India) was administered intramuscularly at a dosage of 0.1 mg/kg body weight to give relief from pain. Ceftriaxone sodium with Tazobactum (Inj. INTACEF TAZO®, Intas pharmaceuticals Ltd., Ahmedabad, India) at a dosage of 4 mg/kg was administered intravenously to avoid secondary infections from an obstructive mass and from the affected intestinal wall (Greene *et al*. 2018). An enema wasgiven with 1800 ml liquid paraffin, by inserting a 1.3-cm-diameter flexible tube approximately 4 feet inside the rectum and injecting paraffin with a 400 ml plastic syringeconnected to the tube (Fig. 2). The tube was lubricated with liquid paraffin before insertion to avoid injury to the rectal mucosa.

Figure 2. The assembly of flexible tube and 400ml syringe used for giving liquid paraffin enema.

After the enema, the elephant was encouraged to walk and sit down and stand up a few times. Within two hours of the enema, the bloating started reducing and the elephant released some gas rectally. The frequency and intensity of colic symptoms also reduced. But signs of tenesmus persisted. Within the next two hours, the animal developed bloating again, with the recurrence of severe colic symptoms.

Enema was given again with 35 l lukewarm water mixed with 2 l of liquid paraffin. The enema mixture was kept in a plastic bucket, and a 40-watt submersible electric pump was immersed in it. The 1.3-cm-diameter flexible tube was connected to the pump.The other end of the tube was inserted about 4 feet inside the rectum. The enema mixturewas administered by running the pump (Fig. 3). Approximately one third of the mixture flushed back out. However, the remaining mix was retained inside.

Figure 3. The assembly of bucket, motorised pump and flexible tube used for giving lukewarm water and paraffin mixture enema to the elephant.

The animal was given a long walk of about 4km early morning. She did not defecate during the walk but passed gas rectally, thus relieving the bloating. Fluid therapy was initiated with intravenous administration of 5.5 l of Ringer Lactate (RL, Inven Pharmaceuticals Pvt. Ltd., Dhar, India), 3.5 l of 5% Dextrose Normal Saline (DNS, Inven Pharmaceuticals Pvt. Ltd., Dhar, India), 1 l of multi-electrolytes (K-LYTE, Kunal remedies Pvt. Ltd., Lucknow, India) and 600 ml of amino acids (ASTYMIN-3, Tablets (India) Ltd., Chennai, India).

Around 10:50 am, the animal urinated. The urine volume was less than normal. The rectal temperature at this point was recorded as 36.4ºC. Blood samples were collected for lab analysis. The serum biochemistry and hematologic values were found to be normal. The PCV was 31.5%, indicating that the levels of hydration were optimal with the systemic and rectal administration of fluids (Sarma 2011). A digestive mixture containing 100 g ground ginger, 100 g asafoetida, 100 g ground garlic, 100 g black salt, and 500 g jaggery, was offered to the elephant, which she ate.

Around 3:30 pm (after 17.5 h of therapy), the animal started voluntarily eating a small quantity (2 kg) of fruits. She was then given 800 ml of liquid paraffin, 200 ml of a laxative syrup (CREMAFFIN, Abbott India Limited, Goa, India), and 3 ml digestive tonic (DIGIVET®,Hivet, Haryana, India) orally.

Around 6:10 pm, the elephant started eating sorghum crop fodder and at 9:50 pm she defaecated after 32 h. A single bolus was expelled, which was large, elongated, dry and hard (Fig. 4). The elephant immediately started to drink water and consume fodder normally. Intravenous administration of INTACEF TAZO was repeated along with 12 l of K-LYTE infusion. Liquid paraffin 400 ml and 3 ml of DIGIVET® tonic were given again orally. It was advised to keep the elephant separate from other elephants and allow her to take only 40 kg of sorghum crop fodder with water to drink ad-libitum. Rectal temperature was recorded as 35.8ºC.

Figure 4. The first faecal bolus expelled by the elephant after 32 h without defaecation

During this second night till the next day morning at 9:30 am, the elephant defecated 5 times and drank about 60 l of water. The bloat subsided completely (Fig. 5). She was given long walks both in the morning and evening. She defecated two more times before 5:30 pm during the day. Softened surfaces and borders of the dung balls indicated efficient stool softening action of the liquid paraffin, which helped the obstructing dung mass to pass smoothly through the rectum.

Figure 5. The bloat subsided after the treatment and expulsion of impacting faecal mass

The course of antibiotic and oral liquid paraffin (400 ml) once a day, and 3 ml Digivet thrice a day, was continued for the next 3 days. Oral probiotic (ECOTAS™, Intas pharmaceuticals ltd. Ahmedabad, India) at a dosage of 6 boli once a day, was given for 5 days.

Discussion

Elephants are hindgut fermenters and pass large amounts of low-quality forage through the gastrointestinal tract in a short period of time (Clauss *et al*. 2003). Fermentation and digestion mostly happen in the caecum and colon. The gastrointestinal transit time in captive Asian elephants is 21–55 h (Dierenfeld 2006). Asian Elephants usually drink 200–255 l of water a day, consuming 50–60 l at a time (Cheeran 2009).

Intestinal impaction, either complete or partial, is known to be caused by undigested food, ingestion of dirt, foreign bodies, clay, or sand that becomes impacted in the gastrointestinal tract. Old elephants are prone to impaction from partially digested food due to problems in mastication resulting from worn out teeth (Greene *et al*. 2018). Consumption of large amounts of feed with high fiber content in a short period of time also causes obstruction, leading to colic. There luctance to drink water due to the colic results in dehydration thus aggravating the intestinal stasis (Dumonceaux 2006). Cases have also been reported resulting from the consumption of large amounts of raw rice paddy (Sarma 2011). Once dehydration arises, it is followed by electrolyte imbalance, endotoxic shock, and circulatory collapse. The condition may lead to recumbency and death in the case of per acute colic.

The first vital sign noticed in intestinal impaction is a lack of defecation for a periodlonger than normal, which is accompanied in many cases by bloating of the abdomen. A healthy elephant defecates about 15–20 times a day (Cheeran 2009). If an elephant hasnot defecated for long, it may be suffering from a serious digestive disorder, such as intestinal impaction, intussusception or volvulus. Following the lack of defecation, animalwill stop feeding and show signs of colic. Based on the severity of the symptoms and duration, the colic could be per-acute (severe), acute (moderate) or chronic (mild). The typical symptoms of colic in elephants are kicking the belly, frequently sitting down and getting up, lying down and flipping over from side to side. In severe colic, these symptomsare accompanied by frequent opening of mouth (yawning appearance) and holding andbiting the trunk in the mouth. Elephants, showing such symptoms with the absence of appetite and defecation for a considerable time, could have partial or complete intestinal obstruction, volvulus or intussusception. The history is vital in differentiating between these. For example, if the animal was noted eating soil, or there was a sudden change in diet, overeating, drinking less water, etc., obstruction is likely. Elephants suffering from arthritis or lameness with reduced movement and lack of exercise may also be prone to reduced gut motility and thus impaction. On the other hand, a history ofincidents such as falls, symptoms of aerophagia or sudden changes in diet may indicate volvulus or intussusception (Wiedner *et al*. 2012). In most cases, we have observed that impaction and colic occurred mainly due to factors like reduced gut movement, dehydration, or inadequate exercise, rather than from ingested items.

The first step in suspected intestinal impaction is a per-rectal examination to check for an obstructive mass in the rectum. In many cases, a large dry dung mass stuck in the anterior portion of the rectum maybe found. This mass can be slowly and gently pulled out by lubricating it with liquid paraffin. The examination also helps assess rectal motility. The administration of analgesics such as NSAIDS during initial stages of treatment helps reduce colic, and thus to calm down the elephant, which makes further treatment easier.

If the obstructive mass is not within reach during the per-rectal examination, further treatment should be carried out. In most such cases, elephants are not receptive to oral medications. Thus, an enema with a stool softener should be given after administration of an NSAID. Liquid paraffin is excellent for this purpose. Intravenous fluid therapy must be initiated as soon as possible to avoid severe dehydration and for electrolyte replacement as well as for intravenous administration of medications. In elephants, the large volumes of fluid required for maintenance is a challenge. As per the recommended dose of 40–60 ml/kg/day, an adult elephant would need to be administered 120–160 l of fluids in 24 h, which is challenging under field conditions. Therefore, intravenous fluid administration can be supported by rectal administration of fluids (Mikota 2006). Rectal administration of a lukewarm solution of oral rehydration salts such as ORS (Cipla, Mumbai, India) in water, is efficient for maintaining fluid and electrolyte balance. In cases of suspected infection, a course of systemic antibiotics needs to be given for 3 ormore days depending on the time taken by the animal for recovery.

Clinical recovery is generally rapid after the elephant expels the obstructing mass, which in most cases is a large ball of dry, undigested food material. Depending on the size and quantity of the obstructing mass, and the part of the intestines it was stuck in, it may take several hours after initiation of treatment for the elephant to expel the mass. The longest time we have observed for the expulsion of an obstructing mass was 46 h. Even after complete clinical recovery, continuation of oral rehydration solution is recommended for a few days to avoid the recurrence of impaction from dehydration, especially in hot climatic conditions. During recovery, it is advisable not to put the elephant back on standard quantities of diet immediately but to give lesser quantities for the first 24 h. Routine exercise must be encouraged to promote normal gut motility. Giving exercise walks, or if that is not possible, distributing feed in different locations of the enclosure to encourage walking is a good practice (Ullrey *et al*. 1997).

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Art – 230. REUNION WITH THE MOTHER: A SUCCESSFUL REHABILITATION STRATEGY FOR DISPLACED WILD RUSTY-SPOTTED CAT *Prionailurus rubiginosus* (I. GEOFFROY SAINT-HILAIRE, 1831) (MAMMALIA: CARNIVORA: FELIDAE) KITTENS

Ajay Deshmukh, Yaduraj Khadpekar, **Mahendra Dhore** and **M.V. Baijuraj**

Abstract

One of the common challenges for wildlife rehabilitators and conservationists is dealing with displaced young animals, needing intervention and help. Most commonly, such displaced animals are moved to zoos or rescue centers where they are hand-raised. In some cases, the hand-raised animals are rehabilitated back in the wild following suitable protocols. For young animals that are not injured or ill, however, reuniting them with their mothers in the wild might be the best option. There are few reports on such reunion efforts. We report successful reunions of 26 Rusty-spotted Cat *Prionailurus rubiginosus* kittens with their mothers in the period of six years in the Junnar Forest Division, Maharashtra, India. The kittens found by the villagers were examined for injuries or signs of sickness, and physiological parameters were recorded. If found healthy, they were placed in a plastic basket at the same location in the evening of the same day for a reunion with their mothers. In all cases, the mother cat was in the vicinity and took the kittens away after a brief period. The success of reunion effort was confirmed by direct observation or vocalization of the kittens combined with the presence of pugmarks of an adult cat at the site, or just by the presence and appearance of pugmarks. The results of our efforts show that displaced kittens of small wild cats can be successfully reunited with their mothers, provided that the time gap between separation and reunion effort is minimized.

Keywords: Displaced wildlife, human-wildlife interaction, small wild cat, wildlife rehabilitation, wildlife rescue.

Introduction

Displacement and separation of young animals from their mothers is not an uncommon occurrence in the wild. Conservationists and wildlife rehabilitators around the world frequently come across young wild animals without their mother, needing help.

There are a number of reasons why the young animals may get separated from their mothers, such as natural calamities (Barman et al. 2014), human activities (McTurk & Spelman 2005), death of the mother due to hunting (Pajetnov & Pajetnov1998) or natural causes (Singh et al. 2011). The common strategies followed for such rescued young animals are hand-raising them to rehabilitate to the wild, or taking them permanently to a captive facility such as a zoo or a lifetime care facility and hand-raise them there. Hand-raised animals have been successfully rehabilitated in the wild such as in case of Asian Elephants *Elephas maximus* (Perera et al. 2018), Greater One-horned Rhinoceros *Rhinoceros unicornis* (Barman et al. 2014), Common Wombat *Vombatus ursinus* (Saran et al. 2011), and Giant Otter *Pteronura brasiliensis* (McTurk & Spelman 2005).

Another option that is less frequently followed, is the immediate or soonest possible reunion of the displaced young animal with its mother. For the young animals that are temporarily separated from their mothers and are not injured or ill, this might be the best suitable option (Sparks & Casey 1998). Indeed, such reunions have been successfully accomplished for infants of wildlife as diverse as the Western Chimpanzee *Pan troglodytes verus* (Pruetz & Kante 2010) and the Giant Otter (Lima & Marmontel 2011). In our knowledge, however, organised efforts for the reunion of cubs or kittens of wild cats with their mothers have not been reported so far.

The Rusty-spotted Cat *Prionailurus rubiginosus* is the smallest cat species in Asia (Menon 2014; Nayak et al. 2017). It is resident in India, Nepal, and Sri Lanka (Mukherjee et al. 2016). The population in India is thought to be fragmented as intensiveirrigated agriculture negatively impacted its prime habitat; dry and moist deciduous forests (Mukherjee et al. 2016). Although the data on the current population trend are scarce, the species is currently categorised as Near Threatened on the IUCN Red List (Mukherjee et al. 2016). The authors have come across many displaced Rusty-spotted Catkittens that were in a situation where they could be reunited with their mothers. Exceptfor one report on the possible natural reunion of a kitten with its mother (Sharma 2007),there is no other recordof reuniting Rusty-spotted Cat kittens with their mothers in situ. Therefore, this may be the first report on successfully reuniting multiple Rusty-spotted Cat kittens with their mothers in the wild.

Study Area

All the reunions occurred within the Junnar Forest Division in Pune District in the state of Maharashtra, India (Fig. 1). The terrain of the area is made up of the northern part of the Western Ghats with hills and valleys. The hills do not have many large trees but are mostly grassy with boulders. The most common occupation in the area is farming, and the crops of sugarcane, grapes, and onion dominate the cultivated parts of the valleys (Athreya et al. 2011). Due to the large areas of sugarcane cultivation (Image 1), which provide suitable habitat and cover for the Leopard *Panthera pardus*, the division is known for high Leopard density and a close co-existence of Leopards and humans (Jhamvar-Shingote & Schuett 2013). Although there are no published records of Rusty-spotted Cat in the division before this report, the species has been recorded during camera trap surveys in adjoining areas (Athreya et al. 2016).

A wildlife rescue team lead by Ajay Deshmukh and Mahendra Dhore had been working in the study area since 2009 for the mitigation of human-Leopard conflict, and thus are well-known to the local villagers and the forest department officials.

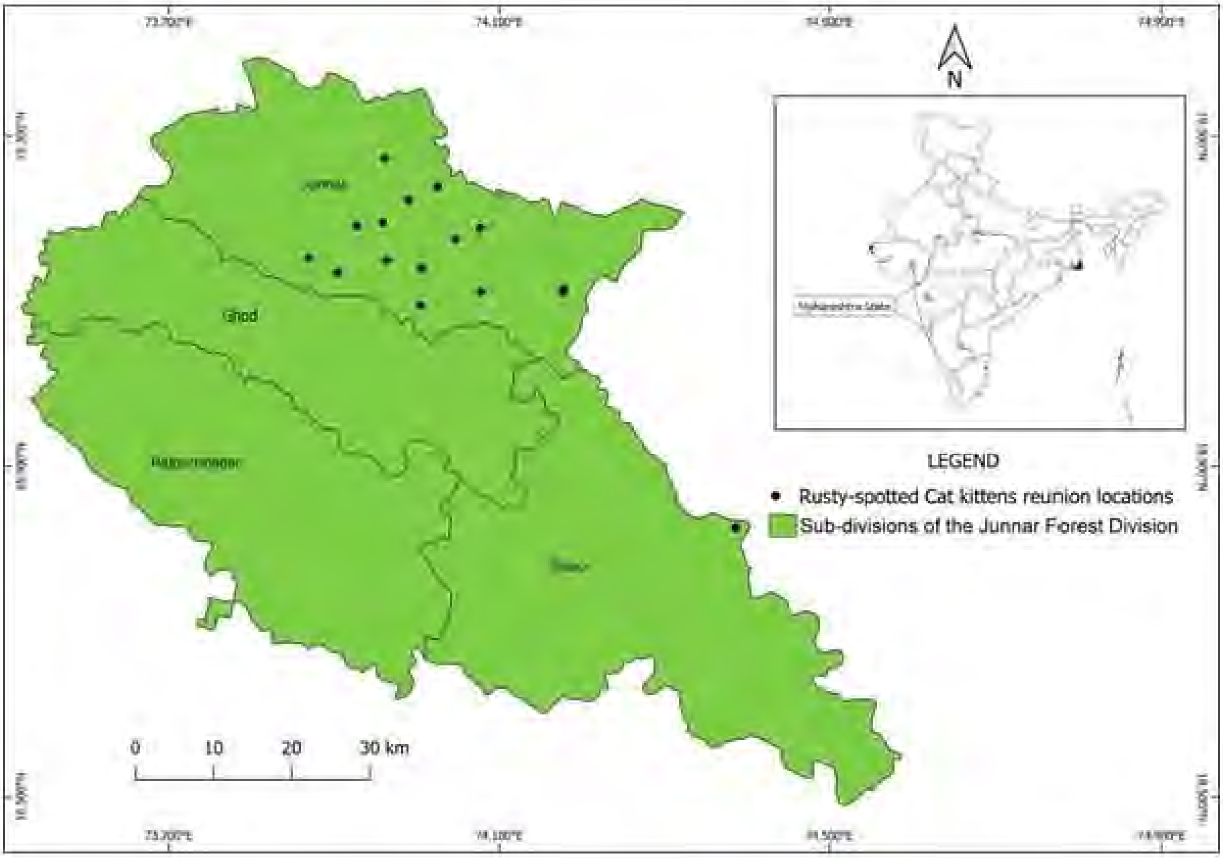

Figure 1. Junnar Forest Division in the state of Maharashtra, India, where all the reunions of Rusty-spotted Cat kittens with their mothers were carried out. The sections inside the main map indicate the sub-divisions within the Junnar Forest Division. The green portion in the small inset map shows the location of the Junnar Forest Division in India.

Image 1. Habitat with sugarcane fields in the study area. © Akash Dolas, Wildlife SOS.

Materials And Methods

When the kittens were handed over to the rescue team, they were first placed in a plastic basket and moved away from the location. They were transported to either the nearest forest department office or to the rescue team office. There they were checked for any visible injuries. Their ages were estimated based on their body size and locomotor abilities described by Dmoch (1997). Physiological parameters such as rectal temperature, heart rate (HR; heart beats/minute), and respiration rate (RR; respirations/minute) were recorded. Once the kittens were found to be healthy through this examination, they were left undisturbed in the basket in a quiet area. No attempts were made to feed them before reunion. For the reunion attempt with the mother, they were taken in the evening to the exact spot where they were found. The basket with the kittens was left on the ground at the location. This procedure was always carried out after 18.00h to minimise the possibility of disturbance by human activities, and considering the crepuscular and nocturnal activity pattern of Rusty- spotted Cat (Nimalrathna et al. 2019; Bora et al. 2020). When possible, the rescue team of two to three members observed the kittens with the help of spotlights from a distance of 200–300 m until the mother cameand picked them up. At other times, the rescue team moved 500– 1,000 m away and went back periodically to check if the kittens were safe. If the kittens were not in the basket any more, the area was examined for pugmarks and signs of predation, to make sure that the kittens had been picked up by the mother. The reunion was confirmed by the direct observation of an adult female picking up and carrying the kittens away, or by the mewing sounds of the kittens combined with the presence of pugmarks of an adult Rusty-spotted Cat at the location, or just by the presence and appearance of pugmarks. For all the reunion events, we recorded the approximate time when the kittens were first spotted, the time when they were kept at the location for the reunion, and the estimated time when the mother took them.

Results

A total of 26 kittens were reunited with their mothers in 18 reunion events between 2014 and 2019 (Table 1). Eight of these reunions involved a litter of two kittens. Out of these, four litters included a male and a female kitten. Their estimated ages ranged between 30 and 60days.

Table 1. Temporal details of reunion events with approximate time when kittens were spotted by the villagers (Rescue time), time when the kittens were taken to the location for reunion (Return time), and estimated time when the kittens were picked up by the mother cat (Reunion time).

Reunion event	Month and year	Rescue time	Return time	Reunion time
1	iv.2014	14.30h	18.45h	22.30h
2	x.2014	13.15h	18.20h	21.20h
3	xi.2014	13.20h	18.20h	21.20h
4	iv.2015	14.45h	18.35h	21.35h
5	x.2015	14.40h	18.30h	21.30h
6	xi.2015	14.45h	18.35h	21.35h
7	v.2016	14.05h	18.10h	22.30h
8	ix.2016	14.20h	18.20h	22.40h
9	xii.2016	14.10h	18.35h	20.35h
10	iv.2017	13.20h	18.20h	22.00h
11	x.2017	12.25h	18.00h	20.00h
12	xi.2017	13.30h	18.10h	19.15h
13	iv.2018	13.25h	18.35h	21.35h
14	x.2018	13.35h	18.40h	21.40h
15	xi.2018	13.25h	18.35h	21.35h
16	iv.2019	15.20h	19.10h	21.10h
17	viii.2019	11.30h	18.15h	22.15h
18	viii.2019	13.10h	18.05h	22.05h

The presence of kittens was reported to the rescue team by either the villagers or the forest department staff. In all the cases, the kittens were first spotted by the villagers and reported to either the forest department or to the rescue team. All kittens were found in sugarcane fields (Image 2). In instances when the rescue team was directly informed by villagers, the team conveyed the information to the relevant forest department officials for further actions and coordination. It is important to mention here that in about 80% of instances, the kittens were picked up and handled by the villagers before the rescue team could reach the location. At other times, even though the kittens were not picked up by the villagers, they had to be removed from the location to avoid stress from human activities, crowding, noise, and possibility of handling by the villagers.

Image 2. A Rusty-spotted Cat kitten that was found in a sugarcane field © Akash Dolas, Wildlife SOS.

During the assessment of physiological parameters, the minimum rectal temperature recorded was 37.8^{o}C while the maximum was 38.9^{o}C. The mean rectal temperature among all kittens was 38.4^{o}C (n=26). RR ranged between 16–40, and the HR was within the range of 126–180. It was not possible to measure the body weights of all the kittens, however, the body weights of the kittens that were weighed (n=10) ranged from 100g to 300g. The highest number of kittens were found in the early summer or early winter during the sugarcane harvesting season (Fig. 2). No kittens were reported in late winter or late summer. The villagers spotted the kittens during mid-day between 11.00 and 15.30 h. All the reunions of the rescued kittens occurred at late evening or night on the same day they were found. Among all the reunion efforts, the maximum time gap between finding a kitten and taking it back for the reunion, was >6h 45min. After the kittens were brought to the location for the reunion, the minimum time recorded for a successful reunion was 1h 5 min, while the maximum was 4h 20min.

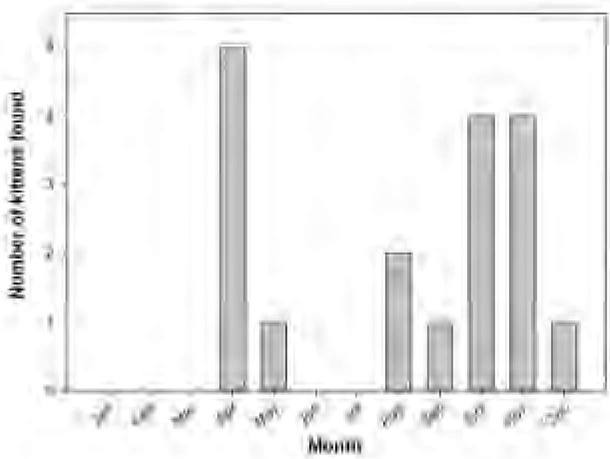

Figure 2. The highest number of displaced Rusty-spotted Cat kittens were found in early summer (April), followed by early winter (October and November).

During the reunions that the rescue team members were able to observe directly, the mother cats were noticed to approach the baskets with the kittens very slowly and cautiously. During approach, they stopped and looked around frequently before continuing the approach. Many times, the mother sat 3–6 m away from the basket for 20 to 30 minutes and kept looking around, before she approached the basket. In some instances, after such pause, the mother walked around the basket keeping some distance and sat down again for some period before approaching the basket. The kittens were noticed to become very active and vocal with a lot of mewing, once they noticed the mother approaching. They kept looking in the direction of the mother until she reached the basket. After reaching the basket, the mothers licked the kittens for some time before picking and carrying them away. When there were more than one kitten, the mother carried away one kitten first, kept it inside the crop cover, and came back for the second kitten with the same cautious approach.

Discussion

All of the 18 reunion attempts were considered to be successful, as the female Rusty-spotted Cats came to pick up the kittens within > 4h 20min. No kitten was lost to predation. We observed that the females remained in the vicinity of the locations where the kittens were found. Under favourable conditions such as same location, late evening hours, and no disturbance from people, they accepted the kittens back and carried them away. The key to successfully reuniting the mother and kittens is to provide this opportunity to them as quickly as possible after separation.

All the kittens were found during the sugarcane harvesting season, indicating that sugarcane fields provide cover for females with kittens and access to prey. Arthreya (2010) also reported two kittens found during the cutting of sugarcane in October and December 2008 in Ahmednagar District, Maharashtra. Leopard and Sunda Leopard Cats *Prionailurus javanensis* were also found in sugarcane fields, apparently attracted by a wide variety of prey (Jhamvar-Shingote & Schuett 2013; Lorica& Heaney 2013).

The Rusty-spotted Cat exhibits some tolerance for human-modified habitats and the vicinity of human settlements (Nowell & Jackson 1996; Athreya 2010). In one occasional instance, a Rusty-spotted Cat even gave birth in a farmhouse (Nowell & Jackson 1996).

In captivity, the Rusty-spotted Cat gives birth at any time of the year (Dmoch 1997). We came across approximately one to two months old kittens, mostly in April, also in October and November (Fig. 2). This might be due to the seasonality of crop harvesting in the region. The sugarcane harvest in the region generally begins in October with the opening of sugar factories. While in April, the speed of harvest in the last remaining sugarcane fields is increased by farmers as the sugar factories are about to close. This possibly results in an increased sighting of Rusty-spotted Cat kittens by villagers in sugarcane fields. Many times, the kittens were picked up and moved by the farmers as they wanted to urgently continue the sugarcane harvest. In a few cases, the kittens were mistaken by the villagers as Leopard cubs and were, therefore, moved away from the field for their own safety. In captivity, Rusty-spotted Cat kittens start moving about at the age of one month but frequently get tired and fall asleep (Dmoch 1997). Weaning begins at the age of 35 to 40 days, but suckling is continued in some cases until two months of age (Dmoch 1997). Information on the maternal care and development of kittens of the

Rusty-spotted Cat in the wild is lacking. We speculate that the kittens were spotted by the people when the mother had left them for a brief period for hunting and feeding. In each of these events, the rescue team members made efforts to communicate with the villagers and farmers involved and give them information aboutRusty-spotted Cat and Leopards. The members also requested the villagers not to handleand remove the kittens they come across and inform forest department officials about their presence. Although there is no information available on normal physiological parameters in Rusty-spotted Cat, the parameters recorded in the rescued kittens were considered to be healthy as compared to the Domestic Cat *Felis catus* (Eldredge et al. 2011). None of the kittens exhibited any sign of sickness and were considered to be healthy and fit for the reunion.

Minimizing the time of separation between kittens and mothers appears to be an important contributing factor for a successful reunion. Leopard females in the wild are known to accept their cubs after a separation period as long as six days (Ajay Deshmukh unpub.). During such long periods, however, attempts were made on each night for the reunion. Our experiences from similar reunion attempts for displaced Leopard cubs indicate that the frequency of the mother returning to the location to look for cubs reduces with the increasing time gap between the separation and the reunion attempt. In case of the reunited Rusty-spotted Cat kittens, all the reunions happened on the evening of the same day. The mother cats were in the vicinity of the location where the kittens were found and returned on presumably hearing the kittens' vocalizations. None of the reunion events needed deployment of any artificial means to attract the mother tothe kittens. We conclude that displaced Rusty-spotted Cat kittens can be successfully reunited with their mothers, provided that the time gap between the finding of kittens and reunion attempt is minimized.

We do not have any evidence that a delay of more than 24 hours would reduce the chance for a successful reunion. We, however, recommend that any reunion attempt should be made in the evening to minimize the disturbance from human activities, and that the handling of kittens before the reunion should be kept minimum. If the reunion does not happen on the same day, attempts should be made on the following nights until the absence of the mother in the vicinity is confirmed. Based on our experience with Rusty-spotted Cats and Leopards, such reunion protocols can be replicated for the kittens or cubs of other wild cat species.

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Art – 231. HAND-REARING OF SLOTH BEAR NEONATES AND ITS NUTRITIONAL REQUIREMENTS - SHARING EXPERIENCES OF FIFTEEN YEAR

A. Sha. Arun, S. llayaraja and **Baiju Raj**

Abstract

The review is based on our study and experiences of past two decades with hand-rearing of over ninty (90) Sloth bear cubs of different age groups (2 weeks - 2 years) which were confiscated or rescued from various situations like forceful early weaning due to illegal poaching, minimizing the chances of the cubs being completely deprived or received minimal quantity of mother's colostrum milk, making them vulnerable to various nutrition-based health issues, in addition to physiological and psychological stress. The data is based on the study and development of balanced diet for sloth bear cubs according to their essential body nutrients, body weight and age.

Keywords: Hand rearing; *Melursus ursinus;* neonate; nutrition, sloth bear

Introduction

In India, sloth bears have been widely used for 400 years old barbaric practice of 'dancing bears'. Thousands of sloth bear cubs were poached mercilessly and the mother bears brutally killed so that poachers could meet the demands created by the Kalandar (madaris) community. Wildlife SOS is an organization known for eradicating this practice by rescuing and rehabilitating the animal as well as community. Sloth bears *(Melursus ursinus)* are the largest insectivores on this planet. The sloth bear is a protected species under the Indian Wildlife (Protection) Act, 1972. The latest population statistics reveal that the remaining/ existing sloth bear population is around 8000 in the wild. The sloth bears are classified as vulnerable in IUCN Red List of Threatened Species and are protected under Schedule I of The Indian Wildlife (Protection) Act, 1972. They inhabit mainly in Indian sub-continent, the south lowlands of Nepal and Sri Lanka. Their distinctive feeding ecology includes frugivory and myrmecophagy, making them unique in their ecological niche. Sloth bear's lifespan is divided into following categories. Neonate (less than one month old), Sub adult (3-5 years), Adult (between 5-20years) and Geriatric (over 20 years). Sloth bears mate during the beginning of monsoon (May-July) period is around 50-60 days. The documented litter size of sloth bears is 2-3 cubs per litter. Single cub is also common. Average birth weight of sloth bear neonate is around 350-450 grams. Sloth bear cubs are born altricial.

Sloth bear cubs receive maternal care with their mother for 2-3 years, depending on their gender. Sloth bear cubs are born with closed eyes and ears and little fur on the body. They need critical care and nursing from their mother to survive. They open their ears first followed by eyes around 20 -25 days respectively. In captive conditions, intensive care must be taken for cubs, at least for six months, to ensure their survival. Separation anxiety is quite common in sloth bear cubs which are separated from their mother.

Before we proceed for hand-rearing, even if there is a case of trapped or injured mother, attempts must be made to reunite the cubs with their mother because the whole process of weaning, hand-rearing, and adaptation of altered environments will be extremely stressful for the cub. If in case the mother has died due to accident or there are some prior health issues to the cub, then only hand-rearing is advised as per the following standard protocol.

Nursing and Care of Sloth bear cubs

For neonates and cubs, it is mandatory to have a strict minimal quarantine of thirty days, this period can also be considered as a socialization period in the rehabilitation centre if we are dealing with more than one cub. It helps to prevent any contagious diseases that a new one may be harboring. The mortality rate is exceptionally low in an ideal rearing condition (3-5%). The cubs must be monitored constantly by the keeper and Veterinarian, every time before the feeding time, urination and defecation which must be stimulated by gentle rubbing of lower abdomen with cotton, soaked in warm water to simulate the effect of gentle licking by their mother.

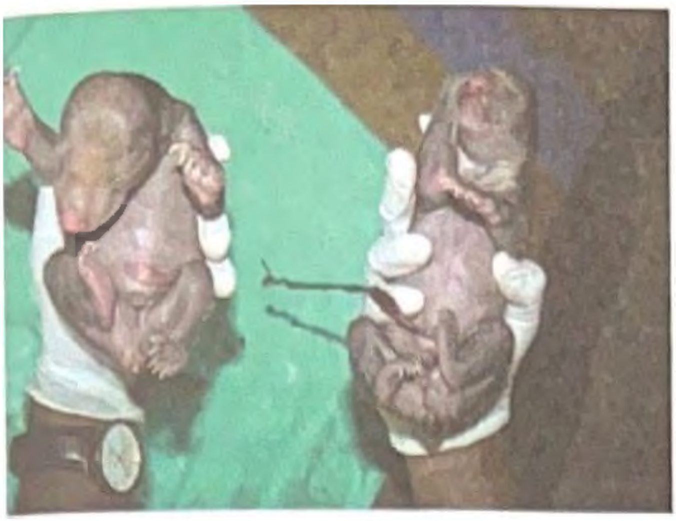

Fig. 1: Sloth bear cubs are born altricial and weighs around 300 grams

a - Brand of Nestle India Ltd., Delhi

Fig. 2: Cubs are fed in equal intervals with dog or human milk replacer

General Precautions

Sloth bear neonates will need an ideal temperature 30-37°C with the humidity range of 60-70 percent for the first two weeks, it can be further reduced, as the time for cubs to exit out of the den with mother. Soft bedding material should be provided. Considering sloth bear's natural birth in cave, a cub's weaning room should be dark throughout the day. Exposing the young ones to bright sunlight in early ages, might damage their retina and may lead to vision impairment. Routine check-up of bodyweight and minimal body morphometry is essential to ensure adequate food intake and growth rate of the hand-reared young ones, there will be 10-15% gain in body weight over every 24-32 hours. Strict hygiene measures must be ensured at all stages of care.

Neonates Nutritional feeding

The lack of information about the milk composition of sloth bears creates another challenge in hand-rearing of cubs. From the latest data, it is known that the average dry matter content in bear's milk is 30-40 percent and has a very high-fat content (40-50 percent); protein content varies from 25-40 percent and very low sugar content (5-10 percent) is there in bear-milk.

For the first three months, sloth bear cubs have to be thoroughly fed with milk (Lactogena 1, 2-42 percent CP and 25 percent fat). This formula must be mixed in 1:2 ratio with warm water. The neonate must be fed more frequently (12 times a day for first week *i.e.* 4-5 ml/ time) in the beginning and the frequency can be reduced as it grows (8 times a day after a week till 6 months *i.e.* 10-15 ml/ time). In between, 5 ml of glucose solution, mixed with electrolytes can be given once in 6 hours, after a week it can be increased up to 10-15 ml.

By the end of three months, they can be fed with mashed fruits (40 grams) and honey (50 grams) along with the milk (Lactogena 3) is recommended for sloth bear cubs. Freshly made milk must be warm (38-39°C) at the time of feeding. By the end of the third month, the expected body weight of the cub is almost 6.5-7.5 kg.

A pediatric oral multivitamin drop can be given 2 times a day (Vitamin A, D, E and B - complex). Cotrimoxazole is an antibiotic which can be used orally as prophylactic for a week or if there is any color change in faeces/ or if there is any respiratory infection. The antibiotic can be chosen/selected through Antibiotic Sensitivity Test (ABST) too. Colic aid is a drop which will avoid any abdominal discomfort (pain /flatulence) and will facilitate voiding motion with ease.

At the age of 6 months, sloth bear cubs will weigh above 20 kilograms and they can be fed 5 times a day. A nutritive feeding at this age will include Finger millet (ragi) porridge (200 ml) and Lactogen-3 milk (2500 ml/day in the 5 intervals) and Calcium supplement (10 ml) orally, fresh fruit juice (watermelon, musk melon, sun melon), (500-600 ml) along with honey (30 grams), wheat porridge (200 ml), Coat conditioner (10 ml) PO as / for skin and coat nutrition. Dates (with or without seed) can be given as nutritional enrichment once or twice a day in between the regular feeding for about 5 to 10 numbers or 50 grams. Also, Colic aid drops (2-3 ml) oral are given to the cubs if any abdominal discomfort or frequent crying is observed in them.

The juvenile at the age around a year, can be continued to be fed 5 times in a day with an increased amount of porridge along with gradual reduction in the amount of milk, honey, dates and fruits adequately to encourage their natural foraging behaviour. Omnivore/ insectivore diets like readymade feed items or pebble feeding or providing them with insects, termites, meal worm etc. can be introduced at least twice a week as a part of their diet.

Adequate exercise Supplements like Calcium, phosphorus and Zinc in addition to other micro and macro minerals along with vitamins can be added to their regular mealor as enrichment diet. For the cubs over 2 years, regular enrichment activities, body weight observation and morphometry of individual bear should be monitored. The feeding practices, depending on the available ingredients to fulfil the energy and protein requirement should be carried out as per/ in accordance with the body weight of the subadult. The total dietary requirement of the animal can be divided into two to three feeds a day. Over feeding should be avoided. The standard body score index of sloth bear should be followed.

Vaccination and deworming

Sloth bears are susceptible to rabies, tetanus, canine adenovirus and leptospirosis, it is recommended to immunize them with anti-rabies and canine multi-component vaccine (including Leptospira) after their three months of age. The gap between the two vaccines should be more than two-three weeks. The first dose of anti-rabies vaccine should be given after 90 days of their birth (12'h week) and canine multicomponent vaccine - (including Leptospira) is recommended for them after 3 months (15'h week). First Deworming on 20'h day. Scheduled deworming can be done once in six months, regular faecal sample checking must be done once in three months.

Conclusion

Hand rearing of neonates are very tricky and challenging though we understand the nutritive value of mother's colostrum. Continuous observation of the young ones who are under hand rearing practice along the line of the mother's behaviour like frequent stimulation of genitalia and perianal region of young ones in order to relieve their nature calls in addition to stimulate the volunteer excretory function of neonate. In case of sloth bears, though the number of feeding is more or less constant for the cubs younger than 6 months, due to the cubs being raided on their mother's back. After 6 months of age, the frequency of the cubs being raided by its mother gets reduced and eventually the exercise of the growing young one's nutritive requirements goes up. So more than quantity of feeding, the quality of feeding is especially important at every growth stage. Based on that Wildlife SOS has standardised the nutritive requirements of the young ones for hand rearing them successfully.

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Art – 232. SUCCESSFUL RESCUE AND REHABILITATION OF AN INJURED WILD SLOTH BEAR TRAPPED IN A POACHER'S SNARE

Arun A. Sha, R. Bakde and **Adhithyan N. K.**

Abstract

An adult male sloth bear of ten years of age was rescued by Karnataka Forests Department, Bellary division was shifted to Wildlife SOS, Bear Rehabilitation Centre with a severely injured left hind limb severed at the level of metatarsals. The paw was putrefied with purulent exudation and loosely hanging broken metatarsals. After immobilizing the bear with medetomidine-ketamine at 0.05 mg kg^{-1} and 3 mg kg^{-1} body weight, respectively the wound edges were debrided for apposition. Necrotic tissues and three remaining metatarsals were removed and four stay sutures were placed. Suturing was accomplished with 2-0 absorbable sutures and the wound was dressed with 5 % povidone iodine and vet wrap. The wound was secured with a fluid bottle, tied to the paw of the sloth bear to prevent the soiling of the wound. Therapeutic management was done using long-acting antibiotics like Benzathine penicillin @ 40,000 IU kg^{-1} q 7 days and Enrofloxacin @ 5 mg kg^{-1} q 72 hour for 20days. Administration of fluids at the rate of 50 ml kg^{1} and opioid analgesics (Tramadol @ 5 mg kg^{-1}) was done at the time of surgery. Thereafter, regular dressing was done with collagen silver sulphadiazine cream and antiseptic powder. Analgesia was achieved by oral supplementation with gabapentin @ 2 g for 10 days. Also, for the prevention of decubitus ulcers, a wooden plank was placed in the bear enclosure. After continuous dressing for 60 days the wound showed remarkable healing. Partial amputations and post-operative management are often lifesaving human interventions that become necessary in human-animal conflict zones.

Key words: Amputation, gabapentin, sloth bear, snare trap

Introduction

Melursus ursinus, commonly known as the Indian Sloth bear, is an endemic inhabitant of the Indian subcontinent with population scattered across several Indian states, but primarily concentrated in Central India and southernmost forest ranges of the Western Ghats (Yoganand et al., 2006). They are primarily confined to moist and dry deciduous forests but can also be found in moist evergreen forests, rocky outcrops, and caves (Kumar et al., 2014; Abdul and Hosseti, 2016). The Indian sloth bear has been considered as a "Vulnerable" species by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List and remains protected under Schedule 1 of the Indian Wildlife (Protection) Act, 1972 (Shanmugam et al.,2008; Singh et al., 2018).

Ecological studies of sloth bear have speculated that the lower metabolic rates and high thermal conductance in them have been associated with their dense fur and myrmecophagic and frugivorous feeding habits (McNab, 1992). This adaptation has also enabled them to survive in sweltering hot climatic conditions by restricting themselves to nocturnal activities. Anatomical features such as a missing pair of upper incisors and protruding loose lips have led to them extensively feeding on termites, ants, and fruits (Shanmugam et al., 2008). Despite these evolutionary adaptations, the sloth bear population is threatened.

Since historical times, illegal sloth bear poaching has been rampant for live animal trade, bear meat, and traditional Chinese medicine. The barbaric practice of "dancing sloth bears" was a significant reason for poaching of bear cubs by the Kalandar community for exhibition and entertainment purposes. (Shanmugam et al., 2008; Palita et al., 2014; Crudge et al., 2020). Traditional Chinese medicines used bear gallbladder and bile for treatment of cardiac problems, eye puffiness, asthma, cancer, burns, liver problems, and impotence (Feng et al., 2009). Reproductive organs of the male sloth bears were considered as aphrodisiacs and used as sex stimulants by the Pardhi-Beheliatribe.

Reports of accidental deaths of Schedule I animals from wildlife corridors and surrounding human settlements have shown a steady rise over the years, owing to the illegal installation of snare traps to protect farmlands from the nuisance caused by wild boars (Gupta, 2017). The Karnataka Forest Department has adopted several conservation efforts in collaboration with the Bannerghatta Biological Park and Wildlife SOS in rehabilitating sloth bears that fall prey to this snare culture. The current case report describes the rescue and rehabilitation of one such sloth bear that was found to be trapped in a deadly snare trap in the Bellary district of Karnataka.

Case Report

Rescue operation

The present paper describes the rescue and rehabilitation of a male wild sloth bear trapped in a poacher's snare in the Bellary district of Karnataka. Wildlife SOS, in collaboration with Karnataka Forest Department, received a call from the Bellary Forest range regarding a distressed sloth bear with a snare wrapped around his foot. Upon reaching the site, the rescue team observed that the snare was tightly fastened around the left foot of the sloth bear, at the level of the metatarsals with severed toes and multiple wounds on the body. After retracting the snare from the bear's ankle, the sloth bear was brought to the Bannerghatta Bear Rescue Centre (BBRC), Bannerghatta Biological Park, for further treatment and therapy.

Fig. 1. Sedation of the injured wild sloth bear for blood collection and radiography

Fig. 2. Injured left hind foot with severed toes and broken metatarsals.

Emergency veterinary care

Upon arrival at BBRC, the sloth bear was sedated using Medetomidine-Ketamine combination @ 0.05 mg kg^{-1} and 3 mg kg^{-1} body weight, respectively, and was subjected to complete health examination. Blood samples were collected, and radiographs of the injured left foot were taken.

The injured leg was flushed with the hydrogen peroxide solution and dressed with collagen-based ointment and antiseptic powders, followed by bandaging to prevent contamination of the wound. In an attempt to stabilise the severely dehydrated bear, immediate treatment with fluids and supplements was initiated. Long-acting Benzathine penicillin @ 40,000 IU kg^{-1} administered for antibiotic cover. Supportive therapy with anti-inflammatory drug (Meloxicam @ 0.2 mg kg^{-1}) and analgesic (Tramadol @ 5 mg kg^{-1}) was undertaken for pain management.

Fig. 3. Cleaning and dressing of the injured foot with hydrogen peroxide followed by sterile bandaging

Stabilization of the patient

The sloth bear was kept under observation for ten days during which the injured leg was regularly cleaned and dressed inside the squeeze cage. Analgesia was achieved by providing Gabapentin @ 5 mg kg^{-1}, and infection was kept under control by long-acting Enrofloxacin @ 5 mg kg^{-1}. The bear was given fruits and honey and was slowly introduced to the cereal-based porridge mixed with milk.

Surgical intervention

Surgical correction of the injured foot was necessary to remove the loosely hanging metatarsals and the surrounding necrotic tissue. The sloth bear was fasted for 12 hours before sedating with Xylazine-Ketamine combination @ 2 mg $kg^{-1}$and 5 mg kg^{-1}, respectively. A series of radiographs were taken to assess the damage to the injured foot.

Fig. 4. Surgical debridement of the wound to remove surrounding necrotic tissue, and broken metatarsals

The leg was thoroughly cleaned with ciprofloxacin solution, and the remaining three loosely hanging broken metatarsal bones were retracted. Debridement of the necrotic tissues was done to facilitate apposition of the wound edges, which were sutured using absorbable 2-0 polyglycolic acid (PGA) suture material.

A total of four loop sutures were placed at the level of the ankle for securing the bandage. The sutured tissue was flushed and dressed with povidone iodine solution and antibiotic powder. Bandaging was done with sterile gauze after placement of cotton padding and covered with vet wrap. The bandaged limb was secured with a fluid bottle to prevent contamination of the wound.

Fig. 5. A three-layer compression bandage was applied over the wound after surgical procedure. Couple of stay sutures were applied to hold the bandage in position

Post-operative management

The sloth bear was housed at the sick animal room for observation post thesurgical procedure. Combination course of Enrofloxacin @ 5 mg kg^{-1} q3 days and Benzathine penicillin @ 40,000 IU kg^{-1}q10 days was given along with antacid (Rantac 0.5mg kg^{-1}). Analgesia was achieved with gabapentin @ 5 mg kg^{-1} orally. Regular cleaning ofthe wound and dressing with silver sulphadiazine collagen- based cream and cipladine antiseptic powder was done through the squeeze cage. The sloth bear was provided witha nutritious cereal based porridge along with milk and honey and fresh fruits as enrichment. Development of decubitus ulcers was controlled by placement of a wooden plank in the enclosure with soft bedding for comfort.

Results And Discussion

An injured wild sloth bear trapped in a poacher's snare was rescued from Bellary district of Karnataka and rehabilitated at the Bannerghatta Bear Rescue Centre, under the Bannerghatta National Park. Preliminary blood testing revealed that the sloth bear was severely dehydrated and anaemic with slightly elevated white blood cells. Serum biochemistry showed hypoproteinemia, elevated liver enzymes, and lactate dehydrogenase levels indicative of tissue damage along with electrolyte imbalances. Radiographic evaluation of the injured leg revealed completely severed metatarsal bones due to the poacher's snare.

Fig. 6. Radiographic image of both normal and damaged digits of hind limb of the injured wild sloth bear

Fig. 7. The injured foot 10 days after the procedure with intact sutures and no exudation

Fig. 8. The gradual formation of granulation tissue and complete closure of the wound after 90 days of dressing

Surgical correction of the left foot resulted in gradual healing of the wound after 90 days during which the leg was regularly cleaned and dressed through the squeeze cage.

The sloth bear was provided the required nourishment with a balanced cereal-based porridge diet along with honey and seasonal fruits, which resulted in weight gain and hastened the recovery of the bear. Supplementation of the sloth bear with oral haematinics and liver tonics resulted in the improvement of the anaemia and body condition.

Fig. 9 (a and b). Complete healing of the found and proper weight bearing was observed by the end of six months

The sloth bear had shown remarkable improvement after three months of therapy. Before re-introducing the sloth bear to the socialisation area along with other bears, he was trained to follow simple commands like “hands out” and “open mouth”, using dates and honey. This practice also termed as "positive conditioning" that helps not only in strengthening of the bond between the keeper and the animal, but also enables the veterinary team to perform simple procedures like blood collection, nail trimming and wound dressing, efficiently. Within a couple of months after complete healing of the wound and stable health, the sloth bear was released into the socialisation area with other bears.

The barbaric practice of using snare traps for poaching of wild animals by poachers has been practiced, for decades, favouring the illegal trade and marketing of animal organs (Lee, 1995; Shepherd and Shepherd, 2010). Several modes of poaching, including firearms, the country-made explosives and muzzle-operated guns, have been widely used by poachers in spite of which wire snares are preferred among poachers due to their easy installation with very meagre investment. Besides this, snare traps are not easily visible in forest patches unless they are being searched for meticulously. These snare traps either kill or amputate the animal, leading to lifelong casualties and the inability for them to be released into the wild again (Hermon, 2017; Balseiro et al., 2020).

Snare trap injuries are very complicated and require prompt veterinary intervention to save the life of the distressed animal. Animals trapped in snares often struggle very hard to free themselves by pulling or biting the wires leading to additional trauma and dental injuries. They suffer from starvation, thirst, strangulation, self-mutilation wounds, excruciating pain, and discomfort, and sometimes, even predation from carnivores (Rochlitz et al., 2010). On several occasions, even before the arrival of the rescue team, local people would end up cutting one end of the snare attached at a tree branch, setting the bear free. Such human interventions cause more harm as the bear still entangled within the trap starts running frantically, aggravating the injuries. Severely injured sloth bears unable to move into deeper areas of forests stay near the forest fringes surrounding human settlements leading to more conflict zones. Rescued animals fight a long battle against physical injuries as well as mental trauma, and have to undergo several correction surgeries, which is why most of the time, they end up losing the functionality of the affected body part.

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Art – 233. OUR JOURNEY FROM A KALANDAR MADARI TO PARA-VETERINARIAN IN A DANCING SLOTH BEAR REHABILITATION CENTRE

Imam Sab and **Saddam Husen**

Kalandar communities are originally gypsy communities with a highly nomadic lifestyle and were famous for their mastery over animals. Initially, these communities took up the profession of Sloth bear fighting in India. This community has performed a 400 years age-old practice of dancing Indian sloth bears. In 2002, Wildlife SOS collaborated with the state forest department, started the rehabilitation of Kalandar communities by educating them and providing alternate livelihood as an extension of the dancing bear rescue project. Over 3,000 families spread out through six states and over 15 villages; have received support to become economically self-sufficient for two decades.

After surrendering my dancing sloth bear in 2004, I worked as an assistant keeper under trained senior animal care staff. Slowly after ten years, my para-veterinarian journey started. As a para-veterinary worker and not having any education, it wasn't easy to understand animal procedures, medicines, surgeries, etc. Gradually, I was trained under several wildlife veterinarians, and it became a regular job because of the core interest in the work. The routine tasks start with arrangements of all the equipment for minor and major surgeries and treatments, maintaining the operation theatre and checking the necessary medicines and consumables, assisting post mortem, and going for the conflict bear rescue operations as and when required. The journey started primarily as an animal performer roaming from villages to villages. It narrowed down as regular salaried staff with settled family and kids' education to a better life.

Art – 234. A CASE OF PENILE FRACTURE AND PARTIAL AMPUTATION OF OS PENIS IN A SLOTH BEAR *(Melursus ursinus)*

Arun A Sha and **Adhithyan N.K**

Sloth bears possess an os penis like their wild canine relatives. It is believed to be an aid in their reproduction and mating success. The average length of the os penis in adult sloth bear is around 16 centimeters. Pathological and congenital affections of genitalia are uncommon in sloth bears, even though geriatric animals are likely to get posthitis and balanitis as their age advances.

A 25 year old male sloth bear housed at Bannerghatta bear rescue centre was showing signs of dysuria, followed by abnormal and excess mobility of penile tissue. Upon close examination, additional clinical abnormalities like crepitus and thickened fracture site was noticed. The plain radiography confirmed a simple, short, oblique fracture at the distal one-third of os penis. A lacerated wound was noticed approximately 15 cms long, extending from base to tip of the prepuce. The bear was immobilized with Xylazine (2mg/kg), and Ketamine (5mg/kg) and partial amputation of os penis and wound reconstruction surgery was performed. The broken tip of the os penis was removed through a 20 mm incision made on the anterio dorsal part of the glans penis without interfering with urethral opening, the incision and extraction of os penis from the corpus spongeosum was performed using an electrocautery to minimize bleeding. The wound was sutured using 3-0 PGA and wound on the prepuce was sutured using a 2-0 PGA. Antibiotics and anti-inflammatory was given for 7 days along with supplementation of serratiopeptidase and vitamin B. The animal had an uneventful recovery.

Art – 235. THERAPEUTIC MANAGEMENT OF BULLAE IN CAPTIVE ASIAN ELEPHANTS *(Elephas maximus)*- A CASE REPORT

Rahul Prasad, **Ilayaraja Selvaraj**, **Arun A. Sha**, Pramod Rajput and **Srinu S. Maharana**

Diagnosis of bullae is an important treatment perspective in Asian elephants. We report here the clinical case of Bullae in rescued captive Asian elephant housed at Elephant conservation and care center, Mathura and its medical intervention. A 50 year old male Asian elephant showed symptoms of pain and fluid filled sac along with watery discharge from ear pinna. Later on, it was tentatively diagnosed as bullae. The bullae were rinsed with normal saline solution and the residual fluids were seized with the help of sterile cotton swab. The elephant was treated topically with a mixture of silver sulfadiazine, Calendula powder and coconut oil in a ratio of 2:1:1 twice a day for 45 days. Oral medication of Meloxicam @0.1mg/kg, Chlorphenamine meleate @0.5mg/kg were given for 25 days and TRP bolus @4/SID until the recovery noticed. The treatment was found to be effective as the animal showed improvement after the course of treatment. The animal was completely recovered after 45 days of treatment.

Art – 236. THERAPEUTIC MANAGEMENT OF VENTRAL EDEMA IN ASIAN ELEPHANT *(Elephas maximus)* - A CASE REPORT

Rahul Prasad, **Illayaraja Selvaraj**, **Arun A Sha** and Shivani Sing

Ventral edema may involve the ventral abdominal wall, the submandibular area, the tissues surrounding the external genitalia, edema associated with parasites, anemia, kidney failure, tuberculosis and chronic diarrhea. A single, specific, underlying etiology has not been identified. Edematous pockets can emerge in any of the respective areas of the elephant's body. Incidence of ventral edema noticed in rescued begging female elephant aged 64 years at Elephant Conservation and Care Center, Wildlife SOS, Mathura. The subcutaneous ventral edema usually exhibits in overnight at abdomen and ventral thoracic region. Animal had mild pain on while touching, walking, sitting and when animal lay down. Applied herbal aerosol spray Wisprec (Eranda, Tulasi, Eucalyptus globules leaf oil topically as anti-inflammatory to reduce inflammation that assist to reduced edematous swelling, orally given Bol. Meloxicam @ 0.1 mg/kg B.Wt. to reduce pain and inflammation also and Tab. Chlorpherinamine maleate @ 0.5 mg/kg B.wt to prevent allergic reactions at morning around 9:00 am . Around 03:00 pm applied Epsom salt ($MgSO_4$) mixed with Glycerin and make thick adhesive paste and applied topically on ventral edema after that an elephant allowed to walk with mahout for approx. 2 km on premises. Same treatment follow-up for next ten days. Reduction in ventral edema noticed from 4th day but complete recovery had been noticed in ten days.

Art – 237. PRELIMINARY STUDY OF CROSS MATCHING OF BLOOD IN INDIAN SLOTH BEAR *(Melursus ursinus)*

Pushkar Banka**, Adhithyan** and **Arun A Sha**

The transfusion of whole blood in sloth bears could provide cardiovascular support and maintains life in critical conditions but transfusion of incompatible blood can lead to fatal reactions. Hence, cross matching of donor and recipient's blood becomes paramount prior to any transfusions. Though transfusion medicine in veterinary science has grown up a lot and practiced well, it is not so common in wildlife practice. Since donor and recipient compatibility needs to be established prior to transfusion, it possesses practical difficulties and challenges to collect blood and cross match them. In order to confirm the same, a technique is being established to allow cross matching of red blood cells and serum in sloth bears. Cross matching was carried out in 14 individuals. Operant conditioning technique was used to collect blood samples from all these individuals. For the major cross matching, few drops of washed red blood cells of the donor were added to a few drops of recipient serum. And for minor cross matching few drops of recipient's washed red blood cells were added to donor's serum. After incubation and centrifugation, the samples were evaluated for presence of macroscopic or microscopic haemolysis or agglutination. Samples showed no agglutination or haemolysis, macroscopically suggesting that donor and recipient would be compatible. But mild clumping of Red Blood Cells under microscope suggested chances of incompatibility. Hence, cross matching should be carried out in sloth bears prior to any blood transfusion and further studies are needed in related areas.

Art – 238. BENEFITS OF OPERANT CONDITIONING IN EFFECTIVE VETERINARY CARE OF CAPTIVE SLOTH BEAR *(Melursus ursinus)*

Karthikeyan C. and **Hussain Basha**

Various behavioral objectives could be achieved through positive conditioning of captive wildlife. Wildlife SOS established four sloth bear rescue and rehabilitation facilities. Once such facility is Bannerghatta bear rescue center and the staff have come up with set of effective practices and achieved voluntary blood collection in 38 Sloth bears in past one year. The animal has been conditioned to give hands out through an opening in the cage and hold trainers hand without pulling and the blood has been collected through cephalic vein for all Sloth bears. They have been introduced to clicker sound through target training. Diluted honey is used as reward which is filled in an empty water bottle and tiny hole is made on the cap. Sloth bear are myrmecophagous, they search, suck ants, termite and other insects. The tiny hole in the bottle acts as a small puzzle feeder and makes the bear completely focused to suck the food, while drawing the blood.

Bears are conditioned to place the hands out and reward puzzle feeder is given through the mesh of the feeding den cage. Each bear will take its own time to get adjusted and desensitized with the touch, clipping the fur and pricked by the needle. The reward makes the bear more focused towards the food and it gives enough time for the vets to collect blood through cephalic vein. The patience of the staff and consistence session on a daily basis plays a vital role in achieving the goal.

Art – 239. CHRONIC KIDNEY DISEASE IN A RESCUED SLOTH BEAR (*Melursus ursinus*) **– A CASE REPORT**

Arun A Sha and **Adhithyan N K**

A seven-year-old rescued sloth bear (*Melursus ursinus*) housed in Wildlife SOS, Bannerghatta Bear Rescue Centre, was diagnosed with chronic kidney disease. Despite all the attempts with treatment, the animal succumbed after six months from the time of diagnosis. The animal showed progressive loss of body condition, loss of appetite, anemia, polyuria, poor coat condition with a change in coat color. The animal was treated symptomatically using hematinics and fluids mixed with electrolytes. A specialized diet containing a reduced amount of protein was fed. Herbal kidney protectants were supplemented along with the diet, and the animal was kept under observation and continuous monitoring. Even after one month of medical and dietary management, the animal's condition deteriorated with high serum creatinine 4.39 mg/dL and BUN 48 mg/dL. The animal started to exhibit anorexia, severe diarrhea and vomiting. Aggressive clinical management was initiated with fluid therapy and parenteral nutrition. Despite all this, the animal went into lateral recumbency along with signs of uremic breath, severe dyspnea and anuria. The serum biochemistry revealed significantly elevated creatinine 17.02mg/dL and BUN 202mg/dL. Haematological parameters also showed a gradual decline over the course of disease. Hb was reduced from 10.6g/dL to 6.7g/dL. Following three days of recumbency, the Sloth bear passed away. Postmortem findings revealed severe dehydration and anemia, pale and atrophied kidneys, congested mesenteric blood vessels and lungs, generalized pallor of the vital organs including liver and intestinal serosa. Histopathological lesions of the kidneys were consistent with chronic kidney failure in canines.

Art – 240. BREECH PRESENTATION – A CASE REPORT OF FOETAL DYSTOKIA IN RHESUS MACAQUE

Prajakta, **Ilayaraja S**, Acharya P.R. Shivani, **Baiju raj MV** and **Arun A. Sha**

A case of dystokia in Rhesus macaque (*Macaca mulata)* was reported at wildlife hospital, Wildlife SOS, Agra with a history of dystokia since 3 hours. An adult female Rhesus macaque was observed with partial expulsion of foetus which was hanging at vaginal opening, severe straining and labour pain. The dead foetus was found with all the four limbs out and head stuck in vaginal passage. Hence, the case was diagnosed for foetal dystokia due to breech presentation. Animal was immobilized by inj. Xylazine @ 2mg/Kg. b.wt. and inj. Ketamine @ 2.5mg/Kg. b.wt. intramuscularly, which occurred on 12^{th} min. The female was restrained in sternal recumbency and 5 ml of liquid paraffin was poured in vaginal passage and the hanging died male foetus was removed applying mild traction by hand. Catheter was inserted in uterine cavity and flushed by inj. Metrogyl @ 60 ml (Total dose). Inj. RL @ 300 ml (Total dose) i/v, inj. Melonex @ 0.2 mg/Kg. b.wt. and inj. Tribivet @ 2ml (Total dose) i/v was given during sedation. Animal was recovered from sedation after 40 min and kept under observation for 3 days. No abnormality was noticed, and on 4^{th} day monkey was released at place of rescue in troops.

Art – 241. IDIOPATHIC EPILEPSY AND ITS MANAGEMENT IN CAPTIVE SLOTH BEARS

Acharya P.R., Shivani, Prajakta, **Ilayaraja S, Baiju raj MV**· and **Arun A.Sha**

At Agra Bear Rescue Facility, two different cases of epilepsy of unknown origin were observed in captive sloth bears. Both cases were observed in adult male sloth bears in peak summer days at afternoon hours and shown almost similar signs. Incoordination, tachycardia, hyperpnoea with harsh and loud rales, left lateral recumbency and hyperthermia were prominent clinically observed signs. Both animals were noticed during period of epilepsy in socialization fields at noon hours and were taken inside den using animal stretcher. Intravenous administration of inj. Ringer lactate was started immediately and inj. Phenytoin sodium @ 2mg/Kg.Bwt. was given along with it. Inj. Meloxicam @ 0.2mg/Kg.Bwt. i/v, inj. Tribivet @ 5 ml (Total dose) i/v & inj. Avil @ 4 ml (Total dose) i/m, was given in both cases. During treatment both animals were shifted to sternal recumbency and assisted ventilation was provided by opening the oral cavity and holding the tongue in craniolateral direction. In Case 1, inj. RL (1 litre, Total dose) was administered over a period of 35 min. Gradual reduction of tachycardia and hyperpnoea was noticed, and complete recovery from episode of epilepsy was observed after one and half hour. In Case 2, inj. RL (700 ml Total dose) was administered over a period of 20 min and complete recovery was noticed on 45th min. After episodes of epilepsy both animals were quarantined, Tab. Phenytoin sodium @ 50 mg O.D. and syrup Nurokind @ 10 ml O.D. was followed for a month.

Art – 242. AN ATTEMPT TO TAKE NORMAL BODY TEMPERATURE IN CAPTIVE SLOTH BEARS

Acharya P.R., Atul Gupta, **Ilayaraja S, Baiju raj MV**· and **Arun A. Sha**

Per-rectal body temperature is one of the major health parameters to diagnose the disease conditions or health status of animals. Taking per-rectal body temperature in captive wild animals is really a task. Two major methods of restraining are followed in India to take per-rectal body temperature and other body parameter collection or treatment in captive wild animals i.e., physical and chemical restraining. In physical method, most commonly used technique is the squeeze cage method, which generally involves lots of struggling and hyperactivity of animals into it. In chemical method, Ketamin HCL and Xylazine HCL are used @ 5 mg/Kg.Bwt. & 2 mg/Kg.Bwt. respectively to restrain the sloth bears. Both drugs are well known for their body temperature altering activity during chemical restrain of animals. In both cases, fluctuation from normal body temperature in captive wild animals is obvious and this is going to affect the diagnosis and treatment of captive sloth bears. Van Vihar Bear Rescue Facility is having a sound population of captive sloth bears. Two female and one male, adult, apparently healthy captive sloth bears were conditioned positively and per-rectal body temperatures were taken for regular observations and those were 98.4 ^{0}F, 98.1 ^{0}F & 98.6 ^{0}F, respectively. All the three sloth bears were taken into normal cage with enough space to move animal in all directions. Honey is used as a reward treat to comfortably stand the animal into cage and per-rectal temperature were taken through a window using digital thermometer for minimum 45 sec. Hence, positive conditioning the captive sloth bears for obtaining per-rectal temperature is best and reliable method compared to physical and chemical restraining which involves high stress to the animal and chemical fluctuation of body temperature.

Art – 243. MANAGEMENT OF UROPHAGIA INDUCED BALANOPOSTHITIS IN CAPTIVE SLOTH BEARS

Acharya P.R., Shivani, Prajakta, **Ilayaraja S, Baiju raj MV**· and **Arun A. Sha**

Agra Bear Rescue Facility is the world's largest rehabilitation facility for rescued sloth bears which accommodating the major population of captive sloth bears in India. Urophagia is considered as one of the vices encountered generally in captive male sloth bears. Balanoposthitis is a common sequel of urophagic behaviour, which in severe cases may affect urogenital system and leads to secondary complications associated with it. In this abstract we are discussing four cases of urophagia induced balanoposthitis in male sloth bears and its management. Case 1&2 were observed as moderate inflammatory swelling at urethral tip with both animals showing intermittent urophagic behaviour. Topical pouring of 2ml Neem Seed Oil (*Azadiracta indica*) is done thrice daily. Remarkable effect is observed with reduced swelling and frequency of urophagia on very next day which supposed to be due to anti-inflammatory activity and acrid taste of NSO. Case 3 was diagnosed with mild balanoposthitis with initial development of urophagic behaviour due to boredom in captivity. Use of structural, environmental and feeding enrichments and positive conditioning of animal showed impressively increased animal activity and reduced urophagic behaviour. Case 4 was a typical urophagia induced balanoposthitis complicated with secondary maggot infestation. Using positive conditioning manual removal of maggots from site was done and flushing of cavity with ice cold normal saline and maggoticidal & fly repellent spray was done. Animal was recovered completely on 24th day uneventfully. However, above four cases in captive sloth bears shown complete recovery, still causes and recurrence of urophagic behaviour is a topic of furthermore study.

Art – 244. SUCCESSFUL MANAGEMENT AND POST-OPERATIVE CARE OF RUPTURED EPULIS IN CAPTIVE DANCING SLOTH BEAR

Acharya P.R., Shivani, Prajakta, **Ilayaraja S, Baiju raj MV** and **Arun A. Sha**

Epulis i.e., swelling of gums, in rescued dancing sloth bear is one of common oral cavity affections. A case is reported at Agra Bear Rescue Facility with rupture of epulis due to infighting in captive sloth bears. A 25 years old, male sloth bear was observed with bleeding and hanging hard tissue mass from oral cavity at right premolar region. Using positive conditioning topical spraying of inj. Adrenaline and 10% lidocaine spray, hanging mass was removed by forcep providing torsion at pedicle, followed by a thorough oral cavity flushing with chlorhexidine solution. Bolus Styplon @ ½ O.D. for next three days was continued. Antibiotic Tab. Moxikind CV @ 1 Tab. O.D. for next seven days and pain management was done with Tab. Rutoheal @ 1 Tab. B.I.D. & Bolus Melonex @ ½ O.D. for next five days. Addition to these, animal was isolated in a concrete floor den for next seven days to prevent mud digging and foraging activity which may create chance for secondary bacterial infection. Therapeutic diet was planned for a month with only liquid diet for initial three days, i.e. a litre of milk, curd, and watermelon juice was provided by intervals. Forth day onwards, 4 to 4.5 kg semisolid porridge with added milk was provided twice daily and 2 kg pulp of watermelon once daily, was followed for a month. Since the day 0, flushing of oral cavity with chlorhexidine solution after each meal was strictly followed.

Art – 245. ANIMAL WELFARE AND ETHICAL CONCERN IN WILDLIFE VETERINARY PRACTICE IN INDIA

A. Sha Arun

The subject of wild animal welfare and ethics in both captive and free ranging wildlife care is poorly understood and hardly in practice. The upkeep of an animal both physically and behaviourally healthy is a paramount important in wildlife veterinary practice too. In this article, “Animal welfare and ethics in wildlife veterinary practice” I would like to discuss about various disciplines and wildlife husbandry practices involved in wildlife veterinary practice. The primary objectives, including examples and interactive discussions with the intention of reinforcing our awareness, understanding and approach regarding the subject.

With eighteen plus years of experience in rescuing and rehabilitating wild animals which include Sloth bears (*Melursus ursinus*), Leopards (*Panthera pardus*), Indian Elephant (*Elephas maximus indicus*), Snakes of different species (suborder: Serpentes) and Striped hyenas (*Hyaena hyaena*), it would be humbling to discuss the subject of animal welfare & ethics with respect to our wild friends that are perhaps equally deserving if not more than their domestic counterparts in leading a life with dignity, at least in their later years.

The Emergence of Animal Welfare and Ethics:

Ethics stem from the moral dimensions that back our everyday decisions. There is consistent evidence of ‘respect’ towards animals in the Indian sub-continent that can be found in the ancient scriptures. Reference to Kamadhenu, the miraculous ‘all giving’ cow in Hindu mythology and the many animals each attributed as the official mascot of different gods namely the spectacled cobra (*Naja naja*) associated with Lord Shiva, Peacock (*Pavo cristatus*) associated with Lord Subramanya, Garuda the Kite (*Haliastur indus*) associated with Lord Vishnu or even the much hated rodent species like the mice (*Mus booduga*) associated with Lord Ganesh all point towards our reverence towards fellow species and an apparent attempt to co-exist. Modern philosophers and thinkers from the West have debated elaborately on the necessity for being ethical towards animals and thereafter about the degree to which ethics is applicable to animals. While some have discounted the very idea of showing moral consideration to these sentient beings, others have had a more consequentialist or obligatory approach in this regard. Modern ethical theories namely Contractarian theory, Utilitarian theory, the theory of animal rights, relational views and respect for nature each reflect on the three-perspective mentioned earlier.

The science of animal welfare concerns how sentient animals experience their life in terms of their physical functioning, mental state/feelings, and natural behaviours. A change in one of these aspects could very much result in the welfare of the animal being compromised. Application of the modern ethical theories to areas concerning animal welfare will yield a holistic view and help set the tone to the line of action. Breeding dogs/captive wild animals with heritable defects, farming animals for meat, controlling infectious diseases in animals (zoonotic or otherwise) and use of animals in research are some areas to be covered taking into consideration the socio-economic angle. A discussion on standard veterinary practices in the above-mentioned areas of concern shall help in arriving at a common consensus within the veterinary community to better the animals' welfare.

Ethical Concerns

A chapter dedicated to animal welfare and ethics in wild animals will help refine our perspective in understanding the subject considering the rarity of these species where requirements are far more stringent and laws far less lenient, taking into consideration the five freedoms of animal welfare. Legal concerns, Ethical animal exhibits, enclosure designing, hygiene- sanitation, quarantine, disease control, biosecurity, Enrichment of wild animals in captivity to simulate situations in the wild, animal welfare audit for wild animals in captivity, sampling, data collection, research, and publication would be making some interesting examples. Similarly, in free-ranging wildlife, the conservation areas of conflict mitigation measures, rescues, reuniting the orphaned wildlife with mother, rehabilitation, veterinary care, disease prevention, necropsy procedures, various aspects of documentation and dissemination of knowledge would certainly have a strong hold of ethical practice. In general, the challenges in various aspects of following animal welfare and ethical practices and possible solutions are discussed in detail during the presentation.

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Art – 246. COLOUR PERCEPTION AND ATTRACTION STUDY IN CAPTIVE SLOTH BEARS (*Melursus ursinus*)

Prajakta Rao and **Arun A Sha**

The sloth bear is a myrmecophagous bear species with their good sense of smell but eyesight has not been studied much. It is considered that they have an equivalent eyesight as humans; however, based on the number and position of the rods (interpret light) and cones (interpret colour) in their eyes they may not see colours the way humans do. On the other hand, animal coloration studies have been highly researched and the way wild animals mimic and camouflage themselves for their survival, hunting and mating with the functional significance of the color pattern.

An interesting study was conducted to examine whether there is any particular colour attraction in sloth bears which will help to encourage sloth bears in captivity with enclosure enrichments. The quantitative analysis was completed over the period of six months with the sample size of 60 sloth bears from semi-captive environment of Bannerghatta Bear Rescue Centre of Wildlife SOS, Bangalore. The age group between 3 to 28 years, of both the gender (30:30) was studied. We used six colours (Blue, Pink, Green, Yellow, Orange, Red) i.e. 2 colours from each spectrum of colour wavelength (high, medium, low). Outliers were kept at minimum to obtain more accurate data. The results suggest that sloth bears showed a significantly higher attraction at 0.05 confidence interval level towards the colour yellow (for humans) among all the six colours tested. This research could be further undertaken to examine the ability of sloth bears to differentiate between the shades of colours.

Art – 247. IMPACT OF FEEDING CHICKEN MEAT OVER BEEF MEAT WITH BONE ON GASTROINTESTINAL PARASITES IN CAPTIVE INDIAN LEOPARDS (*Panthera pardus fusca*) – A REVIEW

Nikhil S. Bangar, **A. Sha Arun** and **Mahendra B. Dhore**

Leopards (*Panthera pardus*) are the most widespread group of wild felids. However, their population is decreasing in a similar range to other large carnivores and has been classified as vulnerable by the International Union for the Conservation of Nature (IUCN) and recognized the nine subspecies. An observational study was conducted from August 2010 to August 2020 in two phases, i.e. phase 1 (August 2010 to August 2015) and phase 2 (September 2015 to 2020). A total of 532 fecal samples (266 from phase 1 and 266 from phase 2) were collected from captive leopards to assess the impact of feeding on gastrointestinal parasites provided with different feeding patterns on these two phases. During study period of Phase I their diet mainly consists of only beef meat with one day off in a week and in Phase II was fed only on chicken meat with two days off in a week. The prevalence of parasitic infection in the conducted study phase one was 29.69% (79/266), and that of phase two was 8.65% (23/266), with an overall prevalence of 19.17% (102/532) from the total sample studied. Results indicate that leopards suffered from more types of parasites when was fed on beef meat than chicken meat and in both phases of study leopards mostly infected by nematodes preferably *Toxocara* spp. Therefore, it is observed that the chicken meat has some advantage over beef meat with respect to gastrointestinal parasites, so this factor could be considered when the feeding formulation for captive leopards are designed, as ruminants preferably serve as intermediate host for most of the parasites.

Art – 248. SENSITIZING THE ANIMAL CARE STAFF FOR THE IMPORTANCE OF ENVIRONMENTAL ENRICHMENT FOR THE BETTER QUALITY OF LIFE IN CAPTIVE WILDLIFE

Saddam Husen, G M. Vishwanath and **Prakash G P.**

Wildlife SOS has played an important role in rescuing Sloth bears (*Melursus ursinus*) in India, Bannerghatta is the world's second largest Bear Rehab Centre for sloth bears. The rescued sloth bears are accommodated here in a semi-captive environment. In captive environment, enrichments are creatively designed by veterinarians, biologists and animal care staff to ease the transition of getting them used to live in their natural habitat.

Enrichments can be of five types social, occupational, physical, sensory and nutritional. These types of enrichments are regularly being used to reduce the stress in captive wildlife. These wild animals often develop stereotypy like pacing, regurgitation, head weaving, head rotation etc., which are indicative of boredom and distress. It indicates that these wild animals are deprived or left with little opportunities to express their natural behaviors or facing stress. Various structural enrichment like hammocks, elevated platforms, swings, see-saws, puzzle feeders, wobble tree, tree houses, resting dens, etc. have been installed to improve their physical activity. Sloth bears are very good problem solvers and explorers therefore, lot of puzzle feeders, tender coconuts are given to engage them both physically and mentally, hiding feed items in different places to encourage the animals to use their olfactory senses and also to initiate the foraging behavior that would push them more towards their primal instincts.

Art – 249. BIOMASS - BASED FUEL TO MAKE PORRIDGE FOR RESCUED SLOTH BEARS

Allauddin and **Dada peer**

Sloth bears are native to the Indian subcontinent. Wildlife SOS is an organization known for eradicating a 400-year-old "dancing bear" practice in India and providing lifetime care facilities for all these rescued sloth bears. These sloth bears are situated in world's second largest sloth bear rescue centre of Wildlife SOS, in Bangalore. Having witnessed brutal acts of the kalandar community for the initial period of their life, they have lost their canines. Therefore, the semi-liquid diet is provided to maintain their eating habits and health. Their nutritive diet includes porridge made up of Ragi, Jowar, Bajra, Soya as a main ingredient along with Honey, vegetables, eggs, milk and fruits. The food preparation takes three hours for one-time meal and it's been done twice a day in the eco- friendly bear kitchen. Each meal would be around 800 to 900 litres of porridge made at one go in a giant cooking vessel for 72 rescued sloth bears. This initiative aims at reducing green-house emission by disseminating biomass based improved cooking stoves and biomass-based water boiler for cooking bear food. Biomass briquettes are nothing but agricultural and animal waste. The idea is to replace LPG (a fossil fuel) which was used for cooking purposes. Considering LPG is replaced by a biomass cooking stove each year we reduce emissions by approximately 196 $_{t}$CO2. This green kitchen not only reduces emissions but also encourages use of biomass as a sustainable energy resource and prevents firewood utilisation and deforestation activity.

Art – 250. INCIDENCE OF HYPHEMA IN PEAFOWL (*Pavo cristatus*)

P.R. Acharya, **S. Ilayaraja, M.V. Baijuraj**· and **A. Sha Arun**

Abstract

Wildlife SOS team rescued a Peahen of storm victim from a village near Agra with a history of severe trauma at head. Complete health examination revealed it to be case of hyphema. Parenteral and topical treatment using corticosteroids and antibiotics was provided to the bird for six consecutive days. Complete recovery was observed on 6th day. It was concluded that management and successful recovery in traumatic hyphema can be achieved by systemic and topical therapy. The blue peafowl (*Pavo cristatu*s) was declared as a National Bird of India in 1963 and it is kept under Schedule I of Indian Wildlife Protection Act, 1972 so providing utmost importance is mandatory (Kushwaha and Akhilesh, 2016). The species is in least concern category in the international union for conservation of nature (IUCN) red list. The blue peafowl lives especially in dense forest, both wet and dry forest. However, these birds are adopted to agricultural region now a days and even found very close to human sites (Forshaw, 1998), such that they have water at hand. The peafowl are omnivorous with wild peafowls having vast range of food habits which includes plants, berry, seeds, insects and small creatures such as reptile and amphibians (Arshad *et. al.,* 2000; Chopra and kumar, 2014). Freeman *et. al*. (2008) mentioned that bird communities can be significantly affected by severe storms due to potential destruction of food resources. Any physical injury happened due to natural calamities will further increase the difficulties of their survivability. The illegal poaching for feathers, meat trade and mass mortality due to discriminate application of pesticides and herbicides in crop fields are remain as major causes of the recent decline in peafowl population (Ramesh and McGowan, 2009). In this article, we discussed about successful rescue and rehabilitation of an injured peahen of storm victim, which was rescued by Wildlife SOS rescue team at Agra.

Key Words: Eye injury, Hyphema, Peahen, Storm victim.

Case History, Observations and Discussion

Wildlife SOS team rescued a Peahen which is reported as storm victim from a village near Agra with a history of severe trauma at head. Complete health examination revealed that bird is suffering from impaired vision due to right sided buphthalmos and hyphema i.e.bleeding in the anterior chamber (Fig.1) (Jason Lavy, 2007). Radiological examination not revealed any bone fracture (Fig.5). Good vision is important in birds as it directly influence on flight, feeding and breeding (Bayon *et. al.,* 2007). Hyphema in peafowl is one of the important ocular issues which can cause partial to complete loss of vision if not treated properly thus predisposes the bird as easy prey.

Parenteral and topical treatment provided to the bird for six consecutive days. On day 0, bird was treated with injection dexamethasone @ 2 mg (Total dose), injection neurokind @ 0.4 ml (Total dose), injection gentamicin @ 5mg/kg.bwt. and topical eye drop Tobra D (Tobramycin + dexamethasone). Noureddin *et. al.* (2015) recommended topical and systemic corticosteriods to increase patient comfort and decrease inflammation that accompanies hyphemia. Same medication was repeated for six days and progress of bird noticed. Reduced buphthalmos, aggregation of RBC's in streak form towards dorsal aspect of anterior chamber (Fig.2) exposing ventral portion and improved response to menace reflex was observed on day 2. The menace reflex and blink reflex to visual threatwas frequently used method for visual testing (Ballegoij *et. al.,* 2015). Remarkable reduction in buphthalmos and aggregated RBC's, exposing major part of pupil was noticedon day 4 (Fig. 3). Increased response was recorded to menace reflex suggesting that the bird was gaining its vision gradually. On 6th day, complete recovery was observed and redness of eye was totally vanished. However, the bird was under observation for next five more days to ensure the complete recovery before release into suitable habitat.

Fig. 1: Bleeding in anterior chamber of eye on Day 0.

Fig. 2: Aggregation of RBCs in streak form on Day 2.

Fig. 3: Remarkable reduction in RBCs on Day 4.

Fig. 4: Complete recovery on Day6.

Fig. 5: Radiograph on Day 0.

Conclusion

Natural calamities are affecting the survivability of Indian peafowl by causing serious health issues or by destroying the food habits. Traumatic hyphemia is one of them, hindering the proper vision and making the bird prone as a prey for predators. Management and successful recovery from the condition can be possible by systemic and topical approach.

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Art – 251. GROSS AND HISTOPATHOLOGICAL LESIONS ASSOCIATED WITH TUBERCULOSIS IN TWO SLOTH BEAR (*Melursus ursinus*) IN INDIA

N. Gowri Menon, K. C. Bipin, P. M. Deepa, R. L. Rathish, M. Pradeep and **A. Sha Arun**

Abstract

Post-mortem examination of two sloth bears which died in Bannerghatta Bear Rescue Centre, Bengaluru, Karnataka, were performed. Both the animals were anorectic and had considerable weight loss before death. Representative lung tissue samples were subjected to histopathology and staining. The lung tissues of the animals had diffuse congestion and subpleural petechial hemorrhages. In addition, small nodules of varied diameters were seen scattered on the lung lobes of both animals. On histopathological examination, the lung tissue of one of the animals showed extensive proliferation of blood vessels. Congestion and subpleural hemorrhages were seen in both cases. Few macrophages and epithelioid cells were seen scattered adjacent to a bronchiole. Kinyoun's acid fast staining of the histological sections revealed numerous acid fast bacilli indicative of tuberculosis.

Key words: Tuberculosis, sloth bear, post-mortem

Sloth bear (*Melursus ursinus*) is one of the four species of bears which are found in India. They are classified as vulnerable species according to the International Union for Conservation of Nature and included in schedule I of the Indian Wildlife Protection Act, 1972. Tuberculosis (TB), caused by *Mycobacterium tuberculosis* is a significant cause of morbidity and mortality in both wild and domestic animals worldwide (Lecu and Ball, 2011). Emergence of tuberculosis in sloth bears could be due to the spill over of *Mycobacterium tuberculosis* into this species because of their prolonged contact with humans. A significant number of sloth bears were poached by a community of people known as the Kalandars, and trained for street shows. There is inadequacy of diagnostic assays for detecting tuberculosis in these animals. Moreover, the affected bears appear apparently healthy and exhibit only mild or vague clinical signs in the terminal stages, which make diagnosis and treatment difficult. Hence diagnosis of tuberculosis in this species during post-mortem examination is important for considering preventive measures in live animals against TB.

Materials and methods

Two sloth bears, which died naturally at Bannerghatta Bear Rescue Centre, formed the sample of the study. Both the bears exhibited anorexia, isolation from the group and considerable weight loss before death. A thorough post-mortem examination of the animals was carried out and representative samples from lungs were collected in 10 percent neutral buffered formalin for histopathology.

The formalin fixed samples were washed overnight, processed, cut at five micron thickness and stained with haematoxylin and eosin as per Suvarna *et al.*, 2012. Special staining technique, Kinyoun's acid fast staining, was done to demonstrate the presence of the acid-fast bacteria. The staining procedure is briefed below.

The tissue sections were deparaffinized and hydrated to distilled water after which it was immersed in Kinyoun's carbol fuchsin and incubated at 50°C for one hour. The slides were differentiated in two changes of one per cent acid alcohol after washing in running tap water. Counter staining was done using methylene blue solution and the slides were dehydrated in 95 and 100 per cent alcohol sequentially and cleared in two changes of xylene. The stained sections, after drying completely, were mounted in DPX and viewed under oil immersion objective (100x) in a compound microscope.

Fluorescent staining technique using auramine was also performed to further confirm the presence of acid-fast bacilli. Staining technique was done as per Suvarna *et al.,* 2012 with slight modifications. The tissue sections were deparaffinized and hydrated to distilled water after which the slides were flooded with phenolic auramine – O and placed at 60°C for10 minutes. It was then rinsed off with distilled water and decolourised using one per cent acid alcohol for 2 minutes. Counterstaining was done using Potassium permanganate solution for five minutes. The slides were then dehydrated in 95 and 100 per cent alcohol sequentially and cleared in two changes of xylene. The stained sections, after drying completely, were mounted in DPX and viewed under oil immersion objective (100X) of fluorescent microscope (Carl Ziess®).

Results and discussion

On gross examination, the carcasses of both the animals appeared emaciated. Diffuse congestion along with subpleural petechial hemorrhages were evident in all lung lobes of the first animal (Fig. 1). This finding is in agreement with Pereira (2016) who also reported presence of areas of congestion and hemorrhage as post-mortem findings in TB affected sloth bears. Small nodules of two centimeter diameter were seen scattered on both lung lobes. The right cranial lobe showed two distinct dark red colored nodules of three to four centimeter diameter. This is in agreement with Hedau and Kamdi(2016) who reported the same in a sloth bear carcass suspected to have died of TB.

The second animal showed multiple nodules of two to three centimeter diameter scattered over the parenchyma of both left and right lung lobes (Fig. 2). In addition, left lung lobes had diffuse areas of congestion and mild edema. The present findings are in agreement with Hunter (2011) who reported that granulomas were characteristic lesions of primary TB in immune-competent individuals.

Microscopically, extensive proliferation of capillaries along with severe congestionand hemorrhages were found in the lung tissue sample of the first animal, suggestive of haemangioma (Fig. 3), whereas the lung tissue sample from second animal showed focal areas of macrophages and epithelioid cell proliferation adjacent to a bronchiole (Fig.4). The typical tubercular granuloma with central caseous necrosis and surrounding inflammatory cells as described by Fefar *et al.*(2012) was not present in both the samples. However, Hernandez-Pando *et al.* (2000) had reported the presence of *M. tuberculosis* inlungs without typical histopathologic lesions of TB in case of human beings.

Both the tissue sections showed the presence of numerous acid-fast bacilli when stained using Kinyoun's acid fast staining technique (Fig. 5). This is in agreement with Stephenson and Byard (2020) who reported that TB could be confirmed histologically by acid fast staining of suspected tissue sections. Rishikesavan *et al.* (2010) also confirmed TB in a captive male leopard by the presence of numerous acid-fast bacilli in stained tissue impression smears.

Fluorescent staining for mycobacteria was also carried out in the tissue sections and both tissue samples showed the presence of numerous fluorescent acid-fast bacilli when viewed under oil immersion objective (100X)of fluorescent microscope (Fig. 6). This is in agreement with Bodal *et al.* (2015) who reported that fluorescent staining was highly sensitive and can be used to confirm TB.

In the present study, both acid-fast and fluorescent staining of histological sections showed identical results, which indicate that both these techniques are equally effective in confirming TB in PM samples. However, Kommareddi *et al.* (1984) reported that fluorescent staining using Auramine- O was simpler and had more sensitivity compared to Ziehl Neelsen (ZN) technique, at the same time, less specific than ZN technique.

Fig. 1. Gross picture showing diffuse congestion & subpleural petechial haemorrhages in lung tissue

Fig. 2. Gross picture showing multiple nodular lesions in lung

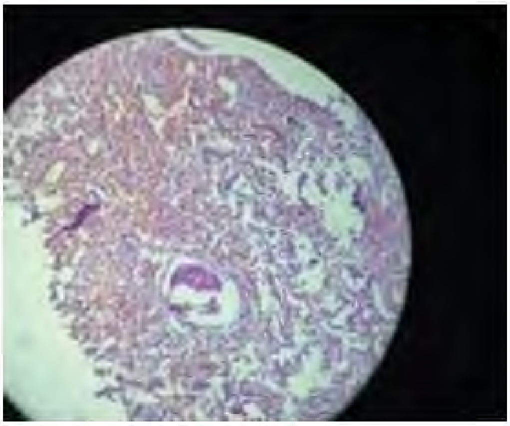

Fig. 3. Capillary proliferation with congestion and haemorrhage, H and E(100x)

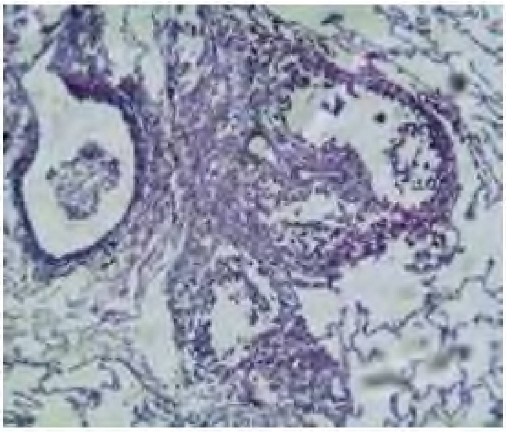

Fig. 4. Lung section with macrophages and epithelioid cells scattered adjacent to bronchiole, H and E (100x)

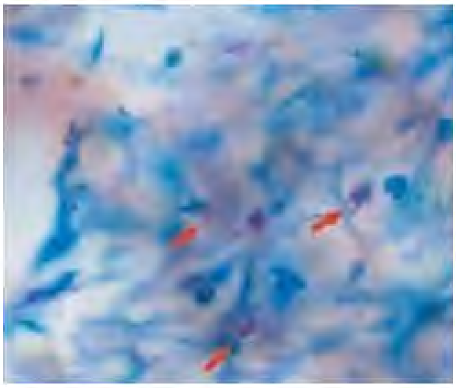

Fig. 5. Lung tissue with acid fast bacilli (arrow), Kinyoun's acid fast staining (1000x)

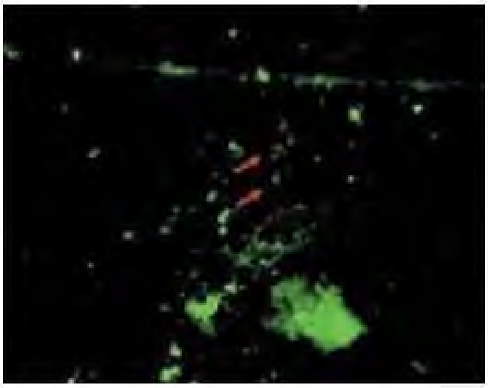

Fig. 6. Lung tissue with acid fast bacilli (arrow), Fluorescent staining (1000x)

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Art – 252. RELOCATION OF A GPS COLLARED CONFLICT SLOTH BEAR *Melursus ursinus* (MAMMALIA: CARNIVORA) IN KARNATAKA, INDIA

Attur Shanmugam Arun, Shanmugavelu Swaminathan, Yogaraj Pannerselvam, Thomas Robert Sharp, Sydney Rae Stephens**, Kartick Satyanarayan & Geeta Seshamani**

Abstract

The relocation of conflict bears has been a tool used widely across the United States and Canada with mixed results. It has also been used in India with Sloth Bears, though without follow-up it remains unknown how successful these relocation efforts have been. We documented the capture and relocation of a conflict female Sloth Bear from a rural area near Bangalore, Karnataka, India to Bannerghatta National Park roughly 30km away. This female bear, approximately six years old, was fitted with a VHF/GPS store-on-board collar, and her movements tracked. She did not attempt to return to her capture location but during the first two- month period after being released she did roam over an area roughly six times that of typical female Sloth Bear home range. Over the subsequent months the area over which she roamed continued to decline. She was least active mid-day and more active in the evening, night, and early morning. During her last few weeks in January, before she was killed by an explosive device just outside of the park, her movement pattern shrank considerably. The post-mortem examination showed that she had been pregnant when killed and would have given birth within the next two weeks. These reduced movements were consistent with those of periparturient female bears or potentially with a bear becoming more acclimated to her new surroundings. The relocation effort appeared successful up until the Sloth Bear was killed by poacher activity.

Keywords: Activity pattern, denning, Bannerghatta, poaching, crop raiding, reproduction.

Introduction

Sloth Bears *Melursus ursinus* are among the least studied bear species in the world and therefore one of the least understood (Garshelis & Steinmetz 2015). They are presently listed as Vulnerable on IUCN's Red List (Dharaiya et al. 2016), and as a Schedule 1 species under the Indian Wildlife Protection Act of 1972. The continued deterioration and fragmentation of habitat outside of protected habitats, where it is thought that the majority of Sloth Bears persist, is presently one of the greatest threats to the species (Dharaiya et al. 2016). The recent and complete extirpation of this species from Bangladesh highlights the concern that fragmented Sloth Bear populations are at risk (Islam 2013). Unfortunately, Sloth Bear-human negative interactions are relatively common and often take the form of bear attacks (Rajpurohit & Krausman 2000). For these reasons, it is imperative to explore viable options for handling 'problem' bear situations, other than simply dispatching the 'problem' bear. The relocation of 'problem' bears is one potential option.

The relocation of 'problem' American Black Bears *Ursus americanus* and Grizzly Bears *Ursus arctos horribilis* has been used as a management tool across North America for decades with mixed success (Linnell et al. 1997). Relocation has also been used in India with 'problem' Sloth Bears, though the success or failure of this management tool has not been well documented. A 'problem' bear is generally defined as a bear that has been involved in repeated bear incidents. A 'bear incident' is defined as an occurrencethat involves a human-bear conflict or episodes (Skrbinšek & Krofel 2015). A human- Sloth Bear conflict usually means a Sloth Bear attacked a person in a defensive manner or behaved aggressively towards people, though it can also mean the bear was involved in crop raiding. The objective of relocation is to move a 'problem' bear to a new area where they are less likely to become engaged in negative interactions with humans. The relocation of a 'problem' bear is generally considered successful if the bear is not involved in subsequent incidents. Success, however, is often at least partially dependent on whether the bear returns to the capture site. Return rates tend to decrease as the relocation distance increases. Return rates are also lower for juvenile bears rather than adult bears (Rogers 1986; Landriault et al. 2009).

Sloth Bears, while generally not attracted to garbage, have conflicts with humans in the form of crop-raiding and attacks. While crop raiding is not a major problem for this species throughout much of its range, attacks are. Sloth Bears are renowned for their aggressive behavior toward humans (Burton 1856; Anderson 1957; Rajpurohit & Krausman 2000). While a Sloth Bear's attack motivation is exclusively defensive, the attacks can inflict serious injuries to the victim and might result in the victim's death (Rajpurohit & Krausman 2000; Bargali et al. 2005; Sharp et al. 2020). Unfortunately, Sloth Bear attacks are relatively common in India and affect hundreds of people annually

(Rajpurohit & Krausman 2000; Bargali et al. 2005; Debata et al.2016; Garcia et al. 2016; Dhamorikar 2017; Singh et al. 2018; Sharp et al. 2020). Given the large number of attacks that occur annually and the vulnerable status of this species (Dharaiya et al. 2016), it is reasonable to consider different management options for 'problem' bears, including relocation; however, there are no data to suggest how successful these relocations in India have been. Is the mortality rate high? Do relocated Sloth Bears attempt to return to their prior location as Grizzly Bears and American Black Bears sometimes do? In short, is relocation a useful management tool for this species?

A female Sloth Bear, believed to have attacked several people in a village roughly 30km from Bannerghatta National Park, was trapped for relocation. Permission was granted to release the bear back to the wild in Bannerghatta National Park with a GPS (store-on- board)/VHF collar. Given the paucity of data on Sloth Bear relocation efforts, as well as Sloth Bear movement and general ecology, the results of these efforts, though based on a single bear, offer valuable insights. This bear was tracked using the VHF transmitter after being released into Bannerghatta National Park. After six and a half months, she was killed by an explosive device illegally set for Wild Boars *Sus scrofa*. At this point, the collar was retrieved, and the store-on-board data downloaded. A post-mortem of the Sloth Bear showed that she had been pregnant when killed. Based on the morphometry and the weight and developmental size of the two fetuses, the female bear would have likely given birth sometime within the following seven to ten days. Very little is known about Sloth Bear breeding in the wild; therefore, movement patterns were also analyzed with respect to those of a periparturient Sloth Bear.

Study Area

Bannerghatta National Park encompasses roughly264km^2 of protected habitat (Fig. 1). The terrain is hilly with elevations ranging 1,245–1,634 m. The valleys are predominantly made up of deciduous forest, while the hillsides and higher elevation areas are covered in scrubland. The temperature in the park ranges from an average of 15^{o}C in the winter to an average of 30^{o}C in the summer, and it gets between 625 and 1,607 mm of rainfall annually (Ramachandra & Setturu 2019). The Suvarnamukhi River is the largest perennial river running through the park.

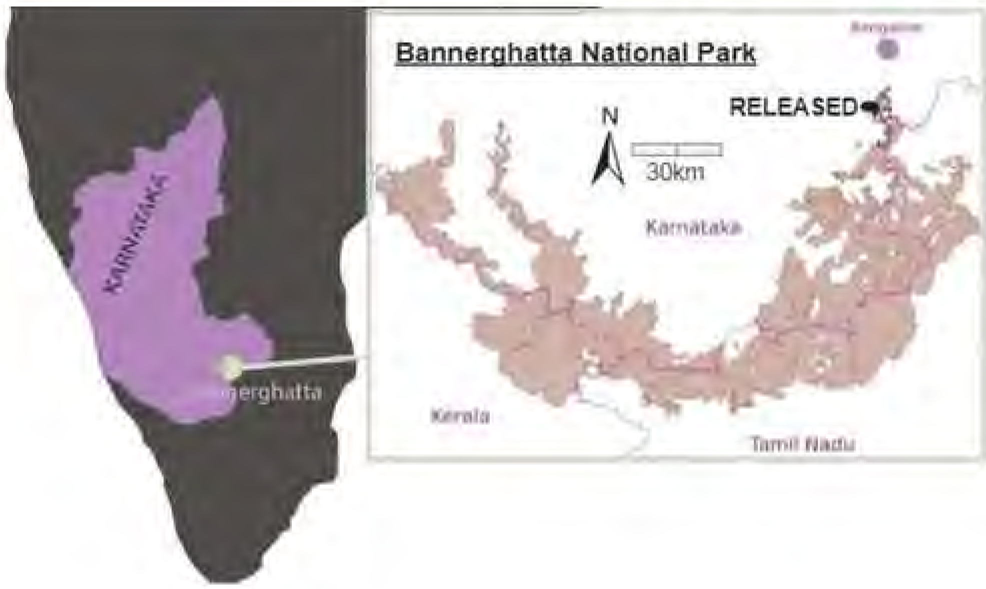

Figure 1. Bannerghatta National Park location within India, and the northeast-most section of the park (inlet map) where the Sloth Bear was released and GPS points were collected.

The southern end of Bannerghatta National Park connects to the Talli Reserve Forest and Bilikal Forest. The park also functions as part of an important Asian Elephant *Elephas maximus* corridor which also connects to the Biligirirangana Hills and the Sathyamangalam Forest. The park not only contains Sloth Bears and elephants, but other large mammals including Leopards *Panthera pardus*, Sambar *Rusa unicolor*, and occasionally even Bengal Tigers *Panthera tigris*. Medium and small mammals that live in the park include Dholes *Cuon alpinus*, Golden Jackals *Canis aureus*, Indian Porcupines *Hystrix indica*, and Indian Pangolins *Manis crassicaudata*. Many species of birds and reptiles also occur in the park including Peafowls *Pavo cristatus*, Mugger Crocodiles *Crocodylus palustris*, and Rock Pythons *Python molurus*.

Materials and Methods

A young adult female Sloth Bear was trapped at night near a village, roughly 30km from Bannerghatta National Park, on 17 March 2017 using a barrel trap baited with honey and fruit. The bear was taken to a remote enclosure at the Wildlife SOS, Bannerghatta Bear Rescue Centre and was given a general health check, and a blood sample was collected, to make sure she was fit to be released back into the wild. She was kept in isolation from other bears, and human interaction was kept to a bare minimum in order to prevent any habituation behaviors. Permission was eventually granted by local authorities to release her in Bannerghatta National Park. The bear was fitted with a Veltronic Aerospace Vertex store-on-board GPS/VHF collar before being released at 07.20h on 30 June 2017. To get a general idea of her movement, the bear was tracked daily, homing in on the pulsed radio signals from the VHF transmitter in the collar, using a receiver and directional antenna. The tracking sessions were completed in the morning, roughly 75%of the time, and in the evening, roughly 25% of thetime. When the bear was found dead, we collected the collar and downloaded the GPS data for further analysis. Additionally, a post-mortem was conducted on the bear, primarily to determine the general health of the bear at the time of her demise. When it was discovered that thebear had been pregnant at the time of death, we checked the progesterone levels in her blood from when she was first captured in an effort to determine with certainty whether she had been impregnated before or after she was captured. Sloth Bears have delayed implantation (Puschmann et al. 1977) which make identifying the time of copulation difficult to ascertain simply by knowing the date, or approximate date, of when the cubs were born. We analyzed the Sloth Bear's movement and frequency of presence by splitting the GPS data points into three time periods. These categories were chosen based on: limited telemetry data gathered before the GPS data were available, a preliminary assessment of the GPS data to note any obvious change in movement rates, and finally the estimation of when the pregnancy would have become active (when the blastocyst implanted) based on the morphometry and weight of the fetuses during the post-mortem. The first period was made up of the initial two months (30 June through 31 August), when the bear was first acclimating to its new surroundings. The second period was made up to the middle three months (01 September through 30 November), after the bear had some time to acclimate and explore her surroundings. The third period was made up of the last month and a half (01 December 2017 through 17 January 2018), in what we call the periparturient period.

We generated maps representing the Sloth Bear's movement and frequency of presence in a given area using ArcGIS Pro 2.2.1. We recorded coordinates once hourly, with 24 counts per day, and an average daily success rate of 89.7% (~2 missed points, SD: 13.7%), with33.5% of days having all 24 points recorded and error evenly distributed across the whole sampling period.In total, 4,848 locations were uploaded from the GPS collar, with 4,289 (88.5%) non-blank recordings used for analysis over 202 days. The release period (30 June – 31 August 2017) had an 87.9% overall success rate, the acclimation period (September– November 2017) had an 87.5% overall success rate, and the periparturient period (01 December 2017–17 January 2018) had a 91.1% overall success rate. We rendered hotspot representation by using the geoprocessing spatial analyst tool: kernel density, which uses the quadratic kernel function. This method creates a search radius around a point that is classified based on the sum of GPS collar counts within that circular area. Point counts of GPS locations, reported in decimal degrees, were classified into eight bins using the geometric interval method, where warmer colors progressing from red, yellow, to orange, convey high visitation\number of GPS collar counts- and greens convey little to one-time visitation. Each map's high and low densities are respective to the designated period and not standardized across the three time periods.

Results

General Movement Pattern

Once released, the Sloth Bear did not appear to attempt to return to her original capture location. She stayed predominantly within the national park borders, though she did wander outside the park borders (Fig.2). The area she utilized in six and a half months was 71.2km^2, where 54.6km^2 (77%) were within the park and16.6km^2 (23%) were outside of the park. The furthest that she roamed beyond park borders was 2.26kmto the north. She moved an average distance of 5.9km night, with a minimum of 1.0km and a maximum of 14.7km (Fig. 3). While she did move roughly the same amount from August through December, and even more during the acclimation period than during therelease period (Figs. 3, 4, 5), the area over which she moved shrank as time went on. Between 30 June and 30 August, she utilized 63.8km^2 (Fig. 4), between 1 September and 31 November she utilized 31.5 km^2 (Fig. 5) and between1 December and 17 January, during the periparturient period, she utilized a total area of 23.4km^2. (Fig. 6). In January, the last 17 days before she was killed, she moved an average distance of just over 4km a day, and over a smaller area (8.6km^2) than she had in any of the previous sixmonths (Fig. 7).

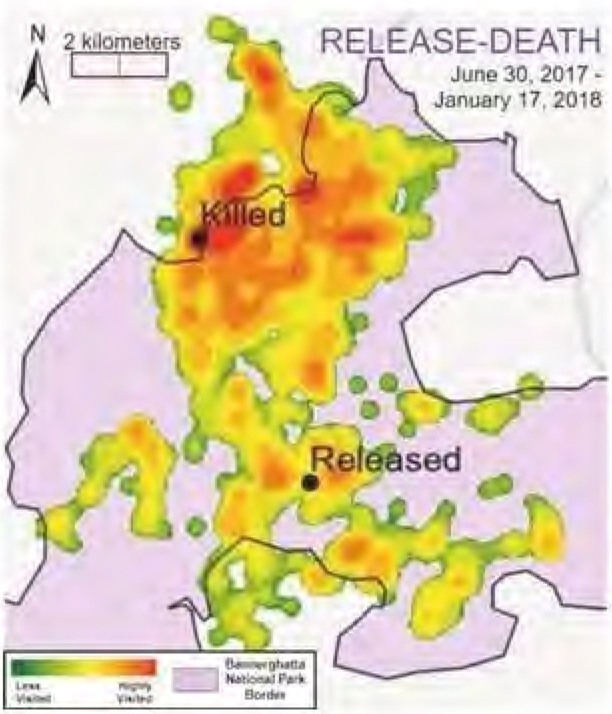

Figure 2. Area utilization by the Sloth Bear over the entire duration of release.

(Days: n= 202, GPS Point Counts: n= 4,289) Hotspot representation rendered by using the geoprocessing spatial analyst tool: kernel density, which uses the quadratic kernel function. Point counts were classified into eight bins (colored) using the geometric interval method.

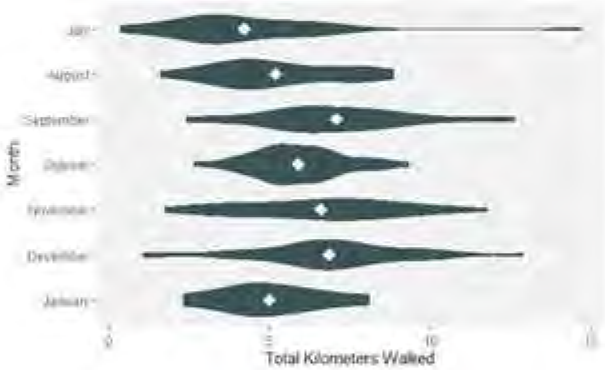

Figure 3. Violin plot of relocated wild Sloth Bear movement per day/per month (km).

Violin plot demonstrates the range of kilometers traveled daily each month, where thicker regions convey a higher number of days spent walking that respective distance. White diamonds represent the mean value for each month (July: n=31, x=4207, SD = 2678; August: n=31, x=5198, SD = 1910; September: n=30, x=7068, SD = 2345; October: n=31, x=5887, SD = 1456; November: n=30, x=6576, SD = 2433; December: n=30, x=6854, SD = 2471; January: n=17, x=4993, SD = 1641).

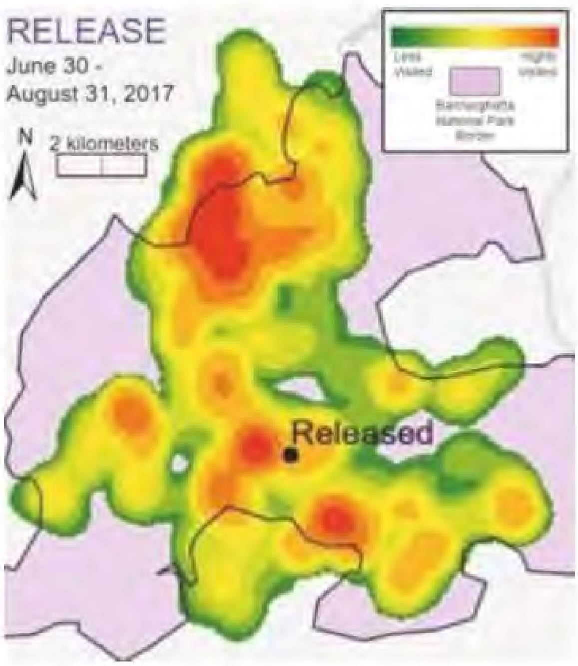

Figure 4. Area utilization by the Sloth Bear during the first two months after release.

(Days: n= 63, GPS Point Counts: n= 1,329) Hotspot representation rendered by using the geoprocessing spatial analyst tool: kernel density, which uses the quadratic kernel function. Point counts were classified into eight bins (colored) using the geometric interval method.

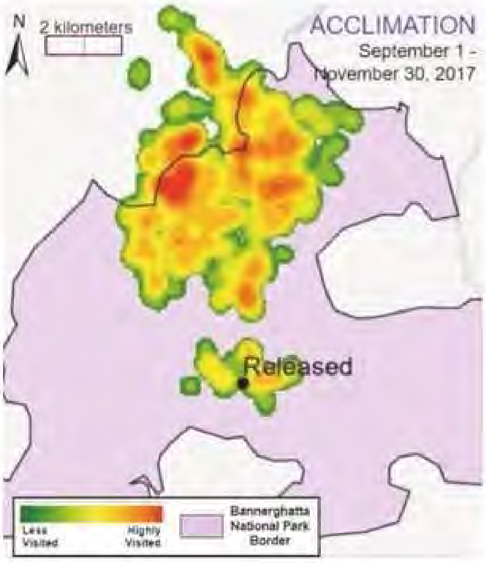

Figure 5. Area utilization by the Sloth Bear during months 3, 4 and 5 in Bannerghatta NP.

(Days: n= 91, GPS Point Counts: n= 1,911) Hotspot representation rendered by using the geoprocessing spatial analyst tool: kernel density, which uses the quadratic kernel function. Point counts were classified into eight bins (colored) using the geometric interval method.

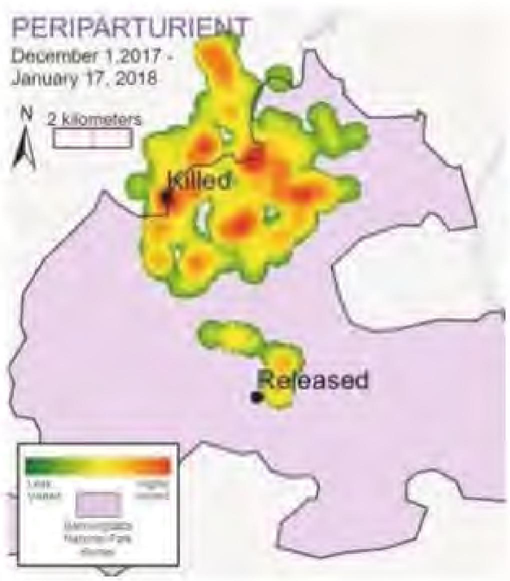

Figure 6. Area utilization by the Sloth Bear during months 6 and 7.

(Days: n= 48, GPS Point Counts: n= 1,049) Hotspot representation rendered by using the geoprocessing spatial analyst tool: kernel density, which uses the quadratic kernel function. Point counts were classified into eight bins (colored) using the geometric interval method.

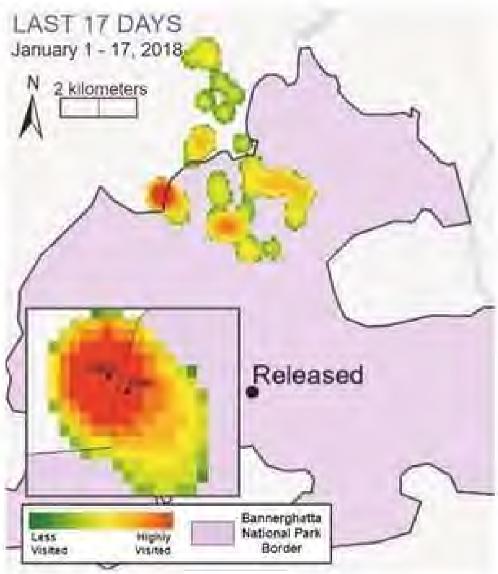

Figure 7. Area utilization by the Sloth Bear for two weeks before death in month 7.

(Days: n= 17, GPS Point Counts: n= 372) Hotspot representation rendered by using the geoprocessing spatial analyst tool: kernel density, which uses the quadratic kernel function. Point counts were classified into eight bins (colored) using the geometric interval method.

Movement was documented south and north of the national park borders, although she eventually settled near the northern border of the park where she spent much of her time. She was photographed multiple times by the use of camera traps and appeared to be a healthy bear (Image 1). She came close to several communities but never, as far as we are aware, had any encounters of consequence with humans. She was found dead just 83m outside of the national park in a fruit orchard (Fig. 7). She had been killed by an explosive device likely set to kill Wild Boars.

***Image 1. Collared Sloth Bear caught in a camera trap*.**

24-Hour Activity Pattern

Diel activity patterns show that she was most active 22.30–04.30 h, with minor peaks at 01.00h and 03.30h, and least active 09.00–15.00h (Fig. 8). This activity pattern did not change substantially throughout the six-and-a-half months post-release. In July, when first released, she was most active 17.30–05.30 h, with activity peaks around 00.45h and 05.00h, and least active 08.00–15.30 h. In January, before her death, she was most active 20.00–05.30 h, with activity peaks at 00.00h and 03.45–04.45 h. She was least active 08.00–15.00 h. The slight changes in peak activity and inactivity during the six-and-a-half months post-release are not correlated with slight changes in sunrise and sunset times.

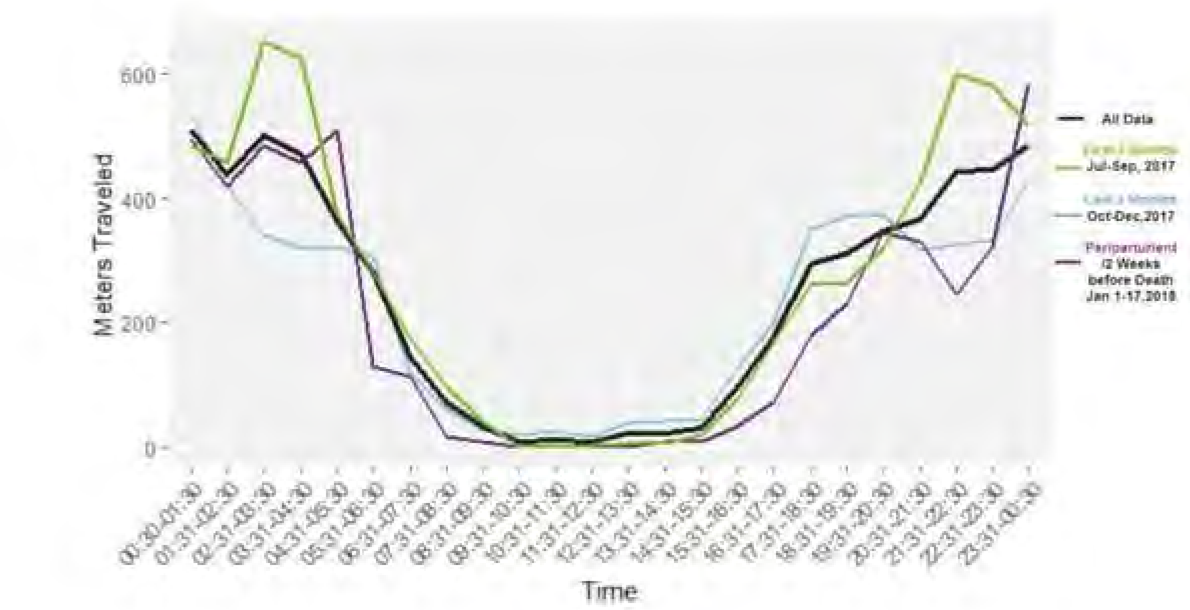

Figure 8. Sloth Bear 24-hour activity pattern per hour

Mean movement (in meters) during 1-hour time blocks for each respective time period, where black is a mean of all data (N=202), green is the mean of the first three months after release (30 June–30 September 2017, n= 93), blue is the mean of the following three months after release (1 October–31 December 2017, n= 92), and purple is the last ~2 weeks before death, during the periparturient time period where implantation is expected to have occurred (1–17 January 2018, n= 17).

Pregnancy and Denning

Necropsy revealed that the Sloth Bear had been pregnant with two cubs. The fetuses were 14 and 15 cm in length, and weighed 60 and 67 g, respectively. We attempted to discern whether she had been impregnated before or after her release by checking progesterone levels in the blood that had been drawn after capture; however, we were unsuccessful due to: 1) lack of access to a baseline of blood progesterone levels in pregnant Sloth Bears, and 2) the delayed implantation in Sloth Bears may cause a delay in raising progesterone levels, as seen in other bear species (Foresman & Daniel 1983). By reviewing the data from the store-on-board GPS unit, we were able to locate multiple resting dens that she had used, including the den she had been using in January. It is likely that this latter den would have been used as the maternal den, which we describe further in the discussion. This den is located in the national park, just 60m from the boundary (Fig. 7).

Discussion

General Movement Patterns

The bear did not appear to attempt to travel back to her capture site though she was only moved 30km. Translocating an American Black Bear or Grizzly Bear only 30km from the trap site would carry with it a relatively high probability that the bear would attempt to return to the trap site (Rogers 1986; Linnel et al. 1997). Sloth Bears, however, have relatively small home ranges compared to these two species and this may affect how far Sloth Bears need to be moved to reduce the likelihood they will return to their capture site area. This Sloth Bear did range over a large area inside and outside of the park borders. Given that the home range for female Sloth Bears in Nepal's Royal Chitwan National Park was estimated at 9.4km^2 (Joshi et al. 1995) and 12.4km^2 in Panna National Park (Yoganand et al. 2005), she appeared to have spent the first two months exploring her new surroundings by utilizing an area roughly six times the size of a typical home range. Interestingly, translocated Grizzly Bears in Alberta, Canada translocated outside of their bear management area of capture, were shown to initially have home ranges roughly 3.25 times that of other resident bears (Milligan et al. 2018). While the presence of other Sloth Bears may be an influencing factor in the dispersal of this individual, the population within Bannerghatta National Park is unknown. It has been speculated that this increase in movement could be linked to increased energetic costs of the bear during the relocation acclimation period (Milligan et al. 2018).

During the Sloth Bear's second three-month period, her activity only utilized an area roughly three times the size of a typical female Sloth Bear's home range, and in December an area only roughly twice the size of a typical home range. This eventual reduction in home range size is consistent with the translocated Grizzly Bears in Alberta, Canada which also saw an overall reduction in home range size as time went on (Milligan 2018). However, the reduction in the size of the Sloth Bear's home range occurred at a much faster rate than it did for the grizzlies in Alberta. The Sloth Bear's movements in January covered an area more comparable to the estimated home range size for an adult female Sloth Bear; however, this reduction may have been more related tothe fact that she was preparing to give birth, rather than a sign she had acclimated to her new surroundings.

24-Hour Activity Pattern

Sloth Bears, though occasionally diurnal, are known to be predominantly crepuscular and nocturnal (Joshiet al. 1999; Chauhan et al. 2004; Yoganand et al. 2005; Ramesh et al. 2013). Subadults and females with cubs, however, may be more active in the morning hours, which may be an attempt to avoid large male Sloth Bears or predators (Joshi et al. 1999). Given that this bear was an adult female without cubs, her activity pattern is consistent with solitary adult females from other studies. There is a longer period of complete inactivity in November, December, and January; however, we cannot be certain whether this is related to the bear's pregnancy, the bear's acclimation to her new surroundings or an unknown variable.

Pregnancy and Denning

Sloth Bear mating generally occurs during April, May, June, and possibly July in this part of the country (Arun et al. 2018a) and cubs are born five to eight months later (Stirling 1993). This bear was captured on 17 March and therefore likely impregnated after her release. Additionally, in early July, while tracking her with VHF technology, it was noted by observing her footprints that she was in the company of a second sloth bear, which could have possibly been her mate. Though generally solitary, Sloth Bears do have a high degree of mutual tolerance for one another (Joshi et al 1999). Therefore, although we cannot be certain when she was impregnated, we believe it to be most likely that she was impregnated after her release back to the wild.

It is not surprising that the mating window in this part of India may be a little wider than previously thought as the Sloth Bear mating season varies slightly by location. In Nepal, they are known to breed May through July (Joshi et al. 1999), and in Sri Lanka, they are thought to breed year-round. If indeed, she had been impregnated post-release, it suggests low stress levels and adjustment to her new surroundings. Whether impregnated before or after release is perhaps less important than the fact the pregnancy was moving forward. The delayed implantation capabilities of the Sloth Bear allow a female to abort and absorb the pregnancy if the animal is physically or environmentally stressed (Mead 1989; Given & Enders 1989). Therefore, the fact that the pregnancy was moving forward suggests that the sloth bear was not overly stressed in her new environment, or at least that the increased energetic costs likely associated with the relocation were still low enough for her to reproduce successfully.

Once implanted, the fetus grows to completion in roughly two months, as is the average time of gestation in bear fetuses (Tsubota et al. 1987; Quest 2001). Since we estimate, she was going to give birth in late January or early February, it seems likely that her pregnancy influenced her movement patterns in December and January. It is also possible that her movements were further reduced in January due to her having identified a maternal den and associated reduction in feeding. Though Sloth Bears in captivity are known to eat within 24 hours of parturition, periparturient appetite is suppressed as parturition approaches (Arun et al. 2018a).

After the bear's death, we inspected the area where she had spent a large amount of her time in January to search for dens. We located several dens, including one within the hotspot. We believe this was likely the den in which she planned to give birth to and raise her cubs since the location was the centre of her activity as she approached parturition. This den is located only 60mfrom the border of the national park. Because this bear spent a significant amount of time just outside the national park in an area with fruit trees, it suggests she might have intentionally chosen to den in the wilderness with food resources, in this case, a fruit orchard, nearby. This Sloth Bear was killed near the Bannerghatta National Park border by an explosive device set by poachers most likely to hunt Wild Boars. These devices are hidden in food and detonate when bitten, thus presenting a risk to non-target species (Arun et al.2018b). Consequently, these devices potentially pose a particular threat to wildlife, which range just outside of protected areas to forage in agricultural areas.

Relocation Conclusions

As stated previously, the relocation of a nuisance bear is generally considered successful if the bear is not involved in any subsequent human-bear conflicts. This is often at least partially dependent on whether the bear returns to their capture site. We believe this Sloth Bear's relocation was successful because: 1) she did not attempt to return to her capture site, 2) she was not involved in human-bear conflicts, other than occasional crop-raiding, 3) she adjusted to her new surroundings and began to establish a home range, 4) she was likely impregnated post-release, 5) her pregnancy was movingforward and we believe she established a maternal den, and 6) she was a healthy bear, based on camera trap photos of her as well as her necropsy.

Given these findings, this relocation effort was deemed a success until the bear was killed. It is also important to note that her death was not the result of a "direct" conflict situation but rather due to a negligent and illegal act not focused on sloth bears. Clearly, this is only one bear, and more documentation is needed in the future in order to determine how successful relocation efforts of Sloth Bears are; however, based on this case study, there is reason to be optimistic.

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Art – 253. MANAGEMENT OF DEGENERATIVE JOINT DISEASE IN AN ADULT CAPTIVE SLOTH BEAR *(Melursus ursinus)*

Arun A. Sha, Riya Bakde and **Adhithyan N.K.**

Abstract

Degenerative joint diseases are commonly encountered in pet practice, but reports among the wild counterparts are few. Management of joint disorders in Sloth bears requires in-depth studies to understand the factors associated with them. The present case reports a 16-year- old rescued dancing male sloth bear housed at the lifetime care facility of Bannerghatta bear rescue centre, showing difficulty in movement and gait abnormalities. The bear was immobilized using xylazine-ketamine combination. Upon general health check- up, blood samples were collected, and complete radiography was performed. Hematobiochemical analysis revealed no significant abnormalities. A series of whole pelvic imaging showed irregular bone density and fragmentation of the femur head and neck and widening joint space indicative of bilateral osteoarthritis of coxo-femoral joint. The bear was supplemented with a joint supplement along with calcium-phosphorus suspension for a period of 3 months. Enrichment activities were done as a means of physiotherapy. The sloth bear showed notable progress with increased mobility and gait pattern. Diagnostic imaging as a tool for early diagnosis of orthopaedic disorders in sloth bears coupled with therapeutics and physiotherapy needs to be further exploited for the betterment of the captively housed wild animals.

Keywords: Degenerative joint disease, Osteoarthritis, Sloth bear, Diagnosis, Treatment

Introduction

Osteoarthritis (OA) or degenerative joint disease (DJD) is a multifactorial, inherited, polygenic disorder involving the synovium, articular cartilage and the underlying bone which can be secondary to congenital developmental abnormalities, instability of the joint or traumatic injuries (Henrotin *et al.,* 2005; Aragon *et al.,* 2007; McCarthy *et al.,* 2007; Runge *et al.,* 2010;Ohlerth *et al.,* 2019). Retrospective studies describing the prevalence of OA in dogs have been frequently reported with very few similar studies in wild animals (Rothschild*et al.,* 1994; Rothschild *et al.,* 1999; Kelly and Murice, 2011 and Selvaraj *et al.,* 2017). Palaeontological investigations on the preserved skeleton specimens of different species of bears reported arthritis as a common finding in the Ursidae family (Fox, 1939; Greer, 1977; Kompanje *et al.,*2000). Radiographic evidence suggestive of osteoarthritis was reported in brown bear *(Ursus arctos)*, black bear *(Ursus americanus)*, polar bear *(Ursus maritimus)*, sun bear *(Helarctos malayanus)* by Follmi(2005) and Aminkov *et al. (*2018) with only one such incidence been reported in sloth bear *(Melursus ursinus)* (Selvaraj *et al.,* 2017). Sloth bears rescued from the barbaric dancing bear tradition maybe prone to OA or DJD owing to the unhygienic and stressful conditions that they were raised in. These bears were trained by the Kalandar community using extremely harsh methods with complete lack of proper nutrition and veterinary care (Selvaraj *et al.,* 2017, Sandilya, 2019). Besides, geriatric sloth bears often develop arthritic joints consistent with degenerative joint disease (Sun bear and Sloth bear care manual, 2019). The present paper describes the diagnosis and therapeutic management of bilateral coxo-femoral osteoarthritis in a rescued dancing male sloth bear.

Materialand Methods

A 16-year-old captive male dancing sloth bear housed under the lifetime care facility of Bannerghatta bear rescue centre was showing recurrent episodes of hindlimb lameness, gait abnormalities and reduced mobility. The sloth bear was reluctant during physical activities such as climbing trees and hammocks present in his socialisation pen. Upon no improvement after preliminary treatment, the bear was sedated for general health examination using xylazine-ketamine combination@ 2mg/kg and 5mg/kg, respectively. Complete radiography of the pelvic region(Fig. 1 and 2) was performed along with blood sampling for laboratory analysis.

Fig. 1: Sloth bear immobilized for radiographic examination.

Fig. 2: Sloth bear positioned (VD view) for radiographic examination.

Fig. 3: Ventro-dorsal radiographs showing bilateral osteoarthritis of coxo-femoral joints.

Fig 4: Ventro-dorsal radiographs of normal hip joint in an adult sloth bear

Therapeutic management of osteoarthritis was attempted by supplementing the sloth bear with chondroitin sulphate tablets (Vendisc®) coupled with calcium and phosphorus suspension for a period of 3 months. In addition, the sloth bear was shifted to a separate area with built in physical structure as a means of providing exercise.

Results and Discussion

Hemato-biochemical analysis revealed no significant abnormalities whereas radiographic examination of the pelvic region showed bilateral degenerative changes in the coxo-femoral joint (Fig. 3).

Remodelling and osteolytic changes of both the femoral heads with flattening of acetabular rim and widened joint space were also evident. The radiographs also showed irregular bone density and fragmentation of the femoral head and neck.

After the course of treatment with the joint supplements, (chondroitin and glucosamine) the bear showed tremendous improvement in activity and mobility. The stress level of the animal had reduced, animal started normal feeding pattern and was showing normal appetite. The sloth bear started involving in enrichment structures and activities like climbing and digging burrows. Serum ALP level showed a slight reduction, and the Haematological parameter were normalised (compared to high HCT and total protein values associated with less food and water intake). Osteoarthritis or Degenerative joint disease is commonly reported in dogs with limited literature available in their wild counterparts. (Rothschild *et al.,*1999; Kelly and Murice, 2011; Follmi, 2005; Aminkov *et al.,*2018). The progression of osteoarthritis can be slow and dependent on several components such as genetics as well as environmental factors. External stressors or traumatic injuries affecting the weight bearing capacity of the subluxated hipjoint may damage the articular cartilage and initiate an inflammatory process subsequently leading to subchondral sclerosis and remodelling of the joint. (Smith *et al.,*2001; Schachner and Lopez, 2015; Iknega *et al.,*2019). Follomi, 2005 and Aminkov *et al.,*2018 radiographically examined bears with gait abnormality and lameness and reported osteoarthritis as the predominant findings in the affected bears. Management of osteoarthritis by supplementation with non-steroidal anti- inflammatory drugs, steroids and addition of joint supplements containing chondroitin sulphate and glucosamine hydrochloride has been shown to decrease cartilage degradation and alleviate the inflammation and pain (Henroitin *et al.,*2005; Aragon *et al.,*2007; Rock, 2007; Oke, 2009; Rychel, 2010; Comblain *et al.,*2016, Sun bear and Sloth bear Care Manual, 2019). Physical rehabilitation can be practised to improve gait function and reduce musculoskeletal pain associated with osteoarthritis. Laser therapy, thermotherapy, transcutaneous electrical nerve stimulation and acupuncture in conjunction with weight management (Rychel, 2010; Schachner and Lopez, 2015) may have positive outcome in arthritic animals. Designing of suitable enrichment structures like a wobble tree with a feeder and tree with honey dispenser may help in assessing thefunctioning of hip joint and vertebrae of the individual bears (Kitchener and Macdonald, 2002 and Law and Reid, 2010) coupled with routine radiographic examination of the bears may increase their longevity in captivity (Sun bear and Sloth bear care manual, 2019).

Summary

Osteoarthritis or degenerative joint disease is a polygenic and multifactorial developmental disorder that requires a multivariate therapeutic approach comprising of nutraceutical medications and physical therapy. In the present study, a rescued dancing sloth bear was radiographically diagnosed with bilateral coxo-femoral osteoarthritis. The affected bear was supplemented with chondroitin sulphate tablets along with calcium phosphorous suspension. Besides therapy, the bear was subjected to enrichment activities that involved honey or peanut butter pasted on tree barks and rocks thus providing him with enough physical exercise. In addition, routine radiographic examination of the sloth bears as a part of their general health examination was made mandatory for better management of geriatric bears under captivity.

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Art – 254. A MULTIPRONGED APPROACH FOR THE DETECTION OF LEPTOSPIROSIS IN CAPTIVE SLOTH BEARS (*Melursus ursinus*) IN AGRA AND BANNERGHATTA SLOTH BEAR RESCUE CENTERS IN INDIA

Karikalan Mathesh, Sabarinath Thankappan, Yosef Deneke, Beena Vamadevan, Chandra Mohan Siddappa, Anil Kumar Sharma, **Ilayaraja Selvaraj, Arun A Sha** and Ashok Kumar

Abstract

Leptospirosis is an exacerbating factor responsible for the drastic decline of sloth bear population in India. In this study, a multipronged approach based on antigen detection using Polymerase Chain Reaction (PCR) employing G1/G2 and LigBF/LigBR primers, antibody detection using Microscopic Agglutination Test (MAT) and recombinant LigBCon1-5 antigen based Latex Agglutination Test (rLigBCon1-5 LAT), serum biochemistry using hepatic (serum glutamate oxalo acetic transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT) and renal biomarkers (blood urea nitrogen (BUN) and Creatinine) and gross/histopathological evidencein liver and kidneys were employed to investigate leptospirosis in captive sloth bears. A total of 133 serum samples collected from Agra (n=113) and Bannerghatta (n=20) sloth bear rescue centers were screened using MAT and rLigBCon1-5 LAT. A total of 87 and 78 sera tested positive by MAT and LAT respectively. Pyrogenes was the leading serovar obtained using MAT followed by Icterohaemorrhagiae, Javanica, Grippotyphosa, Canicola and Tarassovi. The relative sensitivity, specificity and accuracy of rLigBCon1-5 LAT in comparison to MAT were 89.66%, 100% and 93.23% respectively. PCR performed on hepatic and renal tissues showed amplicon of 285 and 219 base pairs for G1/G2 and LigBF/LigBR primers respectively. Gross evidence (icteric liver, severely engorged hepatic sinusoids, congested kidneys with necrotic white spots on sub capsular surface), histopathology (severe hepatic degeneration and tubulointerstitial nephritis) and elevated hepatic/renal biomarkers were suggestive of leptospirosis. This study suggests that rLigBCon1-5 LAT can be employed as a pen-side test for detecting leptospirosis in sloth bears.

Keywords: histopathology, latex agglutination test, leptospira, microscopic agglutination test, serum biochemistry

Sloth bears are classified as vulnerable in the International Union for the Conservation of Nature and Natural Resources (IUCN)Red List of Threatened Species in 2020 [14]. Sloth bear population in India has declined alarmingly due to habitat loss, poaching, the use of bear gall bladder in traditional medicine [33] and predation by tigers [6]. Infectious diseases such as leptospirosis and tuberculosis have also contributed to the decline of sloth bear population [38, 39]. However, reporting of leptospirosis in sloth bears has been rare due to unavailability of penside diagnostics. In India, the onlyinstance where leptospirosis was previously reported in sloth bear was in Nehru Zoological Park, Hyderabad [38].

Microscopic agglutination test (MAT) is the gold standard serological test for detecting leptospirosis [3]. MAT provides information about *Leptospira* serogroups circulating in various animal species in a geographical area [11]. The effectiveness of the killed whole cell leptospiral vaccine is serovar-specific and this serovar-specific protection of killed leptospiral vaccine has been well documented [20]. Vaccination in sloth bears against leptospirosis in India is performed using imported canine vaccines. Hence, ample opportunity exists for developing indigenous leptospira vaccines targeting sloth bears utilizing the information generated by performing MAT in this study.

The inherent pitfalls of MAT have forced disease investigators to search for alternative field-oriented tests [8]. A noteworthy example is Latex Agglutination Test (LAT) which is a highly economical test suited for large-scale screening of sera samples without using sophisticated equipments [32]. The advent of recombinant DNA technology enabled the use of outer membrane proteins (OMPs) which can circumvent shortcomings of MAT when employed in diagnostic assays [22]. Leptospira Immunoglobulin-Like Proteins (Lig proteins) are the serodiagnostic markers for detecting acute leptospirosis and Lig proteins based immunodiagnostic assays would address the under-reporting of leptospirosis [12]. Hence, latex beads coated with first to fiftht andem repeat domains of the conserved region of LigB protein (rLigBCon1-5) has been employed in this study for detection of leptospirosis in sloth bears.

The abnormalities in hepatorenal function serves as an indicator for suspicious case of leptospirosis and based on elevation in the liver function tests such as serum glutamate oxalo acetic transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT) as well as renal function tests such as blood urea nitrogen (BUN) and creatinine, clinicians can develop their own clinical algorithm towards the confirmation of leptospirosis [26, 27]. Hence, serum biochemistry profile of terminally ill sloth bears has been performed to study patterns which will augment disease diagnosis.

A diagnostic PCR assay employing Lig gene was developed for the detection of pathogenic Leptospira interrogans in biological samples with high sensitivity with a detection limit of six leptospires [29]. Several researchers have successfully employed PCR as a diagnostic tool to maximize disease investigation credibility since positive PCR test results in tissue samples in the absence of isolation of the organism by culture attempts have been reported [16, 36]. Hence, in this study diagnostic PCR employing *LigB* gene reported previously [2] has been used to detect leptospirosis in biological samples of sloth bear whose results have been compared with well-established PCR assay [17].

Therefore, the aim of the present study is to conduct a multi-pronged approach for the detection of leptospirosis in sloth bears comprising of antigen and antibody detection which will be supported by serum biochemistry and gross/histopathology findings in hepatic and renal tissues of sloth bears.

Materials and Methods

Collection of sloth bear serum samples

A total of 133 sera samples (113 and 20 sloth bear sera samples from Bear Rescue Centers located in Agra and Bannerghatta, respectively) were submitted to Center for Wildlife, ICAR-IVRI by sloth bear rescue center authorities. Base Map of Sloth bear rescue centers located in Agra and Bannerghatta are depicted in Fig. 1. The rescue center authorities restrained the animals using standard protocols [33]. Briefly, the chemical immobilization of sloth bears was done using a combination of ketamine (5 mg/kg body weight; Ketamil®, Troy Laboratories, Smitfield, NSW, Australia) and xylazine (2 mg/kg body weight; Xylazil®, Troy Laboratories) delivered using a blow pipe on unsuspecting sloth bears thereby causing minimal stress to the animals. Blood was drawn from the jugular vein 10–15 min after immobilization using a 20-guage sterile hypodermic needle in vacutainer (Becton Dickinson, Franklin Lakes, NJ, USA). Serum was separated by centrifugation at 2,000 rpm for 20 min and stored at −20°C until used.

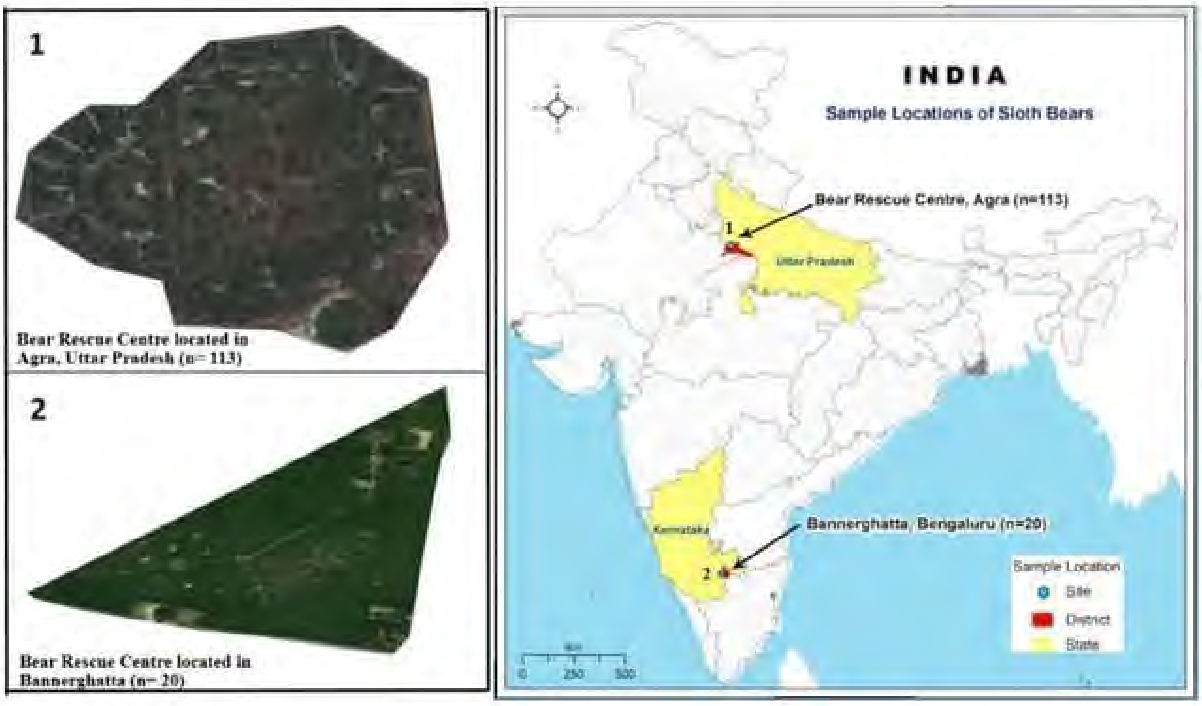

Fig. 1. Base map of Sloth bear rescue centers located in Agra and Bannerghatta from where sera samples were collected (Map was generated using ArcGIS 10.5 software).

Histopathology

Tissues such as liver and kidneys of all the dead sloth bears were fixed in 10% NBF (Neutral Buffered Formalin) and processed for histopathology following standard procedures [23]. Briefly, the tissues were kept under running tap water overnight and transferred to an automatic tissue processor (Leica Biosystems, Wetzler, Hesse, Germany) for further processing. Tissues were dehydrated in ascending grades of ethanol, cleared in xylene and embedded with melted paraffin wax (Merck, Germany) (melting point 60°C to 62°C), and paraffin blocks of the tissues were made. These tissue blocks were trimmed and sectioned at 5 µm thickness using a microtome (Thermo Shandon, Woonsocket, RI, USA) placed on glass slides and were stained with hematoxylin and eosin.

Serum biochemistry

Serum samples of eleven sloth bears that succumbed to leptospirosis were subjected for serum biochemistry evaluation to determine the elevation of hepatic and renal markers. The hepatic biomarkers such as SGOT and SGPT and renal biomarkers such as creatinine and serum urea level for determining BUN were estimated using Modified IFCC method, Reitman and Frankel's method, Alkaline picrate method and modified Berthelot method, respectively, according to the instructions provided by the manufacturer (Coral Clinical Systems, Tulip Diagnostics Private Limited, Verna, Goa, India).

PCR amplification of liver and kidney tissue samples

Nucleic acids were extracted from sloth bear liver and kidney samples (25 mg each) using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions which would yield 10–30 μg DNA from liver and 15–30 μg DNA from kidney. PCR was performed using two sets of primers i.e. LigBF/LigBR (F3/B3) (5′-AACCGGTCTGGTAGGATT-3′ and 5′-GAATCGGGGACGATGGAT-3′) and G1/G2 (5′-CTGAATCGCTGTATAAAAGT-3′ and 5′-GGAAAACAAATGGTCGGAAG-3′). The reaction conditions for LigBF/LigBR (F3/B3) and G1/G2 were followed as described by [2] and [17] respectively. Briefly, both the PCR amplifications were carried out using 1.5 mM $MgCl_2$ buffer, 200 μM of each dNTP, 20 pM of each primer, 1 U of recombinant Taq DNA Polymerase (Genetix Biotech Asia Pvt. Ltd., New Delhi, India), and 10 ng of genomic DNA. PCR Amplification using G1/G2 and LigBF/LigBR primers was done using initial cycle of 95°C for 5 min followed by 30 amplification cycles with denaturation at 94°C for 1 min, annealing at 55°C and 54°C for 1 min for G1/G2 and LigBF/LigBR (F3/B3) respectively and extension at 72°C for 1 min with a final extension of 72°C for 7 min and 5 min for G1/G2 and LigBF/LigBR (F3/B3) respectively. In both the PCR tests, saprophytic leptospires (*Leptospira biflexa* serovar Patoc) was used as negative control and *Leptospira interrogans* serovar Icterohaemorrhagiae was used as positive control. The PCR product was visualized using UV trans-illuminator (Bio-Rad, Hercules, CA, USA) after electrophoresis in 1.5% agarose gel stained with ethidium bromide (0.1 μg/ml).

MAT

MAT was performed for screening of anti-leptospiral antibodies in sloth bears as described previously [37]. Briefly, serum samples were diluted 1:50 in phosphate buffered saline (PBS) and a volume of leptospiral antigen, equal to the diluted serum volume, was added to each well, making the final serum dilution 1/100 in the screening test. Four–eight day old live leptospiral antigens (approx. 2×10^8 leptospires/ml) of 16 leptospiral serovars (Leptospira interrogans serovar Australis strain Ballico, *L. interrogans* serovar Autumnalis strain Akiyami A, *L. interrogans* serovar Ballum strain S102, *L. interrogans* serovar Bataviae strain van Tienen, *L. interrogans* serovar Canicola strain Hond Utrecht IV, *L. kirschneri* serovar Cynopteri strain 3522C, *L. interrogans* serovar Djasiman strain Djasiman, *L. kirschneri* serovar Grippotyphosa strain Moskva V, *L. borgpetersenii* serovar Hardjo strain Hardjoprajitno, *L. interrogans* serovar Hebdomadis strain Hebdomadis, *L. interrogans* serovar Icterohaemorrhagiae strain RGA, *L. borgpetersenii* serovar Javanica strain Veldrat Batavia 46, *L. noguchii* serovar Louisiana strain LSU 1945, *L. interrogans* serovar Pomona strain Pomona, *L. interrogans*serovar Pyrogenes strain Salinem and *L. borgpetersenii* serovar Tarassovi strain Perepelitsin) were used for performing MAT. The microtiter plates were incubated for 2 hr at 29°C and the serum-antigen mixtures were examined using dark field microscopy. A positive outcome in MAT suggestive of exposure/seropositivity was defined as any single serum sample found to have >50% reduction in the number of free non- agglutinable leptospires in the test when compared to the control at 1 in 100 serum dilution for at least one leptospiral serovar.

Induction of expression and purification of recombinant LigBCon1-5 antigen

Escherichia coli (*E. coli*) M15 strain harboring recombinant plasmid pQE30-LigBCon1-5 was used for rLigBCon1-5 antigen production as described previously [13]. *E. coli* M15 strain was grown in Luria Bertani broth (Difco, Sparks, MD, USA) till the spectrometric reading at OD600 nm reached 0.5–0.7. The cells were then induced with 1 mM Isopropyl β-D-1- thiogalactopyranoside (IPTG) (Sigma-Aldrich, St. Louis, MO, USA) and allowed to grow further for 6 hr at 37°C. Cells were harvested and the proteins analyzed by Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Purification of recombinant protein was done using nickel–nitrilotriacetic acid (Ni-NTA) agarose affinity chromatography (Qiagen) as per instructions of the manufacturer and analysis of purified recombinant protein was done on SDS-PAGE.

rLigBCon1-5 based LAT

Latex beads were sensitized with rLigBCon1-5 antigen as described previously [13] with slight modifications. A 10% suspension of latex particles (0.8 μm diameter, Sigma-Aldrich,) was washed thrice with glycine buffered saline (Glycine 0.1 M, NaCl 0.17

M; pH 8.2). Finally, the latex beads were made into a 2% suspension with glycinebuffered saline which was later mixed with an equal volume of rLigBCon1-5 antigen (1 mg/ml) diluted in the same buffer. The mixture was incubated at 37°C for 6 hr in a shaking platform to ensure constant mixing. The sensitized latex beads were further blocked with Bovine Serum Albumin (Difco) (5 mg/ml) and incubated overnight. Latex beads were centrifuged and the pellet was finally resuspended in glycine buffered salineas a 2% suspension containing 0.02% sodium azide. The sensitized latex beads were stored at 4°C until use. LAT was performed on glass slides by mixing equal volume of serum (20 µl) and sensitized beads (20 µl). The result was read within 2 min. Samples were considered positive when there is formation of agglutination. A score of 3+ve, 2+veand 1+ve were designated to sera which showed agglutination within 30 sec, 30 sec to1 min and 2 min respectively as described previously [34]. Samples were considered negative if no agglutination was observed.

Statistical analysis

The evaluation of rLigBCon1-5 LAT for detection of anti-leptospira antibodies in sloth bear as compared with MAT was determined using Kappa statistics. The relative sensitivity, specificity and accuracy (in percentage) of rLigBCon1-5 LAT in comparison to MAT has been calculated as described previously [13]. The calculation of predictive values (in percent) for positive and negative test results was done as per standard method [18].

Results

MAT

The most frequently encountered leptospiral serovar in sloth bears was Pyrogenes (n=64) followed by Icterohaemorrhagiae (n=59), Grippotyphosa (n=29), Javanica (n=25), Canicola (n=9) and Tarassovi (n=2). The leptospiral serovar distribution in Agra and Bannerghatta sloth bear rescue centers are shown in Table 1. Out of eleven dead sloth bears which succumbed to leptospirosis, seven sloth bears hada sera titer of 1in 800 while four showed a sera titer of 1in 400.

Table 1. Leptospira serovars and their antibody titer found in bear rescue centers located in Agra and Bannerghatta

Leptospira serovars whose agglutinins are present in sloth bear sera samples	Sloth bears seropositive by MATin Agra sloth bear rescue center					Sloth bears seropositive by MAT in Bannerghatta sloth bear rescue center				
	Total positive[a]	1 in 100	1 in 200	1 in 400	1 in 800	Total positive b	1 in 100	1 in 200	1 in 400	1 in 800
Pyrogenes	64	32	20	11	1	0	0	0	0	0
Icterohaemorrhagiae	56	17	22	10	7	3	1	1	0	1
Javanica	25	22	2	1	0	0	0	0	0	0
Grippotyphosa	23	22	1	0	0	6	4	2	0	0
Canicola	9	7	2	0	0	0	0	0	0	0
Tarassovi	2	2	0	0	0	0	0	0	0	0

a) Cumulative figure of sera positive for various serovars (179) exceed 79 (total Microscopic Agglutination Test (MAT) positive sera in Agra bear rescue center) as several sera (n=55) reacted with multiple leptospiral serovars. b) Cumulative figure of sera positive for various serovars (9) exceed 8 (total MATpositive sera in Bannerghatta bear rescue center) as one sera (n=1) reacted with multiple leptospiral serovars.

Serum biochemistry of dead sloth bears

SGOT (IU/l) levels suggestive of liver function were found to be elevated in all the dead sloth bears while SGPT (IU/l) levels showed marginal increase in most dead sloth bears as shown in Table 2. Moreover, Creatinine (mg/dl) and BUN (mg/dl) levels were also found to be elevated in all the dead sloth bears as shown in Table 2. One noteworthy finding was the disproportional exaggerated elevation in SGOT levels and the significantly higher SGOT/SGPT ratio during the terminal stages of the infection in all the dead sloth bears. A positive correlation was also observed between MAT titer and SGOT/SGPT ratio since dead sloth bears which had MAT titer of 1 in 800 had SGOT/SGPT ratio in the range of 3.42–7.19 while dead sloth bears which had MAT titer of 1in 400 had SGOT/SGPT ratio in the range of 2.59–3.58.

Table 2. Serum biochemistry profile of dead sloth bears

Serial No of Dead Sloth Bear (DSB)[a]	MAT titer	SGOT[b] (IU/l)	Normal SGOT Level (mean± SD) (IU/l)	SGPT[c] (IU/l)	Normal SGPT Level (mean± SD) (IU/l)	SGOT/SGPT Ratio	Normal SGOT/SGPT Ratio (mean± SD)	BUN[d] (mg/dl)	Normal BUN (mean± SD) (mg/dl)	Creatinine (mg/dl)	Normal Creatinine level (mean± SD) (mg/dl)
DSB No 1	1 in 800	276	56.37± 27.02	43.0	36.78 ± 7.52	6.42	1.53 ± 0.35	31.9	6.24 ± 1.49	1.8	1.08 ± 0.24
DSB No 2	1 in 800	196		39.0		5.03		26.0		1.6	
DSB No 3	1 in 800	345		48.0		7.19		26.5		2.1	
DSB No 4	1 in 800	208		42.0		4.95		40.5		2.0	
DSB No 5	1 in 800	165		40.5		4.07		32.0		1.8	
DSB No 6	1 in 800	174		45.0		3.87		36.0		1.9	
DSB No 7	1 in 800	135		39.5		3.42		36.0		1.7	
DSB No 8	1 in 400	129		36.0		3.58		28.1		1.6	
DSB No 9	1 in 400	114		44.0		2.59		29.5		1.6	
DSB No 10	1 in 400	112		38.5		2.91		35.5		1.6	
DSB No 11	1 in 400	125		39.5		3.16		26.0		1.9	

a)The names of dead sloth bears are kept confidential following request from bear rescue park authorities. b) SGOT stands for serum glutamate oxalo acetictransaminase. c) SGPT stands for serum glutamate pyruvic transaminase. d) BUN stands for blood urea nitrogen.

Gross and histopathology findings of liver and kidneys

All the dead sloth bears had mucous membranes which were icteric in nature and post mortem revealed serosanguinous fluid present inside the abdominal cavity (Supplementary Fig. 1). The most significant postmortem finding in the dead sloth bears was the gross appearance of liver which was yellowish in color due to jaundice in six animals (Fig. 2A) while liver of five sloth bears were highly congested and haemorrhagic with soft and friable consistency (Fig. 2B). The gall bladders of all the dead sloth bears were highly distended (Supplementary Fig. 2). The histopathology of liver sections revealed severely engorged sinusoids (Fig. 2C and 2D) with severe hepatic degeneration (Supplementary Fig. 3) and multifocal areas of hepatic necrosis surrounded by inflammatory cells in liver parenchyma of dead sloth bears (Supplementary Fig. 4). On gross examination, the kidneys appeared soft, swollen and congested with tiny palewhite spots (necrotic foci) on sub capsular surface in four dead sloth bears (Fig. 3A and 3B). The major histopathological findings of renal tissue in sub-acute cases include moderate to severe lymphoplasmacytic and neutrophilic tubulointerstitial nephritis (Supplementary Fig. 5) with tubular degeneration, necrosis, mineralization and mild interstitial fibrosis (Fig. 3C). However, in chronic cases, inflammation is less severe and is accompanied by pronounced interstitial fibrosis and tubular atrophy (Fig. 3D).

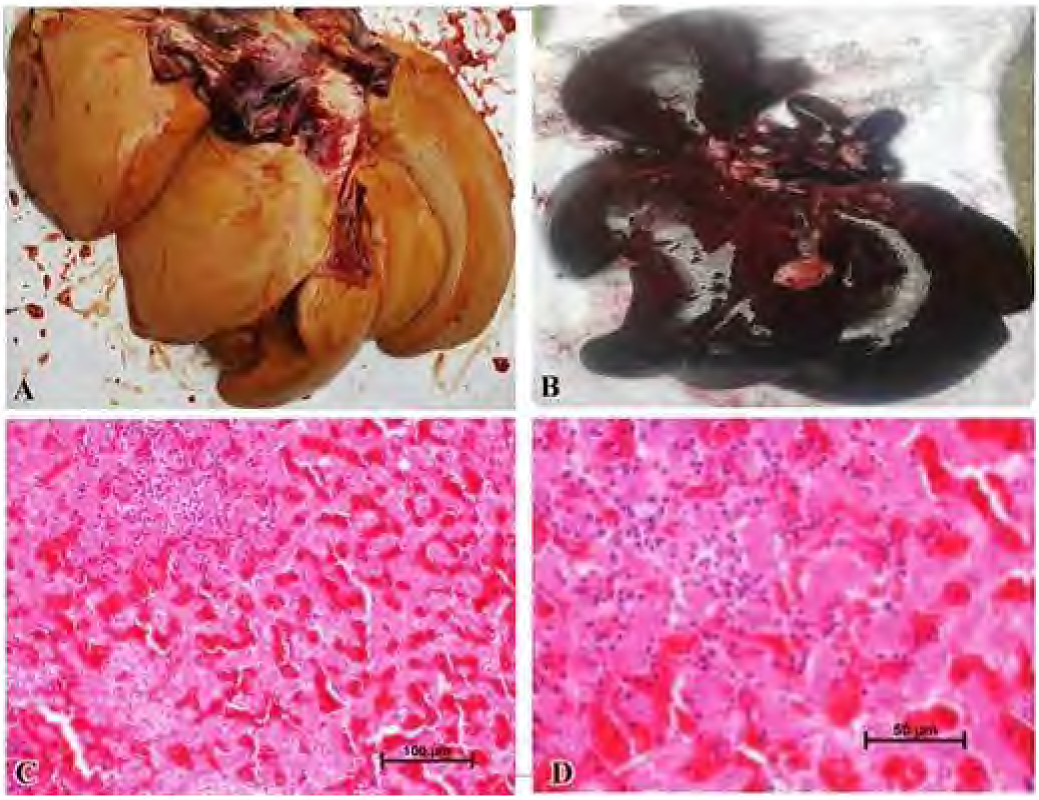

Fig. 2. Gross and histopathology lesions of liver of dead sloth bears. A) Soft and friable icteric liver showing yellowish color due to jaundice and B) highly congested and haemorrhagic liver which were soft and friable. C, D) Liver showing severely engorged sinusoids with degeneration of hepatocytes and infiltration of mononuclear cells in the hepatic parenchyma hematoxylin-eosin (HE) ×200 & HE ×400.

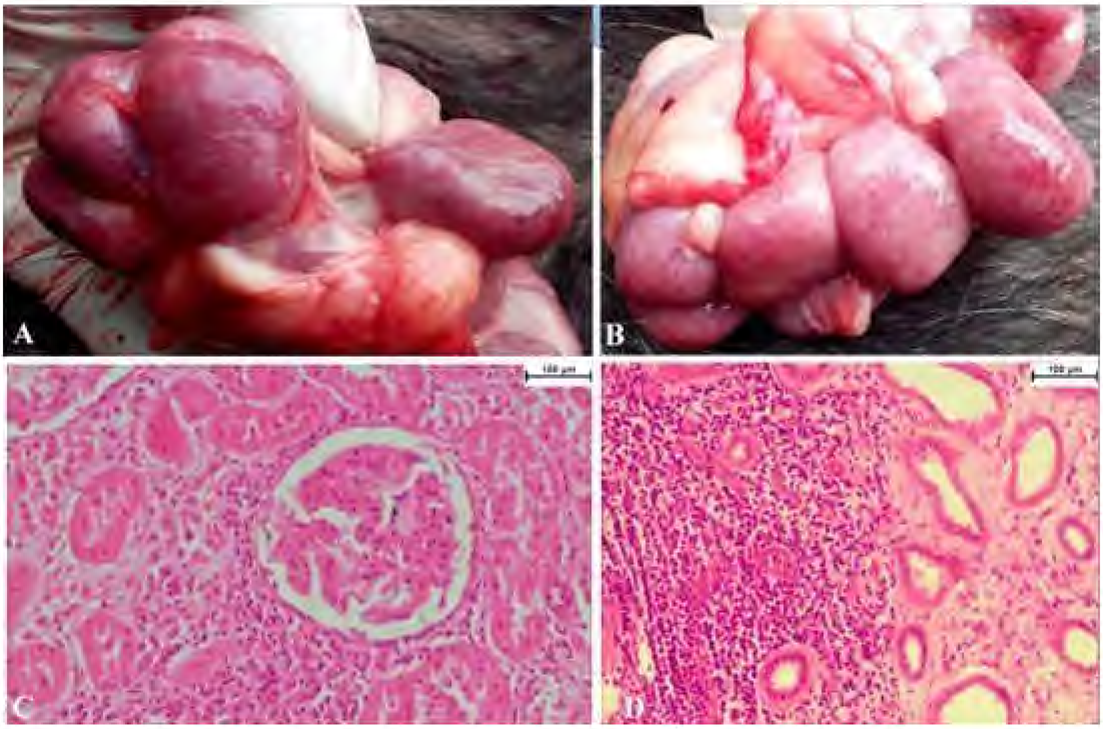

Fig. 3. Gross and histopathology lesions of kidneys of dead sloth bears. A, B) Soft, swollen and congested kidneys with tiny pale white spots (necrotic foci) on subcapsular surface. C) Histopathology of kidneys showing tubular degeneration, necrosis, mineralization and mild interstitial fibrosis hematoxylin-eosin (HE) ×200. D) Histopathology of kidneys in chronic case showing pronounced interstitial fibrosis and tubular atrophy with less severe inflammation HE ×200.

Polymerase chain reaction of tissue samples

PCR amplification of kidney tissue samples using G1/G2 and LigBF/LigBR (F3/B3) primers revealed positive test results as indicated by amplicons of 285 bp and 219 bp respectively. The detection of *Leptospira* sp. DNA in the renal tissue of all dead sloth bears by PCR employing both G1/G2 and LigBF/LigBR (F3/B3) primers provided diagnostic confirmation that leptospirosis was responsible for the mortality of sloth bears in both sloth bear rescue centers due to renal failure. Further, the results obtainedusing PCR helped to corroborate the gross and histopathological findings observed in renal tissue which were suggestive for leptospirosis.

Recombinant LigBCon1-5 antigen expression and utilization in LAT

We have obtained a high-level expression of rLigBCon1-5 protein estimated at approximately 20 mg of protein per liter of IPTG induced culture. LAT developed using rLigBCon1-5 antigen showed a clear-cut agglutination with positive sera which can be visualized easily and can clearly differentiate negative sera showing homogeneous suspension (Fig. 4).

Correlation between MAT titer and LAT score

A positive correlation exists between MAT titer and LAT score. Sloth bear sera (n=23) showing MAT titer ≥1 in 400 gave 3+veLAT score and the agglutination intensity was high with the agglutinins forming a thick halo at the periphery while the center was virtually empty (Fig. 4). Sera samples (n=27) showing 1 in 200 MAT titer gave 2+ve LAT score with moderate agglutination intensity. Sloth bear sera (n=28) showing 1 in 100 MAT titer gave 1+ve LAT score (low intensity of agglutination). Nine sera with1in 100 MAT titer showed no agglutination with rLigBCon1-5 based LAT (Fig. 4). Out of 133 sera sample tested, 87 (65.41%) and 78 (58.65%) sera samples were found positive by MAT and rLigBCon1-5 based LAT respectively. The relative sensitivity, specificity, accuracy, positive and negative predictive values of rLigBCon1-5 based LAT in comparison to MAT were 89.66%, 100%, 93.23%, 100% and 83.64% respectively. Further, Kappa value of 0.86 (95% C.I. 0.77–0.95) for rLigBCon1-5 based LAT indicates high agreement with MAT.

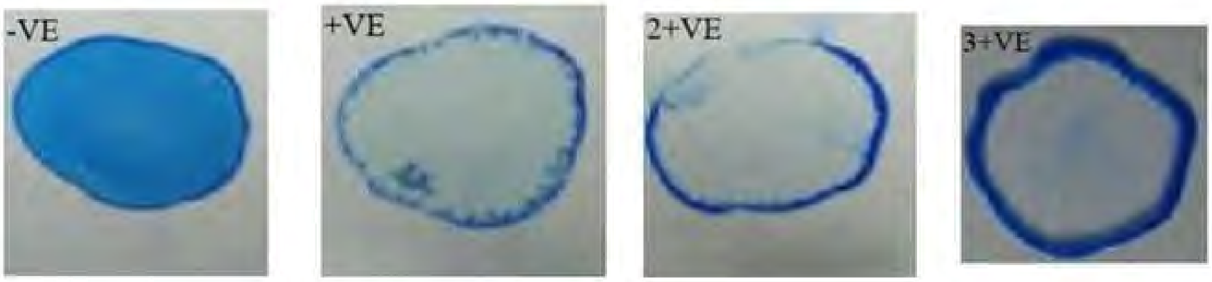

Fig. 4. Latex agglutination test results of sloth bear sera samples using recombinant LigBCon1-5 protein.

Discussion

Sloth bears are enlisted in Appendix I of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) and these endangered animals are protected under Schedule I of the Indian Wildlife Protection Act of 1972 [14]. The five predominant leptospiral serovars (Pyrogenes, Icterohaemorrhagiae, Grippotyphosa, Javanica and Canicola) observed in the present study were also among the five leading serovars reported in seroprevalence studies conducted in four rat species in India [7]. Globally, free living rodents are implicated as the only mammals capable of maintaining close contact with captive wildlife in zoos [19]. This clearly suggests the role of rodents as the main agent for transmission of leptospirosis in both sloth bear rescue centers. There was clear evidence of rodent infestation in granaries as well as sloth bear enclosures. The rodents were attracted to sloth bear enclosures due to the food leftover by sloth bears and the rodent urination on leftover food might be one probable sourceof infection. Hence, a concerted effort for rodent control was launched which included setting up rodent traps and rodenticides at strategic locations especially at entry/exit points within the rescue park premises. The leftover food was immediately disposed off from sloth bear enclosures following meal time.

In order to counteract the post deluge spike in leptospirosis, prophylactic use of doxycycline @5 mg/kg body weight was given prior to monsoon season. Moreover, both the rescue centers started the practice of biannual vaccination with Nobivac DHPPi / RL in captive sloth bears with encouraging results. The dedicated effort of sloth bear rescue center staff ensured that there was no mortality in both rescue centers for the past five years. Further, the MAT titers of recuperating sloth bears have tapered to tolerable levels (MAT titer ≤1 in 100).

In this study, mucous membranes of all the dead sloth bears were icteric in nature. Moreover, the livers of six sloth bears were yellowish in color due to jaundice. The reason is due to invasion of the intercellular junctions of host hepatocytes by pathogenic leptospires which contributed to the disruption of the junction and the subsequent leakage of bile from bile canaliculi contributing to jaundice and elevation in SGOT levels in serum [25].

In this study, pale white spots (necrotic foci) were observed on sub capsular surface of kidneys of four dead sloth bears. The gross findings observed in kidneys in the present study are in concordance with the observations made by [4] who suggested that *Leptospira* spp. were associated with white-spotted bovine kidneys based on PCR results using Lip, 32 primers. The kidneys of sloth bears which died due to sub-acute leptospirosis showed tubulointerstitial nephritis characterized by severe degeneration of proximal convoluted tubules with interstitial and periglomerular infiltration of mononuclear cells and mild fibrosis. Our observations are in concordance with findings of [35] and [30] who observed acute tubulointerstitial nephritis as well as acute tubular necrosis in patients with acute leptospirosis. The typical pathological findings observed in sloth bears which died due to chronic leptospirosis was interstitial and periglomerular fibrosis and infiltration of plasma cells, lymphocytes and few macrophages.Chronic *Leptospira interrogans* infection was studied in a mouse model by

[15] and had suggested that the leptospiral colonization of the kidneys triggers renal fibrosis which is characterized by the pathological accumulation of extra-cellular matrix components that can compromise the kidney functions of patients with leptospirosis.

The serum biochemistry of all the dead sloth bears revealed that both SGOT and SGPT levels were elevated. The mean ± standard deviation for SGOT and SGPT in apparently healthy bears (both sex combined) is 56.37 ± 27.02 IU/l and 36.78 ± 7.52 respectively [9]. However, all the dead sloth bears showed SGOT levels on the higher range of 112–345 IU/l while SGPT levels ranged from 36–48 IU/l. The average SGOT/SGPT ratio in normal sloth bears is approximately 1.53 ± 0.35 [9]. One hallmark feature observed in serum biochemistry in all the eleven sloth bears which succumbed to leptospirosis was the significantly higher SGOT/SGPT ratio during the terminal stagesof the infection. The SGOT levels elevated progressively without a concomitant change of SGPT during the acute disease course in all dead sloth bears. Our experience with regard to SGOT/SGPT levels in terminal stages of leptospirosis suggests that SGOT/SGPTratio can serve as a valuable prognostic parameter for leptospirosis with a ratio of ≥4.0 indicative of a grave prognosis for the disease in sloth bears. We have observed SGOT/SGPT ratio ranging from 2.59–7.19 in sloth bears which succumbed to leptospirosis. The disproportionate and exaggerated elevation in SGOT levels and high SGOT/SGPT ratio in human patients who died due to leptospirosis was also observed by[10]. All the dead sloth bears showed BUN levels on higher range of 26–40.5 mg/dl whereas the normal range of BUN in sloth bear is 6.24 ± 1.49 (95% confidence Interval 5.7–6.7) [9]. BUN is used to evaluate kidney function and elevations in BUN level are often a result of a decrease in Glomerular Filtration Rate (GFR) which clearly suggests kidney damage resulting in decreased excretion of urea in urine [24]. All the dead sloth bears showed creatinine levels on slightly higher range of 1.6–2.1 mg/dl whereas the normal range of creatinine in sloth bear is 1.08 ± 0.24 (95% confidence Interval 1.0–1.15) [9]. An elevation in the serum creatinine concentration usually reflects a reduction in the glomerular filtration rate (GFR) suggestive of kidney damage [31].

Our study clearly suggests that 'Point of care testing' for leptospirosis in both sloth bear rescue centers can be achieved utilizing rLigBCon1-5 based LAT. Our results are in agreement with findings of [5] and [13] where LAT using recombinant LigBCon1-5 protein have yielded high sensitivity and specificity while investigating canine and bovine leptospirosis respectively. In future, we are planning to employ rLigBCon1-5 antigen based LAT to identify sloth bears that contract leptospirosis despite vaccination with Nobivac® DHPPi/RL. Diagnostic assays based on recombinant LigB antigen would be a valuable tool to identify animals that contract leptospirosis despite vaccination [28]. Vaccination of sloth bears with Nobivac® DHPPi/RL (Intervet, Kenilworth, NJ, USA) will generate agglutinins against serogroups Icterohaemorrhagiae and Canicola but fail to confer cross protection against natural infection with serovars belonging to unrelated serogroups. This main drawback of leptospira bacterin based vaccines of limited efficacy spectrum cannot be solved by increasing the amount of protective antigen [1]. Hence, it is of paramount importance to conduct regular epidemiological surveillance in sloth bear rescue centers to unravel temporal variation due to the emergence of new serovars which can become causative agent for leptospirosis in sloth bears despite vaccination. Therefore, the authors of this manuscript are in favor of 'tailored leptospiral vaccines' which was also proposed by [21] which contain serovars representative of those present in the population to be immunized. The MAT results of this study have convinced the authors that the tailored leptospiral vaccine should include serovars Pyrogenes (most frequently encountered serovar), Grippotyphosa and Javanica along with the existing leptospira vaccine serovars such as Icterohaemorrhagiae and Canicola.

In conclusion, the multipronged approach adopted in the present study to diagnose leptospirosis in sloth bears based on antigen detection (using PCR employing G1/G2 primers and LigBF/LigBR (F3/B3) primers), antibody detection (MAT and rLigBCon1-5 based LAT), serum biochemistry profile (SGOT, SGPT, Creatinine and BUN) and gross/histopathological findings of liver and kidney would permit the implementation of intervention strategies in sloth bears based on early case detection and timely initiation of antimicrobial therapy.

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Art – 255. MULTI-DRUG RESISTANT (MDR), EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING AND CARBAPENEM RESISTANT *Escherichia coli* IN RESCUED SLOTH BEARS (*Melursus ursinus*), **INDIA**

O. R. VinodhKumar, M. Karikalan, **S. Ilayaraja, Arun A. Sha**, B. R. Sing, D. K. Sinha, S. Chandra Mohan, B. S. Pruthvishree, A. M. Pawde and A. K. Sharma

Abstract

The study reports the multi-drug resistant (MDR), extended spectrum beta-lactamase (ESBL) producing and carbapenem resistant *Escherichia coli* (CRE) isolated from rescued sloth bear (*Melursus ursinus*), India. Non-duplicate faecal samples from 21 adult rescued sloth bears were collected at once during 2015–2016 and processed for isolation of *E. coli* and anti-bacterial susceptibility pattern. From 21 samples, 45 *E. coli* were isolated and on phenotypic screening, 23 were MDR, 17 were ESBL producers, and five were carbapenem-resistant (CR). Three *E. coli* isolates (6.67%, 3/45) showed no resistance, however 42 isolates (93.33%, 42/45) exhibited resistant to at least one antibiotics. The MDR isolates carried beta-lactamase,chloramphenicol, aminoglycosides, tetracycline, fluroquinolone, and sulphadimidine resistance genes. All the phenotypic ESBL producing isolates harbored *blaCTX*-M genes. On genotypic screening, three CRE (60.0%, 3/5) were positive for *bla*NDM carbapenemase gene and efflux pump-mediated carbapenem resistance was detected in two CRE isolates (40.0%, 2/5) which were negative for carbapenemase genes. The CRE isolates (n = 5) also co-harbored AMR genes like *bla*TEM-1,*bla*AmpC, *qnr*A, *qn*rB, *qnr*S, *tet*A, *tet*B and *sulI*. Virulence screening of the resistant isolates detected the presence of *Stx*1(n = 1), *Stx*2 (n = 3), *eae*A (n = 4) and *hly*A (n = 3) genes. Plasmid incompatibility (Inc) typing revealed that two isolates harboured *bla*NDM-5 gene on Incl1 and one isolate on IncF plasmid. Apart from the NDM gene, the plasmids also carried tetracycline, beta-lactamase and quinolone resistance genes. The plasmid multilocus sequence typing (pMLST) of the *E. coli* Incl1 plasmid showed the Sequence Type (ST) 297. This appears to be the first report of MDR, ESBL producing and *bla*NDM-5 genes on Incl1 and IncF plasmids from rescued sloth bear.

Keywords Sloth bear · Carbapenem · ESBL · *E. coli* · Virulence

Introduction

Antimicrobial resistance is an emerging global threat and needs immediate attention. Genes conferring AMR can spread quickly by horizontal gene transfer to other non-resistant bacteria and thereby disseminating AMR throughout microbial communities (Allen et al. 2010). Wild birds and mammals act as important reservoirs and potential vectors for the spread of resistant bacteria and genetic determinants of AMR (Caroll et al. 2015). Wild animals are not directly exposed to antimicrobials; though, they may be infected with antibiotic-resistant bacteria via the acquisition of resistant bacteria from human sources, agricultural facilities and associated contaminated environments (Dolejska et al. 2007). Anti-microbial agents, such as antibiotics, are used extensively in human and veterinary medicine and often as part of routine agricultural production (Kummerer 2009). The prevalence level of resistant bacteria in the community is often linked with human activity (Bonnedahl et al. 2009; Alroy and Ellis 2011).

Extended spectrum beta-lactamases (ESBL) and/or carbapenemases producing *Escherichia coli* are of great concern worldwide and represent a complex multidimensional problem involving humans, animals and the environment (Atterby et al. 2017). Carbapenems are one of the most important groups of antimicrobials and are considered as a last line of defence for the treatment of severe infections in humans. Though carbapenem resistance in animals is rare, reports are available on carbapenem resistant *E. coli* in animals of India (Pruthvishree et al. 2017, 2018; Nirupamaet al. 2018; Murugan et al. 2019; VinodhKumar et al. 2021).Sloth bears are myrmecophagous and habitually seen as solitary although they are sometimes seen in groups. In India sloth bears are used in circus and by nomadic street performers (Kalandar community). Wildlife SOS, a not-for-profit organization rescue and rehabilitate these animals (Menezes 2021) and we hypothesize that, in these rehabilitation centers the close association of sloth bears among themselves and human may be a driving factor for transfer of resistant bacteria among them. The study was carried out to identify the molecular epidemiology of MDR, ESBL producing and carbapenem resistant *E. coli* isolated from rescued sloth bear. We selected *E. coli* for the AMR pattern in sloth bear since its ubiquitous nature in animals and humans and its easy and inexpensive isolation methods.

Materials and methods

Sample collection and isolation of *E. coli*

A cross-sectional study was conducted on the request of authorities of Agra Bear Rescue Facility (ABRF) for screening of antimicrobial resistance of *E. coli* in rescued sloth bears in India during 2015–16. During the study period the rehabilitation center caged 190 sloth bears and approximately 10% of the healthy animals were randomly selected for bacterial resistance screening. The fecal samples were directly collected from rectum of each sloth bear (n = 21) during the routine health checkup performed under general anesthesia. Non-duplicate samples from apparently healthy adult sloth bears were collected at single time point in sterile transport swabs (HiCulture, HiMedia, India). Following enrichment in MacConkey broth at 37ºC for 6—10 h,a loop full of enriched broth was streaked on MacConkey agar and incubated at 37 °C for 12 -18 h. Based on colony morphology, two to three lactose-fermenting colonies selected were streaked on eosin methylene blue (EMB) agar medium and incubated at 37°C overnight for preliminary characterization. Then, the colonies showing characteristic metallic sheen on EMB agar were selected for oxidase, Voges-Proskauer, methyl red, indole, citrate, urea hydrolysis, catalase, casein hydrolysis, nitrate reduction, and sugar fermentation biochemical tests to confirm their identity. The *E. coli* reference strains used in this study were retrieved from the Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar repository (GenBank accession number: KU318701, KU318691, KU382501).

Antimicrobial susceptibility testing

The biochemically confirmed *E. coli* (n = 45) isolates were tested for antibiotic susceptibility pattern with amoxicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), aztreonam (30 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), cefazolin (30 µg), cefpodoxime (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefixime (5 µg), cefoxitin (30 µg), piperacillin-tazobactam (100/10 µg), cefotaxime/clavulanic acid (30/10 µg), tetracycline (30 µg), nitrofurantoin (300 µg), gentamicin (10 µg), kanamycin(30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg) and norfloxacin (10 µg) by disc diffusion method (Hudzicki 2009). The CLSI (2014) breakpoints were used for interpretation of susceptibility patterns. For phenotypic identification of ESBL producers, the combination disc method using cefotaxime and ceftazidime with and without clavulanic acid was used (Andrews 2003) and the isolates were further confirmed by ESBL E-test (HiMedia, Mumbai, India). Screening for carbapenem resistance was also carried out using the disc diffusion method with meropenem (MEM, 10 μg), imipenem (IMP,10 μg), and ertapenem (ETP,10 μg) disks (BD, Sparks, MD, USA) along with ethylene diamine tetra acetic acid (EDTA 1900 mg/disc) for screening of metallo beta-lactamase (MBL) production (Pruthvishree et al. 2017). A keyhole reaction between carbapenem drug and EDTA was considered positive for metallo beta-lactamase (MBL) production. The minimum inhibitory concentration (MIC) for ceftriaxone, cefotaxime and meropenem was determined using E-test strip (HiMedia, Mumbai, India).

Genotypic screening for antimicrobial resistance (AMR)

Phenotypically resistant *E. coli* isolates were further analyzed for AMR encoding genes. The genomic DNA was extracted from *E. coli* isolates using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) and screened for the presence of antimicrobial resistance genes (ARG), including beta-lactamase, major carbapenemases, sulphonamide, quinolone and tetracycline resistance genes. The isolates were also screened for *Stx*1, *Stx*2, *eae*A and *hly*A virulence genes (Paton and Paton 1998) in order to find the resistant isolates carrying the virulence genes. The PCR reactions were optimized individually in 25 μL volumes, with 10 pmol of each individual primer used (supplementary Table 1). The PCR amplicon from all reactions was electrophoresed in 0.5 X TBE buffer using 1.5% agarose gel and visualized under UV illumination (Syngene, USA). Representative PCR amplicons were purified and sequenced by a commercial sequencing service (Eurofins, Bengaluru, India). The sequencing results were obtained, and similarity searches were performed using the BLAST algorithm (https://blast. ncbi.nlm.nih.gov/Blast.cgi). The sequence data were submitted to GenBank, and accession numbers were obtained.

Table 1 Antibiotic susceptibility pattern, virulence and antibiotic resistance determinants of *E. coli* isolates (n = 45) from rescued sloth bear

S. No.	Isolates	Antibacterial resistance pattern																							Minimum Inhibitory Concentration (μg/mL)			Virulence and antibiotic Resistance determinants	Accession Number
		AMX	AMC	CZ	ATZ	COT	CX	CTX	CFM	CPD	CIP	CAZ	CTR	K	C	GEN	TE	FOX	F/M	TZP	NX	IMP	MRP	ERP	CTX	CTR	MRP		
1	SB-1	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	-	nd
2	SB-2 ESBL	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	8	12	nd	*CTX-M-1,qnrA,AmpC,*catI	nd
3	SB-3 ESBL	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R	S	S	S	12	18	nd	*sull,tetA,ac (3)-IV, eaeA*	nd
4	SB-4	R	S	R	S	R	R	S	S	R	S	S	S	R	S	S	S	S	S	S	R	S	S	S	nd	nd	nd	-	nd
5	SB-5	R	S	R	S	S	R	S	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	nd	nd	nd	floR	nd
6	SB-6	R	R	R	S	R	R	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	nd	nd	nd	*Stx2*	nd
7	SB-7	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	12	16	nd	*CTX-M-15, qnrS, Stx2*	MK559368
8	SB-8ESBL	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	S	R	S	S	S	S	8	12	nd	*AmpC*, *sulI*, floR, *ac*	nd
9	SB-9	R	S	S	S	R	R	S	R	S	R	S	S	R	R	S	S	S	S	R	R	S	S	S	nd	nd	nd	floR,TEM-1	nd
10	SB-10	R	S	S	S	R	S	R	S	S	R	S	S	R	R	R	R	S	S	S	R	S	S	S	nd	nd	nd	floR	nd
11	SB-11	R	S	S	S	S	S	R	S	S	R	S	S	R	R	S	S	S	S	S	S	S	S	S	nd	nd	nd	floR	nd
12	SB-12 ESBL	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S	S	12	16	nd	*CTX-M,qnrS, ac (3)-IV, eae A*	nd
13	SB-13 ESBL	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	32	64	nd	floR,*dhfrI*	nd
14	SB-14	R	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	R	S	S	S	nd	nd	nd	catI	nd
15	SB-15ESBL	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	16	32	nd	*CTX-M,qnrA,sulI,*floR	nd

16	SB-16 ESBL	R	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	8	12	nd	*CTX-M, qnrB,tetA,* hlyA	nd
17	SB-17 ESBL	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	12	16	nd	*CTX-M, qnrS, tetB,* *dhfrI,TEM-1*	MK559374
18	SB-18	R	R	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	nd	nd	nd	catI	nd
19	SB-19ESBL	R	R	R	R	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	S	S	S	8	12	nd	*CTX-M,qnrB,sulII,*catI,	nd
20	SB-20 ESBL	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	S	R	R	S	S	S	16	18	nd	*CTX-M,sulI, dhfrI*	nd
21	SB-22 ESBL	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	S	S	S	S	12	16	nd	*CTX-M,qnrA,TEM-1*	nd
22	SB-23 ESBL	R	R	R	S	R	R	R	R	R	S	R	R	S	R	R	R	R	R	R	R	S	S	S	32	64	nd	*CTX-M,TEM-1,qnrS,sulI,.* *AmpC,tetB,*catI, *ac (3)-IV,dhfrI*	MK559371, MK559373
23	SB-24	R	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	*hlyA*	nd
24	SB-25	S	R	S	S	S	S	R	S	R	R	S	S	R	S	R	R	R	S	S	S	S	S	S	nd	nd	nd	*ac (3)-IV*	nd
25	SB-26	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	-	nd
26	SB27/ 17-CRE	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	8	16	16	*NDM-5, TEM-1,qnrS,sulII,*catI, *ac (3)-IV*	MF136747, MK559369, MK559372
27	SB28/ 12-CRE	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	16	32	32	*NDM-5,CTX-M,TEM-1,AmpC*	MK559365, MK559367, MK559370,
28	SB28/ 16-CRE		R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	16	8	8	*NDM-5, CTX-M,* *Stx1,tetB,*catI, *aph (3′)-IIa*	MK559366
29	SB-29/18-CRE		R	R	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	8	32	16	*tetB*	Efflux pump
30	SB-30 CRE		R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	16	16	32	*CTX-M-,qnrS,floR,*	Efflux pump

																												dhfrI,stx2	
31	SB-31	ESBL	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	16	32	nd	*CTX-M,sulI,tetB, dhfrI*	nd
32	SB-32	ESBL	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	8	8	nd	*CTX-M,qnrB,*catI	nd
33	SB-33	ESBL	R	R	S	S	R	R	R	R	S	R	R	R	S	R	R	R	S	R	R	S	S	S	32	32	nd	*CTX-M,tet A, eaeA*	nd
34	SB-34	ESBL	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	16	16	nd	*CTX-M,sulI,tetA, aph (3′)-IIa*	nd
35	SB-35	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	*sulII*	nd
36	SB-36	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	-	nd
37	SB-37	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S	S	S	R	R	S	S	S	nd	nd	nd	-	nd
38	SB-38	S	S	S	S	S	S	S	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	nd	nd	nd	*aph (3′)-IIa*	nd MK559375
39	SB-39	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	-	nd
40	SB-40	S	S	S	S	S	S	R	S	S	S	R	S	S	S	R	S	S	S	S	R	S	S	S	nd	nd	nd	*ac (3)-IV*	nd
41	SB-41	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	*hlyA*	nd
42	SB-42	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	*eaeA*	nd
43	SB-43	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	nd	nd	nd	*aph (3′)-IIa*	nd
44	SB-44	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	nd	nd	nd	-	nd
45	SB-45	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nd	nd	nd	-	nd

R resistant, *S* sensitive, *AMX* amoxicillin, *AMC* amoxicillin–clavulanic acid, *ATZ* aztreonam, *C* chloramphenicol, *CTR* ceftriaxone, *CPD* cefpodoxime, *CAZ* ceftazidime, *CZ* cefazolin, *CFM* cefixime, *CTX* cefotaxime, *CPM* cefepime, *CIP* ciprofloxacin, *COT* co-trimoxazole, *ERP* ertapenem, *F/M* nitrofurantoin, *FOX* cefoxitin, *GEN* gentamicin, *IMP* imipenem, *K* kanamycin, *NX* nor- floxacin, *MRP* meropenem, *TE* tetracycline, *TZP* piperacillin–tazobactam, *nd* not done

Plasmid replicon and multilocus sequence typing

Plasmid DNA was isolated using a QIA prep Spin miniprepkit (QIAGEN, Helsinki, Finland) from the *bla*NDM positive *E. coli* isolates and PCR based replicon typing (PBRT) was used to determine the plasmid incompatibility groups (Johnson et al. 2007). Plasmid multi locus sequence typing (MLST) was performed using specific primers (https://pubmlst.org/plasmid/primers/) and sequenced by the Sanger method by a commercial sequencing service (Eurofins India Ltd, Bengaluru). The sequences were edited using BioEdit v7.0.5 and queried in the plasmid MLST website (https://pubmlst.org/bigsdb?db=pubmlst_plasmid_seqdef) for an allelic profile, sequence type and clonal complex.

Efflux pump screening

The *E. coli* isolates not showing carbapenemase gene mediated resistance were screened for the presence of active efflux pump by the ethidium bromide cartwheel method in order to find the non-carbapenemase mediated carbapenem resistance (Martins et al. 2013).

Statistical analysis

Antibiotic susceptibility profiles and gene occurrence was converted into binary code. For each antibiotic, 0 denoted susceptibility and “1” denoted resistance. The intermediate resistant isolates were included in resistant category. Similarly, the absence and presence of a resistant gene was denoted as “0” and “1” respectively. Principal component analysis (PCA) was performed using the package Factoextra (version 1.0.6) in R software (version 3.6.1).

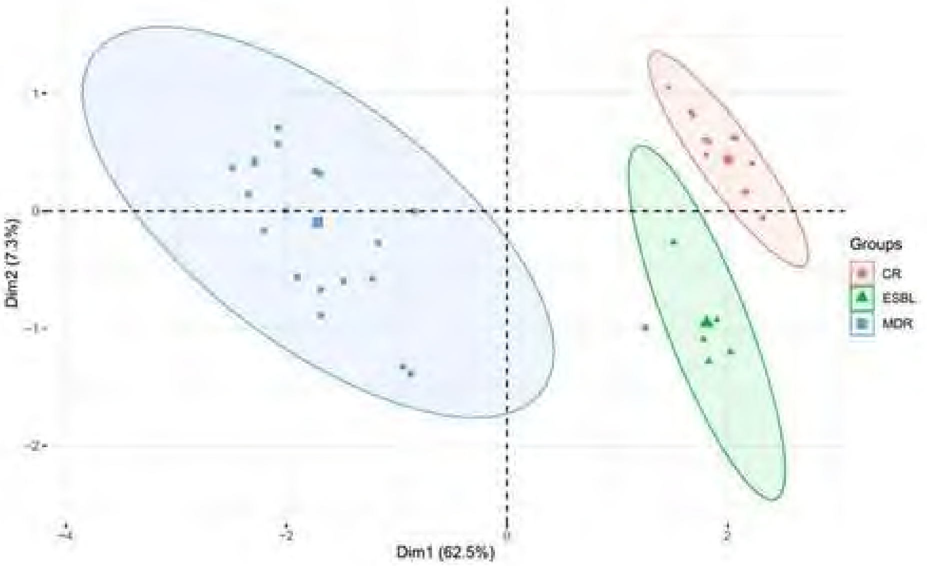

Fig. 1 Principal Component Analysis (PCA) plots of the drug resistant E. coli isolates. The E. coli are encompassed in 95% confidence intervals grouping based on the resistant group [Carbapenem (CR), Extended Beta-lactam (ESBL), Multidrug resistant (MDR)]

Results and discussion

A total of 45 *E. coli* were isolated from 21 sloth bear faecal samples and the antibiograms showed that the isolates were resistant to gentamicin (62.2%, 28/45), cefotaxime (60.0%, 27/45), ceftazidime (60.0%, 27/45), cefotaxime/clavulanic acid (57.8%, 26/45), ceftriaxone (55.6%, 25/45), ceftazidime /clavulanic acid (55.6%, 25/45), norfloxacin (55.6%, 25/45), cefpodoxime (53.3%, 24/45), cefoxitin (53.3%, 24/45), piperacillin/tazobactam (53.3%, 24/45), cefexime (51.1%, 23/45), ciprofloxacin (51.1%, 23/45), tetracycline (51.1%, 23/45),sulphadimidine/trimethoprim combination (46.7%, 21/45), cefepime (46.7%, 21/45), chloramphenicol (42.2%, 19/45), nitrofurantoin (40.0%, 18/45), aztreonam (35.6%, 16/45), meropenem (11.1%, 5/45), imipenem (8.9%, 4/45) and ertapenem (8.9%, 4/45). Of the 45 isolates, 51.1%(23/45) were resistant to more than two classes of screened antibiotics and classified as MDR. Three (6.67%, 3/45) and 42 (93.33%, 42/45) *E. coli* isolates showed no resistance and resistance to at least one antibiotic screened in this study, respectively.

The ESBL screening by combined disc method revealed37.8% (17/45) isolates as ESBL producers (Table 1). Similar kind of antibiotic resistance pattern was documented in *E. coli* isolated from captive blackbuck and leopard of India (VinodhKumar et al. 2021).

Comparable to this study, many authors documented multidrug resistant and ESBL producing *E. coli* in wild animals (Costa et al. 2006; VinodhKumaret al. 2021). Literature reports indicate that wild birds are reservoirs of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase producing *E. coli* (Alcala et al. 2016). Interestingly, a significant percentage of MDR *E. coli* isolates was detected in wild birds and animals such as black-headed gulls (3.3%), starlings (19.2%), herring gulls (3.3%), deer (3.3%) of Ireland (Carroll et al. 2015). In contrast, the present study documented MDR and ESBL producing *E. coli* in rescued sloth bears.

Screening for carbapenem resistance showed that 11.11%(5/45) isolates were resistant to carbapenem drugs and the five carbapenem resistant isolates were MDR and ESBL producers. Interestingly, three carbapenem-resistant isolates showed a keyhole reaction between EDTA and carbapenem drugs, indicating metallo beta-lactamase production (MBL). Metallo-carbapenemases are versatile in hydrolyzing penicillins, cephalosporins, monobactams, and carbapenems (Queenan and Bush 2007). The carbapenem resistant, metallo beta-lactamase producing *E. coli* isolates were reported in piglets, dog, blackbuck and leopard in India (Pruthvishreeet al. 2017, 2018; VinodhKumar et al. 2021).

The phenotypically resistant isolates were analyzed for AMR encoding genes. All the ESBL producing isolates were MDR *E. coli* and genetic screening of the ESBL positive isolates (n = 17) revealed the presence of ESBL encoding genes such as *bla* CTX-M-1 (n = 10), *bla*CTX-M-15 (n = 7) and other antibiotic resistance genes like *bla*TEM (n = 6), *bla*AmpC (n = 4), *qnr*S (n = 6), *qnr*A (n = 3), *qnr*B (n = 3),*sulI* (n = 7), *sulII* (n = 3), *tet*A (n = 4), *tet*B (n = 5). Similarly, most of the ESBL producing *E. coli* isolates in wild birds were also multi-drug resistant and the most common resistant phenotypes were beta-lactams, quinolones, tetracycline, and sulfamethoxazole/trimethoprim (Alcala et al. 2016). The most common ESBL gene in dog, cattle and pig was *bla*CTX-M-1, while in humans *bla*CTX-M-14 and *bla*CTX- M-15 were the dominant genes. However, recent studies have identified CTX-M-1 genes in humans (Madec et al. 2015) and rare identification of CTX-M-15 in pet animals (Dahmen et al. 2013). In the present study, *E. coli* isolated from sloth bear harbored both CTX-M-1 and CTX-M-15 variants indicating sharing of dominant ESBL gene pool among *E. coli* (Zong et al. 2008).

Screening for carbapenemase genes revealed that three isolates which showed phenotypic MBL production were positive for the *bla*NDM-5 gene. The *bla* NDM positive isolates also co-harboured *bla*CTX-M1/15, *bla*TEM-1, *qnr*S, *qnr*A, *qnr*B, *sul*1, *tet*A and *tet*B genes. Two CRE isolates (SB28/ 16-CRE, SB-30 CRE) carried both the virulence and antibiotic resistant genes. Similarly, carbapenem resistant *E. coli* carrying *bla*NDM and virulence genes were reported in captive leopards of India (VinodhKumar et al. 2021).

The efflux pump-mediated carbapenem resistance was detected in two (40%, 2/5) isolates. Similarly, the efflux pump mediated carbapenem resistance was noticed in *E. coli* isolated from piglets and dairy calves in India (Pruthvishreeet al. 2017; Murugan et al. 2019). Chloramphenicol (*cat*I, *flo*R), aminoglycoside (ac (3)-IV, aph (3′)-IIa), quinolone (*qnr*S, *qnr*A, *qnr*B), trimethoprim (*dhfr*I), sulphadimidine (*sul* I, *sul* II) and tetracycline resistance genes (*tet*A, *tet*B) were commonly detected in the phenotypic resistant isolates. Small free-living mammals' fecal samples harbored trimethoprim, ampicillin, ciprofloxacin, and cefotaxime resiatant *E. coli* (Furness et al. 2017). Screening for virulence genes revealed that four ESBL producing (SB-3 ESBL, SB-12 ESBL, SB-16 ESBL, SB-33 ESBL) and two carbapenem- resistant isolate (SB28/ 16-CRE, SB-30 CRE) harbored any of the virulence genes screened (Table 1). In the same way, reports are available on ESBL producing and carbapenem resistant *E. coli* harboring virulence encoding genes in piglets (Pruthvishree et al. 2017, 2018; VinodhKumaret al. 2019, 2021).

Plasmid replicon typing of the three *bla*NDM-5 positive *E. coli* revealed that the NDM gene was on the Incl1 plasmid of two isolates (SB27/ 17-CRE, SB28/ 12-CRE) and on the IncF plasmid of one isolate (SB28/ 16-CRE). The Incl1 and IncF plasmids also co-harbored quinolone, extended-spectrum cephalosporin, sulphonamide and tetracycline resistance genes such as *bla*CTX-M-15, *bla*TEM-1, *tet*A, *sul* I and *qnr*A. In the same way, IncF plasmids have been reported worldwide among *Entero-bacteriaceae* and are one of the most prevalent incompatibility types involved in the transfer of resistance determinants (Yang et al. 2015). Recently, the IncF plasmid carrying multi-drug resistant genes were reported in *Shigella flexneri* isolates from India (Sethuvel et al. 2019). An earlier study also documented the plasmids carrying multiple antibiotic genes in Gram negative bacteria (Ramirez et al. 2014). Similarly, the IncF plasmid encoding beta-lactamase (NDM-1, OXA-1), aminoglycoside (*aacA*4, *aadA*2 and *aacC*2), and extended-spectrum cephalosporin resistance (CTX-M-15) genes in *E. coli* ST131 isolated from humans was reported (Bonnin et al. 2012). Plasmids carrying the AMR genes have the potential to transfer the AMR determinants to non-resistant bacteria (Allen et al. 2010;

Oladeinde et al. 2019).

The plasmid multilocus sequence typing (pMLST) revealed that the *bla*NDM positive *E. coli* belonged toST297. Similarly, a recent study in India documented *bla*VIM gene in *E. coli* isolated from calf belonging to ST 297 (Murugan et al. 2019). Correspondingly, in a recent publication we reported Incl1 plasmid carrying *bla*NDM gene in *E. coli* isolated from captive leopard (VinodhKumaret al. 2021).

The ESBL producing, carbapenem and multidrug resistant *E. coli* were divided into three groups in the principal component analysis (PCA) and confirmed the difference in resistant pattern of the MDR, ESBL producing and carbapenem resistant *E. coli*. The *E. coli* were grouped in to three categories based upon their resistance pattern to antibiotics. The first two dimensions included in PCA map showed the most variance of data (first: 62.5%; second: 7.3%). The third and fourth dimensions, despite being significant, were not plotted due to the complexity of the corresponding output (Fig. 1). Similarly, PCA analysis of ESBL producing *E. coli* isolates were carried out in piglets and pig farm workers indicated a common phenotypic and genotypic resistance pattern among the piglet and the farm workers (Tamtaet al. 2020).

In conclusion, the study documented the presence of MDR, ESBL producing and carbapenem resistant *E.coil* carrying virulence genes from rescued sloth bears in India. The study also witnessed few *E. coli* isolatesco-harboured antimicrobial resistant and virulence genes. We need to focus on these isolates because the use of antibiotics possibly will select for *E. coli* carrying virulence genes and could accelerate the spread of virulence genes within *E. coli* populations. Hence, the *E. coli* iso- lates harboring antibioticresistant with virulence genes are critically important in public health. The sloth bears may be a potential source for spread of these resistant bacteria to its handlers and immediate environment. To the best of our knowledge, this is the first report of IncF and Incl1 type plasmids carrying multidrug-resistant and NDM carbapenemase producing genes in *E. coli* from sloth bear.

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Art – 256. DIAGNOSIS AND MANAGEMENT OF IDIOPATHIC EPISTAXIS IN CAPTIVE SLOTH BEAR

Illayaraja, Shivani Singh and **Pooja Acharya**

Abstract

An adult male sloth bear was observed with intermittent unilateral bleeding from left nostrils during summer. The animal was under intensive monitoring and necessary treatment medication was undertaken for forty consecutive days. Behavior based modification in the feeding schedule needs to be performed in order to address incidents of epistaxis in these animals especially which are hyperactive during adverse climatic conditions i.e., peak summer and winter.

Keywords*:* Behavioral welfare: climate: epistaxis

Introduction

Epistaxis is condition with bleeding from nasal vessel or from those of accessory nasal cavities (Chakrabarti, 2006). Captive wild animals are under great environmental stress and need good protection against environmental factors such as rain, excess heat or cold (Upadhye and Dhoot, 2002). Mortalities due to heat stroke in captivewild animals have been documented by earlier workers (Upadhye and Dhoot. 2002). Incidents of heat stroke in Sloth bear have also been reported by Upadhye (2002). So, Agra Bear Rescue Facility providing advanced veterinary care and welfare to rescue and rehabilitated Sloth bears. However, we encountered a case of epistaxis in Sloth bear and the detailed management practices are discussed below.

Materials and Method

A male sloth bear aged about 08 years was observed with intermittent unilateral bleeding from left nostrils. It was observed that prior to feeding, the bear was hyperactive followed by an increased environmental temperature during summer. Unilateral bleeding was noticed in the left nostril of the animal mostly during consumption of food. On day 0, intramuscularly, hemostyptic injection of n-Butyl alcohol, citric acid and sodium chloride (Revici'' @ 250 mg total dose) and injection Meloxicam (Melonexb) @ 0.5 mg/ kg. b.wt. was given to improve capillary integrity, platelet adhesiveness and to reduce the inflammation. anxiety, the animal was isolated. Cold water was splashed over the head at an interval of every three hours. Additional air cooler was provided by the front of the den and spraying of water with sprinklers was done in and around the enclosure in order to respite from increased atmospheric temperature thus will facilitate the faster recovery. From the second day, bleeding was reduced to half the amount. Orally, Tab. Ethamsylate 500 mg BID for seven days and bolus Melonex[b] @ 0.2 mg/ kg b.wt. However,bleeding was stopped on the 8tb day but recurrence was noticed on 10tb day with mild symptoms, so the oral medication was repeated for one more week. Further it was observed that, a delayed feeding resulting exhibition of hyperactivity in the animal. In order to avoid hyperactivity induced by visual and olfactory senses of said bear, the alternate place was arranged to distribute and coo down the porridge. It was further advised to fed the bear first among the others in the same enclosure.

16'h Day onwards, only blood-tinged mucous was noticed and 22 nd day bleeding was stopped completely. Though the complete recovery observed on 40th day, the bear was kept under observation for the next 30 days and no re- occurrence of epistaxis was noticed.

Results and Discussion

Thirumurugan *et al.* (2014) and Upadhye and Dhoot (2002) reported heat stroke in a sloth bear and its successful treatment. Acute cases of heat stroke can cause death of animals, the temperature goes up and sloth bears are prone to heatstroke (Kachhawaha *et al.,* 2015). In summer, hot weather and lowhumidity turns delicate protective mucosal layer within the nasal cavity to dry which resulting in nose-bleeds. In our reported case, the appetite, urination and defecation of the bear was normal. Hence, the epistaxis was occurred due to hyperactivity followed by increased respiratory rate, dryness of mucous and rupture of blood capillaries at nasal cavity. To upkeep the health of captive sloth bears, intensive veterinary care observation along with understanding behavioral welfare is paramount important to address such incidents.

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Art – 257. COMPARATIVE IMPACT OF CHICKEN AND BEEF FEEDING ON GASTRO-INTESTINAL PARASITES IN CAPTIVE INDIAN LEOPARDS (*Panthera pardus fusca*)

Nikhil S. Bangar and **A. Sha Arun**

Abstract

An observational study was conducted from August, 2010 to August 2020 in two phases. A total of 532 fecal samples (266 from phase 1 and 266 from phase 2) were collected from captive leopards to assess the impact of feeding on gastrointestinal parasites provided with different feeding patterns on these two phases. During the study period of phase 1, their diet mainly consisted of only beef meat with one day off in a week and phase II was fed only on chicken meat with two days off in a week. The prevalence of parasitic infection in phase 1 was 29.69% (79/266) and that of phase II was 8.65% (23/266), with an overall prevalence of 19.17% (102/532). Results indicated that in both phases of study, leopards were mostly infected by nematodes preferably *Toxocara* spp. The leopards suffered from less types of parasites when was fed on chicken meat than beef meat.

Keywords: Beef meat; chicken meat; feeding; gastrointestinal parasites; leopard

Introduction

Leopards (*Panthera pardus*) are the most widespread group of wild felids. However, their population is decreasing in a similar range to other large carnivores due to illegal poaching, depletion of prey, habit fragmentation, conflict with people and indiscriminate killing (Jacobson *et al.,* 2016). The parasitism by different etiological agents like microorganisms, helminths and even arthropods and mostly gastrointestinal parasites is another factor for diminution of this fauna (Dib *et al.,* 2016). The International Union for the Conservation of Nature (IUCN) have classified leopards as vulnerable and recognized the nine sub-species.

An important component for successful conservation and propagation of captive and free-ranging wild felids is balanced nutrition. The felids have got a unique nutritional and metabolic requirement that differs from nutritional requirement of other mammals. For example, they have obligatory dietary requirements for Vitamin D, pre- formed Vitamin A and Niacin (Morris, 2002). Felids appear to have no dietary requirement for carbohydrates and utilize other nutrients for energy (Morris, Trudell and Pencovic, 1977). Estimates for protein requirement for felids, once all needs for essential amino acids are met, ranges from 12.5 percent of dietary calories (Kantorosinski and Morrison, 1988). Felids have a fundamental dietary requirement for two amino acids, Arginine and Taurine which are present freely in animal tissue (Morris, 2002). Also, a source of animal fat to supply arachidonate is an essential component of the diet of cats (Rivers *et al.,*1975). This zoo animal nutritionists take into account this information while formulating diets for captive zoo cats, regardless of body size.

Leopards in their natural habitat predate over a large range of prey which includes ungulates, rodents, primates and birds. In the natural habitat, wild animals range across a large geographical area, have low parasitic exposure and hence are sustained with natural resistance (Kvapil *et al.,* 2017). The dietfor leopards in most of the captive of the Indian sub- continent mainly consists of buffalo meat on the bone(BMB) (Sarode *et al.,* 2019). The gastrointestinal parasites most frequently encountered are *Toxocara, Toxascaris, Strongyloides, Trichuris, Paragonimus, Echinococcus*, *Taenia* and *Eimeria,* (Acharjyo, 2004; Kobbekaduwa *et al.,* 2017; Moudgil *et al.,* 2015). The ruminants, especially cattle and buffalo serve either as a direct or intermediate host for most of these parasites (Bowman, 2014).

The resistance of captive animals to parasitic infections depends upon the type of their feeding and nutritional status (Barbosa *et al.,* 2019; Dib *et al.,* 2016). Beef meal is lower in protein, moisture, Vitamin A (Sarode *et al.,* 2019), Selenium (Barclay *et al.,* 1995; 'Selenium in Foods' 2006), Molybdenum (Gerber *et al.,* 2009), Omega-3 polyunsaturated fatty(de Almeida *et al.,* 2006) with higher in cholesterol content (Nistor *et al.,* 2013) when compared to chicken. Also, buffalo lack carotenoids deposition in their body and carotenoids are present in poultry meat. An impairment of cell-mediated immunity, phagocyte function, complement system and cytokine production is known to be caused by low protein intake (Chandra, 1996; Cornet *et al.,* 2014). Carotenoids and Vitamin A have immune- modulatory effects and an alteration in immune functioning can occur because of dietary manipulations (Mora *et al.,* 2008) and thus can favor multiplication of parasitic and survival in the host body.

The chicken meal can also be easily available throughout India. In contrast to this, buffalo meat is found to have higher calories, Ca, P (Sarode *et al.,* 2019), Fe, Zn (Gerber *et al.,* 2009; Lombardi*et al.,* 2002), Taurine (Morales *et al.,* 1989), Pantothenic acid, Niacin, Vitamin D, Vitamin K and Riboflavin (Schmid and Walther, 2013; Schonfeldt, *et al.,* 2010; Shearer, Bach and Kohlmeier, 1996) than chicken meal. Therefore, it is a matter of interest to see if feeding both buffalo and chicken meat in leopards would provide better nutrients relatively close to their natural habitat and improve the immune status against a parasitic disease. The specific objective of the study was to evaluate the impact of feeding BMB over the only CC on fecal parasite of captive Indian leopards.

Materials and Methods Study Site and Animals

The study was conducted which covers 10 acres of land and is occupied by 33 Leopards from different regions of Maharashtra including males and females. The study included 19 Leopards consist of 14 females and 5 males. All leopards were adults and above 5 years. All leopards selected for study never shown any clinical signs associated with parasitic infection and having good body conditioning score throughout the study period.

Feeding

Leopards from MLRC were fed once daily in the evening when animals returned to night cages. The study was conducted in two phases their diet mainly consists only of beef meat with one day off in a week in phase one which was between August 2010 till August 2015 and from September 2015 till August 2020 was fed only on chicken meat with two days off in a week.

Sampling and Material collection

A total of 532 fecal samples were collected (266 from phase one and 266 from phase two) from the captive leopards of MLRC, Junnar during the study period. About 5-10 gram of fresh fecal matter was collected in the early morning before cleaning directly from the floor of enclosures by the animal caretakers the fecal samples were stored in interlocked polythene bags or plastic containers containing 10% Formalin labeled with the date, time, and sample of collection and were properly sealed. Then the bags or containers were then placed in isothermal containers and were transported to for parasitic examination.

Laboratory Techniques

In laboratory, a portion of fecal matter was processed for qualitative examination like direct, sedimentation and floatation method as per standard protocols given by Bowman (2014). Tentative identification according to morphology was done for the ova, cysts, oocyst and larvae (Bowman, 2014). Then the quantitative estimation was carried out using the Modified McMaster method to determine eggs per gram (EPG).

Results

A total of 566 fecal samples were collected and included in this study, of which, 102 (19.17%) were positive for gastrointestinal parasites. Of these, 79 (14.85%) showed nematodes, 1 (0.37%) trematode, 12 (2.26%) cestodes and 10 (1.88%) protozoa (Table1). The overall intestinal parasitic infection in the conducted study phase one was 29.69% (79/266) out of which 19.17% were *Toxocara* spp, 4.13% *Strongylidae* spp, 2.63% *Diphylidium* spp, 0.75% *Ancylostoma* spp, 0.37% *Coccidia* spp, 0.37% *Uncinaria* spp, 0.37% *Diphylobothrium* spp, 0.37% *Spirocera* spp, 0.37% *Spirometra* spp, 0.37% *Trichuris* spp, 0.37% *Balantidium* spp and 0.37% were *Schistosoma* spp (Table 2). Occurrence of nematode, trematode, cestode and protozoa were 25.2% (67), 0.37% (1), 3.4% (9) and 0.75% (2) (Table 1). Similarly, in phase two the overall gastrointestinal parasitic infection was 8.65% (23/266) out of which 4.13% were *Toxocara* spp, 3.00% *Isospora*, 1.13% *Spirometra* and 0.37% were *Strongylidae* (Table 3). Occurrences of nematode, cestode, and protozoa inthe second phase were 4.51% (12), 1.13% (3) and 3.0% (8) respectively (Table 1). Results indicated that in both phases of study, leopards were mostly infected by nematodes preferably *Toxocara* spp and suffered from more types of parasites when were fed on beef meat than on chicken meat.

Table 1: Occurrence of intestinal parasitic infections in leopards (2010-2020)

Study phase	Sample size	Nematode %	Trematode %	Cestode %	Protozoa %	Total %
Phase 1	266	67 (25.19)	1 (0.37)	9 (3.38)	2 (0.75)	79 (29.69)
Phase 2	266	12 (4.51)	-	3 (1.13)	8 (3.00)	23 (8.65)
Total	532	79 (14.85)	1 (0.37)	12 (2.26)	10 (1.88)	102 (19.17)

Table 2: Prevalence of fecal parasites during study phase I

(August, 2010 - August, 2015)

Parasite	Parasite species	No. of positive case	Prevalence (%)
Nematode	Toxocara *sp.*	51	19.17
	Strongylidae *sp.*	11	4.13
	Ancyclostoma *sp.*	2	0.75
	Uncinaria *sp.*	1	0.37
	Spirocera *sp.*	1	0.37
	Trichuris *sp.*	1	0.37
Trematode	Schistoma *sp.*	1	0.37
Cestode	Diphylidium	7	2.63
	Diphylobothrium	1	0.37
	Spirometra *sp.*	1	0.37
Protozoa	Coccidia *sp.*	1	0.37
	Balantidium coli	1	0.37

Table 3: Prevalence of fecal parasites during study phase II

(September, 2015-August, 2020)

Parasite	Parasite species	No. of positive case	Prevalence (%)
Nematode	Toxocara *sp*	11	4.13
	Strongylidae *sp*	1	0.37
Cestode	Spirometra *sp*	3	1.13
Protozoa	Isospora *sp*	8	3.00

Discussion

A ten year survey on analysis of gastrointestinal parasites from fecal sample of leopards revealed the overall prevalence to be 19.17%. Lower positivity rates in this study when compared to the other reports *i.e.* India 66.6% (Javaregowda, 2015), Sri Lanka 57.8% (Kobbekaduwa *et al.,* 2017), Malaysia 89.3% (Lim, Ngui, Shukri, Rohela and Mat Naim, 2008) and Brazil 65.3% (Barbosa *et al.,* 2019). The lowest prevalence in our study might be due to change in feed with chicken meal from BMB, variation in number of fecal samples screened and other managemental practices adopted.

Among 266 fecal samples screened on phase one and phase two each, 79 (29.69%) and 23 (8.65%) were harboring either helminths or protozoan parasites. It was found that 19.17% and 4.13% of the samples from phase one and phase two were positive with *Toxocara* spp and showed the highest occurrence on both phases compared to other intestinal parasites. This is following Javeregowda (2015) and Kobbekaduwa *et al.*

(2017) who also reported the maximum prevalence with *Toxocara* spp from the fecal samples. Higher intensities of this parasite might be due to the reason that *Toxocara* spp is species-specific and Canidae and Felidae family serve as definite hosts (Bowman, 2014). The presence of rodents in the housing environment could attribute to the complete life cycle as they are the paratenic or intermediate host for *Toxocara* spp. However higher prevalence percentage of *Toxocara* spp during phase one was obtained when compared to phase two. This might be supported by the fact that during grazing, the cattle may be infected with ingestion of embryonated eggs, the larvae of *Toxocara* spp then migrate and locate in the liver, heart, brain, kidneys, and muscle of the host (Okulewicz, Perec-Matysiak, Bunkowska and Hildebrand, 2012). Thus, beef meal may act as a potent source for infecting the captive leopards while poultry farming is reared in the intensive system and thus have a very lower chance of picking embryonated eggs from the ground.

The occurrence of S*trongyloides* nematodes was observed in both phases studied however highest prevalence percentage found in phase one 4.13% when compared to phase two 0.37%. Strongyloides *spp* had not only been reported from various wild felids but also from leopards from different zoological captives of India (Acharjyo, 2004; Moudgil *et al.,* 2015), Italy (Fagiolini *et al.,* 2010), Bangladesh (Ahasan, Iqbal and Azam, 2010). The parasite has a direct life cycle and the host gets infected when free- living larvae penetrate the skin and migrate throughout the host body (Bowman, 2014). In a housing environment, soil and wooden floors may favor the clogging of the eggs and larvae to the surface and get transmitted to susceptible animals. This parasite particularly can undergo autoinfection so that repeated generations of development in the same host individual may occur even after a change in feed (Viney and Lok, 2007). However, host immune response has a deleterious effect on parasite and the role of protein, Vitamin A and carotenoid from chicken meal might have better immunomodulatory effect on the host (Chandra, 1996; Cornet *et al.,* 2014; Mora *et al.,* 2008) as these components are higher in the chicken meal when compared to the beef meal (Sarode *et al.,* 2019).

It was found that 0.37% and 1.13% of the fecal sample were infected with cestode, *Spirometra* y from phase one and phase two respectively. *Spirometra* spp is one of the common gastrointestinal cestode parasites of leopards and was reported from different zoological gardens of India (Gawande *et al.,* 2007; Javaregowda, 2015; Parsani *et al.,* 2001), Malaysia (Lim *et al.,* 2008), Bangladesh (Raja *et al.,* 2014). Poultry has the nature of picking copepods from the environment which are the first intermediate host of the parasite so the prevalence percentage may be higher in a chicken meal in our findings. Buffalo may accidentally ingest infected copepods while grazing and ingestion of chicken and beef meal contaminated with plerocercoid larvae may act as a source of infection to leopards. A higher prevalence of coccidian oocyst was observed in a sample of phase two (3.00%) than phase one (0.37%). In captive wild felids. The incidence of coccidiosis has also been reported by many workers from different parts of the world (Barbosa *et al.,* 2019; Patton and Rabinowitz, 1994; Ravindran *et al.,* 2011). Leopards mayget infected after the ingestion of sporozoites from the mammalian paratenic host and poultry meat or ingestion of sporulated oocyst from the environment (Patton and Rabinowitz, 1994).

Other isolated parasites from phase one when fed beef meal included nematodes (*Ancylostoma* spp, *Uncinaria* spp, *Spirocera* spp and *Trichuris* spp), trematode (*Schistoma* spp.), cestodes (*Diphylidium* spp. and *Diphylobothrium* spp.) and protozoa (*Balantidium coli*) and were not reported from phase two under chicken meal. Similar parasites were also reported from captive wild felids including leopards on previous studies that rely on the beef meal; *Ancylostoma* spp. and *Uncinaria* spp. (Ravindran *etal.,* 2011; Shirbate and Shirbate, 2019; Suresh *et al.,* 2000), *Spirocera* spp. from order Spiruroida (Hosseini *et al.,* 2019; Patton and Rabinowitz, 1994; Shirbate and Shirbate, 2019), *Trichuris* spp. (Acharjyo, 2004; Kvapil *et al.,* 2017; Moudgil *et al.,* 2015), *Schistoma* spp (Moudgil *et al.,* 2015), *Diphylidium* spp. (Acharjyo, 2004; Ahasan *et al.,* 2010), *Diphylobothrium* spp. (Acharjyo, 2004; Ahasan *et al.,* 2010; Liza *et al.,* 2020; Thawait *et al.,*2014; Varadharajan and Pythan, 1999) and *Balantidium coli* (Raja *et al.,* 2014). Cattle and buffalo in slaughterhouses mostly come from rural vicinity after they are infected, diseased, or immunosuppressed. They can get access to infected eggs, larvae and intermediate hosts of the parasites while grazing on field and open lands. Also, a proper deworming practice is not followed by farmers in a rural village. While poultry farming is more advanced and are under an intensive system and managed successfully in India. The poultry industry is under the supervision of veterinarians, follows routine diagnostic parasitology tests, under good housing conditions and have less chance of acquiring parasitic infection through the environment. Thus, this might be the reason for leopards to be infected with the more types of parasite when fed under beef meal in our study. Besides that, the spread of parasitic agents to captive leopards kept in this institution can be contributed by poor husbandry practices and the presence of rodents, copepods, snails in the house premises.

Conclusion

As from the above findings, it can be concluded that there is a need to implement a zoo management program which includes feeding of chicken meal in combination with the beef meal, followed by proper surveillance of slaughter-house and meat for parasitic infestation, diagnostic parasitic tests followed by specific treatment for each parasite. In addition, the routine cleaning of animal enclosures, daily removal of feces, proper disinfection of premises, sanitizing food and water containers, rodent control, and providing filtered water supply should be prioritized in the program.

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Art – 258. A PRELIMINARY ASSESSMENT OF SNAKES AND MONITOR LIZARDS ENCOUNTERED DURING COVID-19 LOCKDOWN IN AGRA, INDIA

S. Prerna, **Baiju Raj MV, S. Ilayaraja**, and Manoj Chaurasiya

Abstract

India had undergone a strict lockdown in the year 2020 to curtail the spread of novel coronavirus. Human mobility was restricted, and the time spent in and around houses was unlimited. This may have added to the increased frequency of encounters with snakes, monitor lizards, and other urban wildlife. We assessed the data of snakes and monitor lizards rescued during the lockdown in 2020 and compared it with 2018 and 2019 data. During the lockdown, there was an increase in the number of encounters with snakes and monitor lizards, but species diversity, seasonality, and activity pattern remained similar with the previous two years. The rescue activity is also an important opportunity to spread awareness among the public about the local snakes and monitor lizards. The overall impact of the lockdown on snakes and other reptiles will be assessed over the years as more data becomes available.

Keywords: Reptiles, snake, monitor lizard, rescue, conflict, covid-19, awareness.

Introduction

In the year 2020, India had undergone a complete lockdown where human mobility was restricted to curtail the spread of COVID-19. During the pandemic, there have been several interesting wildlife encounters reported throughout our country on social and print media. Urban wildlife that resides around human habitation faced new challenges during this pandemic (Corllet *et al.*2020). Most of us spent time in our homes like never before and managed to engage ourselves in activities like gardening, cleaning, and exploring our surroundings daily. We speculate that this may have led to the increase in the frequency of encounters with snakes, monitor lizards, and other urban wildlife that thrives around us. Moreover, we all know that because of urbanization, encroachment, degradation of natural spaces, and other human disturbances there has been an increase in the number of encounters with snakes (Pareek and Singh 2021; Gayen2019; Roshnath 2017; Purkayastha *et al.* 2011) and with other wildlife. An average of 58000 people dies annually due to snake bites in our country (Suraweera et al. 2020). Yet human snake interactions have received very less attention (Ramesh and Nehru 2019). Snakes have always been associated with our culture and are also a cause of fear among people (Pareek and Singh 2021; Gayen *et al.*2019; Roshnath 2017). Snakesare important (Gibbons*et al*. 2000) because they help us to keep a check on rodent populations (Pandey et al. 2016) and act as prey to some mammals and birds. Likewise, monitor lizards are scavengers that help us to control pests and keep the environment clean. They do not attack humans unless provoked. Although most of the snakes, monitorlizards, and other reptiles are protected under The Wildlife (Protection) Act, 1972, they receive no mercy when sighted (Gayen *et al.*2019; Roshnath 2017). They face many otherchallenges such as illegal trade (Joshi *et al.* 2021; Marshall *et al.* 2020); persecution for superstitious beliefs, killed for their meat, skin, and other body parts, etc.

Study area and methods

Wildlife SOS, a non-government organization, has been running a 24 hour helpline with Uttar Pradesh Forest Department for wildlife rescues in Agra, Mathura, and nearby districts (Figure 1) for more than 15 years. When a call for a rescue is received, the contact details are transferred to the rescuer who notes down the address of the location. After the rescue is over, the rescuer enters all the details in a predesigned form and gets it signed by the caller.

Photographic documentation of the rescue and the species is done on the location itself. The rescued snake or monitor lizard is released immediately or on the same day in a suitable natural area. If the snake or monitor lizard is found injured, it is brought to the hospital for its treatment and released after its recovery. These rescue details are entered in a spreadsheet and also reported to the Uttar Pradesh Forest Department. Translocation of rescued snakes is a topic of research and debate (Ramesh and Nehru 2019; Roshnath 2017; Barve *et al.* 2013). Translocation may have negative impacts on the snakes and as well as on humans. We looked at the patterns of the snakes and monitor lizards encountered and rescued in the years 2018 and 2019. We compared it with patterns during the lockdown of the year 2020. We wanted to know if there was an increase in the number of encounters during the lockdown. Which species of snakes were encountered more? Where did most of the encounters happen? What time of the day did the most encounters occur? Do these patterns tell us something about their ecology and behavior?

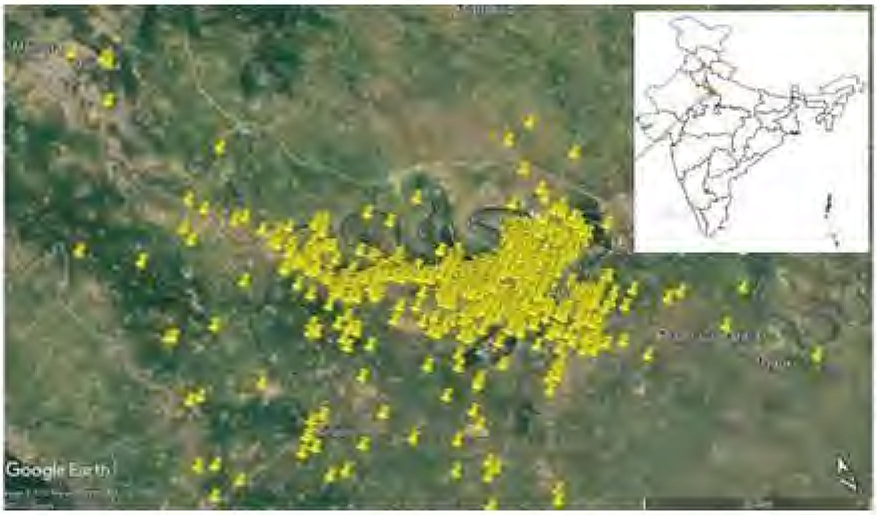

Figure 1. GPS locations of human-reptile (snakes and monitor lizards) encounters in Agra, Mathura and adjoining districts.

Results and discussion

The total number of snakes and monitor lizards rescued in 2018 and 2019 were 309 and 408 respectively. In 2020, we saw that there was a clear increase (564) in the number of calls received for the rescue of snakes and monitor lizards from the city. A maximum number of snakes and monitor lizards were rescued during autumn (September-November) followed by monsoon (July-August) season (Table.1). The pattern remained unchanged throughout these three years. Agra, Mathura, and other adjoining districts are situated on the banks of the river Yamuna. During the monsoon, the river is flooded, and many reptile species migrate to cities and adjoining villages through canals and drainages. Overall, these cities have limited forest patches.

Table 1: Number of rescues in different season

Season	2018	2019	2020
Winter (December-January)	27	43	39
Spring (February-March)	22	21	24
Summer (April-June)	56	56	91
Monsoon (July-August)	84	122	154
Autumn (September-November)	150	166	256

The most common species rescued in these three years (Table.2) were Indian Rat

Snake (*Ptyas mucosa*), Indian Rock Python (*Python molurus)*, Common Wolf Snake *(Lycodon aulicus)*, Spectacled Cobra *(Naja naja)*, Common Indian Monitor *(Varanus bengalensis)*, Common Krait *(Bungarus caeruleus)*, and Checkered keelback *(Xenochrophis piscator)*. Indian Rat snake (Whitaker and Captain 2008) is a diurnal species. It is commonly found throughout the Indian subcontinent. Its diet is eclectic which includes frogs, toads, rats, birds, lizards, and other small vertebrates. It inhabits a wide range of habitats. It is a very fast snake and can escape quickly. Indian Rock Python (Daniel 2002) is diurnal and nocturnal species depending on the extent of human disturbances. Pythons hibernate in the winter season and can be sighted while basking during the day. Being a large-bodied snake, it constricts its prey and prefers to eat mammals and birds. Pythons are forest (dense or open) dwellers or inhabit riverbanks or lakes. Interestingly, of all the pythons rescued from Agra and nearby districts, 33% were from houses, 31% were from farms and fields, 15% from commercial and public facilities, and 9% from the roads, 11% of pythons escaped before their rescue. Common Wolf Snake (Daniel 2002) is nocturnal species. It is commonly found near and in human habitation throughout the country. It can easily climb vertical walls and is mostly rescued from the crevices, ceilings, and roofs. Its diet consists of lizards, geckos, skinks, and mice. Spectacled Cobra (Daniel 2002) is one of the most common venomous snakes found in the country. It is most active during the night but may venture out during the day in search of its prey. Its diet consists of frogs, toads, birds, rodents, snakes, and eggs of invertebrates. It inhabits a wide range of habitats. Common Indian Monitor Lizard (Daniel 2002) is a widely distributed diurnal species. It is a burrow dweller but also inhabits crevices on the ground or rocks. It eats a wide variety of prey like frogs, fish, small turtles to small mammals, and birds. Common Indian Krait (Daniel 2002) is a widely distributed venomous species. It is very active and alert during the night. It is found near human habitation, in farms and fields, in rodent burrows, and termite mounds. Its diet consists mostly of snakes, but it consumes rats and frogs too. Checkered keel back (Whitaker and Captain 2008) is a freshwater snake that is commonly found throughout the country in water bodies and paddy fields. It feeds on frog's eggs, tadpoles, frogs, fishes, and water insects. They are reported to eat rats and birds too. The snake is active during the day and night.

Table 2: Species rescued during the years

Species	2018	2019	2020
Indian Rat Snake (*Ptyas mucosa*)	65	66	101
Indian Rock Python (*Python molurus)*	86	86	93
Common Wolf Snake *(Lycodon aulicus)*	66	84	93
Spectacled Cobra *(Naja naja)*	46	60	71
Common Indian Monitor *(Varanus bengalensis)*	28	33	41
Common Krait *(Bungarus caeruleus)*	22	20	23
Checkered keelback *(Xenochrophis piscator)*	11	8	10
Black Headed Royal Snake *(Spalerosophis atriceps)*	3	3	8
Common Sand Boa *(Gongylophis conicus)*	1	9	6
Common Cat Snake *(Boiga trigonata trigonata)*	2	2	4
Red sand boa *(Eryx johnii)*	7	0	3
Common Kukri Snake *(Oligodon arnensis)*	0	1	0
Russell's Kukri Snake *(Oligodon taeniolatus)*	0	1	0
Leith's Sand snake *(Psammophis leithii)*	0	1	0
Unidentified snakes	2	34	111

In 2020, 20% of the snakes escaped from their location of sight before the rescuer reached. These were recorded and were categorized as unidentified snakes. Snakes could manage to escape because of their elusive nature and if given a chance, they would prefer to slither away from human encounters. It can also be because of the disturbance from the people or the inability of the people to keep a track of its movement because of fear. At times, people just call for the rescue even if they have sighted it in their backyard or near their residence, sometimes a few hours earlier. 80%of the rescues were of non-venomous species. Common Cobra and Common Krait are the only two venomous species of snake rescued from the city. In 2020, 60% of the rescued snakes were from domestic residences. This was similar to 2019 (64%) and 2018(56%) data. Other rescues in the year 2020 were from commercial and public facilities (10%) such as schools, offices, restaurants, warehouses, religious places, shops, showrooms, and even from a hospital, 9% were from farms and fields and around 2% were from the roads. Most of the rescue calls received were concentrated during the day (Figure 2) and that also coincides with the human activity peak hours

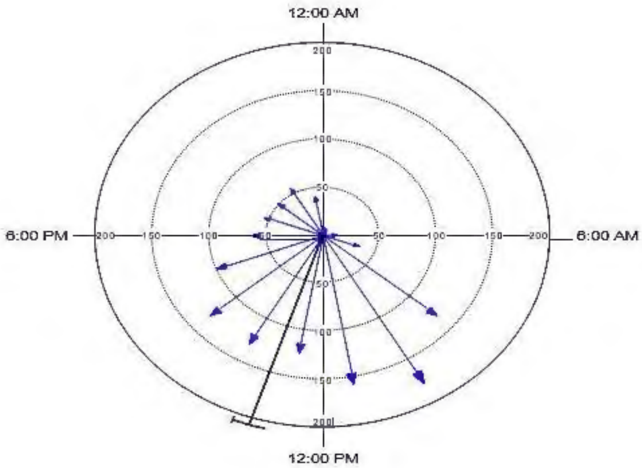

Figure 2. Time of rescue calls received

Conclusions

It may be early to assess the overall impacts of the pandemic and lockdown on the encounters of snakes, monitor lizards and, other wildlife, but such evaluations are possible. Scientific rescue of snakes and monitor lizards provides us an excellent opportunity to interact with the civil society and educate them about the endangered species and the laws. At the same time, we have been able to document the diversity, trends in seasonality and frequency of encounters, activity pattern of the snakes, and monitor lizards. The information can be very useful for further research, develop conservation plans and strategies to manage human-snake interactions effectively.

Pictures showing the role of rescue and awareness activities.

Acknowledgments

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Art – 259. TREATMENT OF TRAUMATIC ESOPHAGEAL RUPTURE IN A GREATWHITE PELICAN (*Pelecanus onocrotalus*): A CASE REPORT

Ilayaraja S, Pooja Acharya**, Baiju Raj MV** and **Arun A Sha**

Abstract

In the present case study, an adult Great White Pelican was presented with an esophageal rupture in the neck region with ingestion of large fish; it was a complete esophageal tear exposing the throat, cervical vertebra, jugular vein, and trachea. The pelican's throat was severely congested with blood clots. Esophageal rupture is very rarely seen in birds. The case was corrected surgically under local anesthesia without intubation. The bird recovered successfully within 15 days without any secondary complications. Avoiding excess handling and providing habitat similar to nature can be extremely useful for the treatment of esophageal rupture in birds.

Keywords: Surgical management, esophageal rupture, great white pelican

Introduction

The esophagus is a flexible musculo-membranous tube that connects the mouth to the crop in avian (Kumar *et al.*, 2016) [1]. Unlike in animals, the large portion of the esophagus lies in the cervical region in birds. The open wounds at the cervical region may lead to tears in the esophagus, trachea, or fistulation of the crop. It is commonly seen in fighting birds, mainlystab and carved wounds are common. We have seen the cases of ingestion of unusual items by wetland birds like plastic, rubber, shells, etc. in urban landscapes (B. Anjan Kumar Prusty *et al.*, 2020) [4] but very barely the cases of oesophageal rupture in birds. In the 1980s, in California, a pair of Great Blue Herons were found freshly deceased with lamprey lodged in their esophagus and they choked to death (https://www.flockingaround.com/post/can-birds- choke). Indian subcontinent plays an important role to host several migratory birds, it is estimated that over a hundred species of beautiful migratory birds fly to India in some bird sanctuaries as there is sufficient availability of depth, quality of water, and availability of food (predator species) and shelter or to escape from extreme winter of their habitat. The great white Pelican (*Pelecanus onocrotalus*) is the heaviest and one of the major winter migratory birds which are visiting peninsular north India by settling down in shallow, freshwater lakes with plenty of fish. Most of these birds settle down in Pakistan, while some fly as high as Nepal. They are carnivores, prefer to be piscivores in nature. Keetam Lake which is located inside Soor Sarovar Bird Sanctuary, Agra, Utter Pradesh is one of the destinations for this giant migratory bird in the winter season (November to mid-March). In February 2019, an injured Great White Pelican (*P. onocrotalus*) was rescued and treated. After surgical correction, the bird showed favorable recovery, which is detailed in the following report.

Surgical Management and Medical Care

In February 2019 evening forest patrolling team rescued an injured great white pelican (*Pelecanus onocrotalus*) to Wildlife Veterinary Hospital which is managed by Wildlife SOS. The rescued bird was showing symptoms like an extended neck with fastened fish in the esophagus (Fig. 1). Close clinical examination revealed a complete tear of the esophagus and a deep cut at neck skin which was almost 8 inches in depth fromthe throat. The deep cut was exposing the severely congested throat, cervical vertebra, and trachea with blood clots. The mucosal layer of the esophagus revealed severe bleeding and congestion with a laceration.

Fig 1: P. onocrotalus with ruptured neck due to the choked fish.

As the throat and trachea were with lacerated injury caused by the previously ingested fish, we decided not to intubate the bird to avoid further damage to the anatomical part of the pelican. The bird was restrained gently with a blindfold. The deep incised wound was flushed with 0.9% sterile isotonic solution which was followed by removal of blood clots and debris (Fig. 2). The edges of incised skin were sprayed with

2% lignocaine solution, the skin edges were brought in opposition and sutured with polyglycolic acid suture no. 4.0 in horizontal mattress pattern followed by application of Himax ointment and 2% lignocaine spray (Fig. 3). Inj. Meloxicam @ 0.2 mg/kg BW and Inj. Enrofloxacin @ 5mg/kg BW (long-acting) was administered intramuscularly.

Fig 2: Lacerated injury at throat, cervical vertebrae and trachea observed after manual restrain

Fig 3: Esophageal tear sutured in horizontal mattress pattern with polyglycolic acid suture

Fig 4: Therapeutic laser application to reduce inflammation and pain management

Post-operative Care and Management

The bird was kept in the observation pen to monitor bird for any sign of respiratory distress, swelling of the throat for post-operative care and management. The bird was provided with ad libitum potable water access by providing an artificial water pond in the observation pen (Fig. 5) Next day morning bird was found active and alert with normal movement in the pen without dropping the head. The bird was maintained on glucose water mixed with multivitamin supplement orally and for pain management and healing, the bird was provided with laser therapy on the surgical site (Fig. 4). for the next two days. On the third day bird seems to be more active and defensive so we decided to offer small size live fish by cutting their fins to prevent any damage to the healing wound, as the bird was with normal activities had live fish on its own without any difficulties which were monitored by a camera trap. The bird was offered with live fish @ 2 kg/day and was kept in the observation area for the next 7 days. From 7 days onwards we offered as such live fish for next 3 days. The bird was recovered uneventfully and released successfully back into the lake (Fig. 6).

Fig 5: Post-operative management; observation pen provided with water access

Fig 6: P. onocrotalus released back into natural habitat after uneventful recovery

Discussion

Esophageal obstruction in seabirds is well documented (Kathryn *et al*., 2004 and B. Anjan Kumar prusty *et al*., 2020) [2, 4] but the cases of esophageal rupture are rarely reported in birds. Spontaneous rupture of the esophagus is a rare and long recognized clinical entity in birds. Ingestion of live fish resulting in airway obstruction is rare and the most fatal occasion (Ben Van Der Hoven *et al*., 2019) [3]. Laser therapy is best for the management of hemostasis in highly vascular areas. The present case is the first reported intentional ingestion of tilapia fish. The osteology and anti-predator behavior of tilapia fish had a bad choice for the drinking game. Tilapia fish have a very sharp dorsal, anal, pectoral, and caudal fin which leads to severe and deep incisions. In the present study it is clear that, after ingestion of the tilapia fish, fish went under distress and tried to come outside the pouch and ended up being stuck in the bird esophagus.

Furthermore, the case illustrates how a reckless drinking game, imitating jackass, wand a fish, and catfish can turn into a critical and dangerous medical situation with serious consequences. When confronted with an ingested fish it is important to identify the specimen for the proper line of treatment. In this case, the decision to operate the bird without intubation and general anesthesia proved to be a reasonable option and resulted in a favorable outcome for the bird.

Acknowledgement

We express our sincere and deepest feelings of gratitude originating from the innermost core of my heart for Mr. Kartick S & Mrs. Geeta Sheshamani Co-Founders, Wildlife SOS for their resolute support and all our animal care staff. Our heartfelt thanks to the Utter Pradesh Forestry Department (UPFD) for their kind support and cooperation.

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Art – 260. SLOTH BEARS AND ANTHROPOGENIC RISKS IN KARNATAKA, INDIA

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

Abstract

Sloth bears (*Melursus ursinus*) fall victim to anthropogenic hazards, including snares, small crude explosive devices, open wells, and roads, as well as gunshots, barbed wire, and mob attacks. While conducting rescue efforts in the southern state of Karnataka, India, Wildlife SOS collected data on 20 snare incidents, 8 crude explosive device incidents, 7 roadkill incidents, 5 open well incidents, 2 gunshot attacks, 1 barbed wire incident, and 1 mob attack that occurred between 2006–2007 and 2013–2019. Female sloth bears fell victim to anthropogenic risks more often than did males (68% vs.32%), and 23% of the females had cubs or were pregnant. This study suggests that anthropogenic risks form a threat to the sloth bear populations in Karnataka, India. Our suggestions to mitigate these threats to sloth bears include stiffer penalties for the use of snares and crude explosive devices, putting lips on all open wells, and working with road agencies and forest departments to construct additional wildlife crossings.

Keywords: India, *Melursus ursinus*, open wells, poaching, roadkill, sloth bear, snares, wildlife hazards

The large and growing human population in India ensures that habitat loss and the encroachment of human activity are the greatest risks to many species of wildlife, including the sloth bear (*Melursus ursinus*). The sloth bear is patchily distributed throughout India, as well as the lowland areas of Nepal and the island of Sri Lanka. Itis listed as Vulnerable on the International Union for Conservation of Nature Red List as a result of habitat loss and habitat deterioration (Dharaiya et al. 2016). Urban development, wild areas converted to agricultural fields, and habitat degradation are all ongoing problems. However, sloth bears are known to persist in areas close to villages and in degraded habitats (Akhtar et al. 2004). This ability may be, at least in part, the result of several specialized characteristics that this species possesses. First, sloth bears are myrmecophagous, and approximately half of their diet consists of termites or ants (Garshelis et al. 1999, Sacco and Van Valkenburgh 2004). Termites and ants often thrive in small patches of disturbed habitat, so sloth bears often have a reliable food source even in the midst of degraded habitats. Secondly, sloth bears have a small home range (Joshi et al. 1995, Akhtar et al. 2006, Ratnayeke et al. 2007) compared with other species of bears. For example, the home range of a female sloth bear has been estimated at 9.4 km^2 in Nepal and 12.4 km^2 in central India, and that of a male sloth bear at 14 km^2 in Nepal and potentially larger in India (Joshi et al. 1995, Yogan and et al. 2005). This is very small when compared with brown bears (*Ursus arctos*), whose home ranges may be >1,000 km^2 (Graham and Stenhouse 2014). Sloth bears have small and often overlapping home ranges, so viable populations are able to persist in a relatively small area for a bear species (Ratnayeke et al. 2007). Thirdly, sloth bears, including adult males and females with dependent young, are socially tolerant of one another (Joshi et al. 1999), which may allow for more bears to occupy a smaller area if there are enough resources. Lastly, sloth bears are largely nocturnal (Joshi et al. 1999, Akhtar et al. 2004, Bargali et al. 2012, Ramesh et al. 2013, Arun et al. 2021), which is beneficial in avoiding direct contact with humans. However, none of these attributes are exceptionally useful in avoiding anthropogenic risks. Wildlife SOS is a nonprofit organization that has been working on sloth bear conflict management for over 25 years. An agreement between the Karnataka Forest Department and Wildlife SOS began with the establishment of a life-time sloth bear care facility in 2005, primarily organized for the rescue and rehabilitation of sloth bears saved from the dancing bear practice. In addition to the captive bear facility, the Karnataka Forest Department entered into an agreement with Wildlife SOS that enabled Wildlife SOS to assist the forest department in the rescueof conflict wild animals within the state. For >15 years, Wildlife SOS has been working in the state of Karnataka rescuing sloth bears from human hazards and documenting these risks. This paper reports on sloth bears that fell victim to anthropogenic hazards between 2006–2007 and 2013–2019. These hazards include snares, crude explosive devices, open wells, roadways, gunshots, barbed wire, and mob attacks.

Study area

The state of Karnataka is very biodiverse, with >12% of the state forested. Karnataka has 3 predominant geographic zones: a coastal region bordered by the Arabian Sea, a hilly region comprising the Western Ghats, and the plains and rocky scrub jungle of the Deccan Plateau (Fig. 1). Sloth bear habitats in Karnataka are considered to be some of the highest quality remaining habitat for the species (Puri et al. 2015). The Western Ghats habitat is largely covered by moist broadleaf forests and is considered one of the world's biodiversity hotspots. The Deccan Plateau and surrounding areas of eastern Karnataka are largely covered by agricultural lands interspersed with protected patches of wilderness. These preserved forests are generally composed of scrub jungles (*Acacia* spp., *Albizzia* spp., *Cassia* spp., and *Ziziphus* spp.) interspersed with boulders and caves. These areas remain unaltered, largely owing to their ruggedness, which makes them un-suitable for agriculture. As these wilderness areas diminished in size and distribution, many species (such as tigers [*Panthera tigris*]) disappeared, whereas others (such as sloth bears and leopards [*P. pardus*]) continued to thrive.

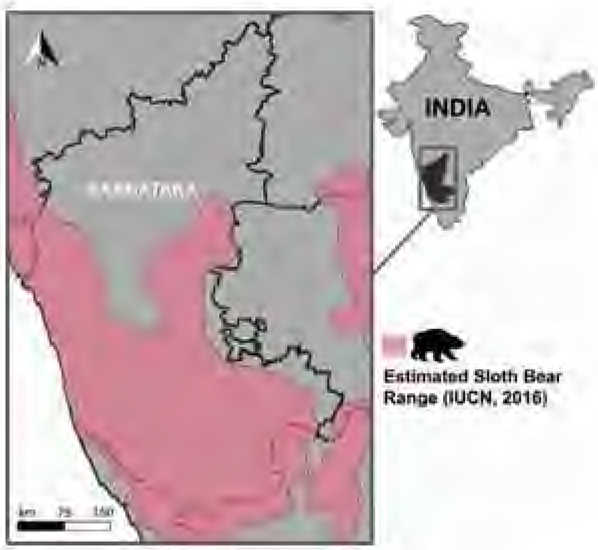

Fig. 1. Location of Karnataka, India, where Wildlife SOS collected data on anthropogenic incidents involving sloth bears (Melursus ursinus) that occurred between 2006–2007 and 2013–2019.

Methods

Wildlife SOS conducts wildlife rescues throughout the state of Karnataka for many species of wildlife, including sloth bears. When information is forwarded by Forest Department officials to Wildlife SOS about an injured, trapped, or dead sloth bear, a rapid response team is sent to rescue the animal or determine the cause of death. All the calls are referred to Wildlife SOS by the Karnataka Forest Department, often by Range Forest Officers. The sloth bear is a schedule I wildlife species; therefore, as a mandate, Forest Department officials are physically present at all bear rescues.

All incidents are documented by date, location of the incident, type of incident, gender of the bear, estimated age of the bear, and the result of the incident. The age of the bear was estimated by personnel involved in the raising of >100 sloth bear cubs to adulthood and >15 years of experience working with sloth bears of all ages at the Wildlife SOS Sloth Bear Rescue Centers. Physical characteristics used to estimate the age included head size, dentition, overall body size, claw color, and sometimes body weight.

Results

We documented 44 events that involved sloth bears subjected to anthropogenic risks. Of these, snares were the most common (45%, *n* = 20), followed by crude explosive devices (18%, *n* = 8), roadkills (16%, *n* = 7), and open wells (11%, *n* = 5). Less common were gunshot (5%, *n* = 2), barbed wire (2%, *n* = 1), and mob attacks (2%, *n* = 1: Fig. 2).

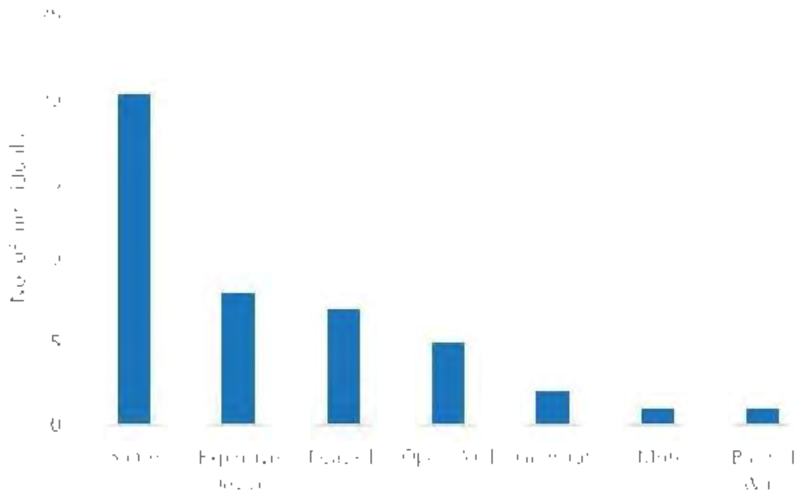

Fig. 2. The number and type of anthropogenic risk incidents that sloth bears (Melursus ursinus) fell victim to from 2006 to 2007 and 2013 to 2019 in Karnataka, India.

Snares

Of the 20 wild sloth bears caught in snares that Wildlife SOS attempted to rescue, 12 (60%) were eventually released back to the forest where they had initially fallen victim to the snares, except for 1 case. Four bears (20%) died in snares, or from the wounds they suffered, and 4 bears (20%) were put into lifetime care at the Wildlife SOS, Bannerghatta Bear Rescue Centre (BBRC), because their injuries were too debilitating to release them back into the wild. Twelve of the 20 bears were female, and 8 were male. Over half of these sloth bears (55%, n 11) were estimated to be ≤2 years old, and 45% (n 9) were estimated to be ≥5 years old.

Ten snares (50%) were in agricultural areas, 3 (15%) were in forest or scrublands, and 7 (35%) did not have the locational descriptions. The average distance of those found in agricultural fields was >2,000 m from forest edges. Two of the 3 snares found in forest or scrublands were<300 m from agricultural fields and 1 was >800 m from an agricultural area.

There was a spike in the number of bears caught in snares (n 12) between the months of August and December (Fig. 3). This period is the main agricultural harvest season, a time when bears enter agricultural areas for crop raiding. Eight bears (40%) were caught in snares outside of the main harvesting season (Fig. 3). Three of them were snared in scrub, 1 in an agricultural area, and 4 were caught in undocumented locations.

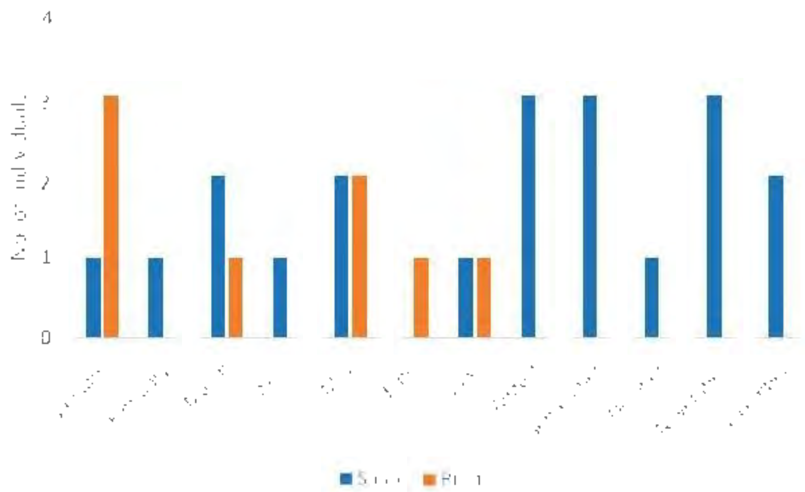

Fig. 3. The number of snare and explosive device blast incidents that sloth bears (Melursus ursinus) fell victim to by month from 2006 to 2007 and 2013 to 2019 in the state of Karnataka, India.

Crude explosive devices

Crude explosive devices killed 8 bears. Seven (88%) were females and only 1 (12%) was male (Fig. 3). All 8 sloth bears were estimated to be ≥4 years old. Seven of the crude explosive devices (88%) were set in agricultural fields. The closest crude explosive device to the forest edge was 40 m from edge and the furthest was >12 km from edge. Only one crude explosive device was set inside of a forest area (12%) and it was <100m from the forest edge. Three bears (38%) were killed in January, two (25%) in May, andone (13%) each in March, June, and July (Fig. 3). The location where each dead sloth bearwas found was likely not the location where the incident occurred because wounded bears often wander from the crude explosive device.

Open wells

Two (40%) of 5 bears, not including cubs, died from injuries sustained from a fall into a well or from being unable to escape the well. Both were females and one had 2 cubs that survived the fall and were rescued by Wildlife SOS. The cubs presently reside at the BBRC. The 3 other bears (60%; 2 females and 1 male) were rescued by Wildlife SOS and eventually released back to the wild. The average distance of these wells from forest edges was 648 m (range = 10–1,900 m).

Roads

Seven bears, not including cubs, died from vehicle collisions. Three incidents occurred within the same 400 m of road in Sulikeri at Hosur Cross, two of which happened within roughly 30 m of one another. One of the 3 deaths involved a mother with cubs, and a second involved an adult female. The third incident involved an adult male and was approximately 400 m from the other 2 incidents. Clearly, these incidents occurred in a movement corridor for bears; also, maternal dens are known to exist within 2 km.

Of the remaining 4 incidents, two involved a mother with cubs, one involved a lone female, and one involved a male. In one of the cases involving a mother with cubs, maternal dens were documented roughly 800 m from the road. The incident with the male sloth bear occurred on a road adjacent to the Shettihalli Wildlife Sanctuary.

Barbed wire fence

One sloth bear was rescued after being entangled in a barbed wire fence. This bear was brought back to the BBRC so its wounds could be treated and given time to heal.It was eventually released back to the wild close to the place it was found.

Gunshots and mob

Two bears were killed by gunshots: 1 male and 1 female. The male was shot by a police officer after the bear attacked a person. The female bear was found dead of a gunshot wound with no other evidence. One male bear, involved in a human–bear conflict, was beaten to death by a mob.

Gender differences

More females (n 30, 68%) than males (n 14, 32%) were injured or killed by human-caused hazards (Fig. 4). Roughly 37% (n 11) of the females were juveniles (<2 yr of age) and roughly 36% (n 5) of the males were juveniles. Additionally, 7 of these females (23%) either had cubs or were pregnant.

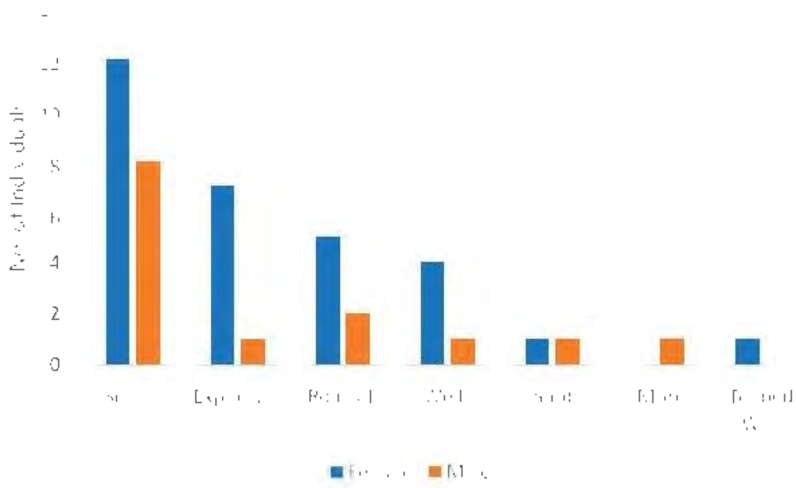

Fig. 4. The number of anthropogenic incidents, from 2006 to 2007 and 2013 to 2019 that sloth bears (Melursus ursinus) fell victim to by gender in the state of Karnataka,India.

Discussion

Snares

Snares, highly effective at capturing wildlife of all types, are used throughout India and are made from widely available and inexpensive products, such as wires and cables (Scotson et al. 2015; Gray et al. 2017; O'Kelly et al. 2018a,b). In Karnataka, snares are generally not baited, nor are they associated with fence lines that are occasionally used to funnel animals to the snare. Snares are generally set for bush meat as food, the primary targets being wild boar (*Sus scrofa*) and Indian hare (*Lepus nigricollis*). Sloth bears are not killed for food, nor are they highly valued for their parts in India. Thus, they appear to be unintentional victims of snares.

Although snares are used year-round in Karnataka, they appear to be most problematic for sloth bears from August through December when crops (mainly peanuts, sunflower seeds, and corn) are most abundant. Farmers attempt to take advantage of the crop raiding as a means to obtain meat by setting more snares in agricultural areas during this season.

Outside of the harvesting seasons, snares are still used but are more often set illegally within forests (Fig. 5).

Fig. 5. Snares and tools collected from sloth bear (Melursus ursinus) habitats in Karnataka, India, where Wildlife SOS collected data on anthropogenic incidents involving the bears that occurred between 2006–2007 and 2013–2019.

Crude explosive devices

Using crude explosive devices to poach wildlife is a crime in India that carries a very stiff penalty (Arun et al.2018). Nevertheless, homemade explosive devices are used in southern India by farmers to remove depredating wildlife and to obtain bush meat, but do not appear to be used with regularity throughout central or northern India. These crude explosive devices are made from mining supplies and are generally hidden within a food item (usually corn or fruit or in the gut and gut waste of poultry or small ruminants [sheep and goat]) that is enticing to herbivores or omnivores. People who use crude explosive devices in Karnataka tend to put them out during the night, largely outside of the cropping season when food is scarcer, and farmers have more time on their hands and pick them up in the morning before cattle or humans might be injured. Unfortunately, these devices are indiscriminate killers.

Open wells

Approximately 8.7 million open wells (as opposed to bore wells) are scattered across the Indian subcontinent (The New Indian Express 2017). An open well is a hole dug far enough to reach the water table and is most common in rural areas. A village may typically have as many as 200 wells (Aggarwal 2000). Open wells range in size from 2 m to 20 m in diameter and 1 m to 70 m in depth (Aggarwal 2000, The New Indian Express 2017). Many of these wells are currently in use (with water present), but a large number have been abandoned as a result of a lack of water (water table drawdown) or saltwater intrusion. Open wells represent an imminent threat to Indian wildlife because they are frequently without barrier walls to prevent animals from falling in; nor do they provide any form of escape for animals that do fall. A wide variety of organisms have been rescued from these wells, including humans, domestic dogs, sheep and cattle, elephants (Elephantidae), tigers, lions (*Panthera leo*), leopards, sloth bears, snakes, and myriad of other fauna. Wildlife falls into dry wells are often killed instantly or suffer lingering deaths if not rescued. Those falling into water-filled wells and not rescued will drown and suffer a painful death, in turn polluting village water supplies (Rajankaret al. 2009). Sloth bears fall into open wells that border protected forests and wells that are >1.5 km from protected habitat.

Roads

Like most wildlife, sloth bears occasionally become victims to vehicle collisions. Being black in color and predominantly nocturnal makes them difficult to notice when crossing a road, even though traffic is less frequent at night in these areas. The fact that 4 of the 7 roadkill situations involved a mother with cubs could potentially mean that the mother was moving more slowly while crossing the road while carrying cubs on her back. In the area where 3 sloth bears were killed within 400 m, the Forest Department and the Road Department are planning to build an underpass for wildlife to allow safe passage.

Guns, barbed wire, and mobs

Guns are rare in India because they are prohibited by law, except with special permits. Although we have only recorded 2 sloth bear deaths by gunshot, one by a policeman and the other unknown, some gun holders do shoot wild boar in the evenings and after dark. It has been reported that some sloth bears have been shot unintentionally because they were mistaken for a wild pig.

Barbed wire can have a negative impact on many species of wildlife, badly maiming or killing, much in the same way that snares do. Additionally, sloth bears are occasionally killed by mobs. Generally, this occurs when a person has been attacked and others mob the bear to halt the attack and neutralize the threat.

Gender

We found that more females than males were injured or killed (68% vs. 32%). Studies of other bear species and human–bear conflict, including crop raiding, damage to property, and attacks, have reported males to be the pre-dominant gender involved (Rogers et al. 1976, Bunnell and Tait 1981, Beckmann and Berger 2003, Merkle 2013). Like other bear species, male sloth bears have larger home ranges and disperse across the landscape more than do female bears (Rogers 1987, Joshi et al. 1995, Yoganandet al. 2005, Støen et al. 2006, Ratnayeke et al. 2007, Zedrosser et al. 2007). Therefore, we expected young males to dominate as victims to these hazards, which was not the case. We also did not expect to see mothers with cubs, or pregnant mothers, involved as often as we did.

Though this is still a relatively small sample size, our findings indicate that females may be more at risk from human hazards than are males. Our findings could also be the direct result of a skewed gender ratio in the wild. Presently the gender ratio of wild sloth bears remains unknown. Alternately, our findings could be the result of social competition among sloth bears. Females may be forced into suboptimal habitat because males are larger (Prater 1986, Garshelis et al. 1999) and are therefore more likely to retain greater holding power over critical resources, such as safe den sites and feeding areas (Ruckstuhl 2007). This may place the females in closer proximity to anthropogenic risks. Finally, recent studies in eastern Karnataka documented that maternal dens are often located near forest edges where protected areas border agricultural areas (Arun et al. 2021; Shanmugavelu, unpublished data). This penchant for mother bears, and pregnant bears, to den near the forest edges may make them more susceptible to the anthropogenic risks that often occur just outside of protected areas.

Management implications

Our findings suggest that anthropogenic risks may have a significant impact on sloth bear populations on the Deccan Plateau. Raising awareness about the threat they pose to wildlife is an important step in mitigating the damage they cause. Snares are the most common anthropogenic risk on the Deccan Plateau, and probably one of the most widely distributed anthropogenic risks across India. Organizing snare patrols to gather snares in or around protected forests, as well as stiffer penalties for using snares, could be useful in combating the use of these indiscriminate traps. We also recommend harsher penalties for poaching with crude explosive devices. Several steps can be taken to help mitigate vehicle–sloth bear collisions, including building underpasses in key areas as well as placing illuminated sloth bear crossing signs at key crossing areas. Additionally, more speed cameras can be placed near key crossing areas, thereby enforcing reduced speeds. When possible, open wells should have a lip associated with them and a means of escape, such as a plank or large branch, if an animal should fall into it. Open wells could also be surrounded by thorny vegetation to keep wildlife out or completely covered if no longer in use.

Acknowledgments

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Art – 261. GRASS SPECIES COMPOSITION IN TROPICAL FOREST OF SOUTHERN INDIA

M. Ashokkumar, S. Swaminathan and R. Nagarajan

Abstract

Grass composition was assessed by plot method (1 m^2; n= 1,749) in three habitats (dry deciduous-DDF, moist deciduous-MDF, and thorn forest-TF) at Mudumalai Tiger Reserve, southern India across different seasons from Jan 2004 to Dec 2007. The grass species richness and availability (percent composition) varied significantly with habitats. Seventy-four species of grasses and sedges were recorded in all three habitats, with a few species common in all habitats. Grass availability varied significantly in different habitats across seasons and was positively influenced by precipitation. Among biotic factors, regeneration and shrub density had a primary influence on grass availability, followed by herb, sedge and weed density. The principal coordinate analysis revealed seven major associations in the tropical forest. There were considerable changes in the composition and association of grasses when compared to the past. Fire resistant species such as *Themeda triandra*, *Heteropogan contortus* and *T. cymbaria* dominated in the DDF. Grass species *Aristida*/*Eragorstis* were recorded in the TF, which were considered as indicators of heavy grazing pressure. Grass species that were reported rare and sporadic in the earlier study were not recorded, which emphasizes better pasture management in the tropical forest. Grass species composition and availability was threatened by invasion of weeds.

Keywords: Graminae, Mudumalai Tiger Reserve, influence of fire on grass, *Themeda triandra*, *Heteropogon contortus*, *Themeda cymbaria*.

Introduction

Grasslands are highly dynamic ecosystems encompassing natural and semi-natural pastures, woodlands and scrubs dominated by grasses (Blair et al. 2014). Grasses are one of the important sources of biodiversity and the primary food source for many herbivores that support ecosystem function, agricultural sustainability, and livelihood for many pastoral communities (Sala & Paruelo 1997; White et al. 2000). In India, 1,506 species of grass belonging to 266 genera were reported (Kellogg et al. 2020). Peninsular

India has maximum diversity and endemism (Karthikeyan 1989). The study of grass species is important since they are sensitive to global warming and altered precipitation patterns and exhibit immediate response to climate change (Knapp & Smith 2001).

Grass species in the Western Ghats are threatened by domestic livestock, mining, windfarms, plantations, canals, and dams have led to degradation and loss of grassland habitats (Vanak 2013). The invasion of exotic species into tropical forest threatens grasslands (Srinivasan 2011; Ashokkumar et al. 2012). Invasions not only affect grass composition but also the foraging efficiency of herbivores (Wilson et al. 2013). Pasture management is essential in protected area management strategies to reduce the human-animal interactions. Although grasses have wide ecological amplitude and several adaptations to withstand trampling, grazing, fire, flood, and drought, they face severe competition for light and nutrients from aggressive wood species and invasive plants in tropical forests.

Mudumalai Tiger Reserve (MTR) is located in the Western Ghats, one of 34 global biodiversity hotspots (Myers et al. 2000). There were no earlier studies on the dynamics of grass species composition and diversity in similar tropical forest in Southern India. Though tree, herb and shrub species were studied in detail (Robert etal. 2002; Nath et al. 2006) information on grass species is lacking in the tropical ecosystem. In addition, the study area also has baseline data on grass species composition studied a decade before (Sivaganesan 1991), which enabled comparison with the present study. Sivaganesan (1991) studied grass composition in the study area in the year 1985, and he has studied grass species composition using strip transects of one kilometer length (n= 20) and laid 1 m^2 plots at every 250 m interval, resulting n sampling of five plots per transect anda total of 100 plots across different vegetation types.

Seasonal changes in the phenology of grass species influence herbivore movement, distribution and abundance (Sivaganesan 1991; Baskaran 1998). Cattle grazing and fire have major impacts on species composition of woody plants (Kodandapani et al. 2008) and grasses. The present study investigated the effect of environmental factors on grass availability (grass abundance) and grass association in tropical forests of Southern India. Studies on the grass association help to understand the grass communities in tropical forest and their dynamics due to climatic and anthropogenic factors.

Study Area

Mudumalai Tiger Reserve (MTR) is located in the Nilgiris District of Tamil Nadu (11º 32´ and 11º 42´ N and76º 20´ and 76º 45´ E). It extends over an area of 321 km^2 and forms a part of the Nilgiris Biosphere Reserve (Figure 1). It is part of a contiguous stretch of forest with Bandipur Tiger Reserve to the north, Segur Reserve Forest to the east, Wayanad Wildlife Sanctuary to the west, and Gudalur forest division to the South. Altitude varies from 485 to 1,226 m with a general elevation of about 900 to 1,000 m. The annual rainfall varies from 1,001 mm to 1,648 mm. The sanctuary receives rain from both south-west (May to August) and north-east (September to December) monsoons. Based on climate seasons can be classified into dry season (January to April), first wet season (south-west monsoon) and second wet season (north-east monsoon). The three major forest types in the study area are tropical moist deciduous forest (MDF), dry deciduous forest (DDF) and tropical thorn forest (TF) (Champion & Seth 1968).

Figure 1. Map showing the location of transect lines used for vegetation sampling at Mudumalai Tiger Reserve.

The major tree species association in MDF is *Lagerstoroemia-Terminalia-Tectona.* The ground flora mainly composed of *Helicteres isora*, *Desmodium* sp., and *Curcuma* sp. The dominant grass species are *Cyrtococcum accrescens*, *C. oxyphyllum*, *Bothriochloa pertusa*, *Oplismensus compositus* and *Oryza meyeriana* occur. Bamboo *Bambusa arundinacea* is very common along the perennial water sources. Swamp vegetation mainly consists of tall grass *Cenchrus hohenackeri*. Tree species in DDF is dominated by *Anogesis latifolia*, *Terminalia crenulata*, *Tectona grandis*, *Diospyrosmontana*, and *Gmelina arborea*. Shrubs include*Helicteres isora*, *Antidesma diandram*, and *Pavetta indica*. Grasses species is dominated by tall perennial rhizomatous grasses such as *Themeda cymbaria*, *Cymbopogon flexuosus*, and *Apluda mutica* in dry deciduous tall grass area. *T. triandra*, *Setaria intermedia*, and *Dicanthium caricosum* are common in short grass area. TF is dominated tree species such as *Acacia* sp., *Albizia* sp., *Premna tomentosa*, *Dalbergia lanceolaria*, and *Ziziphus* sp. The shrub species includes *Acacia pinnata*, *Canthium parviflorum*, *Rhus mysorensis*, and *Mytenus emarginatus*. Grass species in TF includes *Aristida adscencsionis*, *Heteropogon contortus*, and *Tragus mongolorum*. The study area is threatened by habitat degradation from overgrazing and human disturbance.

Methods

Five transects each of three-kilometre length were marked in three habitats (DDF 3; MDF 1, and TF 1; Figure 1). Two transects in Mudumalai range, two transects in Theppakad range and one in Masinagudi range were marked and sampled. The locations of transects were given in the geo referenced study area map (Figure 1). A total of 30 plots (1 m^2) were laid at an interval of 100m in each transect. Transects were sampled two times per season in alternate months. A total of 825 plots were laid in all three vegetation types (DDF 493, MDF 169,TF 103) in different seasons. In addition to this data, grass species composition, which was collected as part of Gaur *Bos gaurus* foragingecology study was used. A total of 924 plots (DDF 669, MDF 110, TF 145) of 1 m^2 were laid in the Gaur foraged areas in different habitats, to assess the forage plant species including grass species and their consumption.

A herbarium of grass species that include both grass and sedges was made for confirmation of the species identity. All specimen vouchers were deposited in the Center for Ecological Sciences, Indian Institute of Sciences, Bangalore. Plant species were identified using Gamble (1935), Saldanha & Nicolson (1976), Saldanha (1984, 1996), Sharma et al. (1977), and Kellogget al. (2020). Grass cover in each quadrat was visually estimated by giving a percent cover. Percent cover was given according to the proportion of area (within the quadrat) covered by grass (Giles 1971; Sivaganesan 1991). The other variables such as grass height, percent green grass, grass texture, and phenology were recorded (Jarman & Sinclair, 1979; Menaut and Cesar 1979; Sivaganesan 1991; Baskaran

1998).

Precipitation data was collected on monthly basis from weather stations located at the different habitats of the study area maintained by Center for Ecological Science, Indian Institute of Science. The information on extant and frequency of fire was collected from forest management plan and studies on fire in the study area (Kodandapani et al. 2008). Grass species richness, mean percent availability and grass height were tested using ANOVA.

The effect of environmental and biotic variable on grass availability was tested using multiple regressions. The relationship between the percent grass availability and environmental factors (habitat, season, precipitation and fire) and biotic factors (shrub, regeneration, herb, sedges, and weed) were investigated using multiple regression. The variations among the habitats, seasons and fire were controlled by entering these predictors as a dichotomous variable.

Grass species association was determined by principal co-ordinate analysis and species association was plotted in Euclidean space. The variables used in the analysis are percent composition of grass, height, habitat, elevation, fire, and spatial locations in the study area. Statistical analyses were performed by using Windows based statistical package *viz.* SPSS 21.0 (SPSS Inc., Chicago, IL, USA) and Past software 3.17 (Hammer et al. 2001).

Results

A total of 74 species of grasses and sedges were recorded in the MTR with a maximum of species in DDF followed by TF and MDF (Table 1). MDF had lower grass species diversity (0.6) than other habitats. Though, species richness was high in TF (3.4), the mean percent availability of grass was less in TF (12.7%) than DDF (19%) and MDF (17%). The species richness and mean percent availability of grass varied significantly among habitats. The equitability of species was equal in all the habitats. While grass species diversity was higher in TF, the abundance of grass was higher in deciduous forests (MDF and DDF).

Table 1. Mean percent grass available (±SD), species richness per plot, diversity and equitability of grass (and sedges) in different habitats of Mudumalai Tiger Reserve.

Habitat[a]		Total number of species	Species richness (S) / plot (±SD)	Mean percent (%) ± SD	Index value	
					Shannon Weiner Diversity (H')	Equitability (J')
DDF (n= 1,162)		61	2.9 ± 1.30	18.8 ± 22.45	0.65 ± 0.40	0.68 ± 0.22
MDF (n= 279)		33	2.7 ± 1.34	17.5 ± 21.67	0.60 ± 0.42	0.69 ± 0.21
TF (n= 248)		53	3.4 ± 1.79	12.7 ± 16.79	0.80 ± 0.45	0.72 ± 0.21
Overall (n= 1,749)		74	3.0 ± 1.42	17.3 ± 21.40	0.67 ± 0.42	0.69 ± 0.22
ANOVA	F		F_{1645}= 20.3	$F_{2,821}$= 14.04	F_{1645}= 20.5	F_{1432}= 5.18
	P		p <0.001	p <0.001	p <0.001	p <0.001

[a] -DDF—Dry Deciduous Forest | MDF—Moist Deciduous Forest | TF—Thorn forest.

Species composition and availability

Grass species composition varied among different habitats. Altogether, 66 grasses and eight species of sedges were recorded in three habitats. There were 21 species were common in all habitats, viz., *Themeda triandra, Oplismenus undulatifolius, Setaria intermedia, S.flavidum*, and *S. pumila* (Table 2). Among different grass species *Perotis indica, Cymbopogon* sp., *Cappillipedium assimile, E. spicatus*, and *Kyllinga* sp. were recordedonly in DDF. Likewise, species such as *Cyrtococcum oxyphyllum, Paspalum conjugatum*, and *Cenchrus polystachios* in MDF and *Bothriochloa* sp. *Eragrostis atrovirens*, *Pseudanthistiria umbellata*, *P. tripheron*, and *Leersia hexandra* were recorded only in TF.

In DDF dominant grass species included both tall and short grass species. Tall grass species include *T. cymbaria*(30%), *I. cylindrica* (13%) and *S. fertilis* (13%) and short grasses were *T. triandra* (27%), *O. undulatifolius* (25%) and *S. intermedia* (22%). In MDF, the dominant species were *C. oxyphyllum, E. indica, C. patens, P.polystachion* and *A. compressus*. Swamp areas of both DDF and MDF were dominated by grass species such as *C. polystachyios*, *A. compressus, I. cylindrica*, and *E. indica*. Dominant grass species in TF were *D. bicornis, P.umbellata, D. caricosum*, and *A. mutica* (Table 2).

Table 2. Percent grass (grass and sedges) available in different habitats of Mudumalai Tiger Reserve during the study period (Data sorted in descending order based on total percent).

S. No	Species	Habitats			Total
		DDF	MDF	TF	
	Grass				
1	*Axonopus compressus*	45.7 ± 39	28.2±31.67	-	33.4±34.34
2	*Cyrtococcum oxyphyllum*	-	33.4± 2.19	-	33.1 ± 22.3
3	*Cenchrus hohenackeri*	27.4 ± 24.17	36.3±33.65	-	33.1 ± 30.6
4	*Themeda cymbaria*	30.4 ± 22.45	25 ± 17.32	-	30.1±22.15
5	*Themeda triandra*	27.2 ± 21.53	20.2± 0.17	23 ± 26.08	26.7±21.68
6	*Oplismenus undulatifolius*	25.1 ± 24.99	4.3 ± 4.27	26.7 ± 24.9	25.3 ± 24.9
7	*Axonopus* sp.	28.4 ± 31.12		6.5 ± 5.58	23.9±29.19
8	*Setaria intermedia*	22.3 ± 22.06	25 ± 7.07	31.4 ± 26.1	23.6±22.78
9	*Pseudanthistiria umbellata*	-	-	23.4±20.47	23.4±20.47
10	*Centotheca lappacea*	-	-	40 ± 0.01	20.5±27.58
11	*Setraria flavidum*	17.5 ± 18.03	2 ± 0.01	25.9±16.92	18.9±18.06
12	*Setaria pumila*	18.5 ± 21.39	28 ± 0.01	17.3±16.38	18.5 ± 21
13	*Enteropogon dolichostachyus*	16.2 ± 18.84	14.1± 4.95	20.7±24.35	16.6±19.42
14	*Eleusine indica*	22.4 ± 31.09	14 ± 13.86	7.4 ± 7.16	16.3±22.74
15	*Cenchrus polystachios*	-	15.6±13.53	-	15.6±13.53
16	*Heteropogon contortus*	19.2 ± 20.03	-	10 ± 13	15.3±17.93
17	*Cyrtococcum accrescens*	8.7 ± 13.06	20.1±21.69	-	15.1 ± 19.3
18	*Setaria verticillata*	-		15 ± 0.1	15 ± 0.1
19	*Imperata cylindrica*	13 ± 13.9	16.2±28.06	-	13.9±18.92
20	*Digitaria* sp.	11.6 ± 12.51	12.3±15.37	16.9 ± 12.8	13.6±12.82
21	*Bothriochloa* sp.	-	-	13.6 ± 7.47	13.6 ± 7.47
22	*Panicum* sp.	14.4 ± 8.46	-	1 ± 0.01	13.1 ± 9.04
23	*Digitaria bicornis*	13.8 ± 21.91	4 ± 1.73	9.8 ± 15.84	12.2±19.82
24	*Digitaria griffithii*	11.9 ± 13.2	5 ± 0.01	12 ± 6.35	11.9±12.65
25	*Perotis indica*	11.9 ± 17.94	-	-	11.9±17.94
26	*Panicum tripheron*	7.8 ± 11.67	-	15.2±14.85	11.8±13.87
27	*Urochloa distachya*	12.2 ± 11.92	12.5±10.61	10.6 ± 9.93	11.8±11.23
28	*Apluda mutica*	9.4 ± 11.32	9.2 ± 13.09	18 ± 18.37	11.8±14.16
29	*Dichanthium caricosum*	10 ± 0.01	5 ± 0.01	13 ± 9.08	11.4 ± 8.02
30	*Eragrostis tenuifolia*	15.8 ± 23.01	-	3.1 ± 2.77	11.4±19.53
31	*Sporobolus fertilis*	13 ± 12.75	-	1 ± 0	11.4±12.54
32	*Ischaemum ciliare*	10.2 ± 10.98	11 ± 15.25	-	10.9 ± 14.7
33	*Setaria palmifolia*	10.8 ± 12.59	1 ± 0.01	10 ± 0.01	10.5±12.33
34	*Eragrosteilla* sp.	11.8 ± 8.67	-	8.2 ± 13.66	10.1±11.39
35	*Eragrosits atrovirens*	-	-	10 ± 7.07	10 ± 7.07

36	*Oplismenus compositus*	6.3 ± 10.4	13.2± 3.87	-	9.9 ± 12.79
37	*Paspalum conjugatum*	-	9 ± 9.64	-	9 ± 9.64
38	*Aristida adscensionis*	8.4 ± 7.6	-	8.8 ± 10.18	8.7 ± 9.89
39	*Cynodon radiatus*	15 ± 0.1	-	2 ± 0.01	8.5 ± 9.19
40	*Echinochloa colona*	6.3 ± 7.51	15 ± 0.1	-	8.5 ± 7.51
41	*Themeda tremula*	7.2 ± 3.13	5 ± 0.1	20 ± 0.1	8.5 ± 5.4
42	*Dactyloctenium aegyptium*	13.9 ± 16.5	-	5.3 ± 7.09	8.5 ± 11.8
43	*Sehima sp.*	7.3 ± 15.34	-	11.5±15.73	8.2 ± 15.23
44	*Tragus mongolorum*	1 ± 0.01	-	8.1 ± 7.74	8 ± 7.73
45	*Sporobolus* sp.	7.8 ± 9.15	7.5 ± 11.22	4.2 ± 3.49	7.5 ± 9.01
46	*Alloteropsis cimicina*	5.4 ± 8.93		24.9 ± 25.6	7.5 ± 13.38
47	*Chrysopogon sp.*	-	-	7.5 ± 9.46	7.5 ± 9.46
48	*Cymbopogon sp.*	7.2 ± 5.18	-	-	7.2 ± 5.18
49	*Cappillipedium assimile*	6.8 ± 3.95	-	-	6.8 ± 3.95
50	*Cynodon dactylon*	7.1 ± 5.73	1 ± 0.01	3.3 ± 2.08	6.4 ± 5.54
51	*Eragrostis* sp.	1 ± 0.01	-	6.6 ± 12.56	6.3 ± 12.22
52	*Oryza meyeriana*	7.3 ± 10.01	4.7 ± 9.64	10 ± 0.1	5.8 ± 9.74
53	*Sporobolus diandrus*	4.8 ± 3.77	10 ± 0.1	-	5.8 ± 4.02
54	*Digitaria abludens*	20 ± 0.1	-	4.5 ± 4.96	5.1 ± 5.65
55	*Elytrophorus spicatus*	5 ± 0.1	-	-	5 ± 0.1
56	*Eragrosits abludens*	-	-	5 ± 0.1	5 ± 0.1
57	*Cenchrus purpureus*	5 ± 0.1		-	5 ± 0.1
58	*Bambusa arundinacea*	5.4 ± 2.88	4.5 ± 4.37	1.7 ± 0.58	4.3 ± 3.74
59	*Arthraxon* sp.	7.3 ± 8.62	-	2.5 ± 1.9	3.1 ± 3.58
60	*Panicum notatum*	-	-	3 ± 0.1	3 ± 0.1
61	*Bothriochloa bladhii*	2 ± 0.1	-	-	2 ± 0.1
62	*Isachne elegance*	2 ± 0	-	-	2 ± 0
63	*Leersia hexandra*	-	-	2 ± 0.1	2 ± 0.1
64	*Arthraxon lancifolia*	-	-	1.5 ± 0.58	1.5 ± 0.58
65	*Mnesithea granularis*	1 ± 0.1	-	1.5 ± 0.55	1.4 ± 0.53
66	*Chrysopogon lawsonii*	1 ± 0.1	-	-	1 ± 0.1
	Sedges				
67	*Kyllinga melanosperma*	15.2 ± 22.46	7.1 ± 7.22	6.2 ± 7.95	12 ± 18.59
68	*Mariscus madraspatanus*	6 ± 8.37	17.4±26.83	2.5 ± 1.22	9.8 ± 17.98
69	*Fimbristylis aestivallis*	7.4 ± 5.87	-	6.2 ± 6.02	7 ± 5.73
70	*Cyperus distans*	4.2 ± 4.91	8.1 ± 13.59	5 ± 0	4.9 ± 7.42
71	*Cyperus rubicundus*	6.2 ± 5	-	3.2 ± 3.75	4.2 ± 4.41
72	*Fimbristylis* sp.	3.7 ± 2.36	-	2.6 ± 2.4	3 ± 2.41
73	*Kyllinga* sp.	2.6 ± 2.78	-	-	2.6 ± 2.78
74	*Kyllinga tenuifolia*	2 ± 0.1	-	1 ± 0	1.3 ± 0.58

DDF—Dry deciduous forest | MDF—Moist deciduous forest | TF—Thorn forest |–Species were not recorded.

The percent grass composition varied significantly across season ($F= 11.6$; $p <0.001$) in different habitats ($F= 13.92$; $p <0.001$). Fire was not recorded in the TF area during the study period. Grass availability was higher in the MDF during dry season (27.7%). The mean percent available grass was highest in first wet season in the DDF (46 %) in the fire burnt areas (Figure 2). Grass availability was low in second wet season in TF. The three-way interaction among fire, habitats and seasons in ANOVA on grass availability was significant. The abundance of grass was higher in the DDF and MDF in wet seasons in the unburnt areas.

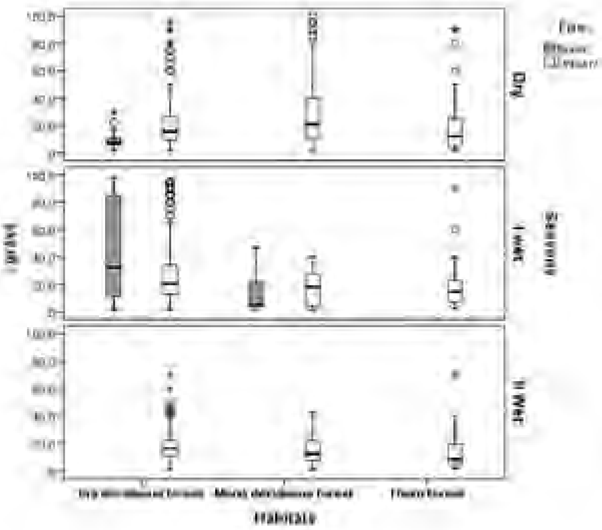

Figure 2. Grass composition (%) in different habitats, seasons, andfire (burnt/unburnt) in Mudumalai Tiger Reserve.

The influence of environmental variables on grass availability

The grass availability had a linear relationship with predictors. The model was highly significant and explained 23% variations in grass availability (%). Previous month precipitation positively influenced grass availability. All the other variables negatively influenced grass availability. From the Standardized Partial Regression Coefficients (SPRC), it was inferred that the shrubs had the primary influence on growth of grasses followed by sedges, regeneration, herbs, and weed (Table 3; Figure 3). Furthermore, the co-efficient of habitat and season indicated that the percent availability of grass reduced significantly among three habitats and seasons. Though, fire negatively influenced grass availability, it was not statistically significant in the model.

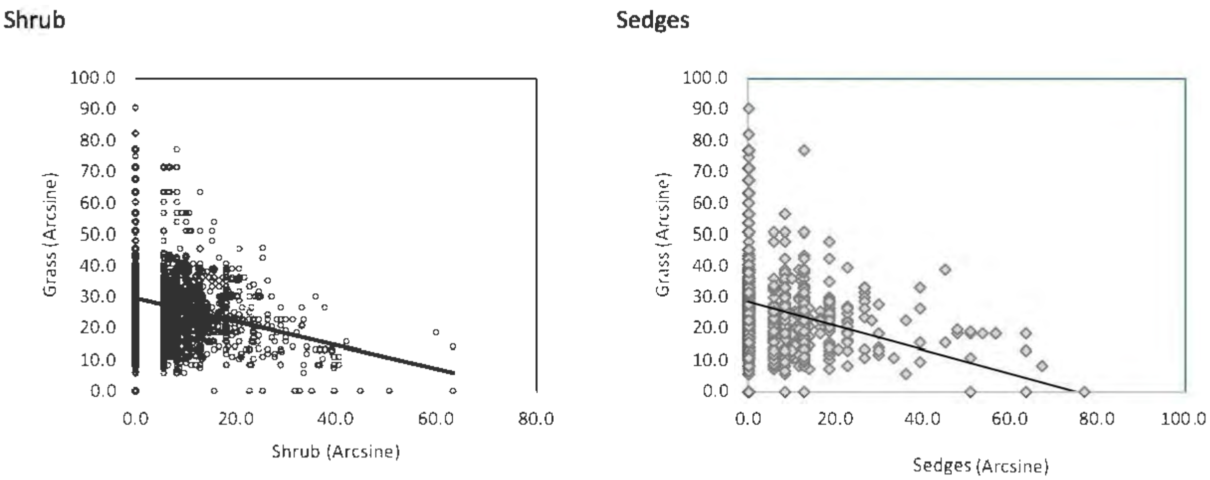

Figure 3. Relation between biotic factors and grass composition at Mudumalai Tiger Reserve.

Table 3. Multiple regression equation to investigate the effect of environmental (habitat, fire and precipitation) and vegetation factors on the grass availability (%) in Mudumalai Tiger Reserve.

Independent variable	Predictor	Coefficients ± SE	SPRC*	t	p	Model (r^2)	Model (p)
Grass (%)	(Constant)	38.17±2.535		15.059	<0.001	23.1	p<0.001
	Fire	-0.76±1.100	-0.015	-.694	0.488		
	Habitat	-3.59±0.414	-0.191	-8.653	<0.001		
	Season	-2.60±0.598	-0.138	-4.353	<0.001		
	Previous month precipitation (mm)	2.82 ± 0.403	0.229	6.998	<0.001		
	Herb (%)	-0.31±0.042	-0.161	-7.286	<0.001		
	Regeneration (%)	-0.52±0.063	-0.179	-8.206	<0.001		
	Sedges (%)	-0.37±0.039	-0.200	-9.341	<0.001		
	Shrub (%)	-0.46±0.038	-0.268	-12.096	<0.001		
	Weed (%)	-0.23±0.043	-0.112	-5.254	<0.001		

Grass species association

Principal coordinate analysis (multidimensional scaling) summarizes inter grass species association based on dissimilarity in a Euclidean space. There were seven distinct clusters formed. Among different variables elevation, height and percent composition collectively contributed 87% of the variance. There were four distinct clusters identified based on elevation and further separation was based on habitat and microhabitat (Figure 4). The first cluster consist of grass species such as *Themeda triandra*, *Setaria intermedia*, *Enteropogon dolichostachyus* and *Oplismenus undulatifolius* in DDF. The second cluster consisted of *Axonopus* sp. (Image1e) and *Bothriochloa bladhii* in riverine forest. The third cluster consisted of thorn forest species such as *Arthraxon, Chrysopogon, Psudanthistiria*, and *Cynodon* sp. Forth cluster consisted of *Cenchrus*, *Sporobolus, Centotheca*, and *Eragrostis* sp. in dry deciduous tall grass at 1,000 m elevation. Fifth cluster composed of *T. cymbaria, Ischaemum, Cyrtococcum*, and *Kyllinga* species in the moist deciduous forest. The sixth cluster composed of *Imperata*, *Echinochloa*, and *Cenchrus hohenackeri* in swamp areas of MDF. Dry deciduous higher elevation regions composed of *Arthraxon, Cappillipedium*, and *Setaria* species.

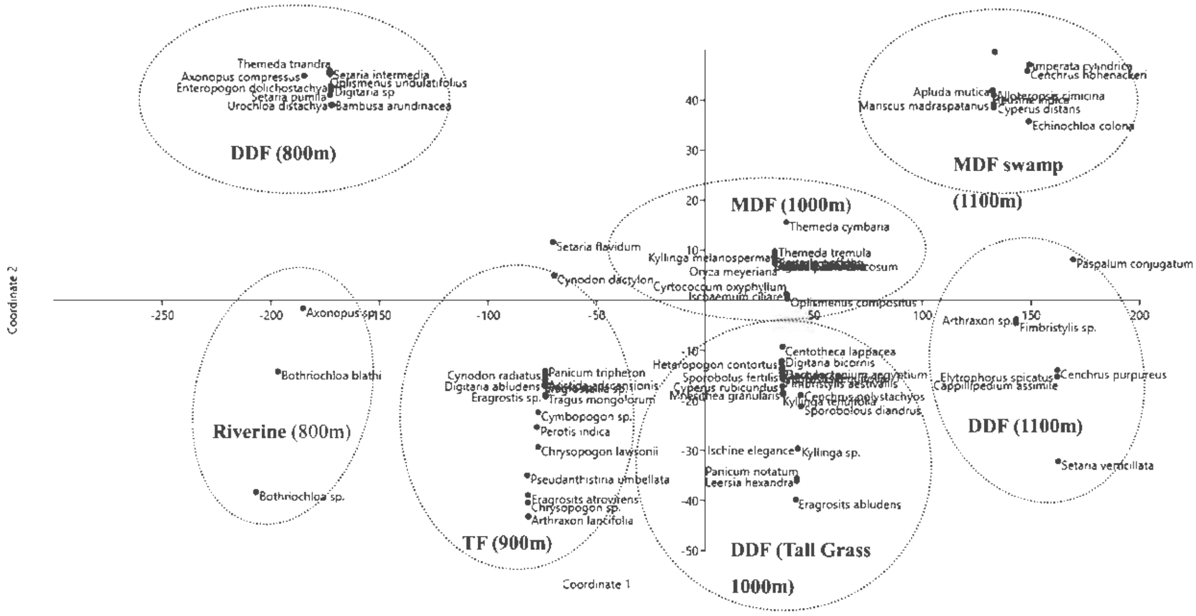

Figure 4. Principal coordinate (PCoA) analysis of grass species association based on dissimilarity in Mudumalai Tiger Reserve (Circles indicate distinct clusters).

Image 1. Grass species composition in the selected habitats of Mudumalai Tiger Reserve: a—Dry deciduous tall grass (Cymbopogon sp.) | b—Dry deciduous tall grass (Themeda-Cymbopogon-Imperata) | c—MDF swamp areas with (Cenchrus-Themeda-Imperata) | d—Setaria palmifolia | e—Axonopus compressus | f—Cyrtococcum oxyphyllum. © M. Ashokkumar

Discussion

A total of 66 species of grasses and eight sedges were recorded in the Mudumalai Tiger Reserve. The number of species recorded was lower than earlier report (75 species) in the study area (Sivaganesan 1991). The marginal variation in the species composition could be due to difference in the area of sampling, earlier study covered greater area of sampling. Sivaganesan (1991) divided the tiger reserve into five zones and did sampling in five transects with 30 plots in each transects with 250m interval. The number of transect in Moist deciduous forest is less than earlier study. Further, there were invasion of exotic weed species such as *Lantana camera* and *Chromolena odorata* in the study area (Ashokkumar et al. 2012; Wilson et al. 2013), which were less and restricted to tourism zone in the study area. Whereas the growth of weeds was extensive and occupied all the grassland patches of DDF and MDF.

Grass species richness, composition varied among habitats, with maximum number of species recorded in DDF followed by TF. *Cymbopogon* sp. found in hill slopes of DDF in the elevation range of 2,000–3,000 m, *P. polystachyon* recorded in swamp areas of MDF in the elevation of above 1,000 m, and *A. adscensionis* found inTF in the elevation less than 600 m. Grass species such as *C. polystachios, L. hexandra*, and *I. cylindrica* were observed in the swamps of MDF and DDF in MTR. This might have been influenced by high moisture content and nutrients of the soil (Skerman & Riveros 1990). Amarasinghe & Pemadasa (1982) have also concluded that the complex interaction of edaphic factors, altitude, precipitation and human disturbance were responsible for a variation on Montane grasslands in Sri Lanka. Thus, the grass composition varied depending on altitudes and moisture content of the soil.

Factors influencing grass composition

Shrubs had the primary influence on the grass growth followed by sedges, regeneration, herbs and weeds. Studies done in Prairie grasslands in Canada indicated that shrubs strongly reduced available soil nitrogen and the secondary growth of shrubs allowed them to accumulate more biomass and height that eventually displaced the grass species (Kochy & Wilson 2000). The grass species *Axonopus* sp. was recorded only in *L. camara* invaded areas. This grass species was originated in United States and this species itself considered as weed (Skerman & Riveros 1990). Therefore, it competes well with weed species. In addition, both *L. camara* and *Axonopus* sp. grow well in humid areas and thus, they do have similar microhabitat preference. The microhabitat preference and weed resistance properties of *Axonopus* sp. enabled successful survival in *L. camara* invaded areas. Grass species that were recorded in *C. odorata* invaded areas (*Cenchrus*, *Setaria*, and *Chrysopogon*) seem to have high alkaline tolerance (Skerman & Riveros 1990). Thus, grass species had species-specific interaction with weed species. The percent availability of grass varied significantly among three habitats and seasons. The seasonal variation in grass availability was due to phenological changes of grass species due to senescence. The phenology of tropical grasses are moisture driven, with germination occurring shortly after the rains of first wet season. Grass senescence occurs in the end of the second wet season or in the early dry season. Both the reproduction and senescence have been influenced by multiple factors such as temperature, rainfall and photoperiod (Blair et al. 2014). Hence the availability of grass was higher in the wet seasons.

The percent grass available was significantly positively correlated with precipitation. Rainfall varied spatiotemporally across vegetation types in the study area. Such a rainfall pattern is ecologically significant and perhaps a boon to the dynamics of the study area. Elephant habitat preference was related to the rainfall in the study area (Sivaganesan 1991). In Africa, several ecologists (Leuthold & Sale 1973; Caughley & Goddard 1975; Leuthold 1976; Eltringham 1979; McNaughton 1985) documented the significance of the rainfall on the habitats and distribution pattern of the larger herbivores. The western part of the study area with MDF receives rainfall during south-west monsoon and eastern part (TF) during north-east monsoon. The grass growth and phenological changes can be seen depending on the precipitation.

Variation in grass composition in the study area

Comparison of grass species composition with earlier study Sivaganesan (1991) revealed that though, there were no changes in the dominant grass species there were considerable changes in the minor grass species composition. The principal coordinate analysis revealed seven distinct clusters of grass species association. Sivaganesan (1991) reported four distinct clusters of grass association in the study area: *Themeda-Cymbopogon-Imperata* in the dry deciduous tall grass area (Image 1a), *Cenchrus-Themeda-Imperata* in the swamp area (Image 1c), *Cyrtococcum-Apluda-Arthraxon*in MDF, and *Themeda-Heteropogon-Digitaria-Apluda* in the TF area. Changes occurred in the grass species composition in all habitats. The percent availability of grass was reduced when compared to past, possibly due to greater extent of invasion of exotic species.

Sivaganesan (1991) indicated that annual fire seems to influence the species association and succession of species at Mudumalai. He reported that fire-resistant species such as *T. triandra*, *H. contortus*, and *T. cymbaria* have survived and dominated the dry deciduous forest. This is unison with his finding that the above species also dominated in DDF based on the present study. The fire frequency was also high (22 incidences per annum), and more area was burnt in DDF (56%) than other habitats (Ashokkumar 2011). Grass species which were reported rare and sporadic in the earlier study were not reported in the present survey, for example *Chionachnekoenigii* in DDF and *Oryza meyeriana* in MDF were not recorded. Similarly, percent composition of *Apluda* sp. and *Arthraxon* sp. were less in MDF. Fewer species were recorded in MDF, butthe mean percent available grass was more in MDF. The dominant grasses in MDF were tall grass species in the swamp areas which grow up to 3 m, and thus their percent composition was higher. Earlier TF was dominated by *T. triandra* and *H. contortus* (Sivaganesan 1991) and these species were poorly represented during the present survey and TF is dominated by *Digitaria* sp., *Pseudanthistiria umbellata.* TFs facing severe pressure due to cattle grazing and removal of cattle dung from the forest floor had severely affected the forest regeneration and nutrient cycle. Earlier studies on livestock populations reported 7,248 cattle in the fringe areas (Silori & Mishra 2001) allowed to free graze in the reserve. Continued grazing affects grass availability and species composition.

Protection from cattle grazing

Grass species *Aristida-Eragrostis* were recorded in the TF which were considered as an indicator species of deteriorated grassland (Skerman & Riveros 1990). Grass species such as *Themeda-Heteropogon-Digitaria-Apluda* were dominant species in thorn forest reported in the past. At present, the quality of grass pastures was too poor to provide any grazing. Severe cattle grazing should be stopped for four or five years to allow the succession to progress towards fair condition represented by *Cynodon dactylon* as the first step toward improvement. Thus, grasslands of TF required protection of pasture from cattle grazing or at least reduction of cattle pressure for at least four to five years to recover. Species reduced by overgrazing can recover if there were no change in the physical environment.

Influence of fire on grass availability

In the study area during the peak of dry season wildfire was common. These, wildfires were set by the villagers to get fresh fodder for their cattle and easy to move around in burnt areas. Fire in grass patches last only for a short time and high temperatures were maintained for only a few seconds. Temperatures at soil level rise steeply to 175–200 °C depending on wind, height, and density, and usually return to ambient temperature within a few minutes (Mondal & Sukumar 2014). The soil temperature at a depth of about two centimeters changes little, varying at most by 14 C. The effect of subterranean portions of grasses is thus slight.

The study area as a whole had a fire-return interval of 3.3 years (Ashokkumar 2011). The vegetation type with the highest mean area burnt was at DDF (*Shorea* sp. dominant) with 56.6%, whereas, TF had the lowest mean area burnt with 14.6%. Forest fires burnt an average of 30% (98 km^2/year) of the forests in each year. Grass biomass was significantly low in burnt areas. Distance from the park boundary was reported as an important factor that predicts the fire-return interval in the study area (Kodandapani et al. 2008). Grass biomass was significantly low in the fire burnt areas of DDF and MDF. Sivaganesan (1991) indicated that the effect of annual fire seems to influence the grass species association and succession of species. On other hand, the annual fire plays an important role in the maintenance of forest stands at deciduous forest and seedling growth. The forest fire scorches the tree seeds of *Tectona grandis* and facilitates the growth by removing a portion of the seed coat (Seth & Kaul 1978). But overall tree species diversity, structure and regeneration were reduced by fire in tropical forest (Kodandapani et al. 2008), further, the results suggest both grass availability and composition altered by fire.

Conclusions

The present study provides baseline information on grass species composition in the tropical forest of southern India. There were considerable changes occurred in the grass species composition when compared to past. Grass association revealed seven major types of association in the tropical deciduous forest. Grasslands of TF were dominated by *Aristida-Eragrostis* indicators of heavy gazing and require protection of pasture from cattle grazing or at least reduction of cattle pressure to recover. Grass composition and availability was positively influenced by rainfall and reduced by fire in the tropical deciduous forest. Further grass availability and composition is threatened by invasion of weeds.

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Art – 262. NON-INVASIVE URINE COLLECTION- FLOOR PIT METHOD IN CAPTIVE SLOTH BEARS (*Melursus ursinus*)

S Ilayraja, Srinu Srikanta Maharana and **Pooja Acharya**

Abstract

Urine plays an important role in understanding metabolic conditions, reproductive status and stress physiology. We report here an effective non-invasive method of urine collection from captive Sloth bears (*Melursus ursinus*). The role of hormonal profiling and urinalysis are described. The pertinency of this method and its purpose for conservation are discussed.

Keywords: sloth bear, non-invasive, sample collection, urine

Introduction

The process of removing waste products from the body followed by cellular metabolism is known as excretion [1]. Like amphibians and marine fishes, mammals excrete urea as toxic wastes hence called ureotelic animals [2]. Urine plays an important indispensable role in clinical diagnosis [3]. Non-invasive urine samples are widely used for urinalysis and physiological assessment like reproduction, stress, and behaviour [3-5].The nature of being stable and obtainability in large quantities non-invasively makes it most important biofluid as compared to others [6]. Since Sloth bears (*Melursus ursinus*) are highly threatened schedule 1 species and considered as vulnerable by IUCN, it is very significant to understand their age- based physiology. There is a severe lack of scientific information about the basic biology of *M. ursinus* even after its wide distribution in Indian subcontinents. There have been defined reports of hematology and biochemistry values in *M. ursinus* but no urologic values [7, 8]. Furthermore, urinalysis have various beneficial aspects still it's a very neglected aspect in veterinary science [3]. It is hoped that the application of this method will facilitate to evaluate health profile of sloth bears in captive settings.

Material and Method

The method reported here was developed for captive Sloth bears under rehabilitation at Agra Bear Rescue Facility, Uttar Pradesh, India. A pit of ≤ 45.72 cm length, 2 cm wide and 1.5 cm deep was made at the end on the concrete flooring of animal's enclosure (figure 1). The pits were made in such a way that the urine will drive downward and get accumulated within it by the effect of gravitational force (figure 1). The ideal time for collection of urine samples was after the morning feeding of individuals. Prior to sample collection, the floor along with the pit was cleaned with disinfectants and distilled water. The pit then wiped by using sterile medical cotton rolls and allowed to air-dry. Soon after the urination, urine was collected from the pit via sterile injection and immediately transferred to an air-tight screw cap vial (figure 2). Urinalysis was performed soon after the collection by using urine test strips and UA analyzer supplied from IDEXX laboratories to avoid the disintegration of casts and other cells. Urine sample filled vials were stored frozen at -20˚C for later analysis.

Fig 1: Yellow coloured arrow indicating the floor pit made at animal's enclosure (a) and accumulation of urine in the pit after urination (b)

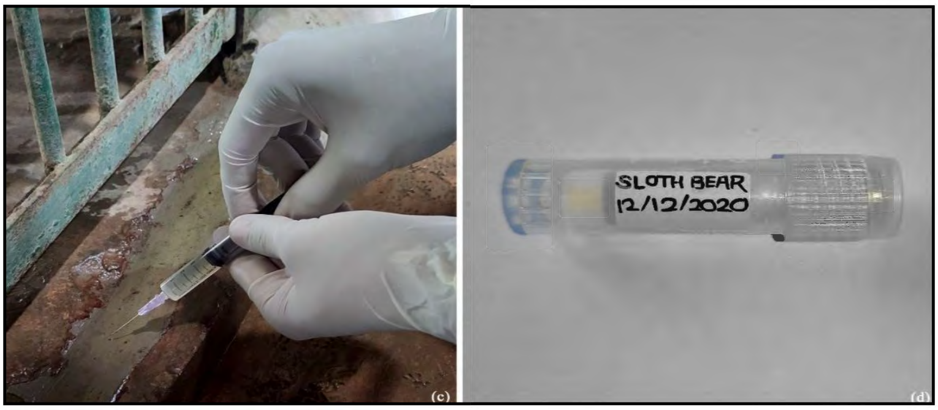

Fig 2: Urine sample collection (c) and storage in labelled air-tight screw cap vial (d)

Results and Discussion

Standard urinalysis parameters viz. colour, clarity, sedimentation, pH, leucocyte, protein, glucose, ketone bodies, urobilinogen, bilirubin, and blood cells were determined to evaluate metabolic conditions. Presence of air bubbles, hairs and accidental contamination by faeces and debris from enclosure floors suspected to be an artifact.

In this scenario, consecutive urinalysis needs to be performed with an uncontaminated sample. In addition to this, presence of leucocytes in urine needs to be confirmed under microscopic examination. However, there have been limited reports of hematology and biochemistry values in sloth bear but no urinalysis values [7, 9]. In most wildlife species and especially in *M. ursinus* repeated blood collection is inconceivable for hormonal analysis [10]. Furthermore, chemical immobilization induces stress in an animal which in turn affects physiological and hormonal profiles [4, 10]. Hence by this method, the animal was allowed in its familiar enclosure for the purpose of urine collection which neither effect it's physiological nor psychological conditions. Quantitative enzyme immunoassays generated urinary oestradiol and progesterone metabolites in *M. ursinus* [11]. Urinary hormone assays and behavioural profiling in *M. ursinus* illustrated the reproductive physiology and behavioural ecology of this iconic species[11, 12]. Earlier studies on the estrous cycle of *M. ursinus* has also observed an exhibition of wild instincts in captive conditions [13]. Reproductive status evaluation by hormonal monitoring for insemination and ovulation could bring successful reproduction in these animals. Even more, corticosteroid studies could be done to gather information about stress levels and so as necessary modification could be done in habitat and enrichments to adrenal activity diminution. By this method, we standardize the process of non-invasive urine sample collection is found to be less complicated in *M. ursinus*. Hence it is believed that the information obtained by this method would be invaluable or ameliorated veterinary care and in situ conservation.

Conclusion

The collection of urine sample by non-invasive method found to be an effective way to monitor metabolic condition and reproductive endocrinology. Procedure of collection may vary with respect to the local condition and the species involved. Advancement in understanding the urologic values of sloth bear will facilitate to offer high quality veterinary care and management.

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Art – 263. SURVEILLANCE AND EXAMINATION OF DENTAL PROBLEMS IN CAPTIVE SLOTH BEARS

S Ilayaraja, M Palanivelrajan**,** MG Jayathnagaraj and **A Sha Arun**

Abstract

Sloth Bear is classified as vulnerable species in the International Union for Conservation of Nature (IUCN) red list data of threatened species under vulnerable animal in 1990 and is a protected wild animal, under the Schedule I of Indian Wildlife Protection Act, 1972 (IWPA,1972). Sloth bears (*Melursus ursinus*) are omnivorous in general. This retrospective study period (from 2006 to 2015) was carried out to survey the dental problems and management measures adopted, in rescued sloth bears in rescued sloth bear from Agra Bear Rescue Facility, Agra, Uttar Pradesh. Out of 224 rescued sloth bears, 11 sloth bears were noticed with dental problems during the study period and the common dental problems of sloth bears were found to be broken canine and incisor, broken canine and incisor with peri-apical abscess, and over-grown gums/tissue lesions. During examinations of oral cavities of bears under study, some of the animals had infected root canals, peri-apical abscesses, and overgrown gums tissues with evidence of infections. From this area, swabs were obtained and subjected to bacterial culture. The bacterial culture revealed Beta hemolytic Streptococci and Anaerobic Bacteroides, Coagulase positive *Staphylococci*, *Pseudomonas* species, beta-hemolytic *Streptococci, Pseudomonas profuse, Klebsiella, etc.* Antibiotic sensitivity tests using 40 antibiotics were used in this study and management-related measures concerning the dental problems encountered and studied in the rescued Sloth bears were revealed in this study.

Keywords: Dental lesions, peri-apical abscess, sloth bears, teeth

1. Introduction

Sloth Bear is classified as vulnerable species in the International Union for Conservation of Nature (IUCN) red list data of threatened species under vulnerable animal in 1990 and protected under Schedule I of the Indian Wildlife Protection Act, 1972 (IWPA,1972). Sloth bears (*Melursus ursinus*) are omnivorous in general, specifically, it is frugivorous, myrmecophagy (feeding on ants and termites) and fond of honey [7, 13, 17]. Their food is rich in natural flavonoids, acids, protein, and low in fat. The oral flora of the animal can be influenced by the microbiome of the ingested prey and other food.

For more than 400 years, these bears are used for the entertainment of peopleby a community of Kalandar gypsies. Because of improper care, unconventional feeding of the animal leads to metabolic insult thus resulting in severe stress, low level of immunity to the animal, and make them more prone to a variety of infectious and non- infectious diseases. Agra Bear Rescue Facility is a unique facility for rescued dancing slothbears managed by an NGO Wildlife SOS in India which rescued above more than 500 dancing Sloth bears (*Melursus ursinus*) throughout India and is located at (27^{0}0'N;77^{0}45'E) Agra, Uttar Pradesh. Hence, for better understanding and to evaluate and necessary treatment, a study was undertaken in rescued dancing sloth bear in Agra Bear Rescue Facility and the objectives of this study were furnished below;

1. Retrospective surveillance of the dental problems in rescued sloth bears in Agra Bear Rescue Facility.
2. Screening of bacterial infections during the occurrence of tooth problems and conducting antibiotic sensitivity tests.

To suggest management related measures

1.2 Review of Literature

1.2.1 Dental problem in bears

Wallach and Boever quoted that tooth injuries were common among the ursids and cracks or break off a tooth always necessitated the repair or extraction because of the occurrences of abscessation or periodontitis and repair of the ursid canine teeth withan exposed pulp cavity might be accomplished by standard root canal techniques that were used for canids [22]. Fowler (1986) quoted that bears are omnivores and consume any food material available [5]. Bears have the dental formula as Ursidae I 3/3, C 1/1, P 4/4, M 2/3 = 42. Dental carries were most prevalent, and the associated predisposing factors were found to be a fracture, malpositioned teeth that create areas of impaction, and improper diet, particularly the carbohydrates. Wenker *et al.* did the retrospective study of dental conditions of captive brown bears with radiographic techniques and compared them with free-ranging Alaskan grizzlies [23]. Radiographic evaluation of per alveolar osteolytic process revealed the greater frequency of dental problems in zoo bears 10 years old. Stereotypical behavior like cage chewing is a suspected cause of canine tooth and secondary alveolar lesions. The bear with the dental problem will always like to prefer a liquid diet like milk, a watery portion of the porridge, and just suckthe juice from the fruits after crushing it. The bear is always in an aggressive mood due to the pain from the affected tooth. The bears always try to be away from the fellow bearand not showing any interest in the enrichment items. The painful dental conditions can considerably influence social behavior and breeding success in zoo animals. Lisa Milella conducted the study on 'Dental disease in rescued dancing sloth bear' and did necessary treatment such as tooth extraction, root canal treatment, excision of overgrown gum tissue etc [14]. When the canines teeth first erupt, many gypsies deliberately smash the teeth without using any kind of anesthesia to disarm the bear and make them look less aggressive for tourists and also not to get any bite from the bear. It is not known at what age their teeth had been fractured, but mostly all four canines and most incisors were fractured and had necrotic pulps. The sloth bear was fed a combination of soft dietary ingredients that are taken in by a sucking action. The traumatic condition caused by the Kalandars by brutally breaking the teeth with iron chisels or stones to avoid biting while make them perform is the main reason for the oro-dentic disorder in captive sloth bears. Stromquist *et al.,* reported that dental and pre-dental health in free-ranging Swedish brown bears (*Ursus arctos*). In oral cavity examination, the bears have a low prevalence of calculus and periodontal disease, and they don't have any caries infection [19].

Fleming and Burn revealed that the captive bears were prone to developing dental pathology due to various reasons, including longevity in captivity, inappropriate diet, trauma, and stereotypical bar-biting. If not detected, this could lead to pain and suffering, with negative welfare-related consequences [4]. Behavioral indices measured included general activity, social behaviors, stereotypic activities, eating-related behaviors, and oro-facial behaviors which were hypothesized to be associated with dental pain.

1.2.2 Dental problems in other wild animals

Valkenburgh stated that the number and position of teeth broken in large carnivores such as African lion, leopard, jaguar, cheetah, puma, spotted hyena, striped hyena, wolf, and African wild dog [21]. Clauss *et al*., carried out the study on "Tooth wear in captive giraffes (*Giraffa camelopardalis*)" and during the study, it was revealed that captive giraffe (*Giraffa camelopardalis*) mostly did not attain the remarkable longevity in captive conditions and frequently had problems associated with low energy intake and fat storage mobilization [2]. Abnormal tooth wear was found as one of the causes suggested as an underlying problem. Jurado *et al.*, stated that tooth structures were often suggested as important factors that were found to be limiting the life span of free-ranging wildlife [12]. Given the frequent occurrence of poor dental health in captive animals reported, one could generally expect tooth health to be the limiting factor in captivity as well. Additionally, it could be assumed that brachydont (browsing) animals were more susceptible to dental health problems than the case with hypsodont (grazing) animals, and systematically increased tooth wear occurred in some browsing species. Jeki and Redrobe opined that poor calcification of the teeth and the bones of the skull predisposed pet rabbits to the occurrence of various types of dental diseases. To achieve the periodical oral cavity examination, there was a need for knowledge on dental problems and proper way of treatment was more important [11].

1.2.3 Restraining of sloth bear for clinical examination

Fowler reported that bears are immobilized with the combination of xylazine hydrochloride and ketamine at the ratio of either 1:2 or 1:1 and the dosages are 5-9 mg/kg of ketamine and 2-2.5 mg/kg of xylazine. Chemical methods of immobilization were used to complete stress-free restraining for effective clinical examination [6]. Injection Xylazine hydrochloride @ 2mg/kg body weight and ketamine hydrochloride @ 5-7 mg/kg body weight was delivered with the help of blow dart by using blowpipe on an unsuspected animal. After the sedation, the bear was shifted to OperationTheater for further clinical examinations and providing general anesthesia after intubation with a size 16-18 mm intratracheal tube based on the size of the bear. Isoflurane 2-3% with oxygen @ 8 lits per minute flow rate was used tomaintain the general anesthesia. Local infiltration anesthesia and regional nerve block were also performed with 2%lignocaine solution in order to achieve complete painmanagement while performing the dental procedure [16]. Fowler coated about bear restraining, that the immature small cubs can be restraint by using a handheld or controlled by nets or snares and mature bears should be handled only by using squeeze cage or by chemical restraining [6].

1.2.4 Oral cavity examination in bears

The sloth bear having only 40 teeth as they lack the upper- middle pair of the incisor like other bear species, the dental formula is I 2/3, C 1/1, P 4/4, M 2/3 = 40. Due to improper care and brutal method of removal of canine and incisor tooth, without the benefit of anesthesia in dancing sloth bearby the kalandar gypsies; the bears always suffered due to different kinds or oro-dentic disorders [14]. Hence, the careful examination of the oral cavity is more important to rule out the oro-dental disorders andprovides a suitable treatment measure. The bear might have minor soft tissue injuries like gingivitis or even have lemon-sized gigantic forms of cementoma, partially broken canines and incisors, completely broken canines with infected root canal with or with periapical abscess. Sometimes, the premolar teeth alsogot damaged, while they were breaking the canine. Hence, for evaluation of the dental damage, subjecting all infected teeth to dental radiographic examination is most essential to make decisions to provide suitable treatment measures such as root canal treatment or tooth extraction. The intraoral, occlusal dental films were used for taking dental radiographs with bisecting angle technique by using a dentalx-ray machine. The tooth which was showing an intact root canal was selected for the RCT procedure and the tooth which was not having an intact root canal and exposed pulp cavity with a black spot on the epical region suggesting developing periapical abscess were selected for complete extraction.

1.2.5 Bacterial load in dental problems

Woods *et al.*, stated that salivary proteases played a role in the adherence of gram-negative bacilli to mammalian buccal epithelial cells, during the occurrence of tooth infections [24].Isogai *et al.*, carried out an epidemiological study on periodontal diseases and some other dental disorders in dogsand found that periodontitis was prevalent among the animals, regardless of their sources and its incidence was found to be increased with age [10]. The lesions were observed to be more severe and more frequent in the premolar and molar regions than in the maxillary and mandibular incisor regions. Missing of teeth was observed at a high and increasing incidence with age and the tooth most commonly lost was the first premolar, followed by the other premolars and molars, where severe periodontitis was frequently found. Calculus was seen on many teeth,and aging aggravated its prevalence and severity. Dental caries were observed in stray animals but not to a serious degree or a significant level. Bacteria recovered from the infected bite wounds were most often reflective of the microbiome of their ingested prey and other food. The mouth was colonized by 200 to 300 bacterial species, but only a limited number of these species participate in dental decay or periodontal disease. Dental decay was due to the irreversible solubilization of tooth minerals by acid produced by certain bacteria that adhere to the tooth surface in bacterial communities known as dental plaque [15]. The new definition of a biofilm is a microbial-derived sessile community characterized by cells that are irreversibly attached to a substratum or interface (biotic, non-biotic) or each other, are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription. Biofilm can form on various surfaces, including biotic surfaces (e.g., teeth, mucosal membranes), medical devices, and household surfaces. In the oral cavity, teeth provide constant humidity and adherent surfaces causing the attachment of extensive deposits of microorganisms. In dogs and cats, mouth normal bacterial microflora was structured in a variety of aerobic, facultative, or strictly anaerobic bacteria [25].

1.2.6 Managemental measures against dental problems in bears

Periodontal disease is the most common infectious disease in humans and animals. 75% to 80% of cats and dogs over 2 years of age exhibit signs of periodontal infection. In animals, the upper premolar teeth (generally the fourth premolar tooth) whose roots are located near the eye socket are mostly affected. These animals are generally presented with swollen eyes and fistula. Treatment includes extraction of the tooth and flushing of the infected site with an antibacterial solution. A drain is required in severely infected cases. so extra-oral and intra-oral extraction of the involved teeth is the successful way to mage the dental problems in animal [1]. Wenker *et al.*, stated that stereotypical behavior like cage chewing, nutritionallyinappropriate diet, and inadequate opportunities for tooth- cleaning activities were responsible for the lack of natural cleaning and the extensive calculus formation resulted in dental problems in zoo bears [23]. Hence, the implementation of species-specific enrichments to prevent stereotypic behavior and to provide the periodical dental checkup, and provide good nutrition was more important in the preventionof multiple dental diseases.

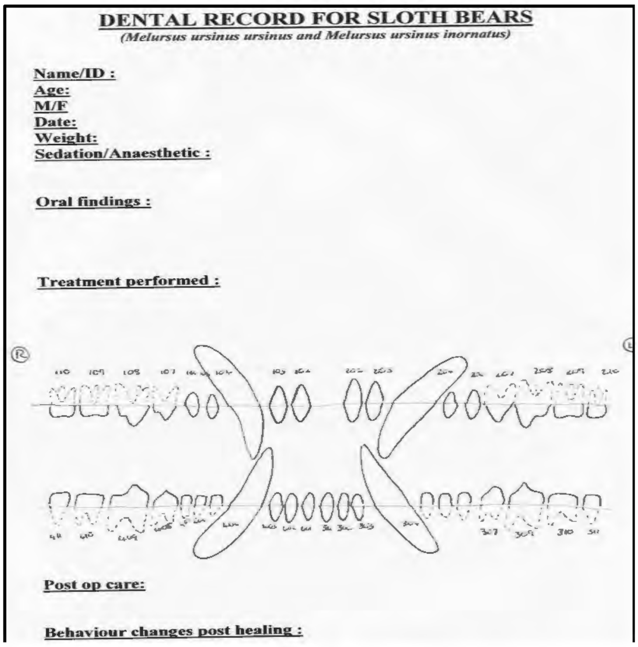
2. Materials and Method

This study was undertaken in rescued sloth bears from all over India and kept in Agra Bear Rescue Facility, Agra, Uttar Pradesh. Samples were collected from 11 rescued sloth bears. The oral cavity with the normal dentition of sloth bear was shown in (figure 1). Retrospective surveillance study data involving the dental problems from 2006 to 2015 concerning the encountered dental problems inthe rescued sloth bears reared at Agra Bear Rescue Facility were collected from January 2015 to May 2015 and a total of 233 cases were documented in this regard.

Fig 1: Oral cavity with normal dentition in sloth bear

2.1. Parameters studied

- Model dental records (figure 2).
- Collection of swabs from suspected cases encountered tooth problems during clinical examination of rescued sloth bears.
- Bacterial culture-related works and antibiotic sensitivity tests were carried out for samples collected from the rescued sloth bears.
- Treatment and management measures adapted to the sloth bears who have tooth problems.



DENTAL RECORD FOR SLOTH BEARS

(Melursus ursinus ursinus and Melursus ursinus inornatus)

Name/ID :
Age:
M/F
Date:
Weight:
Sedation/Anaesthetic :

Oral findings :

Treatment performed :

Post op care:

Behaviour changes post healing :

Fig 2: The model dental record followed in this study

All bears are restrained chemically with the combination of xylazine @ 2mg/kg body weight and Ketamine @ 5-7mg/kgbody weight after sedation animal was shifted to Operation Theater for radiography. For the treatment, the animal was intubated with a suitable endotracheal tube (Size 16/18) and maintained with general anesthesia isoflurane 2-3% along with oxygen 6-7 ltr/min were shown in figure 3.

Fig 3: Bear on the table after intubation

Throughout this study program, buckle swab samples were obtained from the rescued sloth bears with the oro-dental problem (figure 4). Samples were collected in a sterile swab (Himedia sterile swab) for bacterial examination and were properly labeled and sealed and sent to the local pathology lab on the same day. On oral examination, the sloth bear having only 40 teeth as they don't possess the upper-middle pair of the incisor as, like other bear species, the dental formula is I 2/3, C 1/1, P 4/4, M 2/3 = 40. The examination of the oral cavity is more important to rule out the oro- dentaldisorder and provide a suitable treatment measure. The bear may have minor soft tissues injury likes gingivitis to lemon size gaigantiform cementoma, partially broken canine andincisors, completely broken canine with infected root canal with or with peri-apical abscess (figure 5-7). Sometimes the premolar teeth also got damaged while they were breaking the canine. so to evaluate the dental damage, subjecting all infected tooths to dental radiographic examination is more essential to decide to provide suitable treatment such as route canal treatment or tooth extraction. The oral finding recorded inthe dental chart. The intraoral, occlusal dental film was used for taking dental radiograph with bisecting angle technique by using dental x-ray machine (figure 8). The tooth whichwas showing intact root canal selected for RCT (root canal treatment) procedure (figure 9- 11) and the tooth which were not having an intact root canal and exposed pulp cavity with a black spot-on apical region suggesting developing periapical abscess are selected for complete extraction (figure12).

Fig 4: Collecting swab from the Broken and infected tooth

Fig 5: Different kinds of dental issues with bears.

Fig 6: Malocclusion of Teath

Fig 7: The infected molar tooth with swollen gum tissue and enamel dysplasia

Fig 8: Dental Radiographic examination of the bear

Fig 9: (A) Overgrown gum mass covered the fractured tooth (B)Overgrown gum mass with tooth fragments after removal

Fig 10: Periapical abscess due to the infected canine tooth in lower jaw and radiograph showing exposed root canal with developing a periapical abscess.

Fig 11: RCT (Root Canal Treatment) procedure

Fig 12: Tooth extraction procedure

2.1. Analysis of samples for bacterial culture

A Blood Agar is a special and more complex kind of agar that is composed of the requisite rich nutrients along with blood. Blood agar plates are used to culture those bacteria ormicrobes that do not grow easily. Such bacteria are called “fastidious” as they demand a special, enriched nutritional environment as compared to the routine bacteria. Blood agaris, predictably, red in color and opaque. Besides being a rich base for the fastidious bacteria to grow, blood agar also has the function to help differentiate and classify bacteria into three types based on their hemolytic activity on the agar. They are alpha, beta, and gamma-hemolytic bacteria. The gamma-hemolytic bacteria leave the RBC and the mediumas it is (no hemolysis occurs). The alpha-hemolytic bacteria make the medium green in color because of partial hemolysis. Beta-hemolytic bacteria cause complete hemolysis, which makes the medium clear.

Nutrient agar is a general-purpose medium supporting growth of a wide range of non-fastidious organisms. MacConkey's Agar is a specialized bacterial growth medium that is selective for Gram-negative bacteria and can differentiate those Gram-bacteria that can ferment lactose. For antibiotic sensitivity test, the antibiotic disc-like Amikacin, Amoxicillin, Amoxy-Clavulanic acid, Ampicillin, Azithromycin, Cefindinir, Cefixime, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clarithromycin, Clindamycin, Floxacin, Fosfomycin, Fusidic acid, Gatifloxacin, Gentamicin, Imipenem, Ivanz, Kanamycin, Lenozolid, Levofloxacin, Lincomycin, Linezolid, Meropenem, Methicillin, Nalidixic, Netilmicin, Penicillin G, Piperacillin-tazobactam, Pristinamycin, Rifampicin, Sulfamethoxazole, Teicoplanin, Tetracycline, Ticarcillin-clavulanate, Tigecycline, Tobramycin, and Vancomycin were used and the test is done by Kirbay BauerMethod. The results were analyzed scientifically and were documented, and statistical analysis was carried out as perthe methods given by Snedecor and Cochren [18].

3. Results and Discussion

A retrospective study of dental problems in a rescued sloth bear (*Melursus ursinus*) was carried out in Agra Bear Rescue Facility, Agra from 2006 to 2015. Out of 224 rescued sloth bears, 11 sloth bears were noticed with dental problems during the retrospective study period (from 2006 to 2015) and list of problems as given below (Figure 13).

- Broken canine and incisor,
- Broken canine and incisor with peri-apical abscess and
- Overgrown gums/tissue lesions

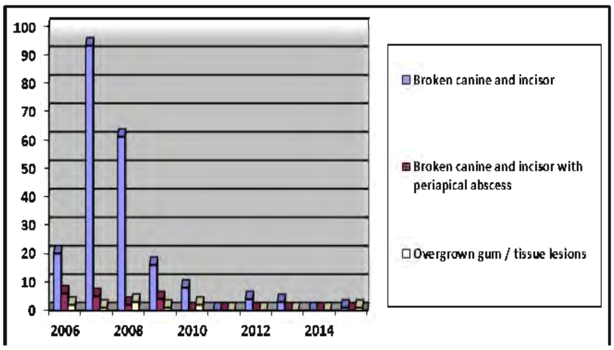


Fig 13: Retrospective surveillance of the dental problems in rescued sloth bears in Agra Bear Rescue Facility

During oral examinations, some of the animals had infected root canals, periapical abscesses, and overgrown gums tissues with infection. From this area, a swab was taken and subjected to bacterial culture in three different agar plates like blood agar, nutrient agar, and Macconkey's agar. The bacterial culture revealed that Beta hemolytic streptococci and Bacteroides anaerobic were 18.18% respectively, Coayagulase positive staphylococci was 36.36% and pseudomonas species, beta-hemolytic streptococci & Pseudomonas profuse, and beta-hemolytic streptococci & klebsiella were 9.09% respectively. The bacterial culture shows a higher rate of infection for Coayagulase positive staphylococci in oro-dental problems of sloth bears (figure 14). In the antibiotic sensitivity test, 40 antibiotics wereused for the evaluation of antibiotic sensitivity against the cultured bacteria was shown in (Table 1). The antibiotic sensitivity test resulted in 100% of imipenem, 63.64% of ceftriaxone, chloramphenicol, and gentamicin, and 54.55% of amikacin and Amox-clavulanic acid was sensitive against the cultured bacteria. Based upon the bacterial culture and antibiotic sensitivity test findings the decisions were made for the further treatment of the dental problems in slothbears. Similarly, the sloth bears had (1) broken teeth and infective root canal without periapical abscess (2) Broken teeth with infected root canal with periapical abscess. In this condition, tooth extraction was done under general anesthesia. For the root canal treatment (RCT), the animal was subjected to radiography in both pre and post-treatment, the oral radiography gives a conclusion about the tooth extraction. The other lesion such as overgrown gum tissue and gigantic form cementoma were excised off surgically and the gum flab was opposed with PGA suture material attached with traumatic cutting edge needles. The post- operative care can be carried out as per the antibiotic sensitivity test along with NSAID like meloxicam @ 0.5 mg/kg body weight I/M injection for 3 to 5 days and maintain the animal with a soft liquid diet and kept the animal in an indoor enclosure. During this study with rescued sloth bears, dental problems were noticed in 11 sloth bears. The findings of dental problems during the retrospective study in sloth bears that were rescued were in accordance with the findings furnished by Liza Milella [14] who reported more or less similar types of dental problems in the rescued sloth bears, and these comprised broken tooth, infected root canal, periapical abscess, overgrown gums/tissue,etc.

Table 1: Antibiotic Sensitivity Tests of the dental problems in rescued sloth bears

n=11

S. No.	Antibiotic used	No. of sensitive samples	%
1	Amikacin	6	54.55
2	Amoxicillin	3	27.27
3	Amoxy-Clavulanic acid	6	54.55
4	Azithromycin	3	54.55
5	Ampicillin	1	9.09
6	Cefindinir	3	27.27
7	Cefixime	3	27.27
8	Ceftriaxone	7	63.64
9	Cefuroxime	2	18.18
10	Clindamycin	3	27.27
11	Chloramphenocol	7	63.64
12	Ciprofloxacin	3	27.27
13	Fosfomycin	1	9.09
14	Gatifloxacin	3	27.27
15	Gentamicin	7	63.64
16	Imipenem	11	100.00
17	Ivanz	2	18.18
18	Kanamycin	2	18.18
19	Methicillin	3	27.27
20	Nalidixic	1	9.09
21	Netlimicin	2	18.18
22	Levofloxacin	3	27.27
23	Lincomycin	3	27.27
24	Linezolid	3	27.27
25	Pristinamycin	1	9.09
26	Ofloxacin	3	27.27
27	Rifampicin	4	36.36
28	Tigecycline	5	45.45
29	Teicoplanin	4	36.36
30	Tetracycline	4	36.36
31	Tobramycin	5	45.45
32	Penicillin G	4	36.36
33	Vancomycin	5	45.45
34	Fusidic acid	1	9.09
35	Sulfamethoxazole	1	9.09
36	Clathrithromycin	2	18.18
37	Meropenem	4	36.36
38	Ticarcillin-clavulanate	1	9.09
39	Piperacillin-tazobactam	3	27.27
40	Lenozolid	1	9.09

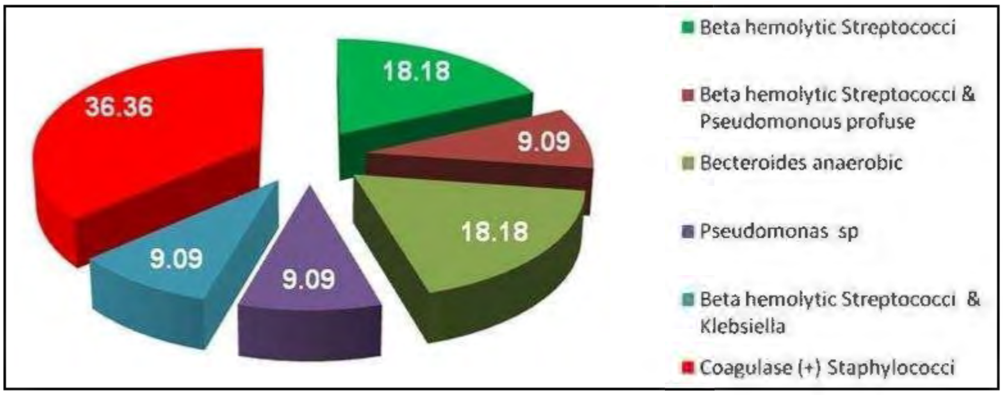


Fig 14: Percentage of bacterial load in the oro-dental problem of rescued sloth bears (n=11)

However, during this study, it was found out that the identical dental problems were found to comprise broken incisors, fractured canine, infected root canal, overgrown gum, and peri-apical abscess and these findings agreed with the reports presented by Fowler as well as Wallach and Boever [5, 22]. In this regard, it was important to mention the report of Stranquist *et al.*, revealing the existence of low prevalence of calculus and periodontal diseases, and further, caries were absent in them [19]. Additionally, Wenker *et al.*, quoted that the bears with dental problems preferred to take liquid diet like milk and the watery portion of the porridge and just sucked the juice from the fruits and did not reveal any interest in the enrichment items under the captive conditions [23], as noticed during the study and the reasons for this might be assigned to the presence of painful dental conditions due to the affected tooth and the affected bears always revealed aggressive behavior. Further, Fleming and Burn indicated the development of dental pathology due to various reasons and the different behavioral changes [4]. Encountering of dental problems in this study was in agreement with the report furnished by Wallach and Boever [22], who stated that tooth injuries were common among the bears and reported on the usage of standard root canal techniques. Isogi *et al.,* found that periodontitis was prevalent among the animals regardless of the sources and the incidences were found to be increased with the age ofthe animals [10]. Zambori *et al.*, stated that in oral cavity teeth provides constant humidity and adherent surfaces causing the attachment of extensive deposit of microorganism [25]. It is noteworthy to mention that the study of dental problems in dancing sloth bears report furnished by Liza Milella about the correcting of dental problems by using dental radiographic examination. It is more essential to decide to provide suitable treatment suchas Root Canal Treatment (RCT) or tooth extraction under general anaesthesia [14]. Encountering the different bacterial fauna in the swabs from the oral cavity in this study was in agreement with the findings of Traub and Leohard [20], who also encountered bacterial organisms like beta-hemolytic streptococci, which was found to be more susceptible to teicoplanin and vancomycin. None of the isolates revealed high-level gentamicin resistance. Hayes (2001) reported about the use of different drugs as done in this study after the carrying out of antibiotic sensitivity tests and it was stated that the oral penicillin remained the drug of choice in most clinical situations, although the more expensive cephalosporins and, perhaps, amoxicillin-clavulanatepotassium provided the superior bacteriologic and clinical cure rates [8]. Encountering of different bacterial organismsin this study with swabs from dental regions of captive sloth bears was in agreement with the reports furnished by Finland *et al.,* who opined about the 69 strains tested and revealed their moderate or high susceptibility to all the antibiotics used, except those belonging to the aminoglycoside and polymyxin groups [3].

3.1 Management measures

In this study on dental diseases, it was found that if rescued sloth bears had tooth problems, it was very difficult to maintain the animal in normal health condition. Due to the dental problems, the sloth bears would not take the normal food. To correct this problem necessary treatments such as tooth extraction, Root canal treatment (RCT), excision of overgrown tissue, etc, needed to be carried out immediately, as supported by Milella (2007) [14]. Since the animals were having severe oro-dental problems for a long time, they might be prone to any kind of infections in the future. However, the endodontic procedures and extraction of the infected tooth were to be carried out in time. Findings revealed the need for continuous care and monitoring to evaluate the overall health status of the bears and successful implementation of endodontic procedures were highly required in related teeth-disease affected cases. Hosey (2013) also reported about the lack of canine and incisor they have not been undergoing the omnivorous diet as like the wild bear and not fit to release into the wild [9]. So, the animals are needed to be kept in lifetime care at rehabilitation centers and advice to provide with a semisolid/soft nutritious diet and fruits. Provision of a soft diet and also a palatable diet needed to be considered in the case of bears with dental diseases. Health-related measures needed to be taken care of whenever a newly rescued sloth bear enters the Bear Rescue Facility at Agra in an intensified manner with careful ruling out of dental problems and this was because of the earlier maintenance of them with poor health status, often.

4. Conclusion

A retrospective study period (from 2006 to 2015) was carried out to survey the dental problems and management measures adopted, in rescued sloth bears in rescued sloth bear from Agra Bear Rescue Facility, Agra, Uttar Pradesh. A retrospective study of dental problems in ten years period revealed the existence of multiple dental problems in captivebears that were rescued. Out of 224 rescued sloth bears, 11 sloth bears were noticed with dental problems during the study period, and the list of problems documented as follows;

- Broken canine and incisor,
- Broken canine and incisor with periapical abscess and
- Overgrown gums/tissue lesions

During oral examinations, some of the animals had infected root canals, periapical abscesses, and overgrown gums tissues with infection. The bacterial culture revealed organisms like Beta hemolytic *streptococci,* Coayagulase positive *Staphylococci*, *Pseudomonas* species, beta- hemolytic *streptococci*, *Pseudomonas profuse,* beta- hemolytic *Streptococci, Klebsiella* sp., etc. in dental lesions. In the antibiotic sensitivity test, 40 antibiotics were used for the evaluation of antibiotic sensitivity against the bacteria. The antibiotic sensitivity test results were also revealed in this study. Management measures pertaining to the findings on dental problems in captive bears were detailed.

From this study, the following conclusions were obtained:

- The common dental problems of sloth bears were broken canine and incisor,broken canine and incisor with periapical abscess, and overgrown gums/tissue lesions.
- The swabs from the oral cavity have different bacterial loads.
- Antibiotics like imipenem, ceftriaxone, chloramphenicol, gentamicin, amikacin, and Amox- clavulanic acid had more effects in bears affected by dental problems.
- The post-operative care could be carried out as per the antibiotic sensitivity tests, along with administration of NSAID like meloxicam @ 0.5 mg/kg body weight by I/M injection, daily for 3 to 5 days.

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iv b) HIGHLIGHTS IN THE COMPENDIUM – 04

Bear Care staffs of Bannerghatta Bear Rescue Center participated and presented their articles in the Online International Conference: Wildcon – 2020 Insights into Wildlife Conflicts, Rescue and Rehabilitation: Challenges & Opportunities for Conservation & 14th Annual Convention of Association of Indian Zoo and Wildlife Veterinarians in December 2020.

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iv c) LIST OF AUTHORS AND THEIR AFFILIATIONS

Author's Name	Affiliation
A.M. Pawde	Center for Wildlife, ICAR - IVRI, U.P.
Aaliya Mir	Manager and Education Officer, J&K Projects, Wildlife SOS, J&K
Abhijit Bhawal	Veterinary Officer, National Zoological Park, New Delhi
Abhishek Kumar Singh	Wildlife Veterinary Officer, Gurugram, Wildlife SOS, Haryana
Acharya P.R.	Wildlife Veterinary Officer, Wildlife SOS, Agra
Adithyan N.K.	Wildlife Veterinary Officer, Agra Bear Rescue Center, Wildlife SOS, Agra
Late Ajay A. Desai	Co-chair Asian Elephant Specialist Group
Ajay Deshmukh	Wildlife Veterinary Officer, Manikdoh Leopard Rescue Center, Wildlife SOS, Junnar
Allauddin	Bear Kitchen staff, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Anil Kumar Sharma	Center for Wildlife, ICAR - IVRI, U.P.
Apsana Rizwan	Institute of Animal Health and Veterinary Biologicals, Bangalore.
Arshvarinder Singh Virk	Wildlife Veterinary Officer, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Arun A. Sha	Director – Research and Veterinary Operations, Wildlife SOS, Bangalore
Arvind Mathur	Senior Veterinary Officer, Nahargarh Biological Park, Rajasthan
Ashish Kumar Jangid	Wildlife Veterinary Officer, Agra Bear Rescue Center, Wildlife SOS, Agra
Ashok Kumar	Krishi Bhawan, ICAR, New Delhi
Atul Gupta	Veterinary Officer, Van Vihar National Park, Bhopal
B. Shrikrishna	Wildlife Veterinary Officer, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
B.R Singh	Division of Epidemiology, ICAR - IVRI, U.P.
B.S. Pruthvishree	Division of Epidemiology, ICAR - IVRI, U.P.
Balasubramanian G.	Wildlife Biologist, Wildlife SOS
Barbara S. Durrant	Institute for Conservation Research, Sandiego Zoo Global, USA
Beena Vamadevan	Veterinary Pathology Division, ICAR – IVRI, U.P.
Bhaskaran Ravi Latha	Department of Veterinary Parasitology, Madras Veterinary College, TNVASU, Chennai, India
C. Sakthivel	Bombay Natural History Society
Chandra Mohan Siddappa	Center for Wildlife, ICAR - IVRI, U.P.
Chandranaik B. Marinaik	Institute of Animal Health and Veterinary Biologicals, Bangalore
D.K. Sinha	Division of Epidemiology, ICAR - IVRI, U.P.
D.T. Pal	ICAR, National Institute of Animal Nutrition and Physiology, Bangalore
Dada peer	Bear Kitchen staff, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
David L. Garshelis	IUCN, Species Survival Commission, Bear Specialist Group
Doddamane Rathnamma	Veterinary College, Bangalore.

G.M. Vishwanath	Animal Keeper, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Geeta Seshamani	Co-founder, Wildlife SOS, Defense Colony, New Delhi
Guntoju Sriram	Wildlife Veterinary Officer, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Hussain Basha	Animal keeper, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Ilayaraja S.	Deputy Director – Veterinary Services, Wildlife SOS, Agra
Imam Sab	Animal Keeper and Paravet, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
John P. Whiteman	Institute for Conservation Research, Sandiego Zoo Global, USA
K. Nagaraja	Institute of Animal Health and Veterinary Biologicals, Bangalore
K. Sudhakar	Bombay Natural History Society
K.C. Bipin	Assistant Professor, KVASU, Kerala
K.V. Jamuna	Professor and Head of the Department of Veterinary Anatomy and Histology, Bangalore Veterinary College
Karikalan Mathesh	Centre for Wildlife Conservation Management and Disease Surveillance, ICAR - IVRI, U.P.
Karine E. Pigeon	Faculty of Environment, University of North BC, Canada
Karthikeyan C.	Animal keeper, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Kartick Satyanarayan	Co-founder and CEO, Wildlife SOS, Defense Colony, New Delhi
Kumanan Vijayarani	Department of Animal Biotechnology, Madras Veterinary College, TVASU, Chennai, India
L. Jose	ICAR – National Institute of Animal Nutrition and Physiology, Bangalore
M. Ashokkumar	Researcher, Field Officer, Bear Conservation Projects, Wildlife SOS
M. Asok Kumar	ICAR – IVRI, U.P.
M. Kamalanathan	Animal Husbandry Department, Tamil Nadu, India
M. Pradeep	Assistant Professor, Department of Veterinary Pathology, KVASU, Kerala
M.L. Satyanarayana	Retired Professor, Department of Veterinary Pathology, Veterinary College, Hebbal
M.V. Baijuraj	Director- Conservation Projects, Wildlife SOS, Agra
Madurai Ganesan Jayathangaraj	Professor and Head of the Department of Wildlife Science, Madras Veterinary College, TVASU, Chennai, India
Mahendra Dhore	Manager, Manikdoh Leopard Rescue Center, Wildlife SOS
Manoj Chaurasiya	Wildlife SOS, Agra
Megan A. Owen	Institute for Conservation Research, San Diego Zoo Global, USA
Megha Sharma	Ph. D Student in Pathology, ICAR – IVRI, U.P.
Muthusami Palanivelrajan	Assistant Professor, Department of Wildlife Science, Madras Veterinary College, Chennai, India
N. Gowri Menon	M.V.Sc Scholar, KVASU, Kerala
N.K.S. Gowda	ICAR, National Institute of Animal Nutrition and Physiology, Bangalore

N.M. Rajashailesha	Associate professor, Department of Veterinary Anatomy, Veterinary college, Hosur.
Nikhil S. Bangar	Wildlife Veterinary Officer, Manikdoh Leopard Rescue Center, Wildlife SOS, Junnar
Nishith Dharaiya	Wildlife and Conservation Biology Research lab, Department of life science, Hemachandracharya North Gujarat University, Patna
Nithin	Wildlife Veterinary Officer, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
O.R. Vinodhkumar	Division of Epidemiology, ICAR - IVRI, U.P.
P. Dandapat	ICAR – IVRI, Kolkata
P.M. Deepa	Assistant Professor and Head i/c, KVASU, Kerala
P.M. Sidharth	Department of Animal Genetics and Breeding, Faculty of Veterinary and Animal Science, WBVAFS., Kolkata
Partha Sarathi Swain	Dept. of Animal Nutrition, National Dairy Research Institute, Bangalore
Pooja Acharya	Wildlife Veterinary Officer, Agra Bear Rescue Center, Wildlife SOS, Agra
Prajakta Rao	Education Officer, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Prakash G.P.	Animal keeper, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Pramod Rajput	Wildlife Veterinary Officer, Elephant Conservation and Care Center, Wildlife SOS, Mathura
Puja Deb	Team Lead – Resource Mobilization & Events, Wildlife SOS, Mumbai
Purushothaman Sridevi	Madras Veterinary College, TANUVAS, Chennai, India
Pushkar Banka	Veterinarian, Maharashtra
R. Bhatta	Director, ICAR - NIANP, Bangalore
R. Nagarajan	P.G, Research Department of Zoology and Wildlife Biology, A.V.C. College
R.L. Rathish	Assistant Professor, KVASU, Kerala
R.V. Prasad	Retired Professor, Department of Veterinary Anatomy and Histology, KVAFSU, Karnataka
Rahul Prasad	Wildlife Veterinary Officer, Elephant Conservation and Care Center, Wildlife SOS, Mathura
Randy Larsen	Professor, Brigham Young University, Provo, USA
Rashid Y. Naqash	Regional Wildlife Warden, Department of Wildlife Protection, Srinagar, J&K, India
Reegan P	Wildlife Biologist, Wildlife SOS
Richa Kumar	Wildlife Biologist, Wildlife SOS
Riya Bakde	Wildlife Veterinary Officer, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
S. Ganga Naik	Professor & Head, Department of Veterinary Anatomy, Veterinary College, Gadag
S. Prerna	Researcher, Wildlife Institute of India, Dehradun
S. Shylaja	Veterinary College, Bangalore
S.K. Bhat	ICAR, National Institute of Animal Nutrition & Physiology, Bangalore

Sabarinath Thankappan	Clinical Bacteriology laboratory, ICAR – IVRI, Uttarakhand
Saddam Husen	Animal Keeper, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Sant Prakash	Department of Zoology, Dayalbagh Educational Institute, Agra, U.P.
Shabir Mir	Veterinary Assistant Surgeon, Animal Husbandry Department, J&K
Shanmugavelu Swaminathan	Head – Wildlife Biologist, Wildlife SOS
Sharma K	Center for Wildlife Conservation, Management and Disease Surveillance, ICAR – IVRI, U.P.
Shiva Prasad Parida	Microbiologist, Wildlife SOS, Agra
Shivani Das	Department of Veterinary Physiology, Odisha University of Agriculture and Technology
Shivani Sing	Wildlife Veterinary Officer, Agra Bear Rescue Facility, Wildlife SOS, Agra
Shruti	Department of Veterinary Anatomy, Histology, KVAFSU, Karnataka
Shubham Pramod Patil	Wildlife Veterinary Officer, Bannerghatta Bear Rescue Center, Bangalore
Siva Sankari G.	Junior Biologist, Wildlife SOS
Sree Lakshmi P.	Ph. D Student in Pathology, ICAR – IVRI, U.P.
Srinu Srikanta Maharana	Senior Research Associate, Agra Bear Rescue Facility, Wildlife SOS, Agra
Suchitra Sahoo	Department of Veterinary & Physiology, Odisha University of Agriculture and Technology, Odisha
Swagat Mohapatra	Department of Veterinary & Physiology, Odisha University of Agriculture and Technology, Odisha
Swostika Priyadarsini	Addl. Veterinary Assistant Surgeon, Barang, F&ARD Department, Govt. of Odisha, Odisha
Sydney Rae Stephens	Biologist, Biological Research and Conservation, Salt lake, USA
Thomas Robert Sharp	Director of Conservation and Research, Wildlife SOS, USA
Tista Joseph	Wildlife Veterinary Officer, Bannerghatta Bear Rescue Center, Wildlife SOS, Bangalore
Tom S. Smith	Brigham Young University, Provo, USA
V. Sejian	ICAR, National Institute of Animal Nutrition and Physiology, Bangalore
V. Girish Kumar	Department of Veterinary Anatomy and Histology, KVAFSU, Bangalore
V.R. Annie	Department of Veterinary Anatomy and Histology, KVAFSU, Bangalore
Venkataravanappa Manjunatha	Institute of Animal Health and Veterinary Biologicals, Bangalore
Yaduraj Khadpekar	Wildlife Veterinary Officer, Wildlife SOS, New Delhi
Yogaraj Pannerselvam	Wildlife Biologist, Wildlife SOS, Bangalore
Yosef Deneke	School of Veterinary Medicine, Jimma University, Ethiopia

Abbreviations:

FLA: Full Length Article **ABT: Abstract**

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Web: www.wildlifesos.org
Mail: research@wildlifesos.org