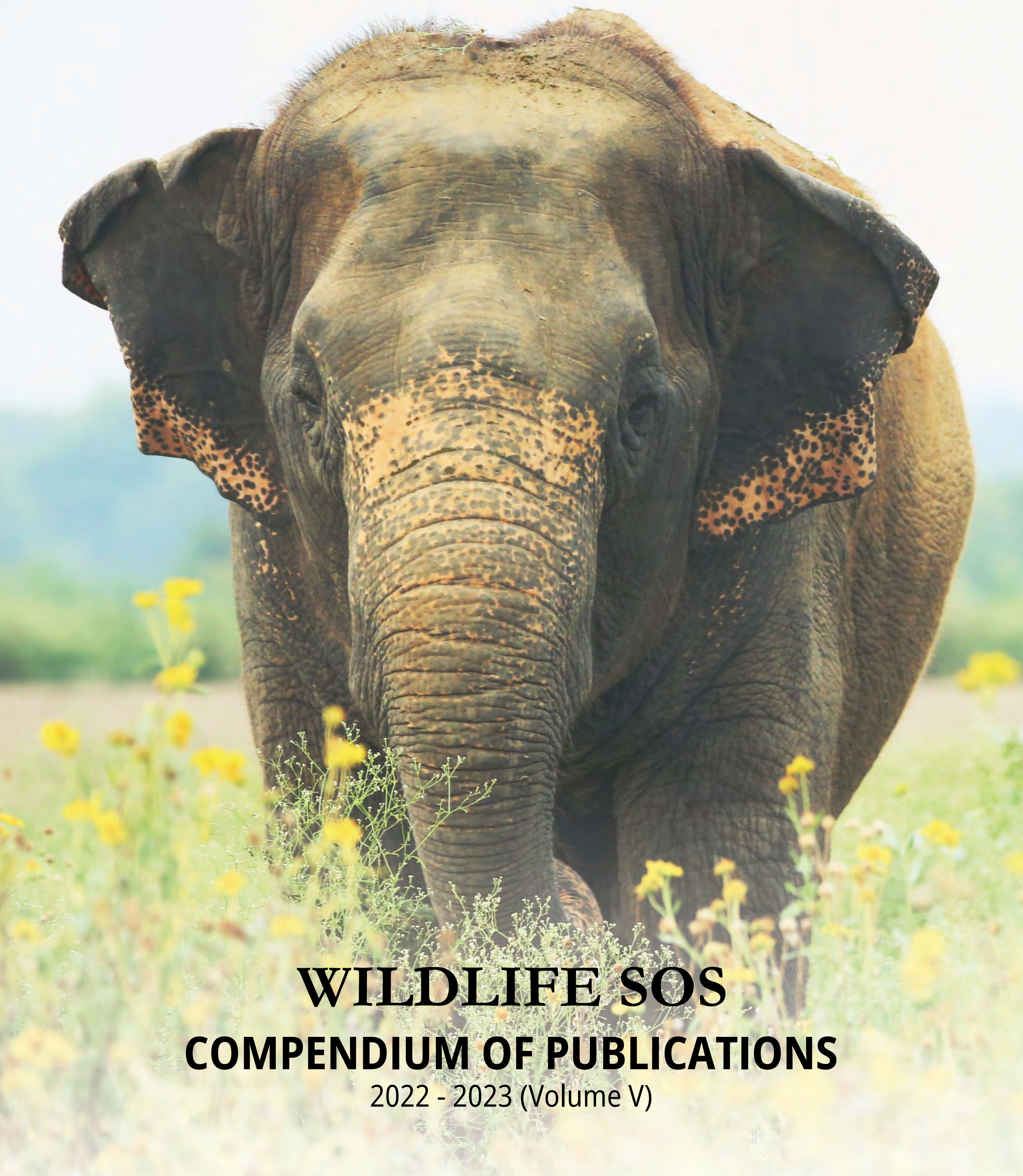


WILDLIFE SOS

COMPENDIUM OF PUBLICATIONS

2022 - 2023 (Volume V)

A COMPENDIUM OF SCIENTIFIC PUBLICATIONS OF WILDLIFE SOS

The review between: 2017 – 2023

Fifth Volume: 2021 – 2023

Volume: 05

Year released: 2023

Compilation Team: Wildlife SOS, BBRC

Cover Design: Mr. Kewal Nawariya, Wildlife SOS

A COMPENDIUM OF SCIENTIFIC PUBLICATIONS OF WILDLIFE SOS

Volume - V

An Official Publication of Wildlife SOS(R)

*** Refer Page No: 324**

NOTE FROM THE CO-FOUNDERS

Dear Friends and Supporters of Wildlife SOS,

Since its humble origins in 1995, Wildlife SOS has continued working tirelessly towards the cause of India's voiceless animals. One of our earliest and most significant endeavours was the abolition of the barbaric practice of 'dancing' bears, that involved excruciating training methods to break the body and spirit of sloth bear cubs poached from the wild. Fortunately, we successfully rescued and rehabilitated over 600 bears. This project not only saved bears but also empowered the Kalandar community, who were originally involved in this practice. We provided them with alternative sustainable livelihoods, demonstrating our commitment to holistic conservation efforts that consider both wildlife and the well-being of the communities intertwined with it.

Even as the 'dancing' bear project remains our landmark endeavour, our conservation efforts extend far beyond its scope. We remain vigilant in our mission to rescue injured begging elephants who are forced to work in oppressive conditions. Through our Manikdoh Leopard Rescue Centre in Junnar, we provide short-term and long-term care for leopards in danger, from man animal conflict situations, and we work in collaboration with the Maharashtra Forest Department. Additionally, we operate a 24-hour rescue hotline in three states and tirelessly attend to distress calls related to wild animals in peril or caught in conflict situations. Finally, we have been actively involved in a variety of activities, such as running helplines for the rescue of reptiles and other urban-wildlife, elephant conservation and care, Himalayan brown bear conservation, human-wildlife conflict mitigation, anti-poaching operations, and habitat conservation.

Wildlife SOS works in collaboration with local communities to combat illegal wildlife trade and trafficking. We express our heartfelt gratitude to all supporters, as it is clear that our mission to protect India's incredible wildlife is far from over. Together, let us forge ahead, driven by an unwavering commitment to a future where humans and wildlife coexist harmoniously, ensuring the survival of India's natural treasures for generations to come.

Geeta Seshamani

Geeta Seshamani
Co-founder & Secretary
Wildlife SOS

Kartick Satyanarayan
Co-founder & CEO
Wildlife SOS

Dr. C. Sreekumar, Ph.D.,
Professor and Head,
Dept. of Wildlife Science,
Madras Veterinary College,
Vepery, Chennai-600007

Ph:+919841906211
Email: sreekumar.c@tanuvas.ac.in

FOREWORD

October 8th, 2023

I am glad to introduce the fifth volume of Wildlife SOS's compendium of research publications for a three year period from 2021 to 2023. This organization has consistently set a benchmark for not only successful rescue and rehabilitation of distressed wildlife in India, but also on-field research on topics of paramount importance to wildlife health and conservation. This compendium stands as a testament to their commitment and dedication to wildlife conservation

This volume, delves into a diverse array of subjects, including anatomy, physiology, clinical medicine, surgery, anaesthesia, pathology, and more, which are topics less studied with hardly any baseline data available otherwise. The research has been carried out meticulously with attention to presenting the facts in an analytical way to benefit the reader. The case studies presented here offer invaluable insights and knowledge for aspiring wildlife veterinarians and conservationists alike, serving as a guide when they encounter similar cases. While this compendium encompasses cases of various species, it provides in-depth coverage of the vital work on sloth bears and elephants, making it an indispensable handbook for veterinarians worldwide who work with these remarkable creatures. A handy compilation of such unique research data will definitely be an asset to researchers and conservationists.

The commitment shown by Wildlife SOS to the rescue, rehabilitation, and conservation of a wide range of wild animals, including sloth bears, elephants, leopards, black bears, brown bears, reptiles and other displaced wildlife, is truly commendable. Furthermore, their active involvement in assisting forest departments of various states, the police and law enforcement agencies in investigations and providing legal support in cases pertaining to wildlife poaching underscores their dedication to protecting India's wildlife.

As we eagerly await the next volume of research studies from Wildlife SOS, I am confident that the organization will continue to nurture the tree of learning and serve as an essential resource for those dedicated to the well-being of wildlife.

C.SREEKUMAR

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,

I am delighted to introduce the latest volume of "A Compendium of Scientific Publications of Wildlife SOS." This publication stands as a testament to the determined commitment of the Wildlife SOS team to the pursuit of research and scientific inquiry, a value deeply ingrained in our organizational culture.

Our heartfelt gratitude extends to our co-founders, whose relentless support has been the keystone of our journey thus far. We trust in their continued encouragement as we forge ahead in these intellectual pursuits.

This compendium is a collective achievement, the fruit of tireless dedication exhibited by our remarkable team. I want to express my special gratitude to Ms Vasavi and team for their invaluable contributions. Their tireless e orts have been instrumental in shaping this volume.

I also extend my thanks to our dedicated animal keepers, whose steadfast cooperation provided the foundation for the research contained within these pages. Your hard work and dedication have not gone unnoticed.

I am also indebted to my peers and colleagues whose positive feedback and encouragement have played a pivotal role in refining our work. Your insights have been invaluable.

Lastly, I would be remiss not to acknowledge the indispensable role played by the various forest departments across the country. Their support has been pivotal to our research endeavours, 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

18	281	2022	Investigating Co-occurrence among Look-alike Species: The Case of Three Bears in Northeast India	117
19	282	2022	Tuberculosis in free-ranging and captive wild animals: Pathological and molecular diagnosis with histomorphological differentiation of granulomatous lesions	154
20	283	2022	Xenografting of tilapia fish skin for snare wound management in wild sloth bear Melursus ursinus): A novel approachh	179
21	284	2022	A study on clinical diagnosis of tuberculosis in free ranging and captive wild animals of India	190
22	285	2022	Sloth bear maternal and resting den locations in eastern Karnataka	203
23	286	2022	Asiatic Black Bear (*Ursus thibetanus*) attacks in Kashmir Valley, India	225
24	287	2022	Colonization of wild Elephants and Human Elephant interface in central Indian landscape	239
25	288	2022	Does Sloth bear (Melursus ursinus) hold a unique individual identity? An effort to establish human-sloth bear conflict mitigation measures through individual recognition	240
26	289	2022	Essentials of Footcare in Captive Elephants	241
27	290	2022	Welfare concerns in managing Elephants in captivity: A Case study	249
28	291	2022	Population status of Sloth bears (*Melursus ursinus*) and other large mammals in Daroji Sloth Bear Sanctuary, Karnataka	255
29	292	2022	Population status of Sloth bears (Melursus ursinus) and other large mammals in Gudekote Sloth Bear Sanctuary, Karnataka	256
30	293	2023	Asian Elephant (*Elephas maximus Linnaeus*) Population Status and Demography in Tropical Forest of Southern India	257
31	294	2023	Intra-abdominal laparoscopic sterilization of macaques: An attempt to mitigate human-primate conflict through an effective animal birth control technique	276
32	295	2023	Critical injuries due to negative interface between wild boar and captive sloth bear in a free ranging area	286
33	296	2023	Poacher's snare as a threat to Indian wildlife: A review	291
34	297	2023	Comparative study on checklist of aerobic microflora of oral and cloacal sample of spectacled cobra; rescued from snake charmers & wild cobra rescued from the conflict situations in Agra, Uttar Pradesh, India	310

Art – 264. GROSS MORPHOLOGY AND HISTOLOGY OF EXTRAOCULAR MUSCLES IN SLOTH BEAR (*Melursus ursinus*)

NM Rajashailesha, RV Prasad**, Arun A Sha,** KV Jamuna, M L Satyanarayana and S Ganga Naik

Abstract

The extra-ocular muscles of are accessory organs of the eye (*Organa oculi accessoria*) which are involved in ocular movement and are located between the sclera and the wall of the orbit. These muscles are classically classified as four rectus, two oblique and one retractor bulbi. Though, their details and potential associations with sloth bear behaviour are limited. The aim of this study is to characterise extraocular muscles in sloth bear from its habitat with behaviour. The right and left eyeball with intact extraocular muscles of six adult sloth bears were collected during post mortem examination of bears that had died due to natural causes and extraocular muscles were dissected and utilized for gross and histomorphological studies. Extraocular muscles of sloth bear comprised of dorsal (*M. rectus dorsalis*), ventral (*M. rectus ventralis*), lateral (*M. rectus lateralis*), medial rectus muscles (*m. rectus medialis*), the dorsal oblique (*M. obliquus dorsalis*) ventral oblique muscles (*M. obliquus ventralis*) and the retractor bulbi muscle (*M. retractor bulbi*) as like in domestic animals. Among extraocular muscles, m. dorsal oblique was the longest, m. lateral rectus was broadest and thinnest muscle and m. retractor bulbi was composed of four fascicles. The extraocular muscles were skeletal in nature, each muscle fascicle presented few homogenous slow twitch muscle fibers and more granular fast twitch muscle fibers. Nerve fiber bundles were more in number around the muscle fascicles in this study.

Keywords: Extraocular muscles, Sloth bear, gross, histology, histochemistry

Introduction

Bears are the youngest of the carnivore family, having arisen from dog-like ancestors during the Eocene Epoch, around 55 million to 38 million years ago. The sloth bear is originally thought to be a bear-like sloth due to its sloth like looks and behaviour. This bear is medium- sized and native only to southern Asia. These bears live in forests of India, Bhutan, Nepal and Bangladesh as well as in the island of Sri Lanka. The estimated number of bears still living in the wild is believed to be somewhere between 7000 and 10000. Sloth bears are classified as "Vulnerable" in the 1996 IUCN (International Union for Conservation of Nature and Natural Resources) Red List of Threatened Animals and is listed on Appendix I of CITES (Convention on the International Trade in Endangered Species of Flora and Fauna). In India trade and export of sloth bears is illegal and the bears are completely protected under Schedule I of the Indian Wildlife Protection Act of 1972 as amended in 1986 (IUCN Red List, Version 2014.2). Bears have swaying head movement because of their habitat and behavior physiology. They have poor near vision (Joshi *et al*, 1995) [10]. They identify termites by using olfaction as a food resource. The present study was conducted on the gross, histological and histochemical studies on the extraocular muscles of sloth bear. Sloth bear are scheduled animals and live in forest, mountains and caves. They are rescued from the human beings who are using these animals as game animals. The objective of this study was to characterize extraocular muscles in sloth bear with behaviour.

Material and Methods

The eyeball with intact extraocular muscles of six adult sloth bears and six whole heads with intact eyes were collected during post-mortem examination of bears that had died due to natural causes at the Wildlife Save Our Soul (WSOS), Bannerghatta Bear Rescue facility Centre (BBRC), Bannerghatta National Park, Bengaluru and were utilized for gross and histomorphological studies.

The eyeballs were removed according to the method of Keller (1975) [12]. The eyelids were sutured together with continuous suture pattern from the medial to lateral canthus and 5 to 10 mililitre of 10 percent Neutral buffered formalin infused into globe by using 18 gauge needle with syringe; the lids were then held and pulled out with an artery forceps and a scalpel knife was used to cut around the periorbital fat as traction was applied to the lids. This exposed the globe and further incision was made around the orbit, to reach the covering orbital fascia and extraocular muscles to reach the optic nerve and severing it. Further the eyeballs with intact orbital fascia and EOM were left in the 10 per cent NBF for 48 hours. The orbital fascia covering the eyeballs were carefully separated with the help of ophthalmic scissor and forceps from the eyeball to locate the EOMs. Carefully individual muscles were separated from the eyeball and length, width and thickness of each muscle were recorded using Vernier calipers, metal scale and non-absorbable thread (Joanna *et al*., 2003) [9]. After studying the morphological details of extraocular muscles, they were fixed in 10 % neutral buffered formalin for further histological and histochemical studies. The extraocular muscle tissues collected from sloth bear were processed by routine alcohol-xylene schedule and paraffin blocks were made (Luna, 1968) [18]. The sections were cut at 5-6 µm thickness and were stained by Haematoxylin and Eosin, Masson's trichrome method for collagen and muscle fibres (Luna, 1968) [18], Phosphotungstic Acid Haematoxylin (PTAH) method for skeletal muscles (Luna, 1968) [18], Gomori's sliver method for reticulum (Luna, 1968) [18], Bielschowsky's method for staining axis cylinders anddendrites (Luna, 1968) [18] and Periodic acid Schiff (PAS) technique for mucopolysaccharides (Luna, 1968) [18] study.

Results and Discussion

Carpenter (1977) [1], Konig and Liebich (2004) [14], Gellat *et.al*. (2013) [4] and Evans and de Lahunta (2013) [3] reported in domestic animals that the muscles important to the function of the eye constitute three groups: intrinsic the extrinsic and the palpebral muscles. The intrinsic muscles regulate the pupillary diameter and the shape of the lens. The palpebral muscle group includes the muscles of the lid and of the head; these regulate the shape and position of the palpebral fissure. The extrinsic muscles are concerned with the movement of the eyeball; rotate the globe around three perpendicular axes. The complex movements require the fine coordination of all these muscles, and they never act alone. In addition, the eyeball can be retracted into the orbit along the optic axis by the retractor muscle of the eyeball.

In the present study extraocular extended from the sclera and to the wall of the orbit. Extraocular muscles in turn were covered by the thick and tough orbital/periorbital fascia. There were four recti muscles: the dorsal, ventral, medial, and lateral rectus (Figs.1&2). These recti muscles were inserted on the sclera posterior to the limbus in right and left eyeball at a distance of 4.2±0.05 mm and 4.3±0.05mm, respectively (Table-2). The dorsal oblique and ventral oblique muscles were inserted in an intermediate area between the insertions of the recti and retractor bulbi muscle. Muscle retractor bulbi was made up of four fascicles and all the four fascicles continued rostrally under recti muscles and formed a cone in appearance (Fig.3). These muscle fascicles surrounded the optic nerve and inserted posterior to recti muscles on the sclera. The space between recti muscles and retractor bulbi muscle was filled with intraperiorbital fat. Similar observations were made by Getty (1975) [5]; Konig and Liebich (2004) [14]; Gellat *et al*. (2013) [4] and Evans and de Lahunta (2013) [3] in domestic animals. The functional significance of these muscles is to move the eyeball in different directions. The integration between voluntary and involuntary control of eye occurs is a subject of continuing research. However, it is known that vestibulo-ocular reflex plays an important role in the involuntary movement of the eye.

The average length of the dorsal rectus muscle on the right and left side was 5.10±0.19 cm and 5.13±0.16 cm, respectively (Fig.4 and Table.1). The average width of the dorsal rectus muscle on the right and left side was 1.11±0.16 cm and 1.11±0.11 cm, respectively. The average thickness of the dorsal rectus muscle on the right and left side was 0.25±0.02 cm and 0.22±0.01 cm. Kaseem *et al*. (1982) [11] assessed the dimensions of the dorsal rectus muscle in dog and it was 3.5 cm in length and 1 cm in thickness. Ridyard (2015) [21] observed 3.82 cm length of the superior rectus muscle in humans.

In the sloth bear the average length of the medial rectus muscle on the right and left side was 4.87±0.13 cm and 4.88±0.14 cm, respectively. The average width of the medial rectus muscle on the right and left side was 1.15±0.1 cm and 1.12±0.11 cm, respectively. The average thickness of the medial rectus muscle on the right and left side was 0.30±0.08 cm and 0.29±0.06 cm, respectively (Table-1). Kaseem *et al*. (1982) [11] mentioned the dimension of the medial rectus muscle in dog and recorded 3.2 cm in length and 0.9 cm in thickness. Whereas Ridyard (2015) [21] observed 3.85 cm length of the medial rectus muscle in humans.

The average length of the lateral rectus muscle on the right and left side was 4.80±0.10 cm and 4.83±0.09 cm, respectively. The average width of the lateral rectus muscle on the right and left side was 1.28±0.09 cm and 1.37±0.09cm, respectively. The average thickness of the lateral rectus muscle on the right and left side was 0.14±0.09 cm and 0.16±0.06 cm, respectively (Table-1). Whereas Kaseem *et al*. (1982) [11] assessed the dimension of the lateral rectus muscle in dog and it was 3.9 cm in length and 0.97 cm in thickness. Ridyard (2015) [21] observed 3.84 cm length of the lateral rectus muscle in humans.

In the sloth bear the average length of the ventral rectus muscle on the right and left side was 4.78±0.11 cm and 4.73±0.12 cm, respectively. The average width of the ventral rectus muscle on the right and left side was 0.77±0.09 cm and 0.73±0.12 cm, respectively. The average thickness of the ventral rectus muscle on the right and left side was 0.20±0.02 cm and 0.22±0.07 cm, respectively (Table-1). Whereas in dog Kaseem *et al*. (1982) [11] reported the dimensions of the ventral rectus muscle as 3.4 cm in length and 0.9 cm in thickness. Whereas Ridyard (2015) [21] observed 3.72 cm length of the inferior rectus muscle in humans.

In the present study the average length of the dorsal oblique muscle on the right and left side was 6.24±0.12 cm and 6.26±0.09 cm, respectively. The average width of the dorsal oblique muscle on the right and left side was 0.29±0.06 cm and 0.30±0.03 cm, respectively (Table-1). The average thickness of the dorsal oblique muscle on the right and left side was 0.16±0.01 cm and 0.12±0.03 cm, respectively. Kaseem *et al*. (1982) [11] recorded the dimension of the dorsal oblique muscle in dog and it was measured 3.1 cm in length and 0.5 cm in thickness. Whereas Ridyard (2015) [21] observed 3.92 cm length of the superior oblique muscle in humans.

Table 1: Morphometry of extraocular muscles in both eyes

Sl. No	Name of muscle	**Right** (cm)			**Left**(cm)		
		Length	**Width**	**Thickness**	**Length**	**Width**	**Thickness**
1	Dorsal rectus	5.10±0.19	1.11±0.16	0.25±0.022	5.13±0.16	1.11±0.11	0.22±0.01
2	Medial rectus	4.87±0.13	1.15±0.1	0.30±0.08	4.88±0.14	1.12±0.11	0.29±0.06
3	Lateral rectus	4.80±0.1	1.28±0.09	0.14±0.09	4.83±0.09	1.37±0.09	0.16±0.06
4	Ventral rectus	4.78±0.11	0.77±0.09	0.2±0.02	4.73±0.12	0.73±0.12	0.22±0.07
5	Dorsal oblique	6.24±0.12	0.29±0.06	0.16±0.01	6.26±0.09	0.3±0.03	0.12±0.03
6	Ventral oblique	5.15±0.13	0.6±0.13	0.16±0.05	5.17±0.09	0.5±0.16	0.18±0.08
7	Retractor bulbi	4.47±0.19	0.47±0.09	0.25±0.04	4.48±0.16	0.45±0.10	0.23±0.07

Table 2: Distance between the limbus to point of insertion of recti muscles of Sloth Bear

Animal number	Right eyeball (mm)	Left eyeball(mm)	P value
1	4.4	4.3	0.20
2	4.1	4.2	
3	4.2	4.2	
4	4.1	4.4	
5	4.3	4.2	
6	4.1	4.5	
Mean±SE	4.2±0.05	4.3±0.05	

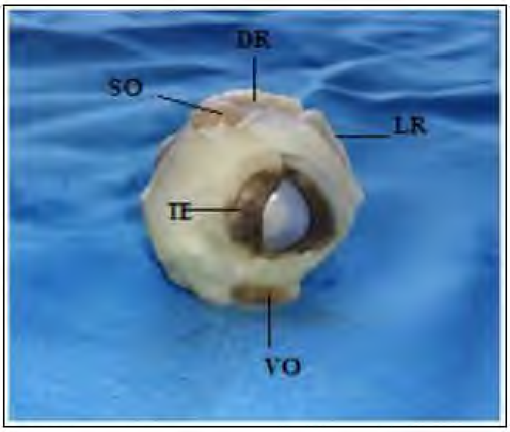


Fig 1: Photograph of sloth bear eyeball showing TE- third eyelid, DR- muscle dorsal rectus, SO-superior oblique, LR- lateral rectus, VO- ventral oblique.

Fig 2: Photograph of lateral view of sloth bear eyeball showing EOM A- lateral rectus, B- dorsal rectus, C- ventral rectus, D- medial rectus, E- dorsal oblique and F- ventral oblique.

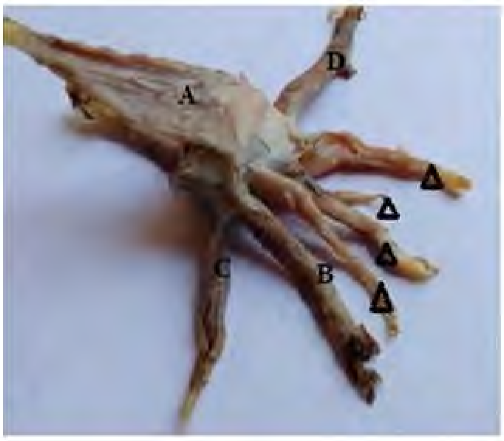


Fig 3: Photograph of sloth bear eyeball showing EOM A- lateral rectus, B- ventral rectus, C- ventral oblique, D- dorsal rectus and open arrow heads showing fascicles of retractor bulbi.

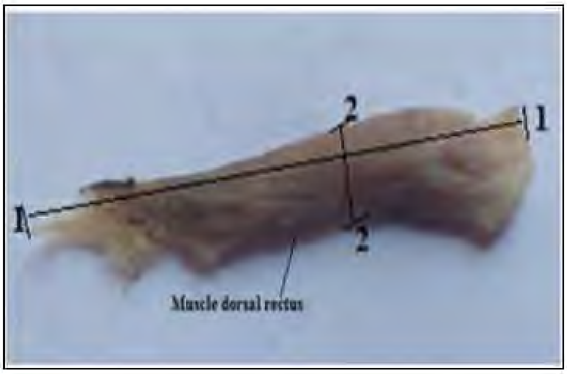


Fig 4: Photograph of muscle dorsal rectus of sloth bear showing 1-1-length of the muscle and 2-2- width of the muscle.

In the sloth bear the average length of the ventral oblique muscle on the right and left side was 5.15±0.13 cm and 5.17±0.09 cm, respectively. The average width of the ventral oblique muscle on the right and left side was 0.6±0.13 cm and 0.5±0.16 cm, respectively. The average thickness of the ventral oblique muscle on the right and left side was 0.16±0.05 cm and 0.18±0.08 cm, respectively (Table-1). Kaseem *et al*. (1982)

[11] described the dimension of the ventral oblique muscle in dog and he recorded that the ventral oblique muscle was 3.6 cm in length and 0.6 cm thick. Whereas Ridyard (2015) [21] observed 2.25 cm length of the inferior oblique muscle in humans.

The average length of the retractor bulbi muscle on the right and left side was 4.47±0.19 cm and 4.48±0.16 cm, respectively. The average width of the retractor bulbi muscle on the right and left side was 0.47±0.09 cm and 0.45±0.10cm, respectively. The average thickness of the retractor bulbi muscle on the right and left side was 0.25±0.04 cm and 0.23±0.07 cm, respectively (Table-1). Kaseem *et al*. (1982)[11] described the dimension of the retractor bulbi muscle in dog and he recorded that ventral oblique muscle was 3.4 cm in length and 0.4 cm thick.

According to Ridyard (2015) [21] muscle retractor bulbi is absent in humans. Ludinghausen *et al*. (1999) [17] observed the variations in the rectus muscles in humans which were thought to be the remnants of retractor bulbi and are responsible for preventing the protrusion of eyeball in most mammals.

The EOMs of sloth bear were oriented unidirectional. Each muscle fiber was separated by endomysium with large number of blood vessels and few nerve fibers (Fig. 5.6.7&12). Between the fascicle nerve fiber bundles and blood vessels were also seen. In cross section muscle fibers appeared oval/round/irregular/polygonal with peripherally located nuclei with prominent nucleolus (Fig. 5.6&7). The muscle fiber showed uniform distribution of cytoplasm without stippled appearance hence they were considered to be fine fibers, which were few in number as compared to other muscle fibers, which were stippled without homogenous cytoplasm and hence they were considered to be coarse fibers. These fibers were more in number with irregular shape (Fig. 8&9). Based on these morphologoical features animal can be classified having slow twitch ocular muscle. Strong PAS positive reaction observed around the individual muscle fiber indicating more of mucopolysaccharide in the connective tissue (Fig. 9 and 10).

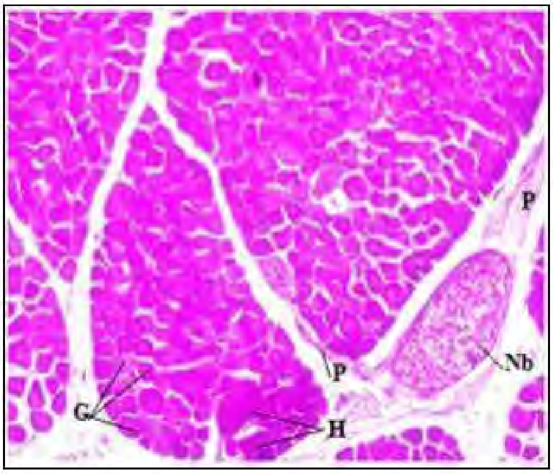


Fig 5: Photomicrograph of transverse section of muscle retractor bulbi showing G- muscle fiber with granular cytoplasm, H- muscle fiber with homogenous cytoplasm, P- perimysium and Nb- nerve bundle. (Haematoxylin and Eosin X100)

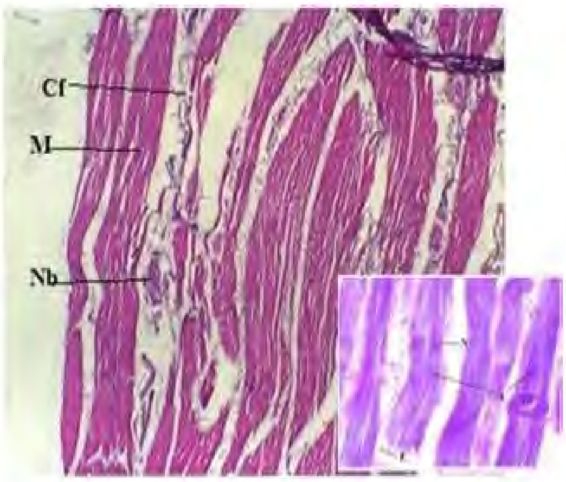


Fig 6: Photomicrograph showing horizotal section of muscle dorsal rectus M- muscle fibers orientation, Nb- nerve bundle and Cf- collagen fibers in perimysium (Haematoxylin and Eosin X100) and inset showing cross striations in lateral rectus muscle under PTAH stain X400

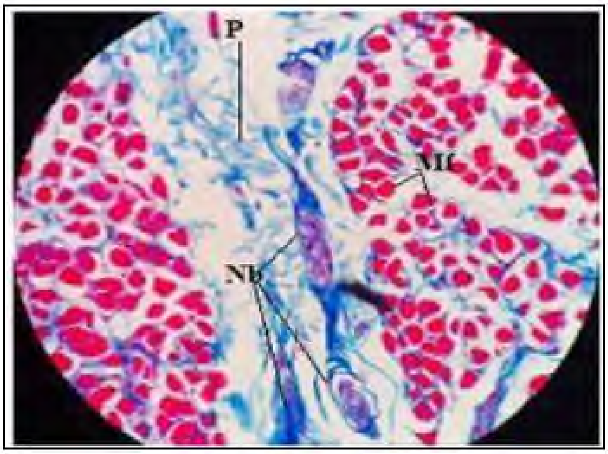


Fig 7: Photomicrograph of transverse section of muscle medial rectus showing P- Collagen fibers in perimysium, Mf- muscle fibers and Nb- nerve bundle (Masson's Trichrome X100)

Fig 8: Photomicrograph of transverse section of muscle ventral rectus showing N- nucleus of muscle fiber, G- granular type of muscle fiber, H- homogenous type and Cf- collagen fiber (Haematoxylin and Eosin X400)

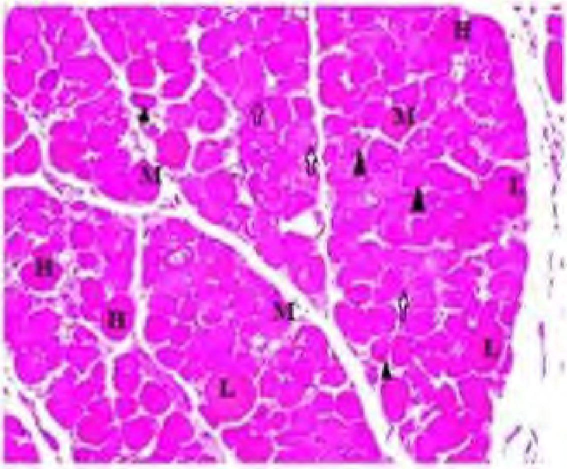


***Fig 9:** Photomicrograph of cross section of muscle ventral oblique showing L- large muscle fibers, M- medium and small muscle fibers (arrow heads) granular (open arrows) and H-nongranular type of muscle fibers (H aematoxylin and Eosin X100)*

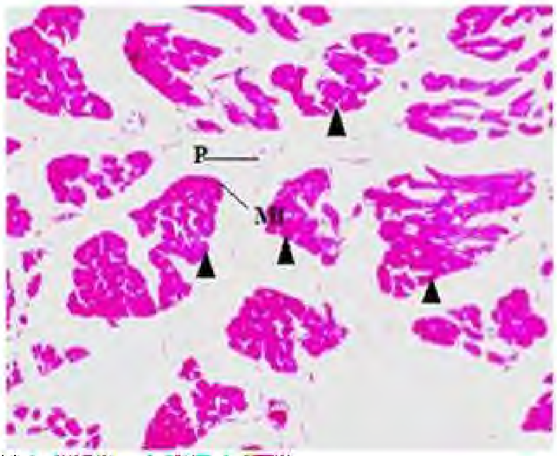


***Fig 10:** Photomicrograph of cross section of muscle ventral rectus showing positive reaction to PAS (arrow heads) P- perimysium and Mf- muscle fiber (Periodic acid Schiff X100)*

Three groups of muscle fibers were observed in sloth bear, large ones have homogenous cytoplasm while, medium and smaller ones have granular cytoplasm (Fig. 8&9). The muscle fibers having granular cytoplasm are considered to be fast twitch whereas non granular muscle fibers (large) which are few in number can be considered as slow twitch. Around the muscle fibers reticular fiber network was seen, which was continuous with perimysium (Fig. 11). Based on the diameter muscle fibers can be divided into large, medium, and small. The medium and small muscle fibers were irregular in shape and showed granular cytoplasm considered to be fast twitch (Fig. 8&9).

In conclusion it can be speculated that in sloth bear fast twitch muscle fibers are more as compared to slow twitch in the EOMs. Therefore, the movement of the eyeball exhausted quickly and need rest. Kruger (1949) [15] identified fast and slow fibers based on the quantity of sarcoplasmic reticulum. Ogata (1960) [20] has classified the muscle fibers based on the activity of oxidative enzymes such as ATPase.

In the present study extraocular muscle fibers also showed the presence of PAS positive activity uniformly. In the present study all EOMs showed strong PAS positive but were negative for acidic and sulfated mucolpolysaccharide. Whereas Lakshmishree *et al.* (2008) [16] identified glycogen content as weak to moderate in EOMs of the buffalo.
Dempsey and Wislocki (1944) [2] suggested that the higher concentrations of glycogen in

tissues accounts for low oxidative activity and provide energy through anaerobic pathway which is similar to the present finding showing higher concentration of glycogen in the EOMs.

The visual activity of the eyeball muscles are controlled by the brain in coordination with the retina as suggested by Jampel (2009) [8].

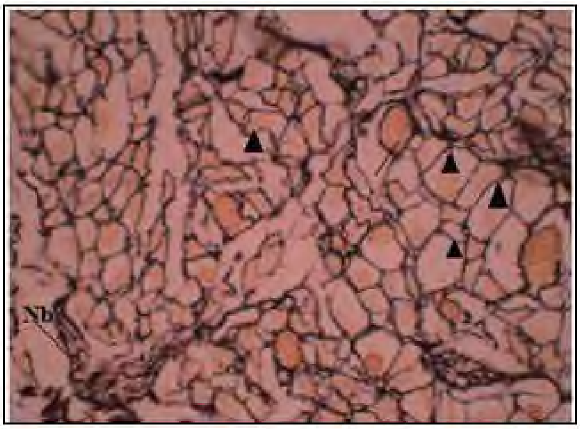


Fig 11: Photomicrograph of transverse section of muscle dorsal oblique showing reticular fibers (arrow heads) and Nb-nerve bundle (Gomori's X400)

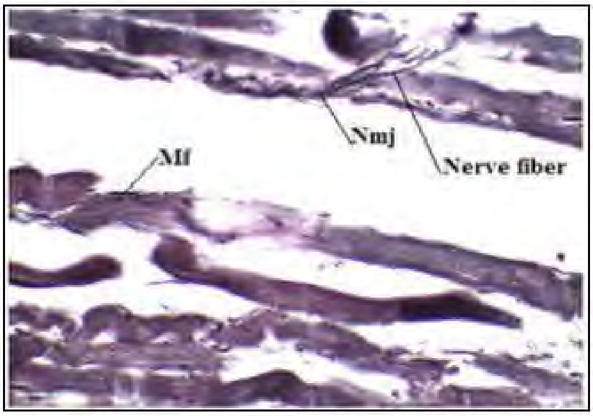


Fig 12: Photomicrograph of horizontal section of muscle medial rectus showing Nmj-neuromuscular junction and Mf-muscle *fiber* (Bielschowsky's X400)

Acknowledgements

Authors are highly thankful to the Wildlife SOS, BBRC, Bannerghatta National Park, Bengaluru and Department of Veterinary Anatomy, Veterinary College, Hassan, Bengaluru and KVAFS University, Bidar, for providing necessary facilities to carry out the study.

References

1. Carpenter RHS. Movements of the Eyes. London: Pion-London 1977.

2. Dempsey EW, Wisocki GB. Endocrinology 1944;35:409.

3. Evans HE, De Lahunta AGC. Miller`s Anatomy of the Dog, 5th *edn*., Elsevier Saunders, WB Saunders Company, Philadelphia, USA 2013, 746-781.

4. Gelatt KN, Gilger BC, Kern TJ. Ophthalmic Anatomy.In: Veterinary Ophthalmology. 5*th* *edn*., Blackwell Publishing Asia, Victoria, Australia 2013, 37-148.

5. Getty R. Sisson and Grossman's the Anatomy of the Domestic animals.5thEdn. W.B. Saunders Company, Philadelphia 1975, 1741-1754.

6. Humason GL. Animal tissue techniques, 4th Edn., W.H. Freeman and company, San Francisco, 1979, 212-213, 297-299.

7. IUCN. International Union for Conservation of Nature red list of threatened animals 2014.

8. Jampel RS. The function of the extraocular muscles, the theory of the coplanarity of the fixation planes. J Neuro. sci 2009;280(1-2):1-9.

9. Joanna K, Maciej J, Monika W, Norbert P. Morphologyof the extra-ocular muscles (*Musculi bulbi*) of the American Staffordshire Terrier during the perinatal period Folia. Morphol 2003;62(4):431-434.

10. Joshi AR, Garshelis DL, Smith JLD. Home ranges of sloth bears in Nepal: Implications for conservation. Journal of Wildlife Management 1995;59(2):204-214.

11. Kassem AM, Hifny A, Misk NA. Some histological and morphometrical studies of the extraocular muscles in dog. Assiut Vet Med J 1982;10(19):25-33.

12. Keller WF. Enucleation of the Eyeball. In: Joseph Bojrab's Current Technique in Small Animal Surgery1975.

13. Kern R. A comparative pharmacologic histologic studyof slow and fast-twitch fibers in the superior rectus muscle of the rabbit*. Invest Ophthalmol 1965;*4:901.

14. Konig HE, Liebich HG. Veterinary anatomy of domestic mammals. Textbook and colour atlas. Stuttgart, Germany 2004, *pp:*579-600.

15. Kruger P. Die innervation de tetanischen und tonischen Faserndel' quergestreiften skeletmuskulatur der wirbeltiere. Anat. Anz 1949;97:169-75. (fide Hodges. 1974.)

16. Lakshmi Shree KT, Prasad RV, Jamuna KV, Krishna Kakade, Dhoolappa M.

Histochemistry of extraocular muscles in buffalo (*Bubalus bubalis*). Ind. J Ani. Sci 2008;78(7):731-733.

17. Ludinghausen VM, Miuram M, Wurzler N. Variations and anomalies of the human orbital muscles. Surg Radiol Anat 1999;21:69-76.

18. Luna LG. Manual of Histological Staining Methods of Armed Forces Institute of Pathology. 3rd edn., McGraw Hill book Co, New York 1968.

19. Nomina Anatomica Veterinaria (NAV). ORGANUM SENSUUM. 6th edition. Published by the EditorialCommittee Hanover (Germany), Ghent (Belgium), Columbia, MO (U.S.A.), Rio de Janeiro (Brazil) 2017, 149.

20. Ogata T. The difference in some labile constituents and some enzymatic activities between the red and the white muscle. J Biochem 1960;47:726-32.

21. Ridyard E. Extraocular muscles: Variation in their anatomy, length and cross-sectional diameter. Int. J Anat Res 2015;3(3):1198-06.

Report – 265. DISTRIBUTION OF SNOW LEOPARD (*Panthera unica*) IN CENTRAL WILDLIFE DIVISION, KASHMIR

S. Swaminathan, A. Sha. Arun, Aliya Mir, Shabir Mir, Ashok Kumar, Thomas Sharp, Reegan P and **Yogaraj P**

Complete report was submitted to Department of Wildlife Protection J&K Government and the report can be accessed via email request at research@wildlifesos.org.

.

Report – 266. HIMALAYAN BROWN BEAR (*Ursus arctos isabellinus*) ECOLOGICAL AND HUMAN-BEAR CONFLICT INVESTIGATION IN KASHMIR WITH SPECIAL REFERENCE TO HABITUATION TO GARBAGE DUMPS IN THE CENTRAL WILDLIFE DIVISION

S. Swaminathan, Dr. A. Sha. Arun, Aliya Mir, Dr. Shabir Mir, Thomas Sharp, Reegan P, Yogaraj P

The complete report was submitted to Department of Wildlife Protection J&K Government. Report is also available online at

http://www.indiaenvironmentportal.org.in/content/474199/himalayan-brown-bear-ursus-arctos-isabellinus-ecological-and-human-bear-conflict-investigation-in-kashmir-with-special-reference-to-bear-habituation-to-garbage-dumps-in-the-central-wildlife-division/

and the report can be accessed via email request at research@wildlifesos.org.

Art – 267. A COMPARATIVE STUDY ON PHYSIOLOGICAL PLASMA MINERAL MAPPING OF SLOTH BEARS *(Melursus ursinus)* IN *IN-SITU* VIS-A-VIS SEMI CAPTIVITY CONDITIONS

A.Sha. Arun, S.K. Bhat, L. Jose, N.K.S. Gowda, D.T. Pal, P.S. Swain and P.M. Sidharth

Abstract

To determine if habitat influenced the physiological plasma mineral profiles in sloth bears, a study was conducted to establish the normal physiological plasma mineral profiles of free-ranging and semi-captive Indian sloth bears. Plasma samples from 28 sloth bears were analysed for their mineral concentrations. The concentration of microminerals (mg L^{-1}) namely Zn, Co, Fe, Mn, Cr, Cu respectively were 1.56±0.90; 0.85±0.42; 3.85±1.11; 0.29±0.27; 0.45±0.01; 1.00±0.85; and macro minerals (mg dL^{-1}) like Mg, Ca, Na, K, P were 2.06±0.72; 8.89±2.28; 42.3±5.96; 10.7±2.04; 23.2±3.55, respectively. It was found that plasma of free ranging sloth bears showed higher concentrations of Zn, Fe, Mn, Cu, Mg, Na, K, whereas, samples of bears in semi captivity showed higher Co, Ca, P, and Cr levels, which signifies that free ranging animal have a choice over their feeding but in semi-captivity, it is controlled and man-made.

Key words: Free ranging, habitat, mineral profiles, semi-captivity, Sloth bear

Introduction

The Sloth bear is endemic to the Indian sub-continent and found in India, Sri Lanka, Nepal, Bhutan, and Bangladesh. In India, sloth bears are distributed from the southern tip of the Western Ghats to the foothills of the Himalayas. Sloth bears (*Melursus ursinus*) have been listed as vulnerable by the IUCN red list with the estimates of about 20,000 or fewer individuals and less than 10,000 adults existing in the wild. The wild population of sloth bears has declined by 30 – 49% in the last 30 years (Graesli et al., 1999). Sloth bears in India have been exploited for generations as “dancing bears”. Wildlife SOS, a non-profit organization working to protect and conserve the natural resources of India, secured the end of the dancing bear trade. Relinquished and confiscated sloth bears now reside in four rescue/rehabilitation centers in India, overseen by Wildlife SOS.

Sloth bears inhabit the tropical and subtropical regions of the Indian subcontinent and are distributed from the foothills of the Himalayas to the southern end of the Western Ghats Mountain range, as well as in the island of Sri Lanka (Prater, 1965). Despite long periods of evolutionary separation, sloth bears have retained their carnivorous morphology but have developed unique physiologic adaptations to live in tropical/ subtropical climates, with substantial reliance on frugivory (feeding on fruits) and myrmecophagy (feeding on insects like termites or ants) (Laurie andSeidensticker, 1977; Gokula et al., 1995; Bargali et al., 2004). Both the micro and macro mineral content in the blood varies according to the feeding habit and available feed resources. Sloth bears have metabolic differences compared to other species of bears. They have been observed to have an overall lower metabolic rate compared to brown and polar bears, and torpor (winter sleep) is unheard of in case of sloth bears (McNab, 1992). Minerals are inorganic components which are required in very minute quantities, yet very important for the normal physiology of animals. Assessment of the plasma mineral profile is an indication of the physiological as well as nutritional status of an animal. Blood mineral profile is important in understanding the impact of disease both at an individual and population level of animals. Macro-minerals namely calcium (Ca), phosphorus (P) and magnesium (Mg) and micro-minerals namely, copper (Cu), zinc (Zn) and cobalt (Co) are essential for fundamental physiological functions like cell morphology, integrity, motility, hormone secretion and metabolism (Kong et al., 2015; Diskin, 2016). In this study, we have tried to establish the reference plasma mineral profile of sloth bears residing in free ranging wild as well as in semi-captivity. We also have tried to observe the change in the plasma mineral profile associated with change in the habitats.

Material And Methods

Study site and animals

Free-ranging wild sloth bears rescued from human animal conflict situations in the state of Karnataka and captive sloth bears rehabilitated at lifetime care centre of Wildlife SOS at Bannerghatta Bear Rescue Centre were utilized for this study. The samples were collected from 28 numbers of sloth bears from both free ranging wild sloth bears and semi-captive sloth bears rehabilitated at the Bannerghatta Bear Rescue Centre, Bannerghatta Biological Park, Bangalore, Karnataka, India. Both these locations are within the documented habitat range for sloth bear species. The sloth bears in the wild were free ranging ones and the ones in captivity were individually rehabilitated under different situations and kept under captivity for more than 7 - 10 years. All the sloth bears were apparently healthy. The adults were having normal behavioural responses and found to be clinically healthy during examination at the time of sampling (as determined by body temperature, hydration, heart/respiration rate, and a detailed external physical examination).

Sampling procedure

Each bear was sampled only once for this study. Sloth bears were immobilized using a ketamine-xylazine combination; ketamine hydrochloride (5 mg kg^{-1} body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg kg^{-1} body weight; Troy Laboratories Pty Ltd., Smithfield, New South Wales 2164, Australia). These drugs were administered using a distance projectile drug delivery system. Blood was collected from the jugular vein within 10 min after immobilization using a 20-gauge sterile hypodermic needle in vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA), with heparin for mineral studies. Samples were immediately stored on cool packs at 4–8 °C and transported to the testing laboratory.

Laboratory analysis

1 ml of the plasma sample was taken and 1 ml of 5N HCl was added to it. The volume was made to 10 ml by adding double distilled water (1:10 dilution). Mineral contents in plasma samples were estimated using Optima 8000 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES;Perkin Elmer, Shelton CT#064840, USA).

Statistical analysis

After quantifying the mineral concentration, the collected data was tabulated. Descriptive statistical analysis like mean ± SD (Standard deviation), SEM (Standard error mean), range and 95% CL (Confidence level) of mean were calculated for captive, wild and entire population respectively. The significance of difference in the mean value based on habitat was performed by independent t-test for normally distributed data and non- parametric Mann Whitney U-test for variables that violated the normality. The normality test was conducted by Kolmogorov-Smirnov and Shapiro- Wilk test. The homogeneity of variance of normally distributed variable was conducted by Levene's test and for non- parametric data by Kruskal-Willis one-way ANOVA test. All processing of data was conducted with the software packages Microsoft Excel 2010 for data storage and SPSS version 21 for statistical analysis. The P-values $P<0.05$ and $P<0.01$ with an alpha level of 95% were assumed as statistically significant (*) and highly significant (**) respectively.

Results And Discussion

Basic descriptive statistical analysis on different mineral concentrations of entire (semi- captive and free range) populations (Table 1) were established as a standardised value. Mean value of each micro-mineral and macro-mineral parameter based on habitat (Table 3) were compared to evaluate the significance difference between each other. Comparing the mean of these mineral concentrations, it was found that Zn, Fe, Mn, Cu, Mg, Na and K were higher in wild population, whereas Co, Cr, Ca and P were higher in captive populations (Fig. 1 and 2). By normality test it was determined that Zn, Co, Fe, Mn, Cu, Mg, Na, K, Cr and Ca were deviating from the normal distribution curve by the Normality test as shown in Table 2. Mean (± S.D.) of Cu was found to be significantly higher in wild (1.74±0.92) as compared to captive populations (0.594±0.46) (Table 3). Mean (± S.D.) of Mg was found to be significantly higher in the wild conditions (2.57±0.79) as compared to captive. (1.78±0.51) (Table 3). Mean (± S.D.) of Nawas found to be significantly higher in wild habitat(45.5±6.30) as compared to captivity (40.4±5.06) (Table 3). Mean (± S.D.) of Zn, Fe, Mn and K were found to be higher in the wild (1.68±1.35), (4.21±1.55), (0.40±0.43) and (11.6±2.53) as compared to captivity (1.49±0.56), (3.65±0.76), (0.22±.06) and (10.2±1.60), respectively but not significantly related (Table 3). Mean (± S.D.) of Co(0.93±0.40) and Cr (0.45±0.01) were found to be significantly higher in semi-captivity as compared to wild habitat (0.71± 0.42) and (0.44±.01) respectively (Table 3). Mean (± S.D.) of Ca and K were observed to be higher in captivity (9.20±2.49)and (23.8±4.22) as compared to wild habitat (8.33± 1.83) and (22.1± 1.45), respectively but not significantly related with each other (Table 3).

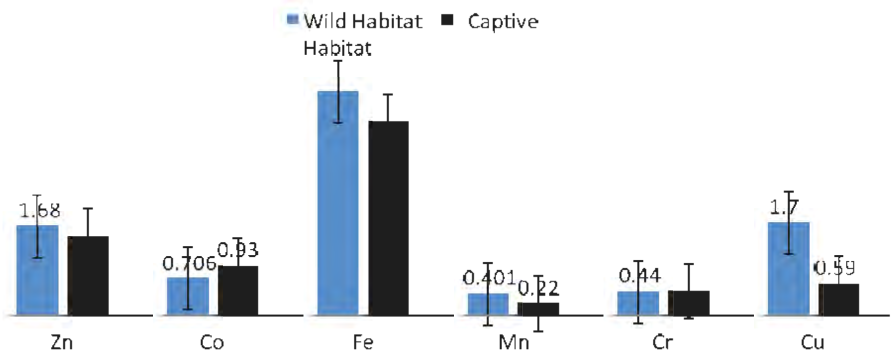


Fig. 1. Comparative study of micro-minerals with respect to habitat

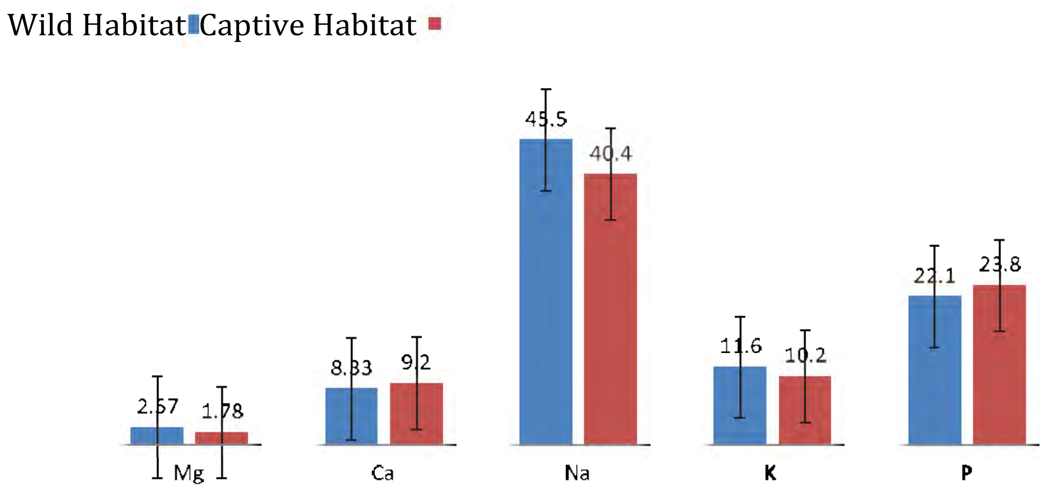


Fig. 2. A comparative study of macro-minerals with respect to habitat

Table 1. Statistical analysis of mineral concentrations of entire (semi-captive and free range) Sloth bear population

		Range	95%CL of Mean				
Parameters	**Sample Size**	**Mean ± SD**	**SEM**	**Minimum**	**Maximum**	**LCL**	**UCL**
Zn (mg L^{-1})	28	1.56±0.90	0.17	0.32	4.14	1.26	1.90
Co (mg L^{-1})	28	0.85± 0.42	0.08	0.37	1.23	0.70	1.00
Fe (mg L^{-1})	28	3.85±1.11	0.21	2.40	7.80	3.49	4.25
Mn (mg L^{-1})	28	0.29± 0.27	0.05	0.14	1.62	0.22	0.40
Cr (mg L^{-1})	28	0.45± 0.01	0.00	0.43	0.48	0.44	0.46
Cu (mg L^{-1})	28	1.00±0.85	0.16	0.01	2.76	0.70	1.31
Mg (mg dL^{-1})	28	2.06±0.72	0.14	1.11	3.81	1.83	2.32
Ca (mg dL^{-1})	28	8.89±2.28	0.43	5.97	15.4	8.09	9.72
Na (mg dL^{-1})	28	42.3±5.96	1.12	26.5	51.0	39.9	44.4
K (mg dL^{-1})	28	10.7±2.04	0.38	8.08	16.5	10.0	11.4
P (mg dL^{-1})	28	23.2±3.55	0.67	16.2	28.7	21.8	24.5

Table 2. Normality test of mineral concentrations of Sloth bear population

Parameters	Kolmogorov-Smirnov[a]	Shapiro-Wilk
Zn (mg L^{-1})	0.001	0.001**
Co (mg L^{-1})	0.000	0.000**
Fe (mg L^{-1})	0.180	0.002**
Mn (mg L^{-1})	0.000	0.000**
Cr (mg L^{-1})	0.000	0.002**
Cu (mg L^{-1})	0.200#	0.012*
Mg (mg dL^{-1})	0.004	0.002**
Ca (mg dL^{-1})	0.000	0.001**
Na (mg dL^{-1})	0.200#	0.040*
K (mg dL^{-1})	0.200#	0.041*
P (mg dL^{-1})	0.200#	0.359

Table 3. Analysis of Homogeneity of Variance and comparison of mean mineral value of sloth bear population based on habitat

Parameters	Free Ranging Wild				Captive				f-value	p-value
	Sample Size	Mean±S.D.	95% CL of Mean		Sample Size	Mean±S.D.	95% CL of Mean			
			LCL	UCL			LCL	UCL		
Zn (mg L^{-1})	10	1.68±1.35	0.98	2.57	18	1.49±0.56	1.25	1.75	[a]0.737	[d]0.759
Co (mg L^{-1})	10	0.710.42±	0.46	0.95	18	0.93±0.40	0.75	1.12	[a]0.026*	[d]0.024*
Fe (mg L^{-1})	10	4.21±1.55	3.35	5.17	18	3.65±0.76	3.33	4.00	[a]0.314	[d]0.332
Mn (mg L^{-1})	10	0.40±0.43	0.24	0.69	18	0.22±0.06	0.19	0.25	[a]0.061	[d]0.064
Cr (mg L^{-1})	10	0.44±0.01	0.44	0.44	18	0.45±0.01	0.45	0.46	[a]0.005**	[d]0.004**
Cu (mg L^{-1})	10	1.74±0.92	1.17	2.30	18	0.59±0.46	0.38	0.79	[a]0.003**	[d]0.003**
Mg (mg L^{-1})	10	2.57±0.79	2.09	3.06	18	1.78±0.506	1.58	2.02	[a]0.003**	[d]0.003**
Ca (mg L^{-1})	10	8.33±1.83	7.27	9.40	18	9.20±2.49	8.16	10.3	[a]0.472	[d]0.494
Na (mg L^{-1})	10	45.5±6.30	40.9	48.6	18	40.4±5.06	37.8	42.7	[a]0.005**	[d]0.003**
K (mg L^{-1})	10	11.6±2.53	10.1	13.0	18	10.2±1.60	9.51	11.0	[a]0.137	[d]0.146
P (mg L^{-1})	10	22.1±1.45	21.3	22.9	18	23.8±4.22	21.8	25.7	[b]0.001	[c]0.131

On analyzing the homogeneity of variance, it was found that concentrations of Co, Cr, Cu, Mg and Na were found to be significant ($p<0.01$) with respect to habitat while other parameters were found to have no significant effect.

Literature pertaining to the wild Sloth bear plasma mineral profile is very limited. Perhaps, this is the first study of its kind where we have tried to establish the normal physiological range of sloth bears and its variation when they are free ranging or when kept in semi captivity. Mineral content in the plasma varies according to the feeding habit and available feed resources. Both the micro and macro minerals differ in blood samples of the sloth bears under different habitats, which can be attributed to the availability of the feed sources. Plasma of free ranging bears showed a higher Zn, Fe, Mn, Cu, Mg,Na, K, whereas, that in semi captivity showed higher Co, Ca, P and similar Cr level, which signifies that the animals in free ranging have a choice over their feeding, but in semi captivity it is somewhat controlled. However, in semi captivity, they had free access to the forest apart from being fed.

Graesli et al. (2014, 2015) studied the change in the macro-mineral profile in brown bears. The plasma calcium content in this study was lower than the reported values of Graesli et al. (2015) in brown bears inhabiting in Sweden which might be due to the change in the geographical location, or the type of bear studied. This can be correlated to the availability of feed and the animal's feeding habits as well. Regarding Mg, the result obtained in the present study corroborates with the reported values of Graesli et al. (2014, 2015).

Conclusion

Considering the plasma mineral status in free ranging and semi-captive habitat, a significant change in Co ($P<0.05$), Cr ($P<0.01$), Mg ($P<0.01$), Na ($P<0.01$) was observed, which may be attributed to the availability of feed resources and the selective feeding behaviour of the animal. There was no change ($P>0.05$) in other mineral status.

For assessing the animal health, plasma mineral status is an important indicator. An attempt was made to establish the reference values of plasma mineral profile for apparently healthy Indian sloth bears.

Acknowledgement

We greatly appreciate the support of K. Satyanarayan and G. Seshamani of Wildlife SOS, who made these studies possible. We thank the authorities of Karnataka Forest Department and Bannerghatta Biological Park for their kind co-operation.

References

Bargali, H.S. 2005. Characteristics of sloth bear attacks and human casualties in North Bilaspur Forest Division, Chhattisgarh, India. *Ursus* 16: 263–267.

Diskin, M., Waters, S., Parr, M. and Kenny, D. 2016. Pregnancy losses in cattle: potential for improvement. *Reprod. Fertil. Dev.* 28: 83–93.

Gokula, V., Sivaganesan, N. and Varadarajan, M. 1995. Food of the sloth bear (*Melursus ursinus*) in Mundanthurai Plateau, Tamil Nadu. *J. Bombay Nat.Hist. Soc.* 92: 408–410.

Graesli, A.R., Evans, A.L., Fahlman, A., Bertelsen, M.F., Blanc, S. and Arnemo, J.M. 2015. Seasonal variation in haematological and biochemical variables in free-ranging sub adult brown bears (*Ursus arctos*) in Sweden. *BMC Vet. Res.* 11(1): 301.

Graesli, A.R., Fahlman, A., Evans, A.L., Bertelsen, M.F.,Arnemo, J.M. and Nielsen, S.S. 2014. Hematological and biochemical reference intervals for free-ranging brown bears (*Ursus arctos*) in Sweden. *BMC Vet. Res.* 10(1): 183.

Kong, B.Y, Duncan, F.E., Que, E.L, Xu, Y., Vogt, S., O'Halloran, T.V. and Woodruff, T.K. 2015. The inorganic anatomy of the mammalian preimplantation embryo and the requirement of zinc during the first mitotic divisions. *Dev. Dynam.* 244:935–947.

Laurie, A. and Seidensticker, J. 1977. Behavioral ecology of the sloth bear (*Melursus ursinus*). *J. Zool. London*182: 187–204.

McNab, B.K. 1992. Rate of metabolism in the termite- eating sloth bear (*Melursus ursinus*). *J. Mammol.* 73: 168–172.

Page, C.D. 1986. Sloth bear immobilization with a ketamine-xylazine combination: reversal with yohimbine. *J. Am. Vet. Med. Assoc*. 189(9):1050- 1051.

Prater, S. H. 1965. *The Book of Indian Animals*. Bombay Natural History Society and Prince of Wales Museum of Western India publication, Bombay, Maharashtra, India pp. 324–325.

Art – 268. FEEDING ECOLOGY OF INDIAN LEOPARD (*Panthera pardus fusca*)IN THE HIREKALLUGUDDA SLOTH BEAR SANCTUARY (PROPOSED SANCTUARY) IN ARASIKERE, KARNATAKA, INDIA

Yogaraj Pannerselvam, Reegan P, Attur Shanmugam Arun, Kartick Satyanarayan, Geeta Seshamani and **Shanmugavelu Swaminathan**

Abstract

The feeding ecology of the Indian leopard (*Panthera pardus fusca*) was studied from February 2016 to September 2016 a total of 54 scat samples. These leopards feed on a variety of wild and domestic animals. Diet composition of the leopard will be primarily studied through scat analysis will be collected from well-defined sampling areas within the study period. A total of 12 prey species were successfully identified based on the microscopic hair analysis, percentage frequency of occurrence of prey hair, bones, or claws, in the scats and estimation of standard error using a regression equation and relative biomass consumed also recorded. However, the data regarding their diet composition is scanty and scat analysis revealed that the wild animals (45.00%) was their preferred diet, and domestic species including with dog and livestock were more frequently consumed (52.00%) while Sambar deer (23.70 %) and Goat (21.30 %) was most commonly used as diet during seasonal variations. Other species include porcupine, wild boar, hare, monkeys, peafowl, spurfowl, and mongoose some domestic species of sheep, cow, and some unknown species. Furthermore, the questioner's survey is recorded for human-leopard conflicts with investigations to cattle watchers also assessed.

Keywords: Arasikere, Leopard scat, Prey species, Livestock, Conflict.

Introduction

The Leopard (*Panthera pardus fusca*) has a wide range distribution from Africa to l parts of western and central Asia, in the Indian subcontinent to southeast and east Asia. The leopard is a large carnivore species listed as Vulnerable on the IUCN Red List because of human pressures and forest range fragmentations. It is reported that the leopard is found in almost every kind of habitat from the rain forest of the tropical forests, grassland plains to deserts and temperate region (Kitchener, 1991; Nowell & Jackson, 1996). In India, leopard is endangered with half of the estimated 14,000 leopards living outside protected areas. Given the threats the animals face today ranging from conflict with human and habitat destruction due to explosion for the human pressures, loss of wild prey, poaching for skins, bones, claw, and poisoning carcasses of livestock killed by leopards to places threats to the leopards (Kitchener, 1991). Therefore, the populations living nearby of the human habitations are on the edge of extinction(Carter *et al*., 2012). In India, where the border between jungle area and rural inhabitations could be a range, the leopards stay within the human habitat area. Such as very low density of wild prey, which has, as a result, required leopards depredate attack to humans and livestock, is that the ultimate cause of conflict with local villages and human habitat area (Schaller *et al*., 1988). The animal moved one place to a different area according to the availability of livestock and wild prey and leopard's preferences and incidences of depredation (Knowlton *et al*., 1999; Meriggi & Lovari, 1996). Leopards are considered as ecological generalists instead of specialists and reported feeding on large or small prey, they will be efficient scavengers and that they aren't averse to preying on domestic stock, a behavior that brings them into direct conflict with humans (Bailey, 1993). This is often mainly true in a country like India, where the human population is extremely high together with one in every of the highest density of livestock in the world (Robinson *et al*., 2014). Availability of prey is one of the most critical cyclic of broad carnivore distribution, but wild prey may occur at very low densities in human-habitat (Karanth *et al*., 2004; Khorozyan *et al*., 2015). Livestock depredation by carnivores has become an essential hurdle in the conservation of predators at the top of the food chain. One of the critical aspects of the conservation of wild carnivores in human-dominated landscapes is the attitude of the affected people (Rahalkar, 2008; Thavarajah, 2008).

According to Lal, (1989), most of India's livestock depend on forests for its grazing requirements and when the leopards share their forest and non-forest area habitats with domestic livestock there is inevitably some leopard predation on livestock. In India leopard food habitats had been studied by Johnsingh (1983); Karanth, 1993; Kumaraguru, 2002; Schaller, 1967; Swaminathan *et al*.,2002; Venkataraman *et al*., 1995) the major prey species reported being chital, sambar and few percent of the livestock. Studies reports that leopard had more diverse diet ranging from lower size classes of animals to medium-sized wild species in Protected Areas of South Asia (Eisenberg & Lockhart, 1972; Rabinowitz, 1989; Seidensticker, 1990). Livestock predation is recorded in all the Project Tiger Reserves irrespective of adequate wild prey available in them (Sawarkar, 1986). Negi *et al*., (1996) reported that 61 human beings were killed and injured by man-eating leopards whereas fifty leopards were killed in the Garhwal district of the U.P. between 1986 and 1996.

Recent studies on leopard attacks on humans and livestock have been conducted in Arasikere. The primary food for the leopard hunts the forest presently atherogenic presser increased near human settlements to predate on livestock and domestic animals in areas with abundant wild prey. These conflicts pose a major problem not only in Arasikere but also result to develop recommendations for control with conflict and mitigating human-leopard conflict in the Hassan district. Hirekallugudda sloth bear sanctuary (13° 22′N and 76° 17′E), Hassan District, Karnataka, with an expanse of ca. 15491.09 ha. state of the South-Western part of leopards at a large geographical scale, across Karnataka State in southern India (Athreya *et al*., 2015). The Sanctuary (Figure. 1) is small, isolated area, about Nagapuri-Hirekallugudda reserve forest surroundings 27 fringe villages along borders. The habitat is mainly rocky, with large boulders, dry deciduous scrub, and southern thorn forests. The area has an elevation range between 510-1100 m above mean sea level. The vegetation in this region exists in a degraded state although a forestation program had ensured prominent plants in individual pockets. The study area has a semi-arid climate characterized by hot summers (24.2°– 42°C) during April– June and low rainfall (500-800 mm) from June to November. The rising conservation conflicts in the study area can be attributed to leopard evoked livestock injuries leading to more than 32 villages getting affected in the last four years in the region and also huge livestock loss in 22 villages affected in the previous 3 years protected area reserved for the conservation of wild animals and reduce human wildlife conflict based on scientific knowledge.

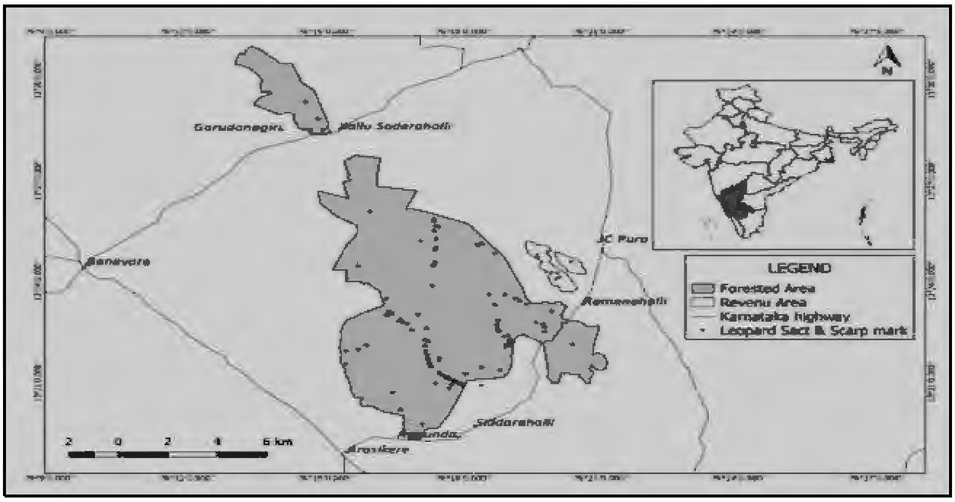


Figure 1. Map showing the location of the study area Hirekallugudda Sloth bear sanctuary (proposed sanctuary) in Arasikere, Hassan district, Karnataka.

Materials And Methods

Leopard scat collection

Leopard scats were collected whenever encountered in the study area during from February 2016 to September 2016 the study period, interviews were conducted with the cattle watchers and village peoples to get preliminary information on the presence of leopard in the area followed by signs survey various flat livestock trails, or footpath terrains available within the field areas collection of the leopard scats. The scats were collected in paper bags and the date, locality, and Global Positioning System (GPS) locations were noted. Sampling sites would be selected based on different levels of human disturbance.

Leopard Scat analysis

The diet composition of the leopard will be primarily studied through the scat analysis technique, as this is a non-invasive method that easily provides large samples (Johnsingh, 1983; Karanth & Sunquist, 1995). A total of 75 scat samples were collected in the field but a sample size of 54 Scat will be taken from well-defined sampling transect areas within the study period. Each scat was then triturated with water and then passed through a sieve. All the scat was weighed to observe the percentage of each part. Prey species were identified based on the component microscopic scat contents that were then teased apart with forceps, and undigested prey remains such as hair, bones, skin, claws, mandible, and other materials were separated for species identification. Unprocessed prey hair which remained in the scat after washing was used for the identification of prey species as described by Grobler & Wilson, (1972); Mukherjee *et al*., (1994). The scat analysis method was chosen to estimate the proportion of different prey species consumed by a leopard (Link & Karanth, 1994; Schaller, 1967; Sunquist, 1981). The single scat taken with one bowl put the water washed then after used forceps five-time randomly take the sample further they were placed on the glass slides, hairs were randomly observed the percentage of each part that diet species. The prey composition of the predator scats was extrapolated in terms of the prey frequency of occurrence in the scat samples (FO), calculated by equation-E (Karanth & Sunquist, 1995; Mizutani, 1999; Pikonov & Korkishko, 1992; Ramakrishnan *et al*., 1999). FO= (ni/N) 100 equation (E) Where ni is the number of scats where a given in the prey species residue occurs and N is the total number of all scat samples.

Relative Biomass Calculation

The relative biomass consumption was estimated by using the linear relationship developed by Y = 1.98 + 0.035X; where Y = weight of prey consumed per scat, and X = average weight of the prey species the applied in the form of a correction factor, to convert frequency of occurrence to relative biomass consumed. As the frequency of occurrence does not give the exact estimation relative biomass was recorded.

Human-Leopard Conflict

For analyzing, leopard-cattle conflicts, interviews of the attack viewers was conducted for cattle watchers using a standard questionnaire. Based on the village surveys, key findings to incidents of livestock depredation within forest, site visits at the time of cattle grazing about 1 km from the buffer area in Hirekallugudda-Arasikere. The information was collected under locations of livestock depredation that were visited and data on species, season, GPS location, etc. were recorded. Identification of predator was based on the direct sightings at cattle watching time, observations of villagers and collected information based on questioners.

Results And Discussion

In the present study of feeding ecology Leopard 540 prey items (12 species) were identified in the study period. About 45% of the species devoured by leopards were of wild origin during study most of the prey species were domestic livestock while domestic species constituted to be 52% of the leopard diet including dog and livestock animals (Figure. 2). About three percent of the leopard prey species were unidentifiable. Taghdisi *et al*., (2013) also reported that wild ungulate including wild sheep, wild goats, and wild pig are amongst the most preferable food items of leopards accounting for 85.98% of their consumed biomass compared to 3.26% biomass consumed for livestock. Three species of domestic animals, i.e. goat, cow, and local domestic dog, were identified from the scat samples of Leopard, of which goat was the most preferred dietary species during the season Feb-Sept 2016.

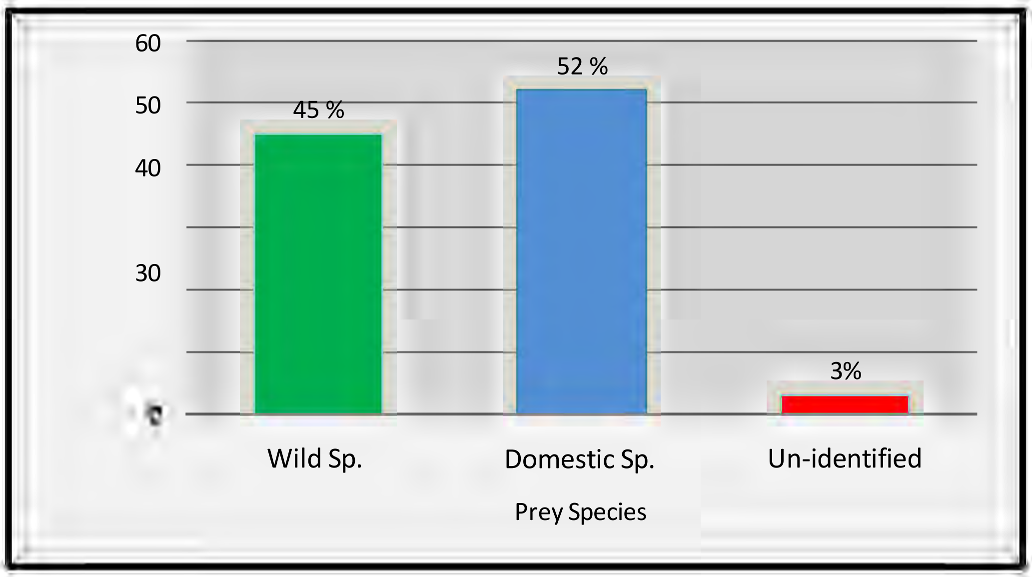


Figure 2. Percentage occurrence of prey species of Indian leopard in Hirekallugudda Sloth bear sanctuary (proposed sanctuary) Arasikere.

During the present study, the dietary preference of the leopards was studied by the relative frequency percentage calculated based on leopard scat collected (n:54). The study exposed that there was a tremendous significant variation in the leopard scats processed comprising of a total of 12 prey items with their percent wise occurrence given in (Table. 1) the present study focused on the dietary preferences of a leopard during the period of Feb-Sept 2016. It was found that Sambar deer was the most preferred prey species (23.70%) of the leopard diet. Contradictory reports have been published by Mukherjee *et al*., (1994). It was reported that leopards most frequently preyed on chital meat (64.7%). Ecosystems with cohabitation of humans and livestock include the highest density of domestic animals like a goat (21.30%) and Sheep (13.15 %) which may be another prey species level to leopard attacks. In the present study, one of the important prey occurrences of Domestic dog (12.59%) of the cattle watchers used the dog for guarding their cattle while grazing in the forest buffer zone since leopards can be easily hunted by domestic dogs, where leopard attacks were by far the dominant cause of death. The most abundant and readily available prey species was the Sambar deer followed by wild boar (7.78%) Black-naped hare (3.52%) and other prey species (Figure 3). Followed by native prey species, Porcupine, Un-identified, and Peafowl (2.96%) had a higher occurrence than mongoose and bonnet macaque (0.74 and 0.93%) in low occurrence leopard scats sampled. Besides the above animals, it was also noted that Hare was one of the preferred prey items that had a 7.96% frequency of occurrence. Due to Porcupine and peafowl being confined, their rate in the leopard scats was limited however dog and peafowl had a similar appearance. Concerning livestock prey species, goat (21.30 percent) and cow (5.19 percent) were preferred species high occurrence of the scat. However, (Mukherjee *et al.*, 1994) reported an 18 percent occurrence of livestock in leopard scat comprising of buffalo and cow with no presence of goat. Livestock depredation in form of calves of cow and buffalo and kids and scavenging on kills of a cow made by a lion was noted in two instances. Spurfowl (2.22%) was a significantly less common prey species devoured by leopards. In conclusion, the prey selection nature of the leopard was found to be drastically variable and was based solely on the availability of the prey species ranging from fowl to sambar deer.

Table 1. Occurrences of Prey Frequency based on hair in Indian leopard scat (n=54) in Hirekallugudda Sloth bear sanctuary (proposed sanctuary) Arasikere.

S.No	Species	Frequency	Occurrence (%)	Mean	SE
1	Black-naped hare	19	3.52	2.11	0.21
2	Indian Porcupine	16	2.96	2.67	0.46
3	Sambar deer	128	23.7	8.53	0.45
4	Wild boar	42	7.78	5.25	0.8
5	Ruddy Mongoose	4	0.74	1.33	0.41
6	Bonnet Macaque	5	0.93	2.5	0.71
7	Spurfowl	12	2.22	1.71	0.31
8	Peafowl	68	2.96	2.67	1.01
9	Sheep	16	13.15	8.88	0.68
10	Goat	16	21.3	8.21	0.72
11	Cow	71	5.19	9.33	0.82
12	Domestic Dog	115	12.59	6.8	0.6
13	Un-identified	28	2.96	2.67	0.37
	Total	540			

In terms of biomass consumed, livestock dominated by contributing 50.7% and flowed by wild animals reaming part of the 49.3% half each percentage relative biomass. Sambar dear is the single most important prey species for leopards in the study area, making up 31.66% of the total biomass consumed (Table 2). Domestic animals for sheep, Goat and cow followed with percentages of 11.05, 19.57 and 10.7% respectively. Wild boar and Dog are also important, with 8.29 and 9.73% of relative biomass consumed (Table 2), Leopards preferred medium-sized prey but balance diet very small mammals for Black-naped hare, Indian porcupine, ruddy mongoose, and bonnet macaque also most important prey 2.32, 2.49, 0.50 and 0.66% of the biomass consumed (Table 2) and spurfowl and peafowl was the contributor among birds (Table 2). The study also evaluated 50 of the leopard attacks cases that occurred between 2012 and 2016. Interviews of the affected people in the village mostly included the cattle herders. The interviews revealed that leopard attacks on domestic animals were a frequent scenario for the past four years. It was revealed that the relaxed, laid-back attitude of the farmers while herding, grazing livestock in forest areas was responsible for most of the attacks. Among the big cat species, leopards are more widely distributed in human habitat area owing to their feeding habit, highly adaptable hunting, and hermit-like nature. The five crucial essential prey species selected were taken from both superficial and deeper regions of the forest-based on the information received from the villagers. The study revealed that sheep (38 %), goat (24 %), domestic dogs (22 %), and cow (16%) were the most commonly attacked by leopards (Figure 4). Most of the attacks (about 70%) took place while the livestock was grazing during broad daylight, and 30% of the animals were attacked outside the forest also. Livestock attacks by leopards were more likely if dogs were present in the household and if the livestock protection in that grazing times it is attacked more that area.

Table 2. Calculation of biomass consumption (kg) Indian leopard in Hirekallugudda Sloth bears sanctuary (proposed sanctuary) Arasikere.

S. No	Species	Average weight (kg)	Biomass per scat	Frequency of occurrence	Biomass consumed	Percentage (%) Consumption
1	Black-naped hare	2	2.05	3.52	7.21	2.32
2	Indian Porcupine	18	2.61	2.96	7.73	2.49
3	Sambar deer	62	4.15	23.70	98.37	31.66
4	Wild boar	38	3.31	7.78	25.74	8.29
5	Ruddy Mongoose	3	2.09	0.74	1.54	0.50
6	Bonnet Macaque	7	2.23	0.93	2.06	0.66
7	Spurfowl	2	2.05	2.22	4.56	1.47
8	Peafowl	2	2.05	2.96	6.07	1.96
9	Sheep	18	2.61	13.15	34.32	11.05
10	Goat	25	2.86	21.30	60.80	19.57
11	Cow	120	6.18	5.19	32.04	10.31
12	Domestic Dog	12	2.40	12.59	30.22	9.73

Based on the questionnaire, the results of the survey conducted among local villagers revealed that leopard attacks were seasonal. The main reason for the inside of the forest February to August that time summer season middle of the not get food in outside villages so more cattle entry with forest and another one rainy season for June to November heavy rain greens start in forest food availability high density cattle grazing resulting in increased human and animal conflicts. Leopard attacks on cattle were recorded throughout the year; however, more (45.0 %) occurred from May to August season high attacks, followed by January to February (30.0 %), winter (20.0 %), and November to December (5.0 %) agriculture harvesting time cattle low entry within forest (Figure 5). Most attacks occurred around the forest and agricultural fields that occurred around forests and edges. The incidence of attacks was noted mostly around agricultural fields that were located around forest zones. More strikes occurred outside the protected area when people were working in agricultural fields and leading their livestock to graze within the territorial forest.

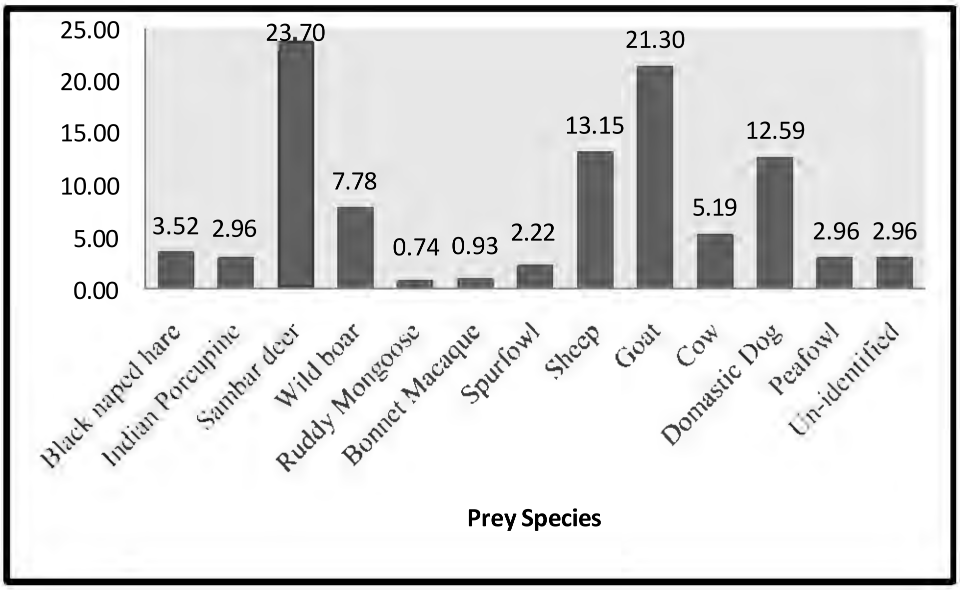


Figure 3. Percentage of prey species based on Indian leopard scat (n=54) in Hirekallugudda Sloth bear Sanctuary (proposed sanctuary) Arasikere.

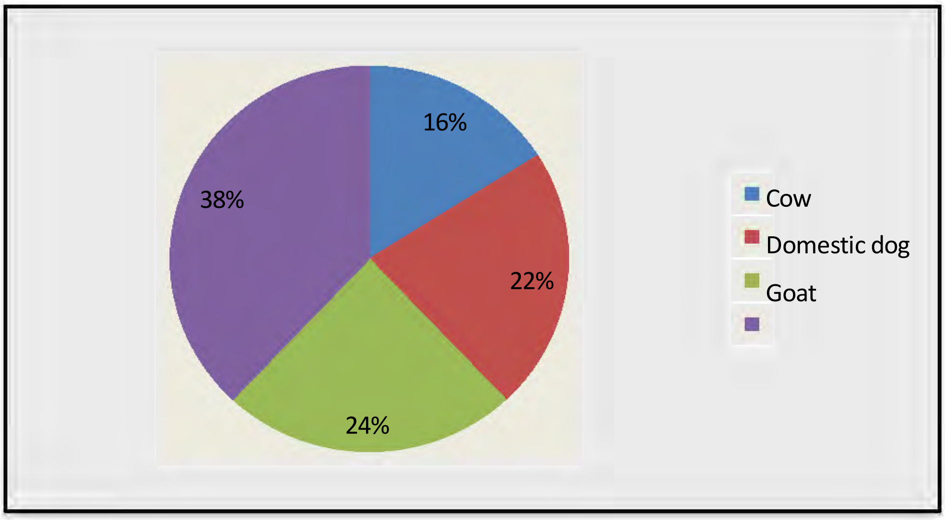


Figure 4. Pie charts display relative domestic of percentage four food categories by a Indian leopard in Hirekallugudda Sloth bear sanctuary (proposed sanctuary) Arasikere.

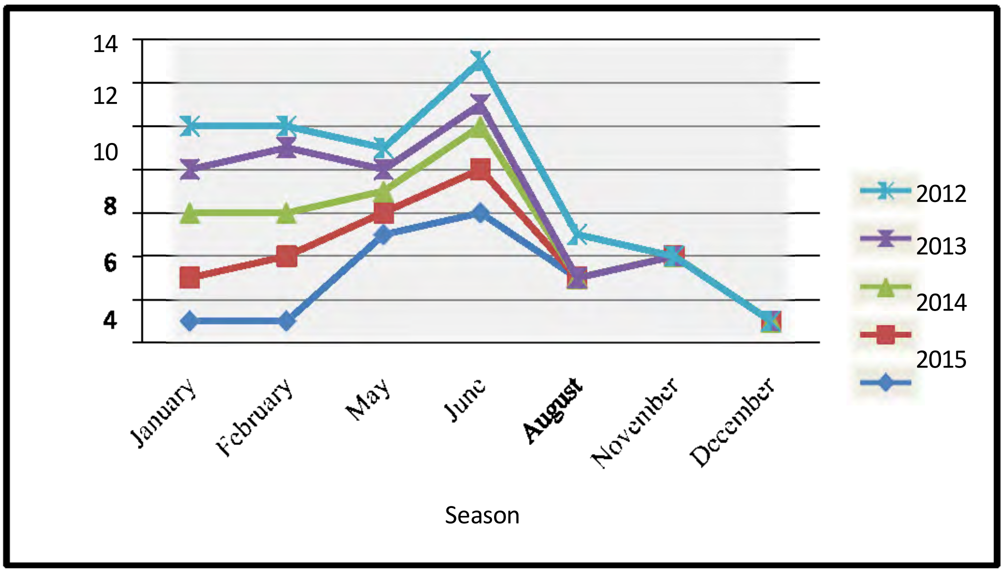


Figure 5. The charts display relative domestic animals of number of attack categories by a Indian leopard in Hirekallugudda Sloth bear sanctuary (proposed sanctuary) Arasikere.

The scats were found to be distributed mainly along the footpath or track, with very few scats located further inside the forest. The main buffer zone area was Aggund, Ramanahalli, and JC Pura which were working cattle entry points and therefore was prone to higher leopard attacks. The village small isolated area that kind of nearest village 1< km as the bulk of the diet consists of abundantly found. The present study focuses on the diet of leopard, and it represents the overall diet range study period sambar deer has the highest frequency (23.70%) in the leopard diet. Contradictory reports have been published by they reported chital has the highest rate (64.7%) in leopard diet followed by sambar (20.2%), and other prey species. The study also revealed that the leopard's major food item in the area was sambar (*Rusaunicolor*) which is a large deer of distribution in Arasikere. Vegetation loss due to clearing by farmers, cutting by shepherds for fodder, and villagers for firewood collection. It is reducing green cover and flora. This leads to multiple issues and adverse effects on the ecosystem. Revival of degraded habitats is one of our focus areas. Among the significant wild species preyed upon, the second most common animal was the wild boar (7.78 %) related to cow (5.19%). The other species of lagomorph found in Arasikere were remains of the relatively abundant black-naped hare (3.52 %) in scats representing the leopard diet. Mukherjee *et al*., (1994); Sankar & Johnsingh, (2002) (personal communication) also found little hare to be preyed commonly by leopards. Karanth & Sunquist, (1995) estimated that only about 5 % of the leopard's prey in Nagarhole comprised of a hare.

The present study noted that primates and small animals were not as important as a component of the leopard diet. Arboreal prey (Un- identified, spurfowl, peafowl, and macaque) represented 0.93 to 2.96 (Table 2) percent of prey taken. Peafowl remains (in one case Mongoose 0.74 %) were found in scats and were only a minor component of the leopard's diet. John Seidensticker, (1983) found that an abundant and diverse prey base in Chitawan resulted in the leopards taking macaques only occasionally, while (Schaller, 1967) found leopards to be killing langur frequently in Kanha Tiger Reserve. Livestock has been recorded as a significant component of the diet. Goats were the most commonly consumed species of domestic animals (Athreya*et al*., 2015) reported 224 goats killed by leopards in the five-year period, followed by the cow, sheep, and domestic dog, and, interestingly, the relative proportions were almost identical as reported losses among interviewed households. Around 87% of leopard diet in the human-dominated landscape supported by domestic animals where only few species of wild animals were recorded (Athreya *et al*., 2016). Human use landscapes for a very long-time watching hunt to dog become important component of their diet (Athreya *et al*., 2015).

Conclusion

This study shows baseline data on the feeding ecology of leopards in Arasikere sanctuary (proposed sanctuary). It is supporting the prey base of the Leopard 12 species of wild and domestic recorded. The similarity of the two sources of data indicates that the scat analyses provided reliable results. Hence, the utilization of domestic animals seems to have increased over time. The cause of this increase is probably related to the fact that leopards are more frequently getting into contact with humans and their livestock and dog mostly found around human habitation area and cattle grazing time so simply attacked. Our data propose that making noise at cattle grazing time while moving through leopard habitat helps to avoid leopard encounters and attacks. The cattle entered the forest supporting for leopard but in results at the same time the economically loss of money in formers. Water sources in the localities of leopard den sites should be protected prey wild species or if lacking, could be provided. Restoration measures could include reducing the causes of degradation, facilitating regeneration of plants, and planting trees species including key food plants to augment cover and food for Sambar deer in those habitats. The domestic species control measures improve habitat quality and indirectly conserve the leopards. The conservation of such habitat and prey species is important for area supported with leopard of population.

Acknowledgement

We would like to acknowledge the Wildlife SOS (WSOS) and Karnataka Forest Department for their support and encouragement, A. K. Singh, IFSCCF Hassan Forest Division and Arasikere Range Forest officers and village peoples. We thank the Wildlife SOS BBRC team who extended their support for gathering Microscope.

References

Athreya, V., Oddesn, M., Linnell, J. D., Krishnaswamy, J.,& Karanth, K. U. (2016). A cat among the dogs: leopard Panthera pardus diet in a human-dominated landscape in western Maharashtra, India. *Oryx, 50*(1), 156-162.

Athreya, V., Srivathsa, A., Puri, M., Karanth, K. K., Kumar, N. S., & Karanth, K. U. (2015). Spotted in the news: using media reports to examine leopard distribution, depredation, and management practices outside protected areas in Southern India. *PLoS One, 10*(11), e0142647.

Bailey, T. N. (1993). *The African leopard*: Columbia University Press. New York.

Carter, N. H., Riley, S. J., & Liu, J. (2012). Utility of a psychological framework for carnivore conservation. *Oryx, 46*(4), 525-535.

Eisenberg, J. F., & Lockhart, M. (1972). An ecological reconnaissance of Wilpattu National Park, Ceylon. *Smithsonian Contributions to Zoology*. 101, 1-118.

Grobler, J. H., & Wilson, V. J. (1972). *Food of the Leopard Panthera Pardus (Linn.) in the Rhodes Matopos National Park, Rhodesia*: National Museums of Rhodesia. *Arnoldia*, 35, 1-9.

Johnsingh, A. (1983). Large mammalian prey--predators in Bandipur. *Journal of the Bombay Natural History Society*. 8, 1-57.

Karanth, K. U. (1993). The relevance of big cat numbers to their conservation. *Cat News, 19*, 11-12.

Karanth, K. U., Nichols, J. D., Kumar, N. S., Link, W. A.,& Hines, J. E. (2004). Tigers and their prey: predicting carnivore densities from prey abundance. *Proceedings of the National Academy of Sciences, 101*(14), 4854- 4858.

Karanth, K. U., & Sunquist, M. E. (1995). Prey selection by tiger, leopard and dhole in tropical forests. *Journal of Animal Ecology*, 439-450.

Khorozyan, I., Ghoddousi, A., Soofi, M., & Waltert, M. (2015). Big cats kill more livestock when wild prey reaches a minimum threshold. *Biological Conservation, 192*, 268-275.

Kitchener, A. (1991). *The natural history of the wild cats*: Christopher Helm, A and C Black, London.

Knowlton, F. F., Gese, E. M., & Jaeger, M. M. (1999). Coyote depredation control: an interface between biology and management. 52, 398-412.

Kumaraguru, A. (2002). The influence of prey species diversity and densities in different vegetation types on the foraging ecology and community structure of large carnivores in Indira Gandhi Wildlife Sanctuary and National Park. Report submitted to Tamil Nadu Forest Department, Tamil Nadu, India.

Lal, J. (1989). India's Forests: Myth and Reality, Natraj Publication, Dehra Dun.

Link, W., & Karanth, K. U. (1994). Correcting for over dispersion in tests of prey selectivity. *Ecology, 75*(8), 2456-2459.

Meriggi, A., & Lovari, S. (1996). A review of wolf predation in southern Europe: does the wolf prefer wild prey to livestock? *Journal of Applied Ecology*,1561-1571.

Mizutani, F. (1999). Impact of leopards on a working ranch in Laikipia, Kenya. *African Journal of Ecology, 37*(2), 211-225.

Mukherjee, B., Heberlein, L. T., & Levitt, K. N. (1994). Network intrusion detection. *IEEE Network, 8*(3), 26- 41.

Mukherjee, S., Goyal, S., & Chellam, R. (1994). Standardisation of scat analysis techniques for leopard (*Panthera pardus*) in Gir National Park, Western India.*Mammalia,* 58, 139-143.

Negi, S., Matsukura, M., Mizuno, M., Miyake, K., & Minami, N. (1996). Synthesis of (2R)-1-(4-Chloro-2- pyridyl)-2-(2-pyridyl) ethylamine: a selective oxime reduction and crystallization-induced asymmetric transformation. *Synthesis, 1996*(08), 991-996.

Nowell, K., & Jackson, P. (1996). *Wild cats: status survey and conservation action plan* (Vol. 382): IUCN/SSC Cat Specialist Group Switzerland.

Pikonov, D., & Korkishko, V. (1992). The Amur Leopard: Nauka, Moscow.pp.1992.

Rabinowitz, A. (1989). The density and behaviour of large cats in a dry tropical forest mosaic in Huai Kha Khaeng Wildlife Sanctuary, Thailand. *Natural History Bulletin Siam Society, 37*, 235-251.

Rahalkar, K. (2008). *Attitudes of local people to conflict with leopards (Panthera pardus) in an agricultural landscape in Maharashtra, India.* M. Sc. Thesis. Centre for Wildlife Studies and National Centre for Biological Sciences, University of Agricultural Sciences, GKVK campus, Bangalore. pp. 37.

Ramakrishnan, U., Coss, R. G., & Pelkey, N. W. (1999). Tiger decline caused by the reduction of large ungulate prey: evidence from a study of leopard diets in southern India. *Biological Conservation, 89*(2), 113-120.

Robinson, T. P., Wint, G. W., Conchedda, G., Van Boeckel, T. P., Ercoli, V., Palamara, E., Gilbert, M. (2014). Mapping the global distribution of livestock. *PLoS One, 9*(5), e96084.

Sankar, K., & Johnsingh, A. (2002). Food habits of tiger (Panthera tigris) and leopard (Panthera pardus) in Sariska Tiger Reserve, Rajasthan, India, as shown by scat analysis. *Mammalia, 66*(2), 285-288.

Sawarkar, V. (1986). Animal Damage Predation on Domestic Livestock by Large Carnivores. *IndianForester, 112*(10), 858-866.

Schaller, G. (1967). The deer and the tiger–University of Chicago Press. *Chicago and London*.USA.

Schaller, G. B., Junrang, R., & Mingjiang, Q. (1988). Status of the snow leopard *Panthera uncia* in Qinghai and Gansu Provinces, China. *Biological Conservation,45*(3), 179-194.

Seidensticker, J. (1983). Predation by Panthera cats and measures of human influence in habitats of South Asian monkeys. *Internationa Journal of Primatol, 4*(3), 323-326.

Seidensticker, J. (1990). Leopards living at the edge of the Royal Chitwan National Park, Nepal. *Conservation in developing countries: Problems and Prospects*, 415- 423.

Sunquist, M. E. (1981). The social organization of tigers (*Panthera tigris*) in Royal Chitawan National Park, Nepal. *Smithsonian Contributions to Zoology*. 336, 1- 98.

Swaminathan, S., Desai, A., & Daniel, J. (2002). Large Carnivores in Mudumalai Wildlife Sanctuary and National Park. *Report submitted to Bombay Natural History Society, Bombay,* 80pp.

Taghdisi, M., Mohammadi, A., Nourani, E., Shokri, S.,Rezaei, A., & Kaboli, M. (2013). Diet and habitat use of the endangered Persian leopard (*Panthera pardus saxicolor*) in northeastern Iran. *Turkish Journal of Zoology, 37*(5), 554-561.

Thavarajah, N. (2008). Predator-human conflict asinfluenced by livestock depredation. *Grassroots: Newsletter of the Grassland Society of Southern Africa, 8*(3), 11-17.

Venkataraman, A. B., Arumugam, R., & Sukumar, R. (1995). The foraging ecology of dhole (Cuon alpinus) in Mudumalai Sanctuary, southern India. *Journal of Zoology, 237*(4), 543-561.

Art – 269. SURGICAL TREATMENT OF BACULUM FRACTURE IN A CAPTIVE SLOTH BEAR *(Melursus ursinus)*

A. Sha Arun, A. S. Virk and S. P. Patil

Abstract

A rescued dancing male sloth bear *(Melursus ursinus)* neutered according to CZA norms at Wildlife SOS; Bannerghatta Bear Rescue Center (BBRC); aged around 24 years was noticed to exhibit frequent behavior of licking and biting of the penile region. With the aid of radiographic examination, it was confirmed as a novel case of baculum fracture in captive sloth bear. Radiographic examination revealed complete fracture of baculum at its anterior one third. Under general anesthesia with ketamine and xylazine in combination at a dose rate of 5 mg/kg and 2 mg/kg respectively, a surgical procedure was performed which involved removal of the anterior fractured portion of the bone cautiously without causing any damage to urethra. Utmost post- operative care was provided. After the completion of the surgical intervention, the sloth bear recovered and stopped exhibiting the abnormal behaviour of licking and biting.

Key words: Baculum fracture, Sloth Bear *(Melursus ursinus)*

Art – 270. VARIATION IN MANDIBULAR TEETH ANATOMY IN SLOTH BEARS

(Melursus ursinus)

A. Sha Arun and S. P. Patil

Abstract

The order Carnivora includes a remarkable array of feeding types and dental morphologies, ranging from pure meat eaters with large cutting carnassial teeth to frugivores with broad crushing teeth. Sloth bear comes under the family Ursidae along with 8 species of bears present in the world. The type of dentition observed in mammals known as heterodont dentition. They possess different functions and types. From what has been observed so far of sloth bear dentition, they have a dental formula of I2/3, C1/1, Pm4/4, M2/3 x2= 40, lacking the first (central) pair of upper incisors. This special adaptation that makes them unique in the bear family, is to facilitate the sucking feeding pattern of sloth bears when they forage for termites or raid honeycombs. Sloth Bears have an omnivorous diet which requires active use of canines as well as wide for- aging ability (Arun et al 2012). The purpose of this study was to establish normal dental root patterns in sloth bears (*Melursus ursinus)*, using digital dental radiography as a tool. Radiography is a vital diagnostic aid in veterinary dentistry too. Most of the periodontium and root apical meristem can only be visualized by dental radiography (Arun et al 2010). It is important to have knowledge of the anatomy and development of teeth and mouth in order to maintain good dental health. It is only by understanding normal dental development, one can learn to recognize abnormal conditions. Without proper knowledge of the tooth root, further dental procedures and interventions like tooth extraction and endodontic treatment are difficult to plan and execute efficiently. The variation between mandible and maxillary molar root pattern is described in detail in this presentation.

Key words: Mandibular Teeth Anatomy, Sloth bears *(Melursus ursinus)*

Art – 271. RETROSPECTIVE ANALYSIS ON THE MORTALITY PATTERN OF RESCUED CAPTIVE DANCING SLOTH BEARS (*Melursus ursinus*) IN FOUR REHABILITATION CENTRES IN INDIA

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

Abstract

A retrospective study was conducted on the mortality and mortality patterns occurred from 2003 to 2021 (18 years of review) in captive dancing sloth bears at Wildlife SOS rescue centers across India. This study included in four different rescue facilities which includes Agra Bear Rescue Facility (Uttar Pradesh), Bannerghatta Bear Rescue Center (Karnataka), Van Vihar Bear Rescue Facility (Madhya Pradesh) and Purulia Bear Rescue Center (West Bengal). The total number mortality observed was 390 (M 237:F153) sloth bears out of total population of 628 rescued sloth bears which was come around 62.10% and amongst the dominant cause was tuberculosis causing overall mortality of 51.02% (199/628) followed by ageing (11.53%), rabies (11.28%), liver cancer (5.99%), infectious canine hepatitis (4.87%), chronic kidney disease with liver cancer(4.10%), snake bite (2.82%), tuberculosis and liver cancer (1.28%), gall bladder (1.28%), trauma (1.02%), ICH and tuberculosis (0.76%), blood cancer (0.76%), unidentified (0.51%), circulatory failure (0.51%), tuberculosis with chronic kidney disease and liver cancer (0.25%), intussusception (0.25%), hepatorenal failure due to carcinoma (0.25%), enteric abscess (0.25%), congestive heart failure (0.25%), congenital abnormality (0.25%), Chronic kidney disease (0.25%), chronic esophagitis (0.25%) and Brain tuberculosis (0.25%). Highest mortality was recorded in males of 60.76% and females had 39.23% mortality. Overall tuberculosis was the major reason for captive sloth bears mortality in all the facilities accounting 51.02% mortality, the second dominant cause was ageing associated with liver, gall bladder and kidney diseases which accounted 31.25% followed by rabies with 11.28% mortality.

Keywords: Retrospective analysis, Dancing Sloth bears (*Melursus ursinus*), rehabilitation centre

Art – 272. INFRARED THERMOGRAPHY A SIGNIFICANT REMOTE EXAMINATION TOOL IN CAPTIVE ELEPHANT'S HEALTH CARE

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

Abstract

In the veterinary discipline, Infrared thermography (IRT) is a novel remote sensing diagnostic modality that has the properties of determining body surface temperature. For instance, the results of thermal imaging techniques are a temperature product of internal tissue temperature and that of the outer surface of the body. Despite a wide range of practical applications, thermography may also be used to determine infectious diseases, stress levels and to diagnose abnormalities associated with locomotor system. In this study, we have presented few case demonstrations on elephants, where IRT was used for diagnosis and to aid intensive care, welfare and management.

Key Words: Thermography, Disease, Diagnosis, Monitoring, Treatment, Welfare

Introduction

Infrared thermography (IRT) is a safe, modern, non- invasive, non-contact thermal profile visualisation technique that uses thermographic scanning equipment (Cilulko *et al.,* 2012). Thermal or infrared energy is a part of the electromagnetic spectrum that is invisible because its wavelength is too long tobe detected by the human eye; instead, we perceiveit as heat. Unlike visible light, everything with a temperature above absolute zero emits heat. The higher the temperature of an object, the greater the amount of infrared radiation it emits. Even very cold objects, such as ice cubes, emit infrared radiation.

A thermal imaging camera is specially designed to measures heat and only images the emit infrared radiation from an object (Ward & Speakman, 1998). This type of camera allows the temperature of an object to be measured and recorded, to create a thermal image i.e., a thermogram. It makes no difference if it is too dark to see the object with the naked eye: the thermal image will be unaffected.

Thermographic method has found numerous applications not only in industries, but also in human and veterinary medicine, primarily for diagnostic purposes (Mccafferty, 2007). Infrared thermography has historically been used as a veterinary diagnostic tool in horses and can be expanded for use in other species as varied as elephants (Mole *et al.,* 2016). Because of the cost, lack of expertise and insufficient awareness, it is still an unfamiliar technique in India.

Methodology

Elephants in captivity are more prone to various health problems such as degenerative joint diseases, stifle joint hygroma, foot pad inflammation, toenail abscess, shoulder bursitis and temporal adenitis etc. The unfavourable tethering area, lack of exercise and hygiene, ignored footcare, and malnourishment further enhance their health problems under captive condition. The infrared thermography technique plays a vital role in this initial evaluation of health status, though the animal may not cooperate in unfamiliar surroundings and if there is a new mahout.

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

Factors such as wet skin, skin contamination due to dirt, moisture in the fur, windy locations, direct sunlight, and other heat sources will affect the appearance of thermal images and can lead to an error in thermal measurements. So, care should be taken to avoid errors due to the above-mentioned factors.

Result and discussion

Thermography can detect many things that change the normal thermal pattern, as it can show differences in thermal symmetry and abnormally warm or cold areas in patients. If the thermal pattern is not symmetrical or asymmetrical of 1°C or more is often significant and indicates possible pathology such as infection, soft tissue injury, joint problem, nervous dysfunction etc. With the help of thermography, we have successfully diagnosed toenail abscesses, compensatory leg lameness, cutaneous inflammation and hygroma in early stages and provided treatments accordingly.

Thermography is a suitable tool to locate the hidden inflammations as well as evaluate the proper foot care in elephants to restore the overall welfare of the animal.

Conclusion

Elephants are ideal models for thermal imaging studies as their skin is barely covered with hair. As a physiological diagnostic tool, thermography makes it possible 'to see the unseen' before anatomical changes have developed. The diagnosis of localized inflammation would not have been possible without thermography. Since it is portable, easy to use/ learn, not stressful to the animal as it's a non-contact safe remote sensing method and less in coast when compared to digital radiography; it can be considered as an efficient diagnostic tool in the health care of captive elephants.

Acknowledgement

We greatly appreciate the support and encouragement extended by the cofounders, Wildlife SOS. We are also thankful to the animal care staff at Wildlife SOS for their sincere assistance.

References

Cilulko, J., Janiszewski, P., Bogdaszewski, M., & Szczygielska,E. (2012). Infrared thermal imaging in studies of wild animals. European Journal of Wildlife Research, 59, 17–23. https://doi.org/10.1007/s10344-012-0688-1

Mccafferty, D. J. (2007). The value of infrared thermography for research on mammals: Previous applications and future directions. Mammal Review, 37(3), 207–223. https://doi.org/10.1111/j.1365-2907.2007.00111.x

Lynch, C. (2019). Sustainability index (S-Index): A tool for use in the evaluation and planning of institutional animal collections. Zoo biology, 38(1), 12-23.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Redford KH, Jensen DB, and Breheny JJ. 2012. Integrating the captive and the wild. Science 338: 1157–58.

Simon Garnier, Noam Ross, Robert Rudis, Antônio P. Camargo, Marco Sciaini, and Cédric Scherer (2021). Rvision - Colorblind-Friendly Color Maps for R. R package version 0.6.2. Wickham et al., (2019). Welcome to the tidy verse. Journal of Open-Source Software, 4(43), 1686, https://doi.org/10.21105/ joss.01686

Mole, M. A., Rodrigues DA' raujo, S., van Aarde, R. J., Mitchell, D., & Fuller, A. (2016). Coping with heat: Behavioural and physiological responses of savanna elephants in their natural habitat. Conservation Physiology, 4, 11. https://doi.org/10.1093/conphys/cow044

Ward, S., & Speakman, J. R. (1998). Infrared thermography: Principles and applications. Zoology, 101(3), 224–232.

Art – 273. OCCURRENCE OF EXTRAHEPATIC BILIARY TRACT PATHOLOGIES IN REHABILITATED CAPTIVE DANCING SLOTH BEARS (*Melursus ursinus*) AND ITS DIAGNOSTIC CHALLENGES

S Ilayaraja and **Arun A Sha**

Abstract

Sloth bears are large, charismatic mammals that belong to the order carnivora and the family Ursidae. Each mammalian species possesses the unique size and structure of their extrahepatic biliary tract. While gall bladder is present in many species, others are lacking this organ. Sloth bears have a well-developed gall bladder and bile ducts with Penta lobed liver. Since they possess a gall bladder; they are more prone to cholecystolithiasis, cholecystitis, varied degree of sludge formation, and cholangiocarcinoma. In the sloth bears at Wildlife SOS, Agra Bear Rescue Facility, such cases were encountered and provided with disease-specific treatment to stabilize the health condition. Trans abdominal ultrasonography examination was conducted to evaluate liver, gall bladder and ascites to diagnose the extrahepatic biliary tract pathologies along with haemato-biochemical indices and visible external symptoms such as inappetence, general weakness, bulged abdomen, hair loss, allergic dermatitis on limbs, passing mucoid brownish black diarrhoeic faeces and icteric mucous membrane. This study aims to disseminate knowledge on extrahepatic biliary tract pathologies of sloth bears that will help wildlife veterinary professionals, wildlife neophytes to enhance their early diagnosis and treatments as well as scientists in evolutionary biology and oncology for conducting further studies.

Keywords: Biliary tract, carcinoma, ultrasonography, gallbladder, sloth bears

Introduction

The sloth bear *(Melursus ursinus)* 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 [6]. Sloth bears forage on mango, fig, ebony, and other fruits (frugivory), and also on some flowers. However, ants and termites (myrmecophagy), dug out of their cement-hard nest mounds, are a year- round staple. Also, sloth bears climb trees and knock down honeycombs, beetles, grubs, ants, and other insects round out their diet. During food shortages, sloth bears will eat carrion. This bear species is commonly found in the Indian subcontinents and frequently got poached for body parts and also the cubs used to train for illegal street performance by the calendar gypsies. Due to their poor handling, hygiene with malnourishment; they are always in the stage of compromised health. Wildlife SOS is an NGO that started working along with all the state forest departments and rescued all the performing bears from the illegal captivity; housed them in a well-established rescue facility for further care and rehabilitation. However, they are receiving good quality freshly prepared balanced porridge, which is further supplemented with multivitamins, minerals, raw honey with propolis, protein source as diet in morning and evening; seasonally available fruits as part of enrichment in noon time food., 45% of the annual mortality happening due to hepatic disorder.

There have been relatively few reports of tumours in bears (*Ursus* sp.). The most prevalent tumours are hepatic neoplasms i.e. hepatocellular and biliary carcinoma [1, 7, 20, 21, 28]. This captive sloth bears populations are more prone for various degree of extrahepatic biliary tract pathologies while aging and cholangiocellular carcinoma at any stage of life. In this article we dealt with the ultrasonography aided diagnostic approach along with haemato-biochemical indices and visible external symptoms for early diagnosis and provide diseases specific treatment and also to create awareness among the scientists in evolutionary biology and oncology, making further research on molecular genetic level to understand the status of p53 gene amplification in this species. p53 is one of the most well-studied and crucial tumour suppressor genes. p53 serves as the "guardian of the genome" and the "cellular gatekeeper" [12, 14] and any possibility for gene therapy to minimize the occurrence of malignancy in this species as part of conservation medicine.

Materials and methods

In captive bears, neoplasia commonly involves the hepatobiliary and gastrointestinal [2, 3] which led to the compelling need of acquiring more adequate clinical approaches for the evaluation of the hepatic system. Obtaining liver tissue samples by liver biopsy (percutaneous, laparoscopic, surgical, trans jugular) is the most direct approach to the evaluation of hepatic fibrosis in human medicine. However, liver biopsy is associated with potential morbidity and mortality and has several limitations, including sampling error and high inter-observer variability [2, 25]. So, by considering captive management of wild animals, the non- invasive method of health evaluation by using ultrasonography technique, collection blood samples for hematobiochemical analysis is more beneficial and less risk to the patient, that can be achieved after chemical immobilization [2] or by positive reinforcement training [4, 18, 24]. Routine observation for any symptoms and correlate the same perfectly with clinical findings will also be mandatory for an exact diagnosis.

External symptoms

The routine monitoring of the animal activities and visual evaluations always remains a preliminary clue for any further diagnostic approaches. The animal suffering from gall bladder disease/cholangiocarcinoma always exhibits symptoms of bulged abdomen (Figure 1 & 2), icteric mucus membrane due to generalized jaundice, facial/peripheral oedema as according to the severity of the condition (Figure 3 & 4), intermittent vomiting, passing mucoid brownish black diarrhoeic faeces, rarely passing grey colour semisolid faces (Figure 7 & 8). The faecal analysis has always revealed the presence of a parasitic infestation. in the initial stage, there won't be any marked changes in food intake, but as days progress the gradual inappetence and intermittent anorexia will develop, which will lead to leathery and general weakness. Non-parasitic allergic dermatitis lesions around the lips, limbs, and perianal region were also noticed in the bears which have hepaticpathology (Figure 5 & 6).

Fig 1 & 2: Bulged abdomen in Sloth bears due to ascitic fluid

Fig 3: Generalized jaundice ***Fig 4: Facial/peripheral oedema***

Fig 5 & 6: Dermatic lesion on limbs

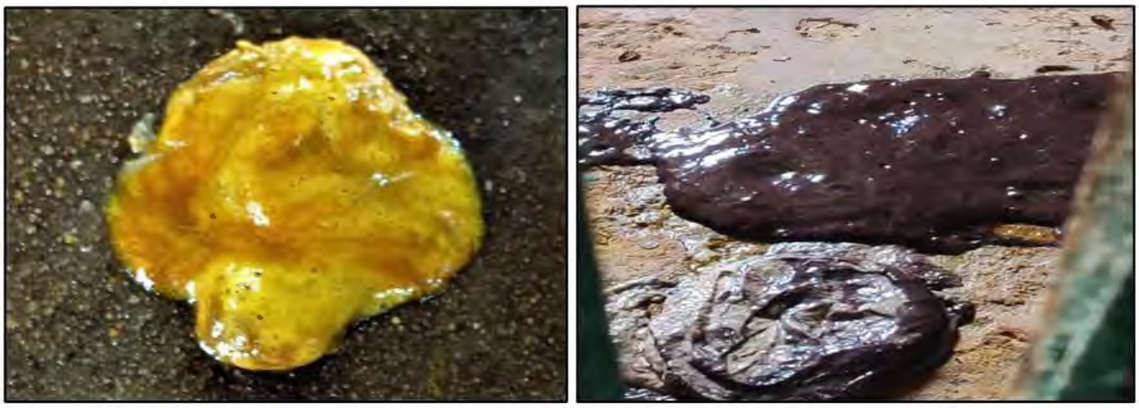


Fig 7 & 8: Faeces appearance and condition in bears affected with hepatic disorders

Hematobiochemical analysis

The blood samples can be obtained from the cephalic vein or saphenous vein or jugular vein for the hematobiochemical analysis. The level of SGPT, SGOT, GGT, ALP, LDH, and total bilirubin is always in the elevated range in animals suffering from hepatic carcinoma. The total protein value will be in the lower range. Low sodium, levels were also observed in most of the cases. In few cases, mild elevation of phosphate ions was noticed.

Transabdominal ultrasonography

Ultrasonography is a well-recognized safe, fast, repeatable method to evaluate gastrointestinal (GI) disease in people. Reference to its use in veterinary medicine is limited, During the previous 20 years, great advances in veterinary abdominal ultrasonography equipment and expertise have occurred and ultrasonography has been commonly in small animal practice and Ultrasonographic descriptions of many chronic GI disorders have been published [13, 23]. Sloth bear has well- developed a hepato-biliary system which consists of Penta lobed liver, gall bladder, and bile ductus (gross image). We can adopt the trans-abdominal ultrasonographic technique as the same is performed in small animals [5, 27]. Any good quality B mode ultrasonography unit with a 3 -5 MHz curve leaner probe is enough to do the ultrasonography in sloth bear (Figure 9 & 10). With the help of ultrasonographic scanning of the abdomen, we can easily find out asities due to peritoneal effusions (Figure 11 & 12), any abdominal mass, gall bladder abnormalities including wall thickening, various degree of proliferation (Figure 13, 14, 15 & 16), presence of pericholecystic fluid (Figure 17 & 18), various stages of sludges (Figure 19 & 20), and hypertrophy of gall bladder and gall stone. Documented photographs of gross PM lesions are incorporated here in this article for better understanding (Figure 21, 22, 23 & 24).

Fig 9 & 10: Ultrasonography machine used for diagnosis in Sloth bear with multi transducer option

Fig 11 & 12: Ascitic fluid present in the abdomen of a Sloth bear appreciated in ultrasonography

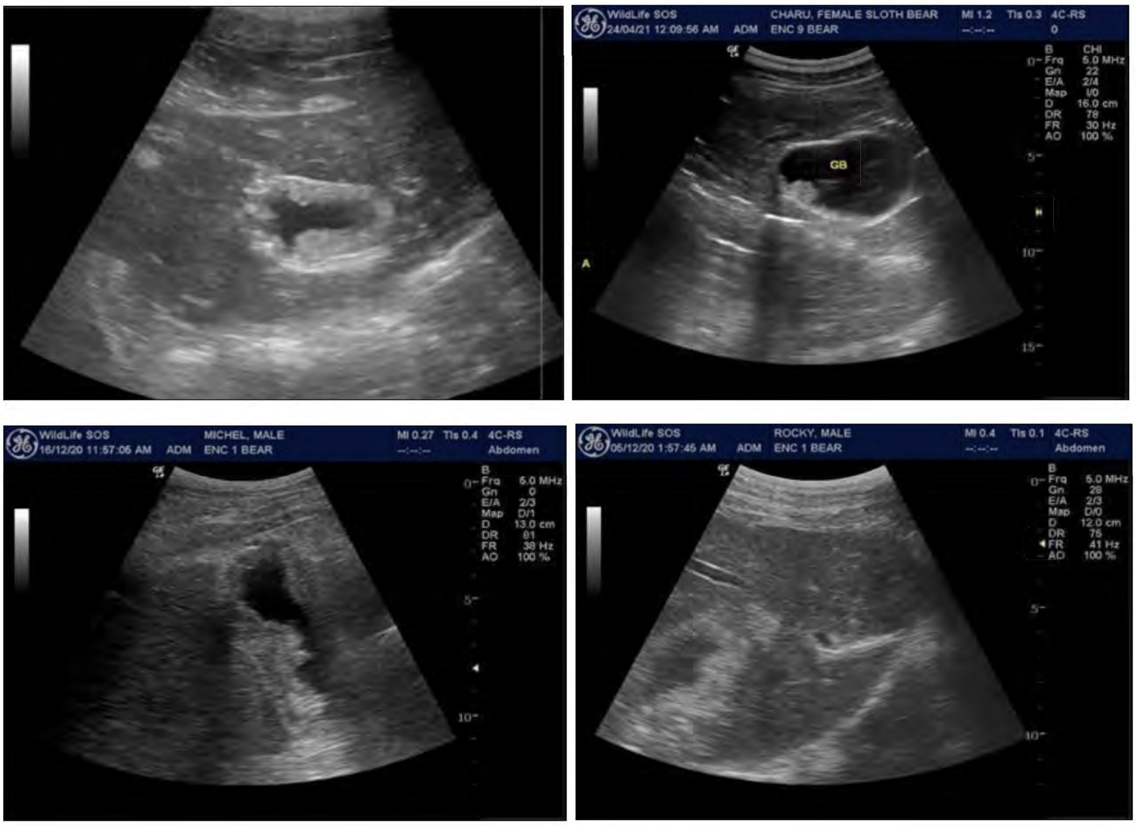


Fig 13, 14, 15 & 16: Various digress of proliferative lesions on the gallbladder wall appreciated in ultrasonography

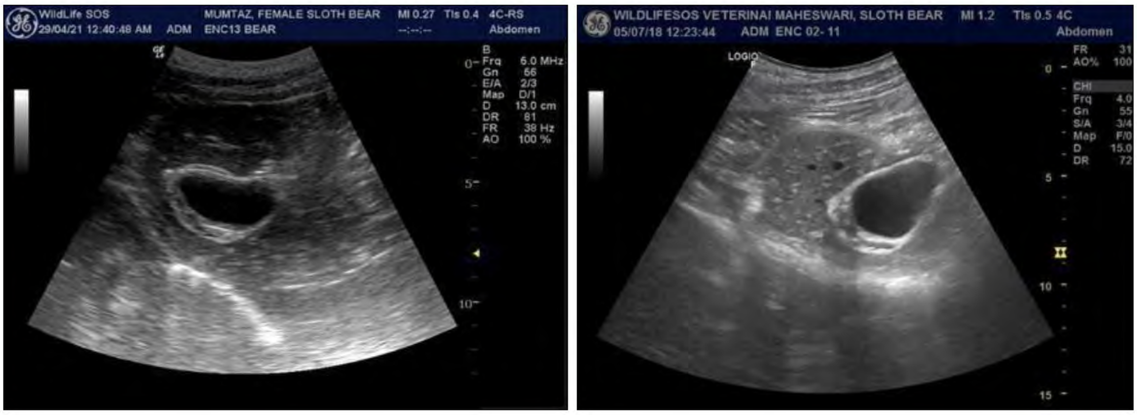


Fig 17 & 18: Arrow showing presence of pericholecystic fluid with hyperechoic gall bladder wall

Fig 19 & 20: Arrow showing presence of cholelith (gall stone) showing sludge inside the gall bladder

Fig 21 & 22: Post mortem examination revealed the presence of cholelith inside the gall bladder and abnormal enlargement of the gall bladder due to sever cholecystitis

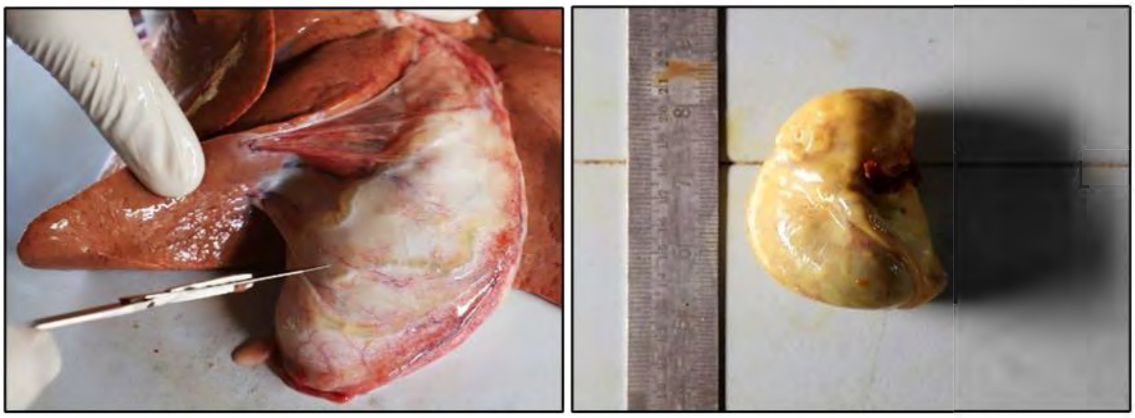


Fig 23 & 24: Congested gall bladder with oedematous wall and icteric, shrunken gall bladder with thick wall and tumour mass

Discussion

The detailed record of cancer occurrence in captive animals remains an important source of information for studies incomparative oncology, as well as providing data on the etiology of neoplastic diseases [17]. Wildlife cancer statistics are, however, highly scattered in the scientific literature and hence challenging to access. Moreover, tumours in wildlife are most commonly detected during post-mortem examination and therefore hard to confirm without histopathological examinations. However, even such analyses can be inaccurate because of high levels of autolysis [17, 19]. Reported the highest tumour prevalence in the carnivores. Similar high cancer prevalence in this group of mammals was mentioned by [15, 16]. More cancer prevalence in the mammal digestive system was recorded by [17] and [15]. In the year 2017, [1] reported that the biliary adenocarcinoma was the leading cause of death for adult sloth bears housed in U.S. zoos with no apparent gender predilection [7]. Reported Biliary and hepatic carcinomas in bears at the San Diego zoological gardens [11]. Also reported the hematobiochemical changes, assists, and jaundice as we mentioned in our findings [22]. Stated that tumours of the liver, bile ducts, and pancreas quite frequently develop in bears, usually in older animals, and also recorded unspecific symptoms such as vomiting, anorexia, weight loss, and abdominal swelling. Hypotnatrema is a common finding in patients with decompensated cirrhosis due to abnormal regulation of body fluid homeostasis [8].

Conclusion

In zoos, bears are considered to be animals that have few problems and are relatively free from disease [9]. This may be attributed either to "an extraordinary resistance against all kinds of diseases or to an unbelievable ability to conceal symptoms of sickness and pain [26]. This statement suggesting that the challenges in disease diagnosis in bears, and insists they need of multi-facet diagnostic approach. The literature reports also evidencing the incidence of extrahepatic biliary tract pathologies in the captive bear population around the globe which is not particular to any location, age, sex, and any specific feeding behaviour exactly. So, this overall reported incidence suggesting that the high chances for the genetic predisposition for neoplasms in this species may be weakening their Cancer defence mechanisms. Using an evolutionary and comparative approach to study cancer defence mechanisms has implications in human health and disease. It can provide new insights into cancer treatment (e.g., p53, immunotherapy) and prevention [10].

Acknowledgements

We greatly appreciate the support extended by Uttar Pradesh Forest department. We sincerely appreciate our cofounders, Wildlife SOS for providing all essential diagnostic facilities in terms of care and management. We are also thankful to the animal care staff at Wildlife SOS for their sincere assistance.

References

1. Anderson KM, Garner MM, Dennis PM. Causes of mortality in sloth bears (Melursus ursinus) housed in U.S. Zoos. Journal of Zoo and Aquarium Research. 2017;6(1):12-15.

2. Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology. 2003;38(6):1449-1457.

3. Bourne DC, Cracknell JM, Bacon HJ. Veterinary issues related to bears (Ursidae). International Zoo Yearbook. 2010;44(1):16-32.

4. Bourne DC, Vila-Garcia G. Wildpro ® Bears: health and management [Internet]. Gateway to wild pro multimedia, 2007.

5. Choi J, Kim A, Keh S, Oh J, Kim H, Yoon J. Comparison between ultrasonographic

and clinical findings in 43 dogs with gallbladder mucoceles. Veterinary Radiology & Ultrasound. 2014;55(2):202-207.

6. Dharaiya N, Bargali HS, Sharp T. Melursus ursinus (amended version of 2016 assessment) [Internet]. International Union for Conservation of Nature and Natural Resources, 2020.

7. Dorn CR. Biliary and hepatic carcinomas in bears at the San Diego zoological gardens. Nature. 1964;202(513):4.

8. Gines P, Cardenas A, Schrier RW. Liver disease and the kidney. in: Schrier RW, ed. diseases of the kidney & urinary tract. Philadelphia, PA: Lippincott Williams & Wilkins. 2006;3:2179-2205.

9. Van der Hage M, Dorrestein GM. Why do bears die in captivity. Bursa, Turkey, 1994, 125-130.

10. Harris VK, Schiffman JD, Boddy AM. Evolution of cancer defense mechanisms across species. Ecology and Evolution of Cancer [Internet]. Elsevier, 2017.

11. Kingston RS, Wright FH. Bile duct carcinoma with widespread metastases in a sloth bear. The Journal of Zoo Animal Medicine. 1985;16(1):16-20.

12. Lane DP. p53, guardian of the genome. Nature. 1992;358(6381):15–16.

13. Leib MS, Larson MM, Panciera DL, Troy GC, Monroe WE, Rossmeisl JH, Forrester SD, Herring ES. Diagnostic utility of abdominal ultrasonography in dogs with chronic vomiting. Journal of Veterinary Internal Medicine. 2010;24(4):803-808.

14. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell. 1997;88(3):323-331.

15. Lombard LS, Witte EJ. Frequency and types of tumors in mammals and birds of the Philadelphia zoological garden. Cancer Research. 1959;19:127-141.

16. ME LG, KB. Nature and rate of neoplasia found in captive wild mammals, birds, and reptiles at necropsy. Journal of the National Cancer Institute. 1977;59(1):185- 198.

17. Madsen T, Arnal A, Vittecoq M, Bernex F, Abadie J, Labrut S, *et al*. Cancer prevalence and etiology in wild and captive animals. Ecology and Evolution of Cancer, 2017, 36.

18. Martinez G. Bear training: a tool that improves care. The Shape of Enrichment.

2016;15(2):9-10.

19. McAloose D, Newton AL. Wildlife cancer: a conservation perspective. Nature Reviews. 2009;9:517- 526.

20. Montali RJ, Jack Hoopes P, Bush M. Extrahepatic biliary carcinomas in Asiatic bears. JNCL. 1981;66(3):603–606.

21. Moulton JE. Bile duct carcinomas in two bears. The Cornell Veterinarian. 1961;51:285-293.

22. Partridge J. Management guidelines for Bears & Raccoons. Bristol BS5 6UQ, U.K.: The Association of British Wild Animal Keepers, 1992.

23. Penninck DG, Nyland TG, Kerr LY, Fisher PE. Ultrasonographic evaluation of gastrointestinal diseases in small animals. Veterinary Radiology. 1990;31(3):134- 141.

24. Perry L, Stevens L, Powell D. Positive reinforcement training for biomedical and reproductive management of Giant pandas. Animal Keepers' Forum. 2006;34:394-401.

25. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, *et al*. Sampling error and intra observer variation in liver biopsy in patients with chronic HCV infection. The American Journal of Gastroenterology. 2002;97(10):2614-2618.

26. Rietschel W. Veterinary aspects of keeping bears in captivity. Bursa, Turkey, 1994, 115-120.

27. Uno T, Okamoto K, Onaka T, Fujita K, Yamamura H, Sakai T. Correlation between ultrasonographic imaging of the gallbladder and gallbladder content in eleven cholecystectomised dogs and their prognoses. J Vet Med Sci. 2009;71(10):1295-1300.

28. Vashist VS, Rattan SK, Gupta BB. Papillary cystadenocarcinoma of the mammary gland with metastases to the gastrointestinal tract in a Himalayan brown bear (Ursus arctos). Journal of Zoo and Wildlife Medicine. 2013;44(2):453-456.

Art – 274. SLOTH BEAR MUZZLE SUCKING AND SALIVA EXCHANGE

Attur Shanmugam Arun, Shanmugavelu Swaminathan, Thomas R Sharp, Yogaraj Pannerselvam, and **Reegan Pushbanathan**

The sloth bear (*Melursus ursinus*) exhibits a host of interesting behaviors not common among bears, mainly due to their specialization for eating termites (Garshelis et al. 1999, Sacco and Van Valkenburgh 2004). Recently we observed, both in captivity and in the wild, what we believe to be an unusual behavior between a mother and cub, as well as between cubs. The behavior was that of a mother sucking on her cub's muzzle, or cubs sucking on each other's muzzle. It was first observed with a group of 4 sloth bear cubs raised at the Wildlife SOS, Bannerghatta Bear Rescue Centre (BBRC), after being rescued from poachers in 2009. In 2017 a similar behavior was recorded on a video camera trap set up at the entrance of a maternal den of a wild sloth bear in the state of Karnataka, India. In this video, the mother is seen sucking on its cub's muzzle for 15–30 seconds. In captivity, each sucking session lasted 7–9minutes and spanned a period of 20–25 days.

Wild sloth bear mother sucking on her cub's muzzle outside of their maternal den, Karnataka, India, 2017.
Photo credit: Still image from camera trap video, Wildlife SOS

We are not presently aware of similar behavior in other bears or other species in the Order Carnivora. We have observed a video of wild American black bear (*Ursus americanus*) cubs licking one another's mouth; however, this behavior did not exhibit the sucking of the whole muzzle that we see in sloth bears. Many canids will regurgitate half-digested food to feed their pups, and the pups will lick at the adult's mouth in anticipation. This behavior is an important step in weaning the young, as this partially digested food is easier for the pups to eat and digest (Malm and Jensen 1993). However, our observations of the sloth bear did not appear to include this kind of regurgitation or feeding, though we cannot be certain that no regurgitation was involved. While captive sloth bear cubs have exhibited the sucking behavior prior to feeding, it was also observed post feeding.

When the behavior was first observed at the rescue centre, we were under the impression that it may be a stereotypic behavior related to their captivity. Yet the observation of this behavior in the wild made us think again. While the behavior may or may not be associated with the regurgitation of food to some limited extent, it most certainly involves the exchange of saliva. Saliva is a complex fluid, which influences oral health through specific and nonspecific physical and chemical properties (Tiwari 2011). We have no information on the composition of saliva in sloth bears, though in humans, saliva is made up of proteins, immunoglobulins, and a variety of enzymes.

Captive sloth bear cubs sucking on each other's muzzles and exchanging saliva, Wildlife SOS, Bannerghatta Bear Rescue Centre, Bangalore, Karnataka, India, 2009.
Photo credit: Lenu Kannan, Wildlife SOS

The behavior may simply be a way to strengthen the bond between mother and cub, or among siblings. Or the behavior may enhance the olfactory capacity of the young ones by stimulating the nerve endings in the muzzle. But if either of these is the primary reason, we would expect the behavior to be present in other bear species. If unique to sloth bears, though admittedly speculative, it may be related to the exchange of oral or gut microbes specific to their myrmecophagous feeding strategy. Specialized gut microbes are necessary for digesting chitin exoskeletons for protein intake. Interestingly, according to Delsuc et al. (2014), the gut microbiomes of placental myrmecophagous (termite and ant eating) mammals have shown a "textbook example of evolutionary convergence driven by extreme diet specialization". That paper clearly highlighted the importance of specific gut microbiota associated with being myrmecophagous. Additionally, it documents that sloth bear gut microbiomes vary significantly from that of other bear species, even other bear specialists, namely the giant panda (*Ailuropoda melanoleuca*), which has a gut microbiome similar to other bear species (Ley et al, 2008, Xue et al. 2015).

Gut microbiota of placental mammals are generally passed on to young during birth and through nursing, but the diet, after weaning, and environmental factors also play a role, though the details are still not fully understood. We do know that captive sloth bears in India have different gut microbiota than wild sloth bears, also in India (Arun 2020). This is not unusual, as differences in gut microbiota between captive and wild individuals of the same species has been shown in other studies (Clayton et al. 2016, Wasimuddin et al. 2017, Gibson et al. 2019, Schmidt et al. 2019). The cause, or causes, for the differences in the gut microbiota between captive and wild sloth bears may have something to do with the diet of the bears, which in captivity often lacks termites; or, it may have something to do with the fact the many of the bears in captivity at one of the Wildlife SOS Bear Rescue Facilities were removed from their mothers prematurely by poachers.

Sloth bears are in many ways the most atypical of all the bear species. The true function of the observed sucking behavior remains unknown. However, the behavior is an unusual one for a bear species and should prompt further investigation.

Literature Cited

Arun, A.S. 2020. Metagenomic profiling of fecal microbial community in carnivorous leopards *(Panthera pardus)* and omnivorous sloth bears *(Melursus ursinus).* Doctoral dissertation, Department of Biotechnology, Jain University, Bangalore. Karnataka, India.

Clayton, J. B., P. Vangay, H. Huang, T. Ward, B.M. Hillmann, G.A. Al-Ghalith, D.A. Travis, H.T. Long, B.V. Tuan, V.V. Minh, F. Cabana, T. Nadler, B. Toddes, T. Murphy, K.E. Glander, T.J.

Johnson, and D. Knights.2016. Captivity humanizes the primate microbiome. Proceedings of the National Academy of Sciences of the United States of America113:10376–10381.

Delsuc, F., J.L. Metcalf, L.W. Parfrey, S.J. Song, A. Gonzalez and R. Knight. 2014. Convergence of gut microbiomes in myrmecophagous mammals. Molecular Ecology 23:1301–1317.

Garshelis, D.L., A.R. Joshi, J.L.D. Smith and C.G. Rice. 1999. Sloth bear conservation action plan. Pages 225–240 *in* C. Servheen, S. Herrero, and B. Peyton, editors. Bears: status survey and conservation action plan. IUCN/SSC Bear and Polar Bear Specialist Groups, Gland, Switzerland and Cambridge, U.K. Gibson, K.M., B.N. Nguyen, L.M. Neumann, M. Miller, P. Buss, S. Daniels, M.J. Ahn, K.A. Crandall and B. Pukazhenthi. 2019. Gut microbiome differences between wild and captive black rhinoceros – implications for rhino health. Scientific Reports 9:7570.http://doi.org/10.1038/s41598-019-43875-3

Ley, R.E., M. Hamady, C. Lozupone. 2008. Evolution of mammals and their gut microbes. Science. 320:1647–1651.

Malm, K., and P. Jensen. 1993. Regurgitation as a weaning strategy—a selective review on an old subject in a new light. Applied Animal Behaviour Science 36:47–64.

Sacco, T. and B. Van Valkenburgh. 2004. Ecomorphological indicators offeeding behaviour in the bears (Carnivora: Ursidae). Journal of Zoology (London) 263:41–54.

Schmidt, E., N. Mykytczuk, and A.I. Schulte-Hostedde. 2019. Effects of the captive and wild environment on diversity of the gut microbiome of deer mice (*Peromyscus maniculatus*). ISME Journal 13:1293–1305.

Tiwari, M. 2011. Science behind human saliva. Journal of Natural Science, Biology, and Medicine 2(1):53–58.

Wasimuddin, S. Menke, J. Metzheimer, S. Thalwitzer, S. Heinrich, B. Wachter, and S. Sommer. 2017. Gut microbiomes of free-ranging and captive Namibian cheetahs: diversity, putative functions, and occurrence of potential pathogens. Molecular Ecology 26:5515–5527.

Xue, Z., W. Zhang, L. Wang, R. Hou, M. Zhang, L. Fei, X. Zhang, H. Huang, L.C. Bridgewater, Y. Jiang, C. Jiang, L. Zhao, X. Pang, Z. Zhang. 2015. The bamboo-eating giant panda harbor a carnivore-like gut microbiota, with excessive seasonal variations. mBio 6(3):e00022-15. doi:10.1128/mBio.00022-15.

.

Report – 275. TECHNICAL RADIO COLLARING AND EARLY WARNING ALERT SYSTEM OF HUMAN-ELEPHANT CONFLICT MITIGATION PROJECT IN CHHATTISGARH STATE

S. Swaminathan, A. Sha. Arun, S. Ilayaraja, Thomas Sharp, Reegan P, Yogaraj P, and **Balasubramaniyan G.**

Complete report was submitted to Chhattisgarh Forest Department
and the report can be accessed via email request at
research@wildlifesos.org.

Art – 276. SLOTH BEAR ATTACKS: REGIONAL DIFFERENCES AND SAFETY MESSAGING

Thomas R. Sharp, Tom S. Smith, Shanmugavelu Swaminathan and **Attur S. Arun**

Sloth bears behave aggressively toward humans when threatened and are among the most dangerous wildlife in India. Safety messaging for those who live in sloth bear country must be accurate to be effective, and messaging may need to be modified to account for regional differences in human-bear relationships. The timing of sloth bear attacks on the Deccan Plateau of Karnataka, both by season and by time of day, deviated enough from those reported in other areas such that it warranted further investigation. We compared data from eight studies of human-sloth bear conflict from across the Indian subcontinent and explored possibilities as to why differences exist. Seasonally all studies reported that human-sloth bear conflict was highest when human activity in the forest was greatest, though the season of highest human activity varied significantly by region (χ^2 = 5921, df = 5, P < 0.001). The time of day that the majority of attacks occurred also varied significantly by region (χ^2 = 666, df = 5, P < 0.001), though human activity was relatively consistent. We speculated that the rate of day attacks on the Deccan Plateau was lower due to the reduced probability of encountering a sleeping bear as they are concealed and secure in shallow caves. Additionally, the rate of attacks was significantly higher at night on the Deccan Plateau because people often to work into night-time. We concluded that slight differences, or different emphasis, to bear safety messaging may be necessary on a regional basis to keep the messaging accurate and effective.

The sloth bear (*Melursus ursinus*) is the most ubiquitous bear species in India and ranges throughout the subcontinent[1]. It is also considered one of the most dangerous wild animals in the region[2, 3.] Several studies have chronicled human-sloth bear conflict, and some have provided safety messaging advice intended to mitigate conflicts[4–6]. Additionally, sloth bear safety messaging has been dispensed through pamphlets, booklets, videos, and workshops to reach those living and working in sloth bear country. For bear safety messaging to be effective it must be accurate and generally it must be short and to the point. The basic rule 'less is more' applies to bear safety messaging as most people will not remember more than three or four key points[7], and perhaps even less when under the duress associated with a sudden bear encounter. And while too much information overly complicates messaging, ignoring regional differences is ill-advised when differences exist. Alaska's interior brown bears, for example, are significantly more aggressive than Alaska's coastal brown bears[8]. The varying levels of aggressiveness are attributed to resource density and habituation with conspecifics and people. Consequently, Denali National Park advises people to maintain at least 400 m distance from brown bears whereas coastal Katmai recommends just 50 m as a safe distance[8].

Bear safety messaging is based on bear behaviors that are both intrinsic and extrinsic to the species. For example, it is known that sloth bears, irrespective of location, have an innate defensive-aggressive response to surprise (sudden) encounters. This intrinsic response is likely due to having co-evolved with tigers, a formidable predator which opportunistically prey on sloth bears[9–12]. Alternately, coastal brown bears are more tolerant of other bears, and humans, than their interior counterparts likely due to being accustomed to a high density of bears in the region[8]. There have been calls to standardize bear safety messaging across the range of bear habitats[13] but we suggest that a 'one size fits all' approach may seek consistency at the risk of safety. Recognizing that a bear's response to human encounters often varies by region is key to providing effective bear safety messaging for people living in a specific area.

Our recent study[6] of sloth bear attacks on the Deccan Plateau differed significantly from other studies of human-sloth bear conflict in terms of the timing of attacks, both by season and by time of day. While some variation is expected between areas, the differences between our study and others were substantial enough to warrant further investigation and for us to consider incorporating significant changes to the safety messaging for living with sloth bears on the Deccan Plateau. In this paper we present those differences and discuss their implications for effective bear safety messaging.

Study area

We studied human-sloth bear conflict in the Indian state of Karnataka on the Deccan Plateau[6]. Sloth bear habitat on the Deccan Plateau is considered some of the highest quality for the species[14]. This area is largely comprised of rocky scrub forest with an abundance of naturally occurring caves (Fig. 1). The climate is semi-arid and characterized by hot summers (24°– 45 °C) during April–June and low rainfall (571–802 mm) from June to November[15]. For this work we compared the seasonal and diurnal timing of sloth bear conflicts on the Deccan Plateau[6] to that reported in seven other studies, five of which were conducted in central India. Central India studies occurred in the states of Chhattisgarh, Odisha, Madya Pradesh, and Maharashtra. Additionally, one study was conducted in the state of Gujarat (west-central) and another in Sri Lanka (Fig. 2).

Figure 1. Sloth bear habitat on the Deccan Plateau.

Figure 2. Sloth bear attack study locations and number of attacks (QGIS Geographic Information System.Version 3.14.0-Pi. QGIS Association. http://www.qgis.org).

Methods

We compared the temporality of bear attacks on the Deccan Plateau, both seasonally and within a 24-h period, to that reported in studies presented in Table 1. Since the definition of seasons varied between studies, we standardized all seasonal data as follows: (1) summer, (2) monsoon and (3) winter. Sri Lanka is generally characterized as having four seasons, not three, two of which are characterized as monsoons and two of which are characterized as inter-monsoons[4], so we did not include data from this study in our seasonal comparisons. However, we did include the fact that in Sri Lanka the majority of attacks occurred during the dry season. If a study reported the number of attacks per season, we used those numbers accordingly. If, however, the number of attacks was reported by month rather than season, we used the following dates to regroup them for Central India: summer (March–June), monsoon (July–October) and winter (November–February). Gujarat's seasons are slightly different from those of central India, and therefore the following dates were used: summer (March–May), monsoon (June–September) and winter (October–February). Rajpurohit and Krausman[16] documented bear attacks by month for both Madya Pradesh and the Bilaspur North Forest Division, a subregion within Madya Pradesh, so we treated these two areas separately.

The time-of-day of the attack was not always reported similarly between studies. We standardized this variable across studies by reclassifying the time-of-day into four categories: (1) dawn (twilight), (2) daylight, (3), dusk (twilight) and (4) dark. If studies used these categories, we used them as is. If, however, the time of day was given in two-hour increments, as reported by three different studies[17–19], we regrouped the data as (1) dawn (twilight) 04:00–07:59, (2) daylight 08:00–15:59, (3) dusk 16:00–19:59 and dark 20:00–03:59., based on the web- site timeanddate.com (timeanddate.com), specifically the Sunrise and Sunset Calendar (time and date/sun). The time-of-day information from the Gujarat study[20] was not used in this study because the data was not presented in a comparable way to the other studies.

Analysis

To test for significant variation in the number of bear attacks by region according to season and time of day, we used the Chi-squared test, comparing expected and observed attack frequencies. We created a set of expected values by redistributing row totals across the 24-h day proportionally. Chi-squared test expected values represented our null hypothesis that time of day had no influence on the frequency of sloth bear attacks across the region.

Table 1. Sloth bear attack studies.

Location	Years	# of Attacks	Authors	Published
Madya Pradesh	1989–1994	735	Rajpurohit and Krausman	2000
Chhattisgarh	1998–2000	137	Bargali et al	2005
Sri Lanka	1938–2004	271	Ratnayeke et al	2014
Gujarat	2008–2009	71	Garcia et al	2016
Odisha	2002–2013	167	Debata et al	2016
Kanha–Pench corridor	2004–2016	166	Dhamorikar et al	2017
Maharashtra	2009–2017	51	Singh et al	2018
Deccan Plateau	1985–2916	180	Sharp et al	2020

Results

Our analysis of regional variations in season and diel timing was based on 1,778 sloth bear attacks that were documented in eight studies. Seasonally we were able to assess 1,191 attacks (Table 2) and compare these findings to our null hypothesis (Table 3). Based on diel timing we were able to assess 995 attacks (Table 4) and compare these findings to our null hypothesis (Table 5).

Table 2. Sloth bear attack incidents by location and season.

Location	Summer	Monsoon	Winter	Total
Madya Pradesh	108	128	71	307
Bilaspur North	26	53	28	107
Chhattisgarh	37	74	26	137
Gujarat	16	11	36	63
Odisha	51	91	59	201
Kanha–Pench corridor	67	58	41	166
Maharashtra	18	26	7	51
Deccan Plateau	49	34	76	159
Total	372	475	344	1191

Table 3. A comparison of actual (observed) and expected seasonal attack counts by location using the Chi-square analysis. A down arrow indicates lower than expected values; an up arrow indicates higher than expected and a horizontal (two headed) arrow means no difference.

Location	Summer	Monsoon	Winter
Madya Pradesh	↑	↑	↓
Bilaspur North	↓	↑	↓
Chhattisgarh	↓	↑	↓
Gujarat	—	↓	↑
Odisha	↓	↑	↓
Kanha–Pench corridor	↑	↑	↓
Maharashtra	↑	↑	↓
Deccan Plateau	↓	↓	↑

Table 4. Sloth bear attacks by location and time of day.

Location	Morning	Day	Evening	Night	Total
Chhattisgarh	62	49	19	5	135
Sri Lanka	30	211	16	14	271
Odisha	94	41	42	15	192
Kanha–Pench corridor	29	104	24	9	166
Maharashtra	9	37	5	0	51
Deccan Plateau	23	40	35	82	180
Total	247	482	141	125	995

Table 5. A comparison of actual (observed) and expected time of day attack counts by location using the Chi-square analysis. A down arrow indicates lower than expected values; an up arrow indicates higher than expected and a horizontal (two headed) arrow means no difference.

Location	Dawn	Day	Dusk	Night
Chhattisgarh	↑	—	—	↓
Sri Lanka	—	↑	↓	↓
Odisha	↑	—	—	↓
Kanha–Pench corridor	—	↑	—	↓
Maharashtra	—	↑	—	↓
Deccan Plateau	—	—	—	—

Season

Compared to our null hypothesis, that there is no seasonality to sloth bear conflict on the Deccan Plateau, we found a higher-than-expected number of attacks in winter (48%), lower-than-expected number of attacks during summer (31%), and significantly fewer attacks during monsoon season (21%: Fig. 3). In contrast, studies that occurred in central India had a higher-than-expected number of attacks during the monsoons and, with the exception of Odisha, a less than expected number of conflicts in the winter. Attack rates in summer varied greatly between study sites and a clear trend was not evident. However, the number of attacks in the Kanha-Pench Corridor was much higher than in the other study areas. Gujarat was the only area that, like the Deccan Plateau, showed an increase in the number of attacks during winter (57%). Gujarat, also like the Deccan Plateau, had the fewest number of attacks during the monsoon season (17%).

The Chi-squared test showed significant deviation within the table (χ^2 = 5921, df = 5, $P < 0.001$) across rows and down columns for seasonal attack frequency across the 8 study areas. A graphical representation of individual count values by location and time of day, as compared to expected values, demonstrate the variation in attack patterns across India (↔ for no variation from expected values, (↑) for values higher than expected, and (↓) for values lower than expected).

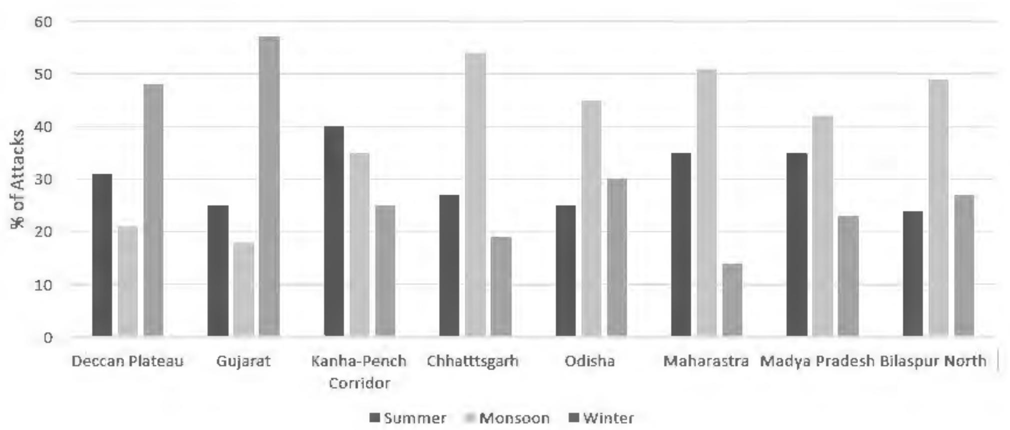


Figure 3. Seasonal percentages of attacks per region as reported in eight locations.

Time of day

Bear attacks on the Deccan Plateau were not affected by time of day, unlike any other regions we analyzed. The Deccan Plateau was also the only area where the number of attacks after dark was higher than at any other time (Fig. 4). Some areas, such as the

Kanha-Pench corridor, Sri Lanka, and Maharashtra, reported more attacks during the day and fewer at night. Chhattisgarh shared similar deviations with respect to night being less than expected. Given these different patterns we ask why they varied so differently between regions.

The Chi-squared test revealed significant deviation within the table (χ^2 = 666, df = 5, $P < 0.001$) across rows and down columns. A representation of individual count values by location and time of day, as compared to expected values, demonstrate the variation in attack patterns across India (↔ for no variation from expected values, (↑) for values higher than expected, and (↓) for values lower than expected). Upon analysis of the chi-square values for each cell, those highlighted contributed largely to significance.

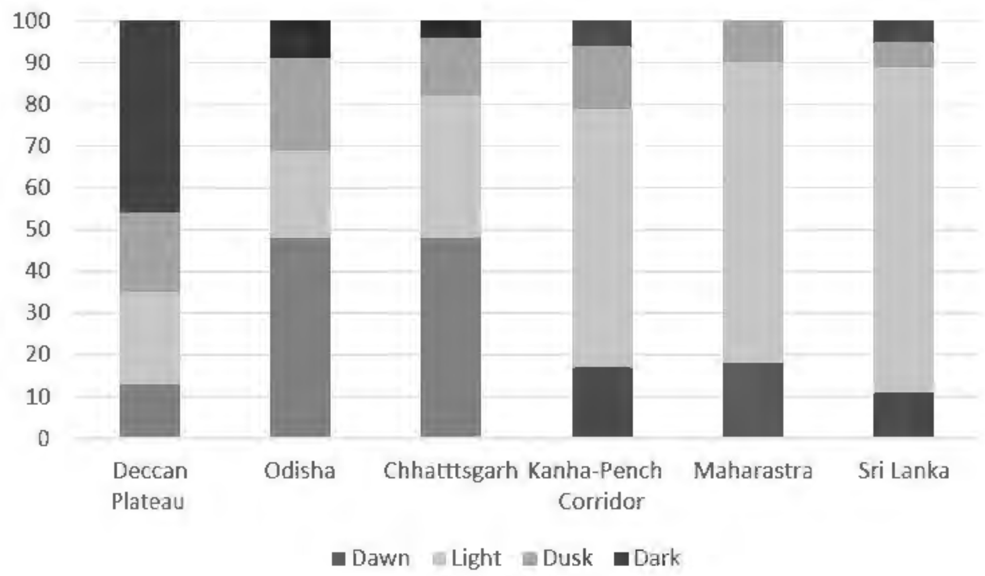


Figure 4. The percentages of sloth bear attacks by time of day and location.

Discussion

Seasonality of human-bear conflict

On the Deccan Plateau and Gujarat, most sloth bear attacks occurred in winter, which differs significantly from the seasonality of attacks reported by other studies. Unlike other study areas, people on the Deccan Plateau and in Gujarat are more active in the forest in winter when monsoons and crop harvests have ended. The higher incidence of attacks during monsoons in central India correlates with the increased presence of people farming and protecting crops from cattle depredation, as well as from bears and other wildlife species grazing in nearby forested areas[5, 16–18]. The Kanha–Pench Corridor study was the only one which documented an increase in sloth bear attacks during summer. This increase is concurrent with an increase of people in the forest that collect mahua flower (*Madhuca* spp) and tendu leaf (*Diospyros* spp)[19]. In Sri Lanka, most attacks occurred in the dry season, coincident with the highest levels of human activity in forested areas. People in Sri Lanka enter forests for alternative sources of income as agriculture activity declines during the dry season[4].

Across all studies, the majority of sloth bear attacks are correlated with the time of year when human activity is greatest in bear habitat. However, the time of year that the peak of human activity occurs in sloth bear habitat varies by region. We conclude that the seasonal activity of bears plays a much smaller role on attack rates than the seasonal activity of humans. Consistent with findings in other studies, human incursion into bear habitat is the primary factor responsible for precipitating conflict[21].

Time of day influences on human–bear conflict

Most studies attributed the time of day that attacks occurred to when most humans were active in the forest[4, 17–20]. However, the Deccan plateau differed in that the majority of attacks occurred after dark when fewer people were active in or near the forest. Working in agricultural areas after dark is a more common practice on the Deccan Plateau than for the other study areas due to the availability of electricity and artificial lighting, though even with artificial lighting human activity after dark on the Deccan Plateau is still substantially less than during daytime. While a contributing factor, we do not feel that the increase in nighttime activity on the Deccan Plateau fully explains the significant increase in attacks during that time period as compared to other areas. We suspect that sloth bear activity patterns on the Deccan Plateau, and how bears use their environment, accounts for the shift in attack timing.

Sloth bears, though potentially active throughout the day, are predominately crepuscular and nocturnal[17, 22–24]. During daytime, sloth bears seek shelter in naturally occurring caves, crevices between big boulders, the spaces between tree roots, beneath fallen trees, or under bushes[1, 25–28]. On the Deccan Plateau, however, sloth bears utilize rocky caves almost exclusively for daytime denning[29]. A cave reduces chance encounters with people and predators while providing a modicum of security, hence the lower incident rate for areas with naturally occurring caves. Conversely, studies conducted in Sri Lanka, Maharashtra and the Kanha-Pench corridor documented more attacks during daytime when people are more active but sloth bears are less active[4, 5, 19]. Large areas where sloth bears are located in Sri Lanka do not have caves for resting, though they do have dense vegetation and tree cavities (S. Ratnayeke, personal communication July 28, 2020). The Dnyanganga Wildlife Sanctuary, in the state of Maharastra, is mostly lower plains forest without rocky caves (N. Dharaiya, personal communication June 25, 2020). The Kanha-Pench corridor landscape is largely comprised of sal (*Shorea* spp) and teak (*Tectona* spp) forests largely devoid of caves[30]. The role of caves in minimizing daylight sloth bear attacks may be best exemplified by an attack in Sri Lanka as quoted in Ratnayeke et al.[4]:

"I was following two of my companions and saw a black form lying at the foot of a clump bushes, about 10 m from me. I called out to my companions. Before I knew it, the impact of the charging bear knocked me off my feet. It happened so fast, I didn't see the bear coming just dust, flying leaves, and the screams and roars of the bear."

Had this bear been in a cave rather than the shade of a bush, it likely would not have felt threatened and reacted defensively. We speculate that during daylight on the Deccan Plateau, sloth bears rest securely within a cave and are not threatened by humans passing nearby. We know that farmers and livestock herders work in relatively close proximity to known den locations without fear of being attacked (S. Shanmugavelu, pers. observation). Clearly, caves afford a level of protection and separation that benefits both bears and humans. Consequently, we suggest this is the most likely explanation as to why there are relatively few attacks on the Deccan Plateau during daytime.

Season and sloth bear safety messaging

Bear attack research and safety messaging often recognizes a seasonal component[17–20, 31] (e.g., more sloth bear attacks occur during the monsoon season than during other seasons). Sloth bears are active year-round, and the rate of attacks is strongly correlated with the level of human activity in the forest. Similarly, in Alaska, Smith and Herrero[32] reported that human-brown bear conflicts were strongly seasonal in their occurrence. Additionally, they reported that attacks occurred most often when both people and bears vied for the same resource, such as salmon or ungulates. Farther north, human-polar bear conflict peaks when bears are on land awaiting freeze up in the fall[33]. Not infrequently, sloth bear safety messaging amounts to little more than general statements such as “when in the forest or in sloth bear country be aware”. In other words, an individual’s odds of being attacked by a sloth bear while in the woods may not significantly vary regardless of season. But, where it has been found to vary by season, this information should be conveyed to the public.

Time of day and sloth bear safety messaging

Sloth bear research and safety messaging often reports and warns of the “most dangerous” time or times of the day to be active in the forest[17–20, 31, 34]. Sloth bear attacks, like grizzly bear or American black bear attacks[33], can occur anytime, day or night[6]. However, due to an abundance of naturally occurring caves on the Deccan Plateau, stumbling across a sleeping sloth bear mid-day is much less likely to occur than it is in Sri Lanka or in the Kanha-Pench corridor. Therefore, regional sloth bear safety messaging should acknowledge this significant difference which will promote bear safety.

The Corbett Foundation[31] and Dharaiya et al.[34] do an admirable job of focusing their safety messaging to a specific regional group of people in their respective publication. This type of regional messaging is necessary for optimizing sloth bear safety messaging efficacy. However, there is also value to non-site-specific sloth bear safety messaging. The short film “Living with Sloth Bears”[35] intentionally addresses general safety messaging that applies to sloth bears across their entire range. Consequently, in the making of this film, we purposely avoided referring to the timing of attacks, seasons or time of day, or other aspects of human-bear conflict because we were aware of significant differences with respect to these variables between locations.

Yet another aspect of bear safety messaging is to keep it simple so that a person, under duress, will remember what to do in the event of a bear encounter Attempting to recall the details of an extended message, especially when being threatened by a bear, can be difficult, if not impossible. Therefore, the trend has been to keep bear messaging as simple as possible, and we agree with it. However, teaching people that work in bear habitat the most likely times of day encounters occur can be beneficial. In summary, there is a time and place to provide detailed information that is regionally specific, and other situations in which to keep messaging simple.

Sloth bear denning ecology on the Deccan Plateau and its role in human–bear conflict

The Deccan Plateau is known as high quality sloth bear habitat, as evidenced by the relatively high density of bears in this area (S. Shanmugavelu, pers. observation). While there is ample food on the Deccan Plateau, the abundanceof caves there sets it apart from other areas within the specie's range. Sloth bears use only caves or cave-like structures on the Deccan Plateau for resting (Shanmugavelu et al. In Print). Caves provide protection from the elements, such as the heat of the day or severe storms, as well as protection from potential predators. Sloth bears do not have many predators and while a cub or very young bear may be at risk from leopards (*Panthera pardus*) or wolves (*Canis lupes pallipes*), the only natural predator of adult sloth bears is the Bengal tiger (*Panthera tigristigris*). Tiger scat studies revealed that sloth bears can comprise up to 2% of their diet[36–39]. Tigers no longer occur on the Deccan Plateau, but the abundance of caves in the area undoubtedly historically benefited sloth bears, perhaps facilitating a higher density than would have been otherwise attainable. Presently, however, an increase in human population and habitat loss represents greater threat to the species.

Conclusions

Bear safety messaging often differs regionally. Periodically, these differences have been the source of discussion and disagreement between bear biologists, park managers and the public. Should bear safety messaging be more standardized so that there is less confusion, or are regional differences in human-bear conflict important to incorporate? While sloth bear safety messaging is still in its early stages of development compared to that of American black and brown bears, results of this study suggest that incorporation of regional differences is important to optimize human safety. While some regional differences in human-bear conflict are due to varying modes of human activity, in other instances differences in messaging are reflective of variation in bear activity and how the species has evolved to modify behaviors to best fit environmental variation. In seeking to ensure human safety in bear habitat, we see variation in messaging as key to helping better prepare people to avoid bear conflict.

Acknowledgements

We would like to thank anonymous reviewers for their thoughtful comments regarding this manuscript. We would also like to thank the following persons for assisting data collection: G. Balasubramaniyam, P. Yogaraj. and P. Reegan. Additionally, we thank Kartick Satyanarayan and Geeta Seshamani the Co-founders of Wildlife SOS (WSOS) for their support and assistance, without which this work would not have been possible. We also thank Sydney Stephens who provided valuable assistance in the creation of our maps.

References

1. Garshelis, D. L., Joshi, A. R., Smith, J. L. D. & Rice, C. G. Sloth bear conservation action plan. In *Bears Status Survey and Conservation Action Plan. IUCN/SSC Bear and Polar Bear Specialist Groups* (eds Servheen, C. *et al.*) 225–240 (IUCN, 1999).

2. Sterndale, R. A. *Natural History of the Mammalia of India and Ceylon* (Thacker, Spink, and Co., 1884).

3. Pillarisett, A. M. Are sloth bear man marauders? In *Two Decades of Project Tiger, Melghat (1973–1993)* (eds Gogate, M. G. & Thorsare, P. J.) (Melghat Tiger Reserve, 1993).

4. Ratnayeke, S., van Manen, F. T., Pieris, R. & Pragash, V. S. J. Challenges of large carnivore conservation: sloth bear attacks in Sri Lanka. *Hum. Ecol.* 42, 467–479 (2014).

5. Singh, N., Sonone, S. & Dharaiya, N. Sloth bear attacks on humans in central India: Implications for species conservation. *Human- Wildlife Interactions* 12, 338–347 (2018).

6. Sharp, T. R. *et al.* Sloth bear attacks on the Deccan Plateau of Karnataka, India. *Ursus* 31, e8. https://doi.org/10.2192/URSUS-D- 18-00027.3 (2020).

7. Cowan, N. The magical mystery four: How is working memory capacity limited, and why?. *Curr. Dir. Psychol. Sci.* 19, 51–57 (2010).

8. Smith, T., Herrero, S. & DeBruyen, T. D. Alaskan brown bears, humans and habituation. *Ursus* 16(1), 1–10 (2005).

9. Littledal, H. E. Bears being eaten by tigers. *J. Bombay Nat. Hist. Soc.* 4, 316 (1889).

10. Cambell, T. J. A tiger eating a bear. *J. Bombay Nat. His. Soc.* 9, 101 (1894).

11. Clutterbuck, P. H. A tiger killing a bear. *J. Bombay Nat. Hist. Soc.* 9, 229–230 (1894).

12. Reddy, H. S., Srinivasulu, C. & Thulsi, K. Prey selection by the Indian tiger *Panthera tigris tigris* in Nagarjunasagar Srisailam Tiger Reserve. *India. Mammalian Biology* 69, 384–391 (2004).

13. Interagency Bear Safety Education Working Group. A Framework for Bear Safety Messages in Alaska. (2017). http://www.adfg. alaska.gov/static/species/livingwithwildlife/bears/pdfs/framework_bear_safety_messages_ alaska.pdf. Accessed 22 Sept 2020.

14. Puri, M., Srivathsa, A., Karanth, K. K., Kumar, N. & Karanth, K. U. Multiscale distribution models for conserving widespread species: The case of sloth bear *Melursus ursinus* in India. *Divers. Distrib.* https://doi.org/10.1111/ddi.12335 (2015).

15. Government of Karnataka, Annual Rainfall Report of 2010. Directorate of Economics and Statistics. 2011, July.

16. Rajpurohit, K. S. & Krausman, P. R. Human–sloth bear conflicts in Madhya Pradesh. *India. Wildlife Society Bulletin* 28, 393–399 (2000).

17. Bargali, H. S., Akhtar, N. & Chauhan, N. P. S. Characteristics of sloth bear attacks and human casualties in North Bilaspur Forest Division, Chhattisgarh. *India. Ursus* 16, 263–267 (2005).

18. Debata, S., Swain, K. K., Sahu, H. K. & Palei, H. S. Human–sloth bear conflict in a human-dominated landscape of northern Odisha. *India. Ursus* 27(2), 90–98. https://doi.org/10.2192/URSUS-D-16-00007.1 (2016).

19. Dhamorikar, A. H., Mehta, P., Bargali, H. & Gore, K. Characteristics of human–sloth bear (*Melursus ursinus*) encounters and the resulting human casualties in the Kanha–Pench corridor, Madhya Pradesh, India. *PLoS ONE* 12(4), e0176612 (2017).

20. Garcia, K. C., Joshi, H. M. & Dharaiya, N. Assessment of human–sloth bear conflicts in North Gujarat, India. *Ursus* 27, 5–10 (2016).

21. Smith, T. S. & Johnson, B. A. Modeling the effects of human activity on Katmai brown bears (*Ursus arctos*) through the use of survival analysis. *Arctic* 57, 161–166 (2004).

22. Joshi, A. R., Smith, J. L. D. & Garshelis, D. L. Sociobiology of the myrmecophagous Sloth

Bear in Nepal. *Can. J. Zool.* 77(11),1690–1704 (1999).

23. Akhtar, N., Bargali, H. S. & Chauhan, N. P. S. Sloth bear habitat use in disturbed and unprotected areas of Madhya Pradesh, India.

24. *Ursus* 15, 203–211 (2004).

25. Bargali, H. S., Akhtar, N. & Chauhan, N. P. S. The sloth bear activity and movement in highly fragmented and disturbed habitat in Central India. *World J. Zool.* 7(4), 312–319 (2012).

26. Eisenberg, J. F. & Lockhart, M. C. An ecological reconnaissance of Wilpattu National Park, Ceylon. *Smithson. Contrib. Zool.* 101, 1–119 (1972).

27. Laurie, A. & Seidensticker, J. Behavioural ecology of the sloth bear (*Melursus ursinus*). *J. Zool.* 182, 187–204 (1977).

28. Desai, A. A., Bhaskaran, N. & Ventaktesh, S. *Behavioural Ecology of the Sloth Bear in Mudumalai Wildlife Sanctuary and National Park* (Tamil Nadu and Bombay Natural History Society Collaborative Project, 1997).

29. Akhtar, N., Bargali, H. S. & Chauhan, N. P. S. Characteristics of sloth bear day dens and use in disturbed habitat of North Bilaspur Forest Division, Chhattisgarh, central India. *Ursus* 18(2), 203–208 (2007).

30. Shanmugavelu, S., Sharp, T., Arun, A., Smith, T. & Larsen, R. Sloth bear maternal and resting den locations in eastern Karnataka.

31. *J. Wildl. Manag.* (in press).

32. Jena, J., Borah, J., Dave, C. & Vattakaven, J. *Lifeline for Tigers: Status and Conservation of the Kanha–Pench Corridor* (WWF-India,2011).

33. The Corbett Foundation. How to avoid conflict with sloth bears—A pictorial handbook. The Corbett Foundation 2018, Mumbai, India.

34. Smith, T. & Herrero, S. Human-bear conflict in Alaska: 1880–2015. *Wildl. Soc. Bull.* 42(2), 254–263 (2018).

35. Wilder, J. M. *et al.* Polar bear attacks on humans: Implications of a changing climate. *J. Wildl.*

Manag. 41, 537–547 (2017).

36. Dharaiya, N., Gadhavi, D. & Shah, V. *Living in the Sloth Bear Landscape: An Information Booklet for Locals* (Wildlife and Conserva- tion Biology Research Lab and Vadodara Wildlife Division, 2019).

37. Living with Sloth Bears. (2020). Directed by Thomas Rowell. Short Film.

38. Biswas, S. & Sankar, K. Prey abundance and food habit of tigers Panthera tigris tigris in Pench National Park, Madhya Pradesh, India. *J. Zool.* 256, 411–420 (2002).

39. Reddy, H. S., Srinivasulu, C. & Thulsi, K. 2004: Prey selection by the Indian tiger *Panthera tigris tigris* in Nagarjunasagar Srisailam Tiger Reserve, India. *Mamm. Biol.* 69, 384–391 (2004).

40. Andheria, A. P., Karanth, K. U. & Kumar, N. S. Diet and prey profiles of three sympatric large carnivores in Bandipur Tiger Reserve, India. *J. Zool.* 273, 169–175 (2007).

41. Kapfer, P. M. *et al.* Fine-scale spatio-temporal variation in tiger Panthera tigris diet: Effect of study duration and extent on estimates of tiger diet in Chitwan National Park, Nepal. *Wildl. Biol.* 17(3), 277–285 (2011).

Art – 277. ISOLATION, CHARACTERIZATION, AND DRUG SENSITIVITY OF *Mycobacterium tuberculosis* IN CAPTIVE SLOTH BEARS (*Melursus ursinus*): UNNATURAL HABITAT WITH HUMAN ENVIRONMENT MAY PREDISPOSE SLOTH BEARS TO TUBERCULOSIS

Chandranaik B. Marinaik, **Arun A. Sha**, Venkataravanappa Manjunatha, S. Shylaja, Doddamane Rathnamma, Apsana Rizwan and K. Nagaraja

We describe the isolation, molecular characterization, and drug sensitivity of *Mycobacterium tuberculosis* recovered from lung tissues of four rescued captive sloth bears (*Melursus ursinus*) at Bannerghatta Biological Park (BBP), Bangalore, India. These bears had lived most of their life with humans in circus companies. They were rescued and housed in the Bear Rescue Center (BRC) of BBP. Upon rescue, they showed signs of unthriftiness, chronic debility, and failed to respond to symptomatic treatments. Over the period of the next 12–14 months, the four sloth bears died and the post-mortem examination revealed nodular lesions in the lungs that showed the presence of acid-fast bacilli. Polymerase chain reaction (PCR), culture, and nucleotide sequencing confirmed the bacilli as *Mycobacterium tuberculosis.* Histopathology of the lungs revealed characteristic granulomatous reaction with caseation. We determined the sensitivity of these isolates to rifampicin and isoniazid drugs by a WHO approved test,Line Probe Assay (LPA) using Genotype MTBDR*plus* VER 2.0. We discuss the role of unnatural habitat with the human environment in predisposing captive sloth bears fortuberculosis (TB). In the absence of any other reliable ante-mortem diagnostic test, this study recommends the use of LPA for early detection of TB in captive wild animals, which will help in taking necessary steps to prevent its further spread to animal caretakers and other susceptible animals in captivity.

Keywords: *Mycobacterium tuberculosis*, captive sloth bears, phylogenetic characterization, human environments, drug sensitivity

Introduction

Tuberculosis (TB), a pandemic, is a highly contagious disease caused by organisms belonging to the genus *Mycobacterium*, which has affected up to one-third of the world's population. The south-east Asia (SEA) region, with 26% of the world population,accounts for 44% morbidity and more than 50% mortality of the global burden of tuberculosis

(1). *Mycobacterium bovis* (*M. bovis*) is widespread in domestic animals and has been extensively documented in both captive and free-ranging wildlife populations [2–4]. A number of wildlife populations have been reported to be endemically infected with *M. bovis*, for example, the European badger (*Meles meles*) in the United Kingdom [5] and the African buffalo (*Syncerus caffer*) in South Africa [2]. These permanent reservoirs of infection pose a serious threat to public health and TB eradication programs. In contrast, though *Mycobacterium tuberculosis* (*M. tuberculosis*) is considered a human pathogen, it has been reported to cause TB in wildlife species living in close contact with humans [6–8].

Sloth bears (*Melursus ursinus*) are myrmecophagous bear species, listed as "vulnerable" (high risk of extinction due to human factors) in the International Union for Conservation of Nature (IUCN) red list [9]. TB has been reported in captive sloth bears, caused by *M. bovis* (10) as well as by *M. tuberculosis* [2, 11]. However, there are no detailed studies on molecular phylogenetic characterization and drug sensitivity of the pathogen causing TB in sloth bears. In this paper, we report the isolation, phylogenetic characterization, and line probe assay (LPA)-based drug sensitivity of *Mycobacterium tuberculosis* isolates from sloth bears that were rescued from circus companies. The findings possibly indicate the role of unnatural human-environment in predisposing captive sloth bears for TB and the necessity of using reliable ante-mortem diagnostic tests for early detection of TB in wildlife species.

Methods

Animals and Collection of Samples

The study included four sloth bears (comprising two males and two females), ~14–16 years of age, which were rescued from circus companies and relocated to the Bear Rescue Center (BRC), Bannerghatta Biological Park (BBP), Bangalore, India. Blood samples were collected from these four sloth bears and were submitted for a total hemogram as per the procedure outlined by Benjamin [12].

The sloth bears under this study did not respond to symptomatic treatments and they died at the BRC. A detailed post-mortem examination was conducted on these four carcasses, and impression smears from lung lesions and lung tissues were collected for microbiological and histopathological examinations.

Processing of Lung Tissues

The lung tissues collected at post-mortem examinations were decontaminated and homogenized with sterile 4% sodium hydroxide by modified Petroff's method [13], and then centrifuged at 10,000 rpm for 20 min. The supernatant was discarded, and the sediment was washed with normal saline and centrifuged at 10,000 rpm for 10 min. The supernatant was discarded, and the sediment was used for staining, DNA extraction, and inoculation to culture media.

Ziehl-Neelsen Acid-Fast Staining

The impression smears made out from the nodular lesions of the lungs were stained for acid-fast bacteria by the Ziehl-Neelsen staining method as described by Quinn and co-workers [14].

Polymerase Chain Reaction

Deoxyribonucleic acid (DNA) from the processed lung tissues was extracted using Qiagen® DNA extraction kits (QIAamp DNA mini kit, catalog no. 51304) as per the procedure outlined by the manufacturer. Primers required for PCRwere synthesized and procured from M/s Bioserve Ltd., Hyderabad, India. Initial identification of *Mycobacterium tuberculosis* complex (MTC) was done by specific amplification of 445-bp conserved fragment on IS6110 MTC using the primers IS6110 F 5′GACCACGACCGAAGAATCCGCTG3′ and IS6110 R 5′CGGACAGGCCGAGTTTGGTCATC3′ [15]. For identification of *Mycobacterium* species by PCR, oligonucleotide primers targeting a unique 12.7 kb insertion sequence responsible for species-specific genomic polymorphism between the closely related *M. bovis* and *M. tuberculosis* were used [15]. The primers for detection of *M. bovis* included the forward primer 5′CACCCCGATGATCTTCTGTT 3′ and reverse primer 5'GCCAGTTTGCATTGCTATT 3′ for amplification of a region of 823-bp on 12.7 kb fragment of *M. bovis* [15]. The primers for detection of *M. tuberculosis* included the forward primer F 5′CACCCCGATGATCTTCTGTT 3′ and reverse primer 5′GACCCGCTGATCAAAGGTAT 3′ for amplification of 389- bp on 12.7 kb fragment of *M. tuberculosis* [15]. The PCR was performed with a total volume of 25 µl, with 5 µl of DNA from the sample, 10 pM each of forward and reverse primers, 25 µM of each deoxynucleoside triphosphate (dNTP), 1.5 unitsof *Taq* DNA polymerase, 10 mM Tris-HCl buffer (pH 8.0), and 1.5 mM $MgCl_2$. The PCR thermal cycling conditions for initial identification of MTC and species-specific PCR included initial denaturation at 94°C for 10 min followed by 30 repeated cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The amplicons were analyzed by gel electrophoresis in 1.5% agarose gel.

Phylogenetic Analysis

Polymerase chain reaction-amplified products were extracted from agarose gel and eluted in 25 µl of nuclease-free water using Qiagen® gel extraction kit as per manufacturer's instructions and were then subjected to nucleotide sequencing at M/s Bioserve Ltd., Hyderabad, India. Nucleotide sequences were aligned with published sequences deposited in GenBank. A phylogenetic tree was constructed, and sequence analysis was performed in MEGA version 6.2, using the Neighbor Joining tree method with 1,000 bootstrap replicates (16).

Histopathological Examination

The lung samples collected during the post-mortem examination were processed and submitted for histopathological examination as per previously described standard procedures (3, 6, 17).

Isolations of Mycobacterial Organisms Using LJ Media

A loopful of processed nodular lung tissue from each of the sloth bears was inoculated onto separate LJ media slants (with glycerol) procured from Hi-media laboratories, Mumbai, India. The slants were incubated at 37°C under 5% CO2 for 8 weeks and were observed at weekly intervals.

Line Probe Assay for Detection of DrugSensitivity

Line probe assay is a WHO approved tool for early detection of MTC, which can simultaneously assess anti-TB drug sensitivity (18). In this study, LPA was performed to assess the sensitivity of these isolates to rifampicin and isoniazid drugs using Genotype MTBDR*plus*VER 2.0 procured from M/s Hain Life Sciences, Germany. The LPA was performed using the procedure outlined by Barnard and co-workers (18) at Karnataka State Tuberculosis Center and Intermediate Reference Laboratory, Bangalore, India. The procedure included extraction of DNA from the decontaminated culture using the GenoLyse kit according to the manufacturer's instructions to generate the substrate for PCR amplification and hybridization using the GenoType MTBDR*plus* (VER 2.0) LPA. For this, 700 µl of the decontaminated sample was centrifuged for 15 min at 10,000 x *g*, and the pellet was resuspended in 100 µl of lysis buffer and incubated for 5 min at 95°C. Thereafter, 100 µl of neutralizing buffer was added to the lysate, mixed, and centrifuged at full speed for 5 min. The top 100 µl of the supernatant was aliquoted into a clean 1.5 ml tube and used for the PCR, and the residual portion was discarded. The PCR mixture was prepared by mixing 10 µl of amplification mix A (AM-A) (which contains the buffer, nucleotides, and DNA polymerase) with 35 µl of amplification mix B (AM-B) (which contains $MgCl_2$, the biotinylated primers, and dye), followed by the addition of 5 µl of the GenoLyse-purified DNA. The PCR amplification for the GenoTypeMTBDR*plus* (v2.0) LPA was done using the PCR program recommended by the manufacturer. Following PCR amplification, the reverse hybridization step and the interpretation of the hybridization results were done as previously described [18].

Result

The four sloth bears under this study, aged approximately 14– 16 years, were living in human environments for more than 12 years in circus companies. They were rescued from these circus companies and were housed at the BRC. After their arrival at the BRC, they showed signs of gradual weakness and debility. The blood samples in the ailing animals revealed leucocytosis with an average white blood cell count of 17.4×10^3 per µl (normal values in sloth bears is $12.1–16.2 \times 10^3$ per µL) with neutrophilia with an average of 92% neutrophils (normal values in sloth bears is 56– 63%), indicative of bacterial infection. The animals were treated symptomatically with antibiotics, analgesics, antipyretics, antihistamines, liver-stimulants, multivitamin supplements, mineral supplements, and intravenous fluid. However, they did not respond to these treatments and over a period of the next 12– 14 months, these four sloth bears died with signs of anorexia, cachexia, severe debility, dyspnea, and dry cough.

On post-mortem examination, the carcasses appeared emaciated with poor body condition. The lungs appeared consolidated with widely disseminated white-yellowish firm nodules with central caseous necrosis distributed throughout the lung parenchyma (Figure 1). Most of the affected areas showed cavitation with central necrosis.

Occasionally, these nodules were calcified. Inter-lobular adhesions and pleural adhesions were observed. The bronchial and mediastinal lymphnodes were enlarged with nodular areas of caseous necrosis and calcification. In all animals, the abdominal cavity was filled with ascetic fluid and showed congestion of the liver, kidney, and spleen. Two animals had hydrothorax. One of the sloth bears had hemorrhagic enteritis. The tubercular lesions were confined to the lungs and regional lymph nodes and did not involve any other organs and/or systems. Histopathological examination of the lungs revealed multiple granulomas, each consisting of central caseum surrounded by epithelioid macrophages and occasional multinucleated giant cells of the Langhan's type, with peripheral recruited lymphocytes and a fibrous capsule (Figure 2).

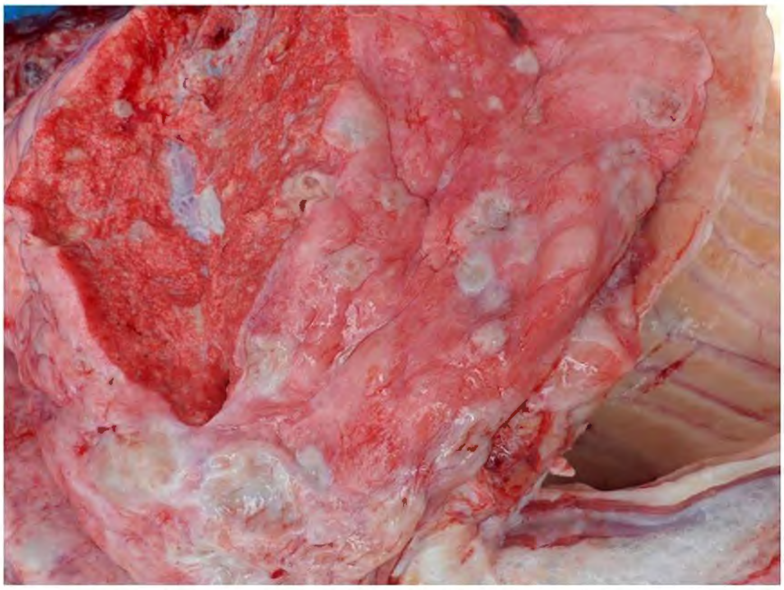


Figure 1 | Lungs showing nodular lesions distributed throughout the parenchyma with central caseous necrosis

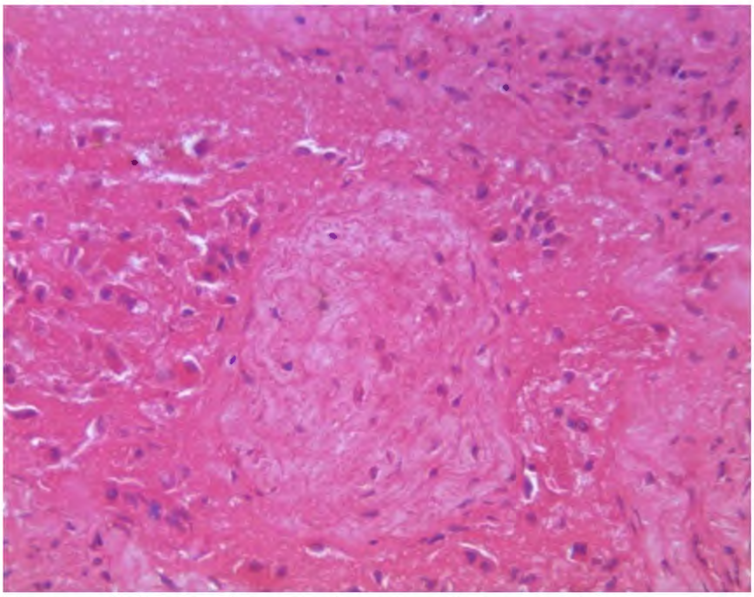


Figure 2 | Histopathology of lungs showing characteristic granulomatous reactions with central caseum, recruited inflammatory cells, and a fibrous capsule.

Impression smears from the cut surfaces of the lungs stained with Ziehl Neelson's method showed bundles of pink- stained acid-fast *Mycobacterium* species (Figure 3A). The DNA extracted from the lung tissue, when subjected for PCR targeting the amplification of a conserved region on "MTC" yielded a specific amplicon of 445-bp, indicating the presence of pathogenic *Mycobacterium* species. The PCR employed for the detection of *M. bovis* did not yield amplicons. The PCR employed for detection of *M. tuberculosis* yielded a specific amplicon of 389- bp (Figure 3B) indicating the presence of *M. tuberculosis* DNA in the lung tissue.

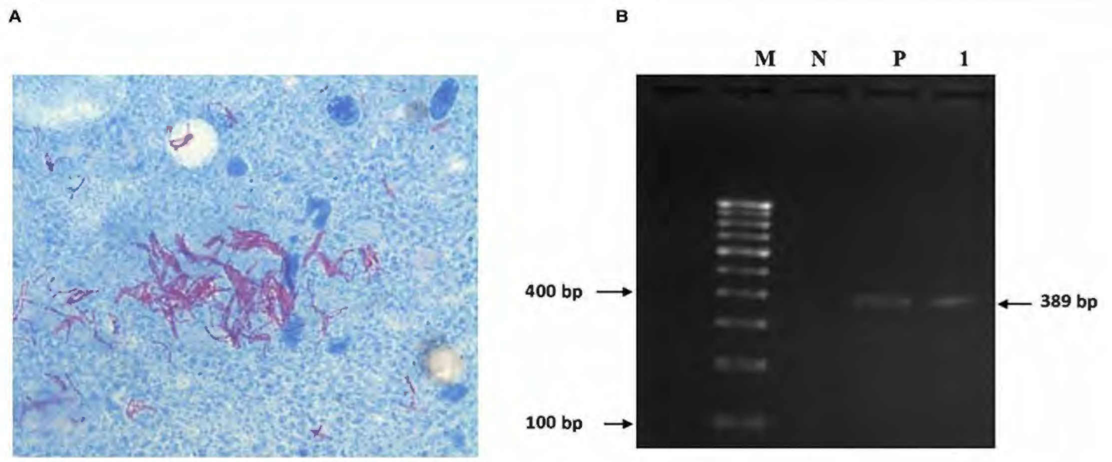


Figure 3 | (A) Impression smears from the cut surfaces of lungs show acid-fast bacilli. (B) Gel showing amplification of 389 bp region of Mycobacterium tuberculosis. Lane 1: DNA extracted from lung lesions of a sloth bear. Lane P, Positive control; Lane N, Negative control; Lane M, 100 bp marker.

The phylogenetic analysis of the *M. tuberculosis*-specific 389- bp nucleotides showed that the isolates had 100% nucleotide sequence identity with *M. tuberculosis* sequences deposited in the GenBank (Figure 4), thereby confirming the pathogen as *M. tuberculosis*. The processed lung tissue samples from each of the sloth bear inoculated on LJ slants yielded growth of buff-colored, rough, dry, raised, irregularly wrinkled colonies. The PCR on the DNA extracted from these colonies confirmed the isolates as *M. tuberculosis.* The LPA employed on the DNA extracted from the colonies on LJ slants further confirmed the isolates as belonging to the MTC group, and the isolates were found to be sensitive to rifampicin and isoniazid, the first line anti-TB drugs.

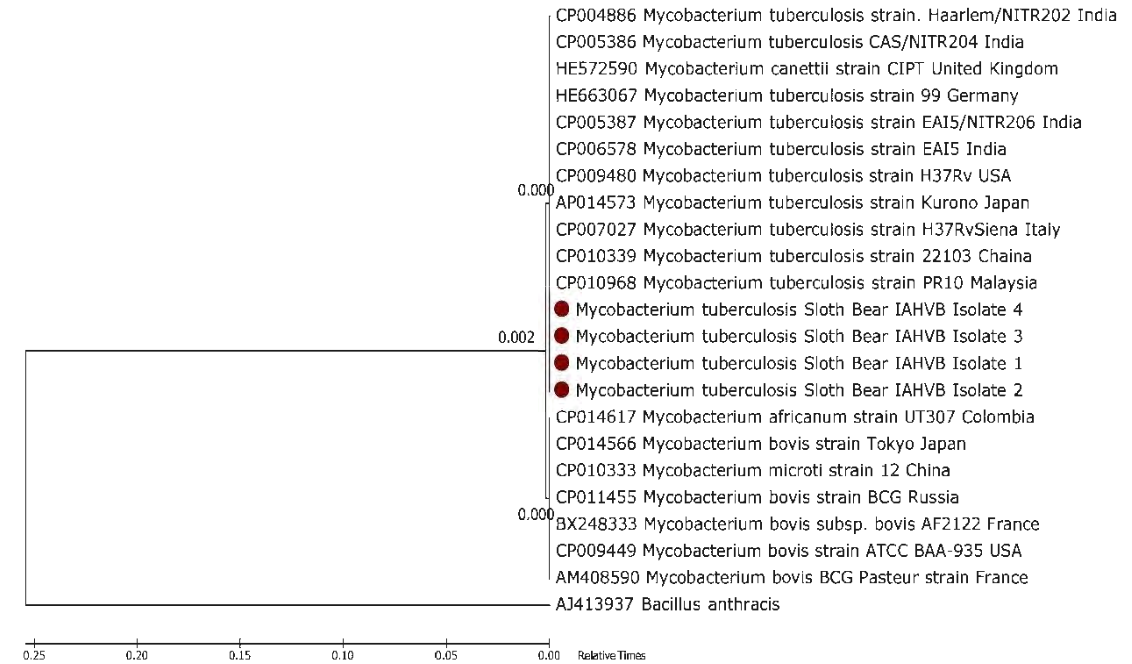


Figure 4 | Phylogenetic analysis of Mycobacterium tuberculosis isolates from sloth bears.

Discussion

Tuberculosis remains one of the major public health concerns in the world. In SEA, it is estimated that about 4.3 million people fell ill with TB in 2019 and about 632,000 people died due to TB in 2019, which is more than half of the global deaths due to TB [1]. India accounts for more than 50% of the prevalence as well as mortality due to TB in SEA [1].

The gross pathology, PCR, and histological findings confirmed TB infection in sloth bears in this study, and these observations were in accordance with the previous reports of TB in wildlife in general [3, 17] and sloth bears in particular [6]. Phylogenetic characterization of *M. tuberculosis* in sloth bears showed 100% sequence homology with *M. tuberculosis* of human origin, possibly indicating the source of the infection.

Since ecologic, environmental, and demographic factors influence the emergence of diseases [19], based on the history of these animals, TB in these animals could be attributed to the following reasons. Prior to rescue, the sloth bears were living in human environments in circus companies for more than 12 years in Indian cities with relatively higher levels of industrial and motor vehicle pollution. Throughout their stay with the circus companies, they were constantly exposed to the general public, who came to watch them, and the animal attendants/circus personnel with whom they lived in very close contact. It is possible that the sloth bears became infected with *M. tuberculosis* from any of these people during their long association with the circus companies. This transmission opportunity exists in India, which accounts for 26% of the world's incident cases and over 20% of world deaths due to TB [1]. It has been reported that the development of TB in an exposed individual is primarily endogenous (host-related), and conditions like malnutrition, indoor air pollution together with over-crowding, which lower the host immune response, accelerate the progression to TB disease [20, 21]. Air pollution impairs macrophage phagocytic function, surface adherence, and bacterial clearance [22]. It is also noted that the sloth bears were more than 14 years of age, which would have predisposed them to rapid progression of the disease as reported in human beings since about 10–15% of those infected go on to develop active disease at later stages of their life [23]. The present study provides evidence that sloth bears living in their unnatural habitats with human environments can harbor *M. tuberculosis* which can become clinical and fatal, thereby emphasizing the role of humans in the emergence of infectious diseases in wildlife populations.

The results of LPA employed in this study not only confirmed the isolates as pathogenic *Mycobacterium* belonging to the MTC group but also found that the isolates were sensitive to rifampicin and isoniazid drugs, which are the most commonly used and readily available human anti-TB drugs. The sloth bears in this study were diagnosed with TB only after their death. If this diagnosis was known prior to death, it would have facilitated better management of the disease, including a possible change in the treatment regimen. In the absence of any other reliable ante-mortem tests for TB diagnosis in sloth bears [24–26] and because LPA can be employed even on the DNA extracted from bronchial washes [18], LPA may be employed for early diagnosis of TB in suspected sloth bears and other captive wildlife species. Alternatively, newer methods like "Xpert MTB/RIF assay," which detects MTC and resistance to rifampicin (RIF) in <2 h in clinical samples like sputum and bronchial washes, can also be used for ante-mortem diagnosis of suspected tuberculosis in sloth bears [27]. Considering the public health significance of TB, the ante-mortem diagnosis will help in implementing the necessary precautions to prevent the spread of TB to animal caretakers and other susceptible animals in captivity.

Although there are similarities between pathogenesis and diagnosis of TB in humans and animals, the disease management strategies are different. Diagnosis is followed by antibiotic therapy in humans, in comparison to test and slaughter for animals

[28], barring some rare instances in captive zoo animals, where they have used anti-TB drugs without much success [29]. Under this global scenario, LPA-based anti-TB drug sensitivity results have limited or no value in the veterinary field. However, LPA data can be used to initiate preliminary research on using anti-TB drugs and assess their efficacy in animals with TB, under required biosafety facilities, which may become useful in saving species in the future.

Data Availability Statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics Statement

Ethical review and approval was not required for the animal study because this research did not require approval and the study was part of the disease process already in the animals under study.

Author Contributions

CM conceived the experiments, performed the experiments, analyzed the data, and wrote the paper. AS, VM, AR, and SS collected the samples, performed the experiments, and contributed to writing the paper. DR and KN performed the experiments and provided the critical reagents. All authors contributed to the paper and approved the submitted version.

Acknowledgments

The authors thank the authorities of Bannerghatta Biological Park, Wildlife SOS, and its staff of the Bear Rescue Centre, Bangalore for their kind help and support for sample collections.The authors also thank Dr. Sangeetha Kodakani and Dr. AnilC from Karnataka State Tuberculosis Centre and Intermediate Reference Laboratory, Bangalore, India for their kind help in conducting the LPA on the samples.

References

1. World Health Organization (WHO). *Global Tuberculosis Report*. Geneva; WHO (2020).

2. Bengis R. Tuberculosis in free-ranging mammals. In: Fowler M, Miller RE, editors. *Zoo and Wildlife Medicine*: *Current Therapy 4,* W.B. Philadelphia; Saunders Company (2000).

3. Marinaik CB, Shivashankar BP, Umashankar KS, Nandini P, Giridhar P, Byregowda SM, et al. *Mycobacterium tuberculosis* infection in free-roaming wild Asian elephant. *Emerg Infe Dis*. (2017) 23:555–7. doi: 10.3201/eid2303.161439

4. Angkwanish T, Vernooij HJCM, Sirimalaisuwan A, Charernpan P, Nielen M, Rutten VPMG. Prevalence and demographic risk factors of mycobacterium tuberculosis infections in captive Asian elephants (elephas maximus) based on serological assays. *Front Vet Sci.* (2021) 8:713663. doi: 10.3389/fvets.2021.713663

5. Gallagher J, Clifton-Hadley RS. Tuberculosis in badgers: a review of the disease and its significance for other animals. *Res Vet Sci.* (2000) 69:203– 17. doi: 10.1053/rvsc.2000.0422

6. Harish BR, Chandranaik BM, Venkatesha MD, Renukaprasad C, Jayakumar SR, Krishnappa G. Tuberculosis in sloth bears (*Melurus ursinus*). *Indian J Vet Pathol.* (2002) 27:129–30.

7. Kathleen AA, Pleydell E, Mark CW, Emily PL, John FCN, Anita LM. Mycobacterium tuberculosis: an emerging disease of free-ranging wildlife. *Emerg Inf Dis.* (2002) 8:598–601. doi: 10.3201/eid0806.010358

8. Obanda V, Poghon J, Yongo M, Mulei I, Ngotho M, Waititu K, et al. First reported case of fatal tuberculosis in a wild African elephant with past human-wildlife contact. *Epidemiol Infe*. (2013) 141:1476– 80. doi: 10.1017/S0950268813000022

9. Dhariza, N., Bargali, H. S., and Sharp, T. (2020). *Melursus ursinus: IUCN Red List of Threatened Species*.

10. Veeraselvam M, Sridhar R, Senthilkumar TMA, Jayathangaraj MG, Perumal, P. Detection of *Mycobacterium bovis* in captive sloth bears (*Melursus ursinus*) by

polymerase chain reaction. *Int J Zoological Res*. (2013) 3:17–20.

11. Rishikesavan R, Arun AS, Chandranaik BM, Basavarajappa K, Giridhar P, Renukaprasad C. Study on prevalence of Tuberculosis in rescued captive sloth bears. *J Vet Pub Health.* (2008) 6:53–4.

12. Benjamin MM. *Outline of Veterinary Clinical Pathology*. 4thEdn. Iowa State University Press, Ames, USA (1998).

13. Srivastava K, Chauhan DS, Gupta P, Singh HB, Sharma VD, Yadav VS. Isolation of *Mycobacterium bovis* and *M. tuberculosis* from cattle of some farms in north India– possible relevance in human health. *Indian J Med Res*. (2008) 128:26–31.

14. Quinn PJ, Markey BK, Leonard FC, Fitzpatrick ES, Fanning S, Hartigan PJ. *Veterinary Microbiology and Microbial Diseases*. *2ndEdn*. United Kingdome; Wiley-Blackwell Publications (2011).

15. Alex K, Rishendra V. PCR-SSCCP analysis in detecting point mutations targeting rpoB, katG and inhA genes for determining multi-drug resistance in *Mycobacterium bovis* and *Mycobacterium tuberculosis* strains. *Indian J Anim Scis*. (2014) 84:1256– 1260.

16. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evolution*. (2013) 30:2725–9. doi: 10.1093/molbev/mst197

17. Rishikesavan R, Chandranaik BM, Swathi B, Roopa S, Giridhar P, Renukaprasad C. Pathoepidemiological study of tuberculosis in panthera pardus. *Zoos Print J.* (2010) 25:28–9.

18. Barnard M, Gey Pittius NC, Van Helden PD, Bosman M, Coetzee G, Warren RM. The diagnostic performance of the genotype MTBDR*plus*version 2 line probe assay is equivalent to that of the xpertMtb/rif assay. *J Clin Microbiol.* (2012) 50:3712–6. doi: 10.1128/JCM. 01958-12

19. Morse S. Factors in the emergence of infectious diseases. *Emerg Inf Dis*. (1995) 1:7–11. doi: 10.3201/eid0101.950102

20. Narasimhan P, Wood J, Macintyre CR, Mathai D. Risk factors for tuberculosis. *Pulmo Med*. (2013) 2013:828939. doi: 10.1155/2013/828939

21. Lonnroth K, Roglic G, Harries AD. Addressing the global diabetes epidemic to improve tuberculosis prevention and care – from evidence to policy and practice. *Lancet Diabetes Endocrinol.* (2014) 2:730–9. doi: 10.1016/S2213-8587(14)7 0109-3

22. Bruce N, Perez-Padilla R, Albalak R. Indoor air pollution in developing countries: a major environmental and public health challenge. *Bull WHO.* (2000) 78:1078–92.

23. Maher D. The natural history of *Mycobacterium tuberculosis* infection in adults. In: Schaaf HS, Zumla A, editors. *Tuberculosis: A Comprehensive Clinical Reference*, Elsevier Health Sciences (2009).

24. Arun AS, David A, Ilayaraja S, Yaduraj K, Anusree SB, Alex PC, et al. *Evaluation of Diagnostic Tests for Ante-Mortem Tuberculosis Screening in Captive Sloth Bears (Melurus ursinus).* 46th American Association of Zoo Veterinarians Annual Conference Walt Disney World, Orlando, FL (2014).

25. Shylaja. *Tuberculosis in wild animals*. Masters (MVSc) thesis submitted to Karnataka Veterinary Animal and Fisheries Sciences University, Karnataka, India (2013).

26. Thomas J, Balseiro A, Gortázar C, Risalde MA. Diagnosis of tuberculosis in wildlife: a systematic review. *Vet Res.* (2021) 52:31–7. doi: 10.1186/s13567-020- 00881-y

27. Centers for Disease Control and Prevention. (2015). *A New Tool to Diagnose Tuberculosis: The Xpert MTB/RIF Assay*. Available online at: https://www.cdc.gov/tb/publications/factsheets/pdf/xpertmtb-rifassayfactsheet_final.pdf

28. Christina M, van Helden Paul D, Goosen Wynand J. TB control in humans and animals in South Africa: a perspective on problems and successes. *Front Vet Sci.* (2018) 5:298–305. doi: 10.3389/fvets.2018.00298

29. Krajewska-Wedzina M, Augustynowicz-Kopec´ E, Weiner M, Szulowski K. Treatment for active tuberculosis in giraffe (*Giraffa camelopardalis*) in a Zoo and potential consequences for public health. *Ann Agric Environ Med*. (2018) 25:593–5. doi: 10.26444/aaem/75685

Art – 278. SURGICAL TREATMENT OF BACULUM FRACTURE IN SLOTH BEAR *(Melursus ursinus)*: A CASE REPORT

A.Sha. Arun, A.S. Virk, and S.P. Patil

Abstract

A rescued dancing male sloth bear (*Melursus ursinus)* neutered according to CZA norms at Wildlife SOS, Bannerghatta bear rescue center, aged around 24 years was noticed to exhibit frequent behavior of licking and biting of penile region. With the aid of radiographic examination, it was confirmed as a novel case of baculum fracture in the sloth bear. Radiographic examination revealed complete fracture of baculum at its anterior one third. Under general anesthesia with ketamine and xylazine in combination at a dose rate of 5 mg kg^{-1} and 2 mg kg^{-1}, respectively, a surgical procedure was performed which involved removal of the anterior fractured portion of the bone without causing any damage to urethra. Utmost post-operative care was provided. After the completion of the surgical intervention, the bear was recovered and stopped exhibiting the abnormal behaviour of licking and biting.

Keywords: Baculum fracture, sloth bear, surgical correction

Introduction

Sloth bear comes under the family Ursidae along with 7 species of bears which include brown bears, polar bears, American black bear, Asian black bears, sun bears, spectacled bears and the giant panda. Baculum also referred to, as heterotopic or extra-skeletal bone is found in the penis of certain placental mammals and primates but absent in humans (Sharir et al., 2011). Anatomically it is in the glans tissues at the distal end of penis and dorsally to the urethra. Generally distal end of corpus cavernosum touches the proximal end of the baculum. The baculum has variations in its shape, size and length in different species. Multiple theories are associated with the significance of baculum but the function of baculum is still not ruled out (Baryshnikov et al., 2003). A study suggests that variation in the length is associated with the taxonomic and behavioral variations, length of baculum varies according to the intromission time in some species (Dixson, 1987). Baculum fracture is amongst the uncommonly observed clinical case with the most probable etiological cause, i.e., aggressive behavior within the species while mating and also snapping associated with the sudden removal of penis during copulation (Bartosiewicz, 2000). One of the male sloth bears, aged around 24 years, was observed with an injury on his penile region (Fig. 1) and the same bear was subjected for a close examination (Fig. 2) after immobilization.

Fig. 1. The sloth bear showing the visible damage of the penile region

Fig. 2. Close examination of injury after immobilisation

Materials and Methods

This procedure was conducted at Wildlife SOS; Bannerghatta bear rescue center on a rescued dancing sloth bear aged 24 with the symptoms of frequent licking and biting of penile region. For restraining and in-depth examination of animal chemical immobilization was preferred. Sedative and anesthetic agent was selected considering the safety of the animal. Xylazine and Ketamine were selected considering their safety and smooth recoveries from the previous observations. Based on the body weight the required dose was calculated, i.e., for 90 kg body weight Ketamine (100 $mgml^{-1}$) @ 5 mg kg^{-1} the calculated dose was 450mg and Xylazine (100 mg ml^{-1}) @ 2 mg kg^{-1} the calculated dose was 180 mg. Hind quarter muscles were the preferred site for the administration of the anesthetic drugs. The drugs were combined and loaded in dart with a capacity of 5 ml with partial dose of Ketamine, i.e., 320 mg and complete dose of Xylazine; the remaining dose of ketamine was administered after animal achieved sternal recumbence for complete induction of anesthesia. Darts were delivered with the aid of blow pipe.

After the confirmation of complete induction of anesthesia, the animal was blind folded and under continuous monitoring of its vital signs, was shifted to the Wildlife SOS, Wildlife Veterinary Hospital at Bannerghatta Bear Rescue Center. Palpation of the suspected penile region emphasized the need for a radiographic confirmation to rule out the facture of the baculum at its anterior one third (Fig. 3A, 3B, 3C).

Fig. 3A. Radiographic image of hip region showing the fracture at one third of the baculum.

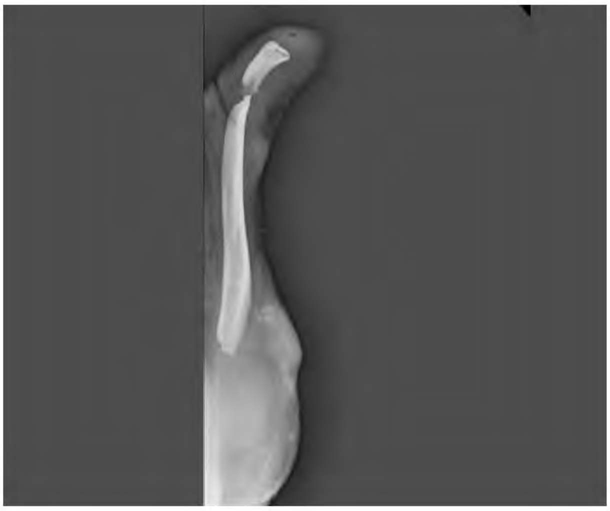


Fig. 3B. Radiographic image of the baculum fracture

Fig. 3C. Radiographic image of the baculum fracture with measurement

For surgical procedure, the sloth bear was intubated with 18 mm endotracheal tube (Fig. 4) and gaseous anesthesia was maintained with 4 liters of oxygen and 2% to 4% isoflurane gas. Intravenous line was started in the saphenous vein.

Fig. 4. Endotracheal intubation for gaseous anaesthesia.

The pelvic area was trimmed and cleaned with spirit and wounds inflicted by biting were cleaned by hydrogen peroxide and 5% povidone iodine and further wound debridement was done. A longitudinal incision of 3-4 cm was made at the site of fracture from the lateral side of the penis by using a No. 3 bp blade. During incision minimal damage to the underlying structures were ensured by displacing the tissues and muscles. The broken proximal part of the penis was removed using forceps (Fig. 5) and, the surrounding tissues were disinfected by using hydrogen peroxide and 5% povidone iodine, followed by the removal of the excessive fibrous tissues. The blunt edges of exposed bone were smoothened by filer to avoid injuries to the tissues and urethra as well. Blood vessels were thermo cauterized to avoid excessive bleeding. The edges were cleaned and sutured by using 2-0 absorbable sutures (Fig. 6) and antibiotic powder was adequately applied.

Fig. 5. Removal of broken piece of baculum

Fig. 6. Post surgical intervention and sutured wound

The post-operative medication regimen was adhered to

- Antibiotic tablet Cephalexin (Hatvet Pharma Private Limited, Meerut, Uttar Pradesh) @ 15 mg kg^{-1} body weight twice daily – to avoid the secondary infections
- Cap. Tramadol (Intas Pharmacueticals Limited, Thaltej, Ahmedabad) 50 mg and combination of enzymatic anti-inflammatory, i.e., Trypsin, Bromelain, Rutoside twice daily to alleviate pain and inflammation.

Results and Discussion

After the surgical correction of the deformity the bear recovered from anesthesia smoothly and was observed to be alert and active exhibiting normal feeding behaviour and was noticed to refrain itself from exhibiting the previous abnormal behaviour of sucking and licking of penile region leading to a healthy recovery of the sloth bear (Fig. 7A) and its radiographic healing was confirmed (Fig. 7B).

Fig. 7A. Post healing of the soft tissue

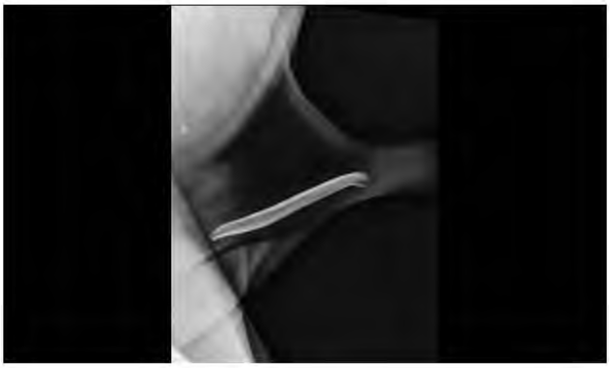


Fig. 7B. Radiographic image of baculum.

Behavioral changes of the animals are the important factors while considering the health status of individual. Behavioral changes in the wild animals are very less known so far and can be assessed rarely to resolve a cause unlike the domestic animals. Assessing the condition or abnormality amongst wild animals needs broader perspective and requires understanding of normal behavior. A specific term called “Zoocosis” is associated with stereotypic behavior in captive wild animals where there is substantial alteration of behavior which includes array of signs including sucking, over grooming or excessive licking and self-mutilation, biting, rocking, head bobbing, vomiting, and regurgitating, coprophilia, coprophagia and circling movements.

In captivity, the life of animals has a remarkable difference between what they exhibit in the wild. Factors like space, human presence, climate, social interactions and diet have a peculiar impact on the animal behavior (Vickery and Mansion, 2005). Thus, while accessing the health issues of the captive animals, the stereotypic behaviors and natural behavior must be considered along with the alterations in behavior associated with illness. There should be a clear understanding of stereotypic, normal and illness associated behavior to get the complete and accurate diagnosis of the illness (Sha et al., 2020). While dealing with health issues and abnormal behavior in the wild animal's implementation of conventional knowledge and modern diagnostic aids can act as liaison to achieve confirmatory diagnosis. However, there must be proper considerations or assumptions to act accordingly, which can minimize the time duration and ensure quick healthy recovery of the distressed animal.

In this study, the observed behavior of sucking was due to baculum fracture resulting in pain and discomfort of the animal. Such pain and discomfort can lead to aggression in the animals. Injuries in the penile region may cause ascending urinary tract infections where the bacteria first invade the urethral mucosa, towards urinary bladder further to kidneys and finally into the circulatory system (Belyayeva and Jeong, 2021). The authors documented that the average length (Fig. 8) of an adult sloth bear baculum falls around 162 mm to 167 mm (n=18).

Fig. 8. Radiographic image of intact baculum of sloth bears

Acknowledgments

The authors are thankful to Executive Director, Bannerghatta Biological Park and Mr. Kartick Satyanarayan and Ms.Geeta Seshamani, Co-Founders, Wildlife SOS, for extending their support during the study. The authors would also like to thank and acknowledge the support of staff at Bannerghatta Bear Rescue Centre for their valuable field assistance during the upkeep of the rescued sloth bears.

References

Bartosiewicz, L. 2000. Baculum fracture in carnivores: Osteological, behavioural and cultural implications.*Int. J. Osteoarchaeol.* 10(6): 447-450.

Baryshnikov, G.F., Bininda-Emonds, O.R. and Abramov,A.V. 2003. Morphological variability andevolution of the baculum (Os Penis) in Mustelidae (Carnivora). *J. Mammal.* 84(2): 673–690.

Belyayeva, M. and Jeong, J.M. 2021. Acute Pyelonephritis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Dixson, A.F. 1987. Baculum length and copulatory behavior in primates. *Am. J. Primatol.* 13(1): 51-60.

https://www.worldatlas.com/articles/what-is-zoochosis. Sha, A.A., Bakde, R. and Adhithyan, N.K. 2020.

Successful rescue and rehabilitation of an injured wild sloth bear trapped in a poacher's snare. *e-planet* 18 (2): 158-163.

Sharir, A., Israeli, D., Milgram, J., Currey, J.D., Monsonego-Ornan, E. and Shahar, R. 2011. The canine baculum: the structure and mechanical properties of an unusual bone. *J. Struct. Biol.* 175(3):451-456.

Vickery, S.S. and Mason, G.J. 2005. Stereotypy and perseverative responding in caged bears: further data and analyses. *Appl. Anim. Behav. Sci.* 91(3-4): 247-260.

Art – 279. SLOTH BEAR'S FECAL TALE: A GROSS OBSERVATION AND INTERPRETATION OF CAPTIVE SLOTH BEAR'S FECES TO UNDERSTAND THE HEALTH STATUS

S Ilayaraja, Arun A Sha, Srinu Srikanta Maharana and **Baiju Raj MV**

Abstract

In the wild, Sloth bears possess a wide range of food habits for which fecal consistency and appearance couldn't be incorporated. However, in captivity, there is a standardized diet formulation concerning their nutritional needs and behavioural welfare. Non-invasive monitoring of fecal samples is paramount to understanding overall health status, including gastrointestinal (GI) disorders, and worm burden etc. Although several studies on scat analysis of sloth bears are available, the gross appearance of feces correlated with health status is largely unexplored. Here we attempted to establish a standardized fecal consistency grading system following the phenotypic appearance of feces cohere with GI health. Our observations revealed the highest prevalence of soft semi-solid feces "always" with no shape and firm and semi-dry feces "often". Comparisons were made with respect to the dietary factors, fecal consistency, health status, and incidence of any abnormality. The current study's findings build an empirical base to understand gastrointestinal health through gross observation of feces.

Keywords: Sloth bear, feces, appearance, consistency, composition, odour, GI health

1. Introduction

A large population of wild animals in captivity is reported to be reduced disease resistance ability and increased susceptibility to parasitic infestation (Dashe & Berhanu, 2020; Varadharajan & Subramanian, 2003) [4, 19]. The gastrointestinal (GI) tract is paramount in maintaining an animal's overall health condition. Gastrointestinal health of captive wildlife could be attributed to various factors such as variability and equilibrium of diet, intestinal permeability, gut microbiota, host immunity and interaction, effective digestion, and absorption (Pietro *et al.*, 2018) [15]. In captivity, persistent non-invasive monitoring of feces condition, appearance, consistency, composition and odour facilitates understanding the gut health and digestive efficiency of animals (Nijboer *et al.*, 2006; Pietro *et al.*, 2018; Whitehouse-Tedd *et al.*, 2015) [15, 12, 21]. Several studies have facilitated fecal consistency and comparative objective grading scale for felids and canids to address animal health and welfare (Cavett *et al.*, 2021; Kerr *et al.*, 2012; Whitehouse-Tedd *et al.*, 2015) [3, 10, 21]. Contrastingly, applying the existing fecal consistency grading scale in domestic dogs and cats could be challenging to captive and free-living wildlife as of the differences in diet. In zoos, bears are considered to have few problems and are relatively free from diseases which may be attributed either to "an extraordinary resistance against all kind of diseases" or to "an unbelievable ability to conceal symptoms of sickness and pain" (Hage & Dorrestein, 1994; Rietschel, 1994) [7, 18]. Hence continually adopting the multifaceted diagnostic approach in terms of addressing veterinary challenges in *Melursus ursinus* could be highly beneficial. So, the voided feces are an important clue to understand the physiological status of an animal that encourages practicing health monitoring through non-invasive sampling, managing species of concern and claiming animal welfare (Dib *et al.*, 2019; Pannoni *et al.*, 2022) [6, 13]. Among ursids, in captive Brown bear (*Ursus arctos*), De Cuyper *et al.* (2021) [5] forms the basis of diet consumption and fecal consistency. To understand home ranges and habitat use by *Melursus ursinus*, there are several studies based on the food habit through scat analysis (Paul & Kumar, 2021; Ramesh *et al.*, 2009) [14, 16].

However, physiology associated with Sloth bears gastrointestinal health through non-invasive monitoring of feces is largely unknown. Here we aim at establishing a fecal consistency grade correlated with gastrointestinal health in Sloth bear (*Melursus ursinus*), with a highly varied omnivorous diet (Bargali *et al.*, 2020) [1]. Despite the large conservation, management, and physiological interest, to our knowledge, apart from *U. arctos*, no such scaling concerning GI health is available for *M. ursinus*. This study has attempted to evaluate GI health and digestive processing through continuous monitoring and gross examination of feces non-invasively.

2. Materials and Methods

The observational study was conducted for two decades i.e., from 2003 to 2022 at Agra Bear Rescue Facility, where over 400 rescued and rehabilitated Sloth bears (age: 2-30 years; body mass: 45-140 kg) are under veterinary care and treatment with an enriched diet of porridge (native grains cooked with quantum sufficient water) and seasonally available fruits. However, in addition to this, bears ingested other vegetation that grew naturally within enclosure premises.

We conducted daily observations of feces at both indoor and outdoor enclosures during the morning and evening feeding. All feces were photo-documented, handled, and collected at the latest 0.16-24 h after defecation. The tactile and visual grading was done when classifying and labelling the feces. Later, to understand gastrointestinal health, veterinary observation i.e., physiology and health condition of the animal along with fecal pictures were interpreted to develop fecal conte of Sloth bears.

Although, we standardized our observations to establish fecal grading scale based on score, appearance, shape, dietary components, and tactility (Table 1). Thereafter, the fecal samples were subjected to parasitological and microbial examination by standard sedimentation, floatation, and culture techniques to understand the exact etiology.

Table 1: Standardized faecal consistency score of *M. ursinus*

Score consistency	1	2	3	4	5
Description	Entirely liquid	Moist to liquid	Moist and viscous faeces	Moist, well-formed, visible cracks	Well-formed, structure-rich material
Shape	Plaques	Viscous liquid with minor areas solid shape	Pile, circular perimeter	Turds, piles	Turds, pile or scattered
Tactile	Poor, no density	Liquid with some viscosity	Soft, squashable, difficult to pick up	Soft, falls apart while picking up	High density, rough, leaves no residue when picked
Possible dietary components	Milk	Vegetation	Fruit and grass	Fruit and vegetable	Fruit, vegetable, grains
Example					

3. Results and discussion

Among the experimental feces and consistency, well-formed feces scored 5 (feces of apparently healthy animal), followed by moist-turd-pile feces (Score 4). An increased proportion of viscous liquid in the feces seemingly led to a decrease in the score (Table 1). Overall, based on diet, a major portion of feces scored 3 (Score 3; Table 1). Our observations suggest that *M. ursinus* diagnosed with verminous enteritis, leads to the passing of mucoid blood-tinged feces followed by intestinal inflammation or ulceration (Figure 1). Symptoms of enteritis may include nausea, vomiting, inappetence, and abdominal pain. However, gastrointestinal parasites are common in sloth bears. Routine monitoring and administering anthelmintic periodically with proper sanitary measures would reduce the parasitic infection (Manjunatha *et al*., 2018) [11]. Some of the feces were grey or brownish-black, whereas the consistency varied from mucoid to watery, revealed the animal was suffering from hepatitis and cholangiocarcinoma (Figure 2). In this condition, visual evaluations suggest those animals exhibit symptoms of the bulged abdomen, ascites, generalized jaundice, inappetence, intermittent vomiting, facial and peripheral oedema based on the severity of the condition (Ilayaraja & Sha, 2021) [8]. Etiological factors such as seasonal changes in diets, coarse and fibrous particles in feed, and excessive dietary carbohydrates expedite the frequent passage of watery feces i.e., diarrhoea (Figure 3). Although, a diarrhoeic condition in an animal could also be associated with physical, chemical, and biological factors (Bhikane & Kawitkar, 2022) [2]. Exceptions would occur during excess milk consumption when the animal passes yellowish diarrhoeic feces. The circumstances of food intolerance and allergies require dietary changes and probiotics. To date, healthy *M. ursinus* has been reported for voiding partially digested (*Cucurbita* spp.,*Daucus carota*, *Cocos nucifera* and *Citrullus lanatus*) or undigested feed particles (seeds of *Phoenix dactylifera, Lantana camara* and *Ziziphus mauritiana*) through defecation (Figure 4). Cases of rectal impaction in *M. ursinus* followed by inappetence, anorexia and constipation revealed excessive consumption of Bengal gram, coconut pieces, and groundnuts with shells (Ilayaraja *et al*., 2012) [9]. To enhance digestive permeability and nutrient absorption the boiled vegetable and fruits should be smashed/slurred properly before offering the animal.

In some cases, we found animal passing greenish mucoid feces due to bacterial enteritis where oral or parenteral antibiotics needs to be given (Figure 6). Including indigestible components, such as hair, followed by trichotillomania ortrichophagia, leads to trichobezoar (Figure 7). An incident of intestinal trichobezoar lead to death of Sloth bear reported the bear was exhibiting symptoms of listless, dull and depressed with poor appetite (Rao & Acharjyo, 1979) [17]. Although, instances of compulsive eating disorder in *M. ursinus* associated with eating soil where they pass muddy colored feces (Figure 5).

Ideally, healthy feces shouldn't have any sort of mucus, blood, and undigested food particles. However, the occasional instance of mucus is normal as it's what allows feces to slide through the rudimentary colon. In addition, the presence of few body hairs in feces is normal because of the physiological process associated with peculiar licking and phonation habit that includes paw sucking in Sloth bears (Rao & Acharjyo, 1979; Weissengruber *et al*., 2001) [17, 20].

Fig 1: Gross appearance of faeces for M. ursinus diagnosed with verminous enteritis

Fig 2: Gross appearance of faeces for M. ursinus diagnosed with Hepatic disorder

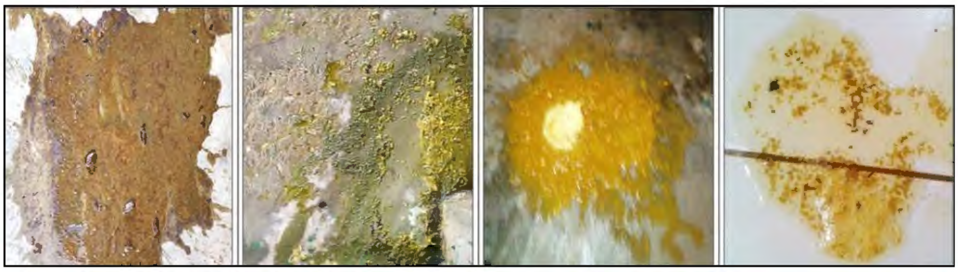


Fig 3: Gross appearance of faeces for M. ursinus diagnosed with Diarrhoea

Fig 4: Gross appearance of faeces for apparently heathy M. ursinus with partially digested and fruit material

Fig 5: Gross appearance of faeces for M. ursinus exhibiting compulsive eating disorder (Pica)

Fig 6: Gross appearance of faeces for M. ursinus diagnosed with bacteria gastroenteritis

Fig 7: Gross appearance of faeces for M. ursinus diagnosed with trichobezoar

Conclusion

This contribution offers a fecal tale correlated with gastrointestinal health for Sloth bear (*M. ursinus*) and can be used in captive settings as a part of monitoring and health assessment. However, apart from this, the fecal consistency and gross appearance may vary according to the animal's diet, age and gender, and environmental conditions. Further studies should be conducted to understand the causes and consequences of dual consistency feces. Yet, based on our observations, we postulate a gross appearance of feces associated with gastrointestinal health. In addition, this observational study would sensitize and make aware animal care staff and biologists to interpret and gather potential clues for veterinarians in addressing health care.

Acknowledgement

We greatly appreciate the support extended by Uttar Pradesh Forest Department. We sincerely appreciate our CEO, Co- Founders, wildlife SOS for unwavering support and encouragement in terms of animal care and management. We are also thankful to the animal care staff at Wildlife SOS for their sincere assistance.

References

1. Bargali HS, Pigeon KE, Akhtar N, Sharp T, Jadav KK. Sloth Bear (*Melursus ursinus*). In V. Penteriani & M. Melletti (Eds.), Bears of the World (1st ed.,). Cambridge University Press, 2020, 99-109. https://doi.org/10.1017/9781108692571.010

2. Bhikane AU, Kawitkar SB. Handbook for veterinary clinicians. Agri-Biovet Press, 2022.

3. Cavett CL, Tonero M, Marks SL, Winston JA, Gilor C, Rudinsky AJ. Consistency of faecal scoring using two canine faecal scoring systems. Journal of Small Animal Practice. 2021;62(3):167-173.https://doi.org/10.1111/jsap.13283

4. Dashe D, Berhanu A. Study on Gastrointestinal Parasitism of Wild Animals in Captivity at the Zoological Garden of Haramaya University, Ethiopia. Open Journal of Veterinary Medicine. 2020;10(9):173-184. https://doi.org/10.4236/ojvm.2020.109015

5. De Cuyper A, Clauss M, Lens L, Strubbe D, Zedrosser A,Steyaert S, *et al*. Grading fecal

consistency in an omnivorous carnivore, the brown bear: Abandoning the concept of uniform feces. Zoo Biology. 2021;40(3):182- 191. https://doi.org/10.1002/zoo.21593

6. Dib LV, Palmer JPS, Lima C de, SCC de, Bastos OMP, Uchôa CMA, *et al*. Noninvasive sampling: Monitoring of wild carnivores and their parasites. In Protected Areas, National Parks and Sustainable Future. Intech Open, 2019. https://doi.org/10.5772/intechopen.85227

7. Hage M van der, Dorrestein GM. Why do bears die in captivity. International conference on aspects of bear conservation, Bursa, Turkey, 1994.

8. Ilayaraja S, Sha AA. Occurrence of extrahepatic biliary tract pathologies in rehabilitated captive dancing sloth bears (*Melursus ursinus*) and its diagnostic challenges. International Journal of Veterinary Sciences and Animal Husbandry. 2021;7(1):9-14. https://doi.org/10.22271/veterinary.2022.v7.i1a.397

9. Ilayaraja S, Sha AA, Khadpekar Y, Jonhson S, Dahe N. Obstipation induced urinary incontinence in a captive sloth bear (*Melursus ursinus*)-A case report, 2012. https://www.researchgate.net/publication/271832230

10. Kerr KR, Vester Boler BM, Morris CL, Liu KJ, Swanson KS. Apparent total tract energy and macronutrient digestibility and fecal fermentative end-product concentrations of domestic cats fed extruded, raw beef- based, and cooked beef-based diets. Journal of Animal Science. 2012;90(2):515-522.https://doi.org/10.2527/jas.2010-3266

11. Manjunatha V, Rout M, Sha AA, Byregowda SM, Joseph T. Coprological analysis for estimating prevalence of gastrointestinal parasites in captive sloth bears (*Melursus ursinus*). Indian J. Anim. Res., 2018, 1-4. https://doi.org/10.18805/ijar.B-3608

12. Nijboer J, Clauss M, Olsthoorn M, Noordermeer W, Huisman TR, Verheyen C, van der Kuilen J, *et al*. Effect of diet on the feces quality in Javan langur (*Trachypithecus auratus*). Journal of Zoo and Wildlife Medicine. 2006;37(3):366–372.https://doi.org/10.1638/05-113.1

13. Pannoni SB, Proffitt KM, Holben WE. Non-invasive monitoring of multiple wildlife health factors by fecal microbiome analysis. Ecology and Evolution. 2022;12(2), e8564.

https://doi.org/10.1002/ece3.8564

14. Paul DK, Kumar G. Feeding Ecology of Sloth Bear (*Melursus ursinus*) in Nawada Forest Division (Bihar) by Scats Analysis. Environment and Ecology. 2021;39(1):1-–9.

15. Pietro C, Viviane V, Estefania PC, Jerome S, Anna- Maria K. Biomarkers of gastrointestinal functionality in animal nutrition and health. Animal Feed Science and Technology. 2018;250:9-31. https://doi.org/10.1016/j.anifeedsci.2018.07.012

16. Ramesh T, Sankar K, Qureshi Q. Additional notes on the diet of sloth bear *Melursus ursinus* in mudumalai tiger reserve as shown by scat analysis. Journal Bombay Natural History Scociety. 2009;106:204-206.

17. Rao AT, Acharjyo LN. Intestinal Trichobezoars in a Captive Sloth Bear. The Journal of Zoo Animal Medicine. 1979;10(2):73-74. https://doi.org/10.2307/20094422

18. Rietschel W. Veterinary aspects of keeping bears in captivity. International conference on aspects of bear conservation, Bursa, Turkey, 1994.

19. Varadharajan A, Subramanian H. Influence of age on the prevalence of parasitic infections among wild mammals in Thrissur Zoo, Thrissur, Kerala. Zoos Print, 2003, 18. https://doi.org/10.11609/JoTT.ZPJ.18.4.1065-6

20. Weissengruber GE, Forstenpointner G, Kübber-Heiss A, Riedelberger K, Schwammer H, Ganzberger K. Occurrence and structure of epipharyngeal pouches inbears (Ursidae). Journal of Anatomy. 2001;198(3):309- 314. https://doi.org/10.1046/j.1469-7580.2001.19830309.x

21. Whitehouse-Tedd KM, Lefebvre SL, Janssens GPJ.Dietary factors associated with faecal consistency and other indicators of gastrointestinal health in the captive cheetah (*Acinonyxjubatus*). PloS One, 2015;10(4):e0120903. https://doi.org/10.1371/journal.pone.0120903

Art – 280. ELECTROCARDIOGRAPHIC AND HAEMATO-BIOCHEMICAL PROFILE OF AN APPARENTLY HEALTHY INDIAN SLOTH BEAR (*Melursus ursinus ursinus*): A CASE REPORT

Attur Shanmugam Arun, Swagat Mohapatra**, Ilayaraja Selvaraj,** Joseph Tista, Suchitra Sahoo, Partha Sarathi Swain, Swostika Priyadarsini and Shivani Das

Abstract

The study aims to report the baseline electrocardiographic and haemato-biochemical values in an adult healthy Indian Sloth bear. The amplitude of P wave was recorded higher in dorsal recumbent position. The Q wave amplitude value was more in right lateral recumbency. R wave amplitude was more in right lateral recumbency whereas R wave duration was more in dorsal recumbency. Higher S wave was obtained in dorsal recumbency. Amplitude and duration of T wave was greater in right lateral recumbency. PR interval and QT interval were more in right lateral recumbency whereas RR interval, PR segment and ST segment were more in dorsal recumbency. Heart rate was higher in right lateral recumbent position. The hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and hematocrit level were comparatively higher in Indian Sloth bear than most domestic animals. However, differential count, eosinophil percentage was found to be higher than most domestic animals. The platelet count in the blood picture was recorded to be higher than most domestic animals. In biochemical analysis under liver function tests, albumin, direct and indirect bilirubin were lower than most domestic animals. Amylase and lipase which were the basic markers of pancreatitis were within normal range. The reported values of serum cardiac biomarkers can be used for assessment of myocardial injury. The result of this study will assist veterinarians in management and conservation of this rare species.

Keywords: Indian Sloth bear, ECG, haematological, biochemical, anaesthesia

1. Introduction

The Sloth bear (*Melursus ursinus ursinus*) is an insectivorous bear species, mostly found in Indian subcontinent (Garshelis *et al.,* 2011) [4]. It is evolved from the ancestral brown bear during the Pleistocene and through convergent evolution. The Indian Sloth

Bear can easily be recognized by its shaggy black coat, long muzzle, protruding lip and by a white V-shaped patch on the chest. It is listed as Vulnerable on the IUCN Red list, mainly due to habitat loss and degradation (Dharaiya *et al.,* 2016) [3]. In Indian conditions, bears reared in semi-captivity are exposed to a variety of biotic and abiotic stress factors. Thermal stress is one of the most pertinent abiotic stressors leading to disturbance of homeostasis in animals (Parida *et al.,* 2020a) [7]. The correlation between stress and cardiovascular diseases has been shown to be independent of cardiovascular risk factors (Steptoe and Kivimaki, 2012) [11]. Mohapatra *et al.,* 2021 [6] reported injury to myocardium, liver and kidney of cattle during heat stress. Previous studied conducted by Parida *et al.,* 2020b [8] also reported the derogatory effects of heat stress on caprine cardiac cells. To cope with high environmental conditions, bears develop various cardiovascular adaptations. This calls for appropriate maintenance of cardiac health in semi- captive Sloth bears.

Electrocardiography (ECG) is a recording of the electrical activity of heart. It is a non-invasive and easily applied technique used in the determination of cardiac hypertrophy, dilatation, arrhythmias and diagnosis of conduction abnormalities (Mohapatra *et al.,* 2015) [13]. There are also reports on variable influence of two different anaesthetic combinations on cardiac function in mice (Hart *et al.,* 2001) [5]. Since it is not possible to record ECG in active bears, breed specific electrocardiographic values under specific anaesthetic combination need to be established for accurate interpretation of ECG in Sloth bears. As per our knowledge there are no reports available for interpreting normal ECG variables in Indian Sloth bears.

The aim of this study was to report the baseline electrocardiographic and haemato biochemical values in an adult healthy Indian Sloth bear, which can be used clinically for effective diagnosis and treatment of cardiac and hematological disorders in Indian Sloth bears which will be helpful for the conservation of this IUCN Red listed wild animal.

2. Materials and Methods

2.1 Ethical approval

Electrocardiography is a non-invasive technique and the study was conducted during routine clinical examination of Indian Sloth bear which is living in a semi-captive state at Wildlife SOS, Bannerghatta Bear Rescue Center, Bengaluru, India. None of the animals were sampled solely for the purpose. The results were obtained by compiling and analysing available ECG and haemato-biochemical reports. So, the study does not mandate ethical approval.

An apparently healthy male Indian Sloth bear aged about nine years was immobilized using a ketamine-xylazine combination, ketamine hydrochloride (5 mg/kg body weight; Ketamil, Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) and xylazine hydrochloride (Xylazil, 2 mg/kg body weight; Troy Laboratories Pty Ltd.). A twelve-lead standard ECG recorder Technocare Medisystems model DP2020 digital single channel ECG recorder was used to record ECG in Indian Sloth bear**.** The electrocardiograph was set with a paper speed of 50 mm/sec and sensitivity of 1 (20 mm = 1 mV). The Sloth bear was positioned in right lateral recumbency (Figure 1) and dorsal recumbency (Figure 2) on an insulated table and the clips of the electrodes fitted to alligator clips were attached to the olecranon and the patella. To minimize the variables associated with anaesthetic depth, the ECG evaluation was conducted once a surgical anaesthetic plane was attended based on lack of pedal reflexes.

Blood sample was collected from jugular vein after immobilisation using a 20 gauge needle in vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA) with and without Ethelyne Diamine Tetraacetic acid (EDTA) for haematology and serology respectively. Samples were immediately stored on cool packs at 4-8 degree centigrade and transported from field location to laboratory. Standard haematological and biochemical parameters were analysed within 24 hours of collection using a haematological analyser Vitros 250. Statistical analysis was performed using Microsoft Excel 2007 and relationships were deemed statistically significant when $p<0.05$ (Student's paired t test).

Fig 1: ECG of an adult Indian Sloth bear taken in right lateral recumbency

Fig 2: ECG of an adult Indian Sloth bear taken in dorsal recumbency

3. Results and Discussion

3.1 Electrocardiographic parameters

The ECG variables obtained in the Indian Sloth bear positioned in right lateral recumbency and dorsal recumbency are represented in table 1. The amplitude of P wave was recorded higher in dorsal recumbent position whereas duration of P wave was similar in both positions. The Q wave amplitude value was more in right lateral recumbency. R wave amplitude was more in right lateral recumbency whereas R wave duration was more in dorsal recumbency. Higher S wave was obtained in dorsal recumbency. Amplitude and duration of T wave was greater in right lateral recumbency. PR interval and QT interval were more in right lateral recumbency whereas RR interval, PR segment and ST segment were more in dorsal recumbency. Heart rate was higher in right lateral recumbent position. The values obtained for ECG variables in the present study were different from those obtained by Cihan *et al.,* 2016 [2] in Brown bears of Turkey. This shows that breed does play an important role in determining the reference range of ECG parameters.

Table 1: Lead II ECG variable in Right Lateral Recumbency and Dorsal Recumbency position of an adult Indian Sloth bear

Lead II	Right LateralRecumbency	Dorsal Recumbency
P amplitude (mV)	0.15	0.20
P duration (sec)	0.08	0.08
Q amplitude (mV)	0.20	0.00

R amplitude (mV)	1.00	0.60
R duration (sec)	0.04	0.08
S amplitude (mV)	0.05	0.20
T amplitude (mV)	0.35	0.30
T duration (sec)	0.10	0.08
PR interval (sec)	0.28	0.12
QT interval (sec)	0.28	0.24
RR interval (sec)	1.28	1.40
PR segment (sec)	0.20	0.24
ST segment (sec)	0.16	0.28
Heart Rate (bpm)	46.87	42.85

Table 2: Serum haematological and biochemical parameters of an adult Indian Sloth bear

Complete Blood Count	
Hb (g/dl)	19.8
RBC (million per mm^3)	7.33
HCT (%)	55.4
MCV (fl)	75.6
MCH (pg)	27.0
MCHC (g/dl)	35.7
RDW (%)	16.3
TLC (million per mm^3)	10.6
Differential Leucocyte Count	
Neutrophil (%)	73.0
Lymphocyte (%)	17.0
Eosinophil (%)	10.0
Monocyte (%)	0
Basophil (%)	0
Platelet (thousand/microlitre)	263.0
Liver Function Tests	
Total Protein (g/dl)	6.7
Albumin (g/dl)	2.7
Globulin (g/dl)	4.0
A:G (Albumin: Globulin) ratio	0.68
AST/SGOT (U/L)	103.0
SGPT/ALT (U/L)	46.0
ALP (U/L)	27.0
GGT (U/L)	34.0
Bilirubin Total (mg/dl)	0.12
Bilirubin Direct (mg/dl)	0.08
Bilirubin Indirect (mg/dl)	0.04
Kidney Function Tests	
Urea (mg/dl)	15.0
Creatinine (mg/dl)	1.24

Uric acid (mg/dl)	1.53
Electrolytes	
Sodium (mg/dl)	126.0
Potassium mmol/L	6.1
Chloride mmol/L	98.0
Biochemical markers of pancreatitis	
Amylase (U/L)	10.05
Lipase (U/L)	24.00
Serum cardiac biomarkers	
c- Reactive Protein (mg/L)	0.20
Lactate Dehydrogenase (U/L)	693.00
Triglyceride (mg/dl)	294.00
Troponin I (qualitative)	Negative

3.2 Haemato-biochemical parameters

The haemato-biochemical parameters in the Indian Sloth bear are represented in table 2. The haemato-biochemical parameters reported by Reece *et al.,* 2015 for domestic animals were taken as reference. The hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and hematocrit level were comparatively higher in Indian sloth bear than reported for most domestic animals. However, in differential count, eosinophil percentage was found to be higher than most domestic animals. The platelet count in the blood picture was recorded to be higher than most domestic animals. Moreover, the values of hematological parameters were within the range obtained by our previous studies on Indian Sloth Bear (Shanmugam *et al.,* 2008) [10].

In biochemical analysis concerning liver function tests, albumin, direct and indirect bilirubin were lower than most domestic animals. Under kidney function test, creatinine values were in agreement with Veeraselvam *et al.,* 2014 [12]. Values obtained for electrolytes in current study are higher than values obtained by Chandra *et al.,* 2018 [1] for Indian Sloth bear. Amylase and lipase which were the basic markers of pancreatitis were within normal range. The values of serum cardiac biomarkers were reported for the first time in this study which can be used for assessment of myocardial injury in bears during cardiac diseases.

4. Conclusion

The study reports baseline reference values of electrocardiographic and haemato-biochemical parameters in an adult Indian Sloth bear. The values may be referred by veterinary while interpreting concerned test results.

5. References

1. Chandra S, Nair S, Gupta R, Karikalan M, Ilayaraja S, Shanmugam A, *et al*. Biochemical Reference Intervals for Semi-captive Sloth Bears (*Melursus ursinus ursinus*) in India. International Journal of Livestock Research. 2018;8(4):311-315.

2. Cihan H, Yilmaz Z, Aytug N. Evaluation of cardiologic functions in captive Eurasian Brown Bears (*Ursus arctos arctos*) in Turkey. Journal of Zoo and Wildlife Medicine. 2016;47(1):120-126.

3. Dharaiya N, Bargali HS, Sharp T. *Melursus ursinus*. The IUCN Red List of Threatened Species, 2016,e.T13143A45033815. http://dx.doi.org/10.2305/ IUCN. UK.2016 - 3.RLTS.T13143A45033815.en

4. Garshelis DL, Joshi AR, Smith JLD, Rice CG. Sloth BearConservation Action Plan, 2011. http://wildpro.twycrosszoo.org/000ADOBES/Bears/Bears_IUCN_ActionPlan/bearsAP_ chapter12.pdf. http://dx.doi.org/10.5455/ijlr.20170923065855.

5. Hart CYT, Burnett Jr JC, Redfield MM. Effects of avertin versus xylazine-ketamine anaesthesia on cardiac function in normal mice. Am J Phy- Heart and Circulatory Physiology. 2001;281(5):H1938-H1945.

6. Mohapatra S, Kundu AK, Mishra SR, Senapati S, Jyotiranjan T, Panda G. HSF 1 and GM-CSF expression, its association with cardiac health, and assessment of organ function during heat stress in crossbred Jersey cattle. Research in Veterinary Science. 2021;139:200- 210.

7. Parida S, Mishra SR, Mishra C, Dalai N, Mohapatra S, Mahapatra APK, *et al*. Impact of heat stress on expression kinetics of HSP27 in cardiac cells of goats. Biological Rhythm Research. 2020a;51(6):925-933. Weblink: DOI: 10.1080/09291016.2018.1564578.

8. Parida S, Mishra SR, Mishra C, Mohapatra S, Dalai N, Mahapatra APK, *et al*. Impact of heat stress on transcriptional abundance of HSP70 in cardiac cells of goat, Animal Biotechnology. 2020b;31(3):223-228.DOI: 10.1080/10495398.2019.1583574.

9. Reece WO, Erickson HH, Jesse P, Goff JP, Uemura EE. Dukes Physiology of Domestic Animals Wiley- Blackwell, 13th edition, 2015, ISBN: 978-1-118-50139-9.

10. Shanmugam AA, Kumar JK, Selvaraj I, Selvaraj V. Hematology of Sloth Bears Melursus ursinus ursinus from Two Locations in India. Journal of Wildlife Diseases. 2008;44(2):509-518.

11. Steptoe A, Kivimaki M. Stress and cardiovascular disease. Nature Reviews Cardiology. 2012;9:360-370.

12. Veeraselvam M, Sridhar R, Perumal P, Jayathangaraj MG. Chemical Immobilization of Sloth Bears (Melursus ursinus) with Ketamine Hydrochloride and Xylazine Hydrochloride: Hematology and Serum Biochemical Values. Vet Med Int. 2014;2014:341047. doi: 10.1155/2014/341047. Epub 2014 Apr 28. PMID: 24876990; PMCID: PMC4020200.

13. Pathak PH, Feng X, Hu P, Mohapatra P. Visible light communication, networking, and sensing: A survey, potential and challenges. IEEE communications surveys & tutorials. 2015 Sep 3;17(4):2047-77.

Art – 281. INVESTIGATING CO-OCCURRENCE AMONG LOOK-ALIKE SPECIES: THE CASE OF THREE BEARS IN NORTHEAST INDIA

David L. Garshelis, Nishith Dharaiya, **Thomas R. Sharp** and Karine E. Pigeon

Abstract

At the most basic level, the assessment of a species' status involves knowing where it occurs. Determining the presence of rare species is difficult and can be further confounded by the presence of a more common look-alike species. We investigated one of the few places in the world where three species of bears have been reported to co-occur at a fine scale: Balpakram National Park, Meghalaya, India. Asiatic black bears (*Ursus thibetanus*) are fairly common, and we sought to determine whether sun bears (*Helarctos malayanus*) and/or sloth bears (*Melursus ursinus*) also resided there. The local Garo language has words for three types of bears, and some local people reported the continued presence of a small type of bear, possibly the sun bear, but the probable extirpation of sloth bears. Because these bears look somewhat alike, local people and government forest officers could not provide convincing accounts of the presence of more than one species. We measured claw marks on climbed trees, a method used to differentiate sun bears from Asiatic black bears where both are known to occur; however, this method turned out to be unreliable for detecting sun bears where their presence was unknown because sun bear-sized marks are not distinguishable from juvenile black bears. We recommend targeted camera trapping near recent purported sightings of the other two bear species.

Keywords: local ecological knowledge; sign survey; rare species; detecting species presence; species misidentification; species coexistence; *Ursus thibetanus*; *Helarctos malayanus*; *Melursus ursinus*

1. Introduction

Bears (Ursidae) are a small family, with only eight species, which range across four continents and the Arctic. Europe and South America are each inhabited by a single extant bear species, whereas multiple species exist in North America and Asia, and in some of places there is wide overlap of two species. However, there is just one place in this global distribution where historically three bear species may have overlapped on a broad scale. Northeast India(hereafter NE India) marks the eastern extremity of the range of sloth bears (*Melursus ursinus*),the western extremity of the range of sun bears (*Helarctos malayanus*) and is near the center of the range of Asiatic black bears (*Ursus thibetanus*; hereafter black bear) (Figure 1). This region thus represents a unique area in terms of bear ecology and conservation.

NE India may possess a distinctive suite of resources that can sustain all three species. In comparison, there are no historical or even fossil records of sun bears in peninsular India, or of sloth bears crossing into present-day Myanmar. The conditions outside of NE India must be different enough to limit the geographic range of these two bears. Several recent studies of bear ecology were conducted in NE India [1–4], but none investigated the limiting ecological factors or even ecological differences among species. Steinmetz et al. [5] identified gradients in fruit and insect abundance as being factors related to the range limits of these three bears.

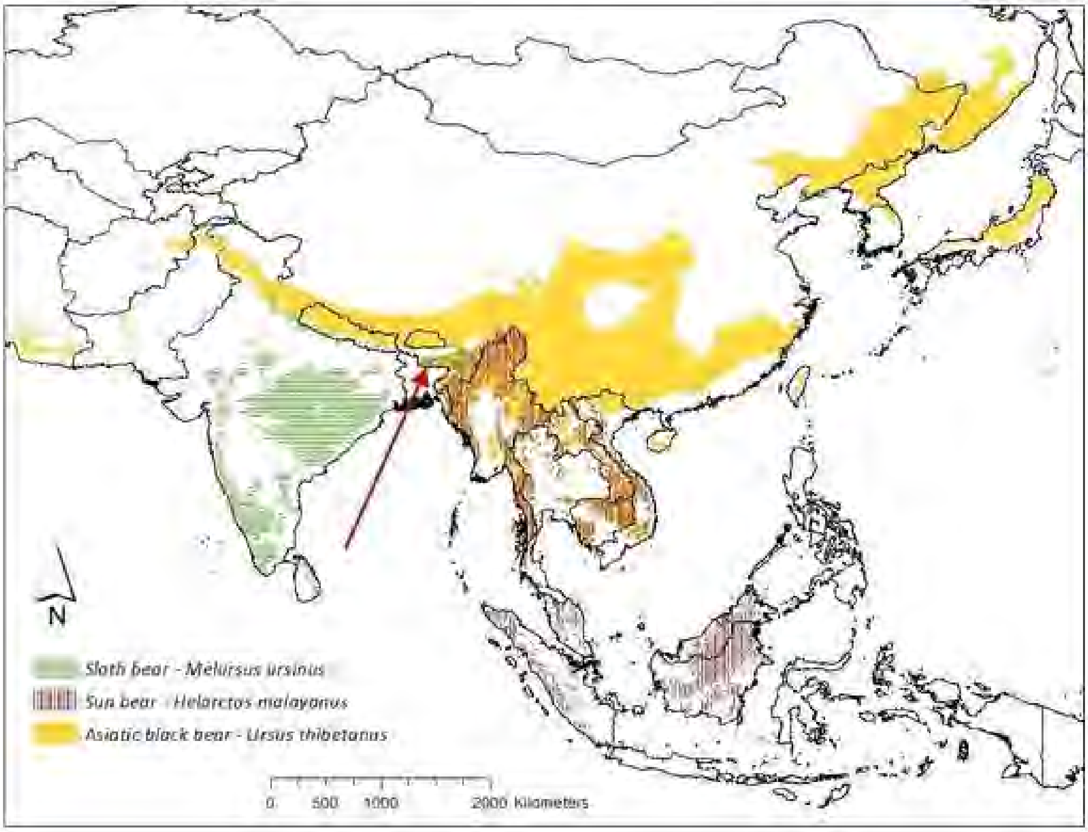


Figure 1. Range map of the three species of Asian bears that converge in Northeast India (red arrow). Depicted here is the maximum present range, combining categories for extant and possibly extant range from the IUCN (International Union for Conservation of Nature) Red List.

Ecological studies have been conducted in Southeast Asia, where sun bears and black bears overlap broadly (Figure 1), and on a fine scale [6–8]. These studies have found remarkably similar fruits in the diet and use of habitat for these two species; however, competition among the species appeared limited by some niche separation. Specifically, sun bears relied more on insects and black bears dominated in fruit-rich montane forests [9,10]. Even at baited camera traps, the two species had similar activity patterns and showed no avoidance of each other, despite black bears being significantly larger [11].

In NE India, records of where bears of each species formerly existed are rather vague, in part due to species misidentifications, and in part due to fragmentary records that are difficult to verify [12,13]. In fact, it is unknown whether all three species actually coexisted on a fine scale anywhere in the region. Sethy et al. [14] attempted to ascertain which protected areas across NE India are now occupied by each of the three species, based on sign surveys and a questionnaire survey of villagers, but they recognized significant uncertainties about identifications of the different bear species from sign or sightings; thus, most sites were classified as unknown presence for sloth bears and sun bears, whereas black bears, considered far more common across the region, were regarded as present in virtually every area. They concluded that the data were not reliable enough to distinguish presence of the rarer two species. Given that bears have long been hunted by people, attack people, and often depredate their crops and livestock, it is rather surprising how little we actually know of their occurrence, and specifically co-occurrence, in this distinctive landscape.

We set out with the purpose of locating a spot in NE India where these three bear species still coexist. That aim fits within a larger goal of understanding the factors limiting bear distribution. Some of these factors may be ecological, and others anthropogenic. It is also possible that the coexistence of these three species, or even two of these species, requires some threshold of resources; if that balance is disrupted, inter-species competition may allow only one species to persist [5]. Hence, there may be downstream conservation implications in finding and examining a place where a species at the edge of its geographic range coexists with another, or conversely, investigating a place where that situation occurred in the recent past but was disrupted by human activities.

In the process of conducting our assessment, it became clear that black bears occurred across much of the region, whereas the other two species were rarer, and only known from a few select spots. Likewise in Bangladesh, just to the southwest of NE India, black bears remain fairly widespread, whereas sloth bears disappeared within the past two decades and sun bears may be hanging on only in a small cross-border population [15,16]. To the north, sun bears also disappeared from southern (Yunnan) China [17], and sloth bears may only be an occasional visitor to southern Bhutan [18], whereas black bears range widely across China and Bhutan. Choudhury [12,13] reviewed the present status of all three of these bear species across NE India and concluded that the sloth bears and sun bears had lost a significant portion of their ranges since the early 1900s.

Hence, to find a point of overlap required a method where we could detect two rare species in the presence of a more common species. All of these species have unique characteristics, yet they are often misidentified from sign, sightings, and even camera trap photos [19,20]. This paper is devoted largely to the methods we used, the problems encountered, and recommendations for the future based on our experiences. Whereas this investigation was specifically about bears, some of the issues that we encountered have wider applicability to assessments of presence of other look-alike species, especially when one is common and others rare.

2. Study Area

2.1. Choice of Study Site

There are three states in NE India, Assam, Meghalaya, and Nagaland, where all three species are reported to potentially occur in the same vicinity [21]. However, the distribution of each species in each state is nebulous at best, often based on unconfirmed reports. A new checklist of the mammals in NE India that includes all three bear species was based on the same repeated sources, none of which have been recently verified [22].

We attempted to gain information on the occurrence of black bears, sloth bears, and sun bears from forest department staff, to identify potential areas where two or all three species might still co-occur. In 2017, we conducted workshops in Assam, Meghalaya, and Nagaland. Surprisingly, among the 80 experienced forest field staff and officers to whom we talked, no one knew how to differentiate the three bear species by sight or sign, and very few were even aware that multiple bear species occurred in the area. However, the best available sources of information [12,13,21,23] led us to focus on

Meghalaya as a place that could have all three species. Checklists of the mammals in this state listed all three bear species, although the information again referred to old sources, with no recent verification [24,25]. The Chief Wildlife Warden of Meghalaya [26] directed us to Balpakram National Park as being a safe place to work and where local staff were willing to assist. This park, in the Garo Hills, also represents the most western historical range limit reported for sun bears, but with no recent documented occurrence; thus, there was a certain appeal in finding out whether this species still exists there.

2.2. Study Area Description

Meghalaya was formed as a state in 1972, by carving out the Jaintia Hills, Khasi Hills, and Garo Hills districts of Assam. The state is bound by Assam to the north and east, and Bangladesh to the south and west. It is the wettest state in India, with a distinct summer monsoon (June–September) followed by a dry winter (November–February) [27]. The state is heavily forested (76%; [28]), with high floral biodiversity and endemism [29,30]. Of the seven states in NE India, Meghalaya has the lowest percentage of state-owned forest (12%) [28], with most forest being community-owned [31]. Some community forests include sacred groves that were preserved for religious and cultural beliefs [32,33]. The three different hill ranges in Meghalaya are home to three tribal ethnic groups, each with their own language. Most of the people rely on traditional agriculture, and practice *jhum* or shifting cultivation as well as terraced cultivation [34]. The hunting of wildlife is prevalent among the tribal people in NE India, both for food and socio-cultural reasons, and this includes bears [35,36].

Balpakram National Park (BNP) is located in the Garo Hills region of southwestern Meghalaya (25°20′–25°30′ N; 90°45′–91°0′ E; Figure 2). The elevations range from <200 m to 1049 m at Chitmang peak. The 220-km^2 park was acquired in pieces, previously being owned by clans of local people. It was established in 1987 and the expansion is continuing, although with some pending legal controversies about acquisitions [37]. Adjoining the park are Rewak and Baghmara Reserve Forests, Siju Wildlife Sanctuary, community forests, and *jhum* agriculture.

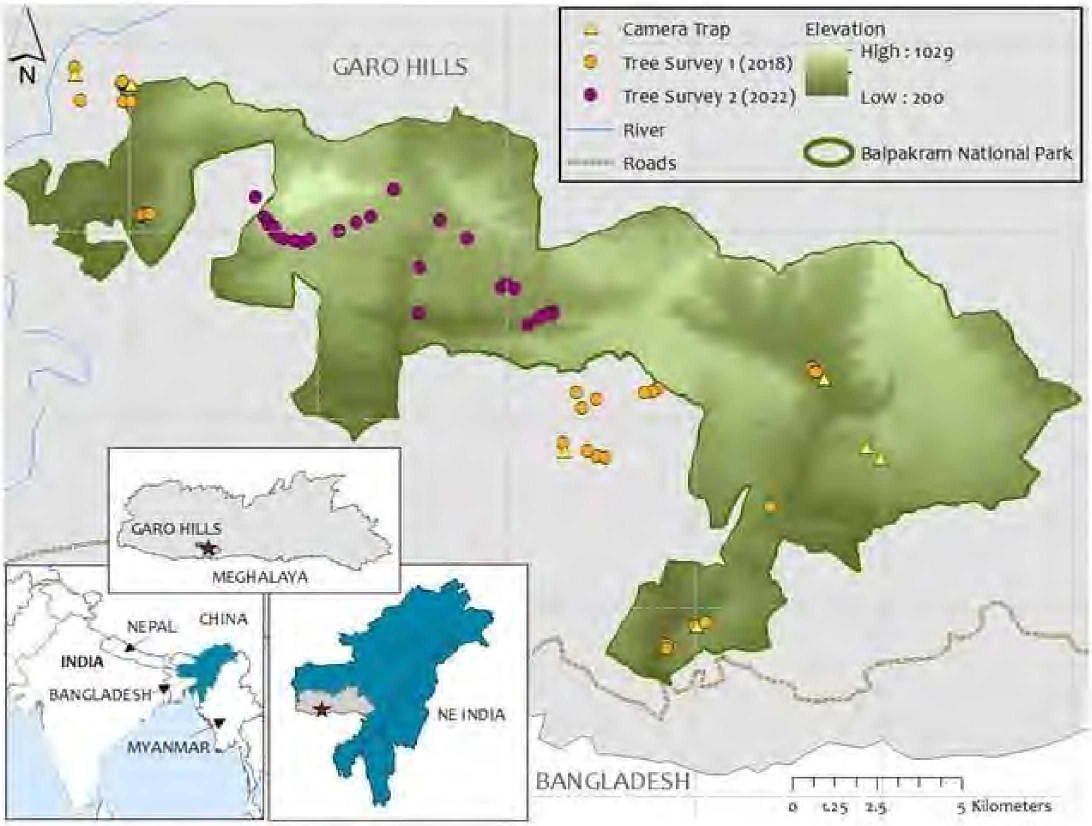


Figure 2. Sites where bear claw-marked trees were found and camera traps set (no bears photographed) in and around Balpakram National Park (star), Meghalaya, India, 2018–2019, and 2022.

Known as the land of eternal wind, Balpakram is sacred to the local Garo people and thought to be inhabited by spirits. Due to poor road access and local tribal customs and mythology, the forests have been relatively undisturbed. Forest types include tropical moist evergreen, tropical semi-evergreen, shola, grassland savanna, tropical moist deciduous, bamboo, and secondary forest resulting from shifting cultivation [37]. These forests contain a high density and diversity of trees [38].

The harvesting of timber, bamboo, firewood, and non-timber forest products is prohibited inside the park, as is grazing livestock and hunting wild animals. According to the local authorities, the extent of illegal hunting is low. The park and the surrounding forested land- scape are home to a wide diversity of wildlife, including primates, such as the threatened stump-tailed macaque (*Macaca arctoides*) and endangered hoolock gibbon (*Hoolock hoolock*),a large population of Asian elephants (*Elephas maximus*), and at least 20 species of carnivores [39]. However, there is no recent evidence of tigers (*Panthera tigris*) [40]. The World Wildlife Fund has designated the entire eastern

Himalayas, including BNP, as a Global 200 ecoregion because of its high biodiversity, and the Indian government has nominated the Garo Hills Conservation Area, including BNP, as a UNESCO World Heritage Site [39]. However, due to the poor road access and few facilities in the park, tourism is low. Access is especially limited during the monsoon season, when the area receives >4000 mm of rainfall.

3. Methods

We used a combination of three methods to attempt to ascertain which bear species were present: surveys of local ecological knowledge; sign surveys; and camera trapping. We conducted our first field season during November 2018 to March 2019. We conducted a second field season during March–April 2022, focused in a specific area (see below).

3.1. Surveys of Local Ecological Knowledge

We conducted interviews with local villagers and forest officers about their knowledge of bears in the area. We asked how often they saw bears, and under what circumstances. We asked how many bear species they believed were present in the area now and in the past, and how they differentiated these species. We did not prompt villagers or officers with photographs or other information, but sometimes asked them afterwards if they could point to the species they were referring to on photographs. We did not use a questionnaire format, but rather carried out an informal discussion, with follow-up questions prompted by their answers. We also asked about their use of the forest, bears and other animals damaging their crops, and to recall any cases of bears being hunted. The interviews required an on-site translation between the local Garo language, Hindi, and English.

3.2. Sign Surveys

We searched for bear sign along human and animal trails and by meandering through the forest. We did not conduct formal transects (with randomized starting points and direction, and fixed width) because our intent was not to quantify sign density but rather to find as much bear sign as possible and distinguish the sign to species. We also visited sites with bear sign that was previously discovered and reported to us by local forest staff. For sloth bear sign, we searched for dug-out termite mounds or scats with termites [41].

We specifically asked people about the places where we might find termite mounds. In addition, knowing that sloth bears often occur in grasslands, we visited the largest grassland savanna in the park, looking for termite mounds.

For black bears and sun bears, we searched for claw marks on climbed trees (Figure 3). Sun bears are significantly smaller than black bears, and in a controlled captive and field study, it was discovered that the spacing of the toes on each individual set of claw marks, especially those on the hind feet, is generally closer together for sun bears than black bears; however, claw mark widths of young black bears are not distinguishable from sun bears [42].

We obtained measurements of hind foot marks that showed at least four of the five claws. We distinguished hind foot marks as being roughly perpendicular to the trunk, whereas front foot marks were somewhat diagonal, resulting from the bear grasping the trunk [42]. We created a permanent template by pressing a sheet of paper over the individual sets of hind foot marks and punching holes with a pencil point over the center of each gouged mark (Figure 3). If the mark was elongated from the claw slipping downward, we punched the hole at the point where the claw stopped slipping and gripped the bark, normally at the bottom of the mark. On this template, we then measured the straight- line distance (width) across sets of four and five claw marks to the nearest millimeter. We measured five-claw width from toe one to five (Figure 3). We measured four-claw width from toe two to five or one to four, whichever was shorter; if only four marks were visible on the tree, we measured the width, even if we could not tell which toes they were from. We sought to measure all of the distinctive marks with four or five claws that were within our reach, and we specifically looked for marks of different sizes on the same tree. We used these measurements to separate which bear species likely left the marks on the trees, as per the key created by Steinmetz and Garshelis [42] (Tables S1–S3, Supplementary Materials). We also estimated the age of each mark [43], and thereby partitioned out the recent (<1 year-old) marks for a separate analysis.

Figure 3. Measurements of hind foot claw marks of bears (marks perpendicular to the tree trunk—left panel) were used to try to distinguish black bears from sun bears. We held a paper over the claw marks and created a permanent template by punching holes at the bottom of each mark (right). On this template we measured spacing of the narrowest span across four claws, and the span across all five claws (if all five claws were visible) (yellow arrows). We distinguished whether claw marks were recent (as shown here), based on the color and extent of regrowth of the bark.

During our 2018–2019 field season (hereafter survey 1), we learned of a wide scale mammalian biodiversity survey of BNP and surrounding landscape that was conducted several years earlier (2012–2015) [44–46]. They employed systematic camera trapping and sign surveys. They also used claw mark measurements on trees in an attempt to differentiate black bears from sun bears, as per Steinmetz and Garshelis [42]. These investigators shared their raw data with us. Those data seemed to show a spatial aggregation of sun bear-sized claw marks in a central portion of the park. However, we learned that these investigators measured the marks differently, making the bear species categorization uncertain. These results prompted us to conduct a follow-up sign survey targeting the area with a high proportion of sun bear-sized marks. In this follow-up survey (2022, hereafter survey 2), we used a precut paper gauge held up to the marks on the tree to quickly assess whether any were sun bear-sized. If only black bear-sized marks were found, one good set was recorded on a paper template. If a sun bear-sized mark was found, it was recorded, and we looked for and recorded all other marks that we could reach from ground level. We sought to determine whether the tree had only been climbed by a smaller bear (i.e., sun bear size) or by a small bear and a larger bear (i.e., black bear size), which could mean either a black bear mother and cub, or a sun bear and a black bear.

Tree identification was difficult because all of the field work was conducted during the non-fruiting season. Typically, a member of the park staff would try to identify the common name of the tree in Garo, and we later attempted to find the corresponding scientific name (some translations provided in [39,47,48]).

3.3. Camera Trapping

We set 11 cameras (Spypoint Force 10, Victoriaville, QC, Canada) in areas close to where we found bear sign or along trails that we expected bears to use (Figure 2). We were not permitted to use lures. The cameras were operational for 10–98 days, from early December 2018 to early March 2019.

4. Results

4.1. Local Ecological Knowledge

We conducted 14 in-depth interviews with the elders in different villages and many more casual discussions with a host of park staff and villagers. Generally, we learned that whereas the park staff were not aware of more than one species of bear, a few villagers thought that there were two different species of bears in the area, and some claimed that there used to be three. The descriptions provided to us (without prompting) of the three species seemed to match those of the black bear (large, black, white crescent chest mark), the sun bear (small, short hair, arboreal, sleeps in a tree nest constructed of broken branches— note: tree nests are also common for black bears, and recognized as such within BNP [49]), and the sloth bear (long shaggy coat, termite-eater, aggressive, attacks people).

We learned that there are Garo words for three different kinds of bears. *Makbil wak*, translated as “pig bear”, is the black bear, which is the most common, according to everyone with whom we spoke. *Makbil merang* was mentioned to us only twice and was said to mean “long-haired bear” or “bear with mane”. Sloth bears have long hair and a distinctive mane, so the Garo word matches these characteristics. *Makbil sarang* was translated to us in various ways, as meaning “bear that eats the first crop of fruit”, “danger bear”, or “bear with orange on chest”, and was considered to be a distinctly smaller bear. Sun bears are much smaller than black bears or sloth bears, but we are not aware of this species eating fruit earlier than black bears—in fact, sun bears are more insectivorous than black bears [9,10,50]. In addition, sun bears are generally unaggressive and thus less dangerous than black bears and far less dangerous than sloth bears. Sun bears often do have an orange chest marking, but we are cautious of this translation from Garo, since the translator had just attended our workshop where we taught park staff the characteristics of these three bears and showed photos of sun bears with orange chest markings.

People reported seeing bears occasionally (but not commonly), especially raiding their farms and orchards, but we heard few complaints about them. The main cash crop was betel nut (fruit of the areca palm, *Areca catechu*), which bears do not eat. However, people also cultivated jackfruit (*Artocarpus heterophyllus*), which is readily eaten by bears in other states in NE India and elsewhere, where it is a source of reported conflicts with people [15,51–53]. Additionally, beyond BNP there were plantations of cashew (*Anacardium occidentale*), which could be attractive to bears. However, people seemed mainly unconcerned about depredations of these crops. From the descriptions of the few crop-raiding bears, all were likely to be black bears. A study of habitat associations by the wildlife in the park suggested that black bears commonly occur in forests near villages and visit *jhum* areas to feed [49].

We became aware of two bear attacks, both reported to be black bears. A man (approx. 40 years old) was attacked in the early morning of 12 November 2020 while walking down a trail towards his *jhum* field, about 500 m from his village. A bear approached on an adjoining trail, and the man ran. The bear pursued, grabbed him, they both fell and rolled. The man played dead, but the bear continued to maul him, scratching his chest. His wife, who was also walking to the field, rushed to help him, and threw a log towards the bear. It did not hit the bear, but nevertheless forced it to run off.

Ten days later, in a village about 150 m away from the first incident, a woman (ap- prox. 60 years old) was working in her *jhum* field in the afternoon when she saw a bear approaching. The bear abruptly attacked, and she fell unconscious. When she regained consciousness, the bear was gone. Although she had significant injuries, she was able to walk back to the village to seek help. She recalled afterwards that she had seen three cubs before the incident and presumed that the bear that attacked her was their mother. We were told that bear attacks are not unusual, yet they are rarely reported.

We found nobody who believed that sloth bears (*Makbil merang*) still existed in or around the park, although a few people indicated they had been present 30 or more years ago and were notably more aggressive than black bears. One man from a village on the northern border of the park, near the Khasi Hills, mentioned that he used to see bears that fit the description of sloth bears in that area as recently as 10 years before (i.e., about 2008), but that he had not seen any since. We were not able to investigate this region, which possibly has the best sloth bear habitat.

A few people mentioned the possible existence of *makbil sarang* (presumably the sun bear). One particularly intriguing story we heard, from multiple sources, was that two sun bear cubs had been confiscated from a village near BNP in 2018 and brought to a nearby zoo. We checked on this and found them to be black bears.

We heard of two people in different villages who had recently seen what they called *makbil sarang*. The villagers had related the sightings to a forest guard, who then told us. We were able to interview one of them in early April 2022, about a sighting 2 months prior. The man was on his way to go fishing, walking along a forest trail, when he spotted a bear in a tree. The bear quickly slid down, and then paused and stood on its hind feet in front of him. The man described the standing bear as only as high as his waist (~1 m), although he said it was not a cub. Both the man and bear ran away in opposite directions. The man indicated that he had previously seen one or more black bears (*makbil wak*) and thought that this bear was different.

4.2. Sign Surveys

The only definitive bear sign we found was claw-marked trees (Figures 2 and 3). We found no diggings or scats that we attributed to bears. In total, we measured 154 sets of claw marks showing at least four claws of which 119 were black bear size, 19 were sun bear size, and 16 were intermediate size.

The appropriate sample unit for the claw mark surveys is the tree, not the individual marks, because we measured a variable number of marks per tree (range one–

six). This variation was due to some of the sets of marks not showing at least four claws, some being the front feet or at least not definitely the back feet (the claw mark key pertains only to the back feet), and some being too high on the tree to measure.

Of the 74 different claw-marked trees, 20 (27%) had two different-sized marks (combinations of black bear, sun bear, and indeterminate). Most striking is that all of the trees where more than one set of marks were measured contained either just black bear size marks, or black bears in combination with smaller marks. We found no claw-marked trees with exclusively sun bear-sized marks (Table 1).

Table 1. Bear claw (climbing) marks found and measured on trees in Balpakram National Park,2018–2022, and fitting the size of sun bears or Asiatic black bears.

Survey No.	Claw Marks Measured	Trees with Marks	Avg. Measured Marks per Tree	% Trees with Sizes of Measured Claw Marks					
				SB Only	SB + ABB	Single ABB	>1 ABB	ABB + I	I Only
1	94	35	2.7	0%	31%	17%	40%	6%	6%
2	60	39	1.5	0%	13%	59%	21%	5%	3%
Combined	154	74	2.1	0%	22%	39%	30%	5%	4%

Survey 1: 2018–2019; survey 2: 2022 (see Figure 2). Claw marks measured = no. of bear claw marks measured and compared to species key [42]. SB = sun bear size mark; ABB = Asiatic black bear size mark; I = indeterminate size, between SB and ABB. Single = only 1 mark measured, but other marks likely occurred on the tree. SB + ABB or ABB + I = combination of different-sized marks.

Survey 1, which was primarily around the edges of the park in the reserve forests, recorded 13 of 35 (37%) trees with two different-sized marks (Table 1). Survey 2, which targeted an area in the park interior where other researchers reported finding sun bear-sized marks, recorded only 7 of 39 trees (18%) with two different-sized marks. We measured less marks per tree in survey 2 (Table 1) because we followed a different protocol, whereby we held a gauge to the various marks on the tree, and if we found no marks smaller than black bear size, we normally measured just a single set. The results did not change significantly when we partitioned out those marks distinguished as recent (<1 year); however, we only aged the marks in survey 1, where we found 18 trees with recent marks, of which 9 (50%) had two different-sized marks.

In one case, a local person brought us to a tree where a few months before he had seen two distinctly different-sized black bears (which he interpreted as either a male and female or a female and large offspring) feeding on a honeycomb. Our measurements of the marks keyed out as black bear and indeterminate. Interestingly, when we measured the width of the central three claw marks of the indeterminate sets, they keyed out to be sun bear size (as per [42]). Furthermore, we found some long sliding marks on this tree apparently caused by the bears hurrying down when they saw the person watching them. We also found another nearby fruiting tree with fresh claw marks matching the size of black bear and sun bear, and broken branches forming a platform (nest).

We were able to identify 49 of the 74 bear-climbed trees to genus or species (Table S4, Supplementary Materials). Identification was hindered because we were not permitted to collect leaf samples, we did not have a botanist on our team, and our local guide used Garo names for the trees, for which we could not always find a corresponding scientific name. Six of the climbed trees were figs (*Ficus* sp.) and 20 were either oaks (Fagaceae: *Castonopsis*) or laurels (Lauraceae: *Litsea*, *Cinnamomum*), all of which produce fruit that is commonly eaten by both black bears and sun bears in other areas [10,54]. In fact, 13 of the 15 genera of climbed trees that we identified were reported previously as food of black bears and sun bears (Table S4, Supplementary Materials).

4.3. Camera Trapping

We set camera-traps at 12 locations for a total of 577 trap nights, and captured 1990 images. We obtained no photos of bears, but 191 (10%) were of people. These involved 32 independent events, of which 9 (28%) parties included people with weapons (guns orlocal weapons).

5. Discussion

Recently, it was discovered that there is at least one place in the world where three species of bears exist together on a local scale. That place is northern Canada, along the southwestern edge of Hudson Bay, where camera traps have revealed the presence of polar bears (*U. maritimus*), American black bears (*U. americanus*), and grizzly (brown) bears(*U. arctos*) all at the same spot, although never together at the same time [55]. Whereas a few grizzly bears have historically occurred in this area, they have become far more common, apparently due to the increased expansion of immigrants southward along the Hudson Bay coastline during the past several decades, possibly related to climate change [56]. However, there is currently no indication of the cooccurrence of resident breeding populations of the three species of bears in this area.

Conversely, our study sought to find a remnant place where resident populations of three species of bears co-occurred historically, and still do. We do not know if there ever was a place where the ranges of three extant bear species overlapped at the level of a forest patch, but if so, it would be NE India (Figure 1). Our study at one site there, in western Meghalaya, revealed that the co-existence of three bears is doubtful. Our study also revealed the difficulties of even detecting whether the site is occupied by two species of bears. Here, we critically examine the information obtained using the methods that we employed.

5.1. Local Ecological Knowledge

Black bears are undoubtedly the most prevalent, if not the only, bear in BNP. We heard this repeatedly in our many discussions with park staff and local people. Thus, it became apparent that we were looking for evidence of one or possibly two rare bear species in an area occupied by a much more common one. Given that most people did not know how many bear species lived in the region and were uncertain how to distinguish bear species by visual sightings or sign, we treated all second-hand evidence with some skepticism, but we attempted to pursue promising leads.

One of the strongest pieces of evidence for the existence of sun bears, which was instrumental in directing us to work in BNP, was the two orphaned cubs that had recently been found there. Our eventual discovery that these cubs were not sun bears underlines the issue of confusion between the species and demonstrates the importance of following up on all accounts of sun bears or sloth bears in the area.

We heard intriguing stories of past observations of both sun bears and sloth bears, suggesting that the three species may have overlapped here in the not-too-distant past (10–30 years ago). That overlap may have been on a macro-scale, but not within the same habitats, as we were told (by one person) that sloth bears were in a relatively close but different area. A few people suggested that sun bears might still occur, although we were told of just one recent, purported first-hand sighting. The description provided by that individual was a very small bear, which he identified as *makbil sarang*, although the bear he described was considerably smaller than a standing adult sun bear. If the standing bear was truly only waist high, as the person indicated, then it must have been a juvenile, in which case it could have been a black bear. Unfortunately, we could not determine from the translation of this interview why the person was so convinced that this bear was not a black bear.

In 2005, an investigator surveyed the local people in the South Garo Hills, including those living around BNP, asking what large mammals they had seen on community lands and what their perceptions were of population trends [57]. Black bears were among three species that most people thought were declining. The sample included 27 hunters, who would have been most familiar with the wildlife, none of whom had seen a sun bear in the previous 5 years.

5.2. Sign

The virtual absence of termite mounds meant that we could not reliably identify whether sloth bears were present based on sign (diggings or scats with termites). Moreover, without this key food source, we felt it was unlikely that sloth bears were present, although we recognize that there are places where sloth bears rely more on ants than on termites [58]. We had hoped to use claw marks to confirm the presence of sun bears, but an important constraint with differentiating black bears and sun bears from claw marks is that small black bears create sun bear-sized marks. Sometimes these small marks are identifiable as being from a cub or yearling, if the mother also climbed the tree.

Interpreting our data cautiously, we assumed that all cases where sun bear-sized marks occurred on the same tree and were about the same age as black bear-sized marks represented a black bear mother and offspring. This interpretation, though, resulted in an unusually high percentage of mother–offspring incidents in the data: 22% overall; 50% for recent marks. Comparatively, only 9% of the bear claw marks were sun bear-size, in a protected area of China occupied only by black bears [42]. One explanation is that our data from along the edges of the park happened to be an area used heavily by females and cubs. Notably, at least one of the two bear attacks that we recorded along the edge of the park was by a female with cubs.

Another explanation for two different-sized marks on the same tree is that different individuals, possibly of two different species, climbed the same trees that had abundant fruit. Certainly, it is not unlikely that a fruiting tree could attract multiple bears at different times. Other studies have shown that black bears and sun bears eat the same fruits, use the same habitats, and can even be photographed at the same site a few days or even hours apart [9–11]. However, it would seem highly unusual that every tree climbed by a sun bear would also have been climbed by a black bear. If some trees are climbed by both species, there should also be some trees climbed only by black bears, which we found, and some trees climbed only by sun bears, which we did not find. This could make sense only if sun bears were much rarer than black bears. We note that the investigators of the mammalian biodiversity survey of BNP mentioned above, who measured claw marks somewhat differently, claimed to have found trees with only sun bear-sized marks [46]. It was their data that directed us to the area targeted in survey 2, where all of the trees that we found with sun bear-sized marks had accompanying black bear marks.

5.3. **Habitat Considerations**

We specifically searched for habitats that seemed suitable for sloth bears, namely sal (*Shorea robusta*) forest and grasslands [59]. In fact, one reason we chose this park as a study site is that it is one of very few places in NE India that has an extensive grassland [60]. However, we found no evidence of sloth bear diggings or termite scats, and although some people told us about places that had termite mounds, we searched there and found very few. We also investigated trees with honeycombs, looking for evidence of bear climbing, since sloth bears are also known to readily consume this food [61–63]. All of the climbed trees with honeycombs that we saw had marks indicative of black bears. We therefore concluded that it was unlikely, based on habitat (i.e., few termites), that sloth bears lived in this park.

Conversely, the habitat features in this park seem amenable to sun bears. Nearly all of the climbed trees that we recorded were fruit trees, known to be part of the sun bear diet in other places (Table S4, Supplementary Materials). Sun bears overlap with black bears throughout most of Southeast Asia at a patch-level scale, and many of their habitat requirements appear to be similar [5]. However, whereas black bear range extends much farther north and west (Figure 1), sun bears have apparently never crossed the Brahmaputra River. The reason for this is not entirely clear, but it seems probable that the alluvial grasslands along this river may have been a significant barrier, as it has been to a host of other Southeast Asian mammals whose distribution is restricted to the east side [64]. In addition to the alluvial corridor associated with the Brahmaputra River, there is a 200-km wide expanse of alluvial habitat within the Brahmaputra–Ganges River delta that separates the Garo Hills of Meghalaya from the Rajmahal Hills of Jharkhand [65]. This so-called Garo–Rajmahal gap imposes range limits not just for a variety of mammalian taxa, but also for birds, reptiles, and fish [66–68]. Thus, from the standpoint of zoo geography it seems reasonable that sun bears would have ranged westward to the Garo Hills (our study site), but no farther. Sloth bears would have crossed the gap because they readily use alluvial habitats, and black bears, which have the widest niche among these three bear species [59], could have bypassed the gap in the hills farther north.

5.4. Limitations of the Methods

Local people readily confirmed the presence of bears and provided some useful information about the context of their interactions with bears. They reported that human– bear conflicts were uncommon. However, after we left the field following survey 1, two people were attacked. The local people and park staff also reported that hunting bears was illegal and rarely occurred, yet our camera traps photographed people coming into the park with guns. Hence, the information we received was not entirely correct, either because people had limited information or because they did not openly share all of the information they had. Nevertheless, no person said that sun bears were common, and most knew nothing about this species.

The best evidence of sun bear presence that we uncovered was a single person who reported seeing one very recently. Whereas we have no reason to believe that this person intentionally tried to deceive us, we cannot discount the possibility that his prior local knowledge of the physical characteristics of a *makbil sarang* influenced what he thought he saw. We were told that a second person also independently saw one, possibly lending more credence to the story, but we were unable to talk to that person. The person we interviewed seemed quite convinced that he saw a different species than the more common black bear. However, we are aware of a number of reports of two species of bears in other places where we know with certainty that there is just one, or even three species where there cannot be more than two [69,70], due to people judging species by erroneous characteristics (e.g., size, coat color, behavior), or being confused by seeing pictures of bear species from other areas, or hearing about different bears from others in the community. There are still many places in the world where persistent stories of large primates not known to science have misled people who have seen bears walking bipedally [71,72].

In the early 1980s, two experienced wildlife scientists working in the Himalayas of Nepal learned of what local people considered to be a different kind of bear, not known to science. It purportedly lived in higher elevations than black bears, was smaller in size, more arboreal, and built more intricate tree nests. The local people had given this bear a distinct name (*rukh balu*), and the researchers began calling it the Nepal tree bear. It was described in a book [73], and there are drawings, which look very much like juvenile black bears [74]. It is now known that only black bears reside in this area [75]. Our point is not that local people are necessarily wrong in differentiating different kinds of bears, but that they may categorize "types" that may not match actual species.

Some of our team had considerable experience in differentiating black bears and sun bear from sign (in Southeast Asia), so we started our study with optimism that measuring claw marks would work to distinguish these two species [42]. One lesson we learned in implementing the technique is that it is imperative to search the tree for different-sized marks, and not just record a single set of clear marks, assuming all marks are the same. This is not foolproof, as many marks do not have the necessary four or five toes needed for species identification. In survey 1, we also measured the widths of the central three claw marks, of which 47% were sun bear-sized, using the Steinmetz and Garshelis key [42], but this declined substantially using four-claw mark sets (17%), and even further with five-claw mark sets (5%). A second important lesson is to record the marks with a paper template, which is a more accurate measure that includes the curvature of the tree (since the bear's paw must bend around the curve) and can serve as a permanent record that can be remeasured.

A final important caveat is that, even if we had found a few trees with only sun bear- sized marks, these could not be definitely categorized as sun bears because young black bears may climb trees as a refuge while their mother remains on the ground. Some might interpret this to mean that the species gauge developed by Steinmetz and Garshelis [42] is not useful, because small marks could be from either species. It is important to recognize that this key was developed for use in an area where both of the species were known to exist, and the purpose was to ascertain whether they used distinct habitats or types of fruits [9,10].In that area, it was discovered that very few small marks occurred in montane habitat, yielding the interpretation that in this habitat, sun bears were excluded by black bears. Our study was the first to try to employ the claw mark gauge to determine whether sun bears exist in an area known to be occupied by black bears. In this case, the interpretation of the marks must be done cautiously to avoid "discovering" sun bears in a place where they never existed or have been extirpated. This would be a serious conservation error. Hence, except in a case where sun bear-sized marks are common and often occur without accompanying larger marks, we suggest that investigators should be wary of using this sign as the sole evidence of the presence of this species.

We have come to believe through this study that the only definitive evidence of the presence of sun bears or sloth bears would be an alive or dead animal, a DNA sample (e.g., from fresh scat), or a clear photo. It is striking that there are no recent specimens and no photos of sun bears anywhere in Meghalaya. An important question is, what level of survey effort would be required to obtain a sun bear photo if this species was much rarer than black bears? Indeed, this is an important question for the detection of any rare species [76].

To investigate this question, it is instructive to look at camera trapping data from other nearby areas. At two study sites in Myanmar, one in the north and one in the west-central, black bears were much rarer than sun bears. Camera trapping revealed ratios of sun: black bear photos of 198:6 and 45:1, averaging 35:1 (Supplementary Materials in [77]). Likewise, a study in Laos obtained photos of sun bears at 104 camera-trap stations, but detected black bears at only 11 stations, giving a ratio of nearly 10:1 with similar detection probabilities for the two species [78]. Note that both studies had a lopsided ratio of photos strongly in favor of sun bears, demonstrating that this species is not harder to camera trap than black bears. In 2012–2015, a research team conducted an extensive camera trapping program across the BNP landscape to document the mammalian biodiversity. In nearly 4000 trap-nights of effort, they obtained only three black bear photos and no sun bear photos [46]. If black bears and sun bears have a roughly equal detection probability, and if (hypothetically) there were 10 as many black bears as sun bears in BNP, then over 12,000 trap-nights would be needed to obtain a single sun bear photo. That would be >3 the effort expended by these investigators, which took 3 years. Likewise, in over 6000 trap-nights, three black bears but no sloth bears were photographed in Manas National Park, Assam [79], although both of these species were known to be present [80].

A sun bear photo might be obtained more quickly if cameras were put in the vicinity of purported sightings by villagers. We think that this strategy—targeted camera trapping immediately following the sighting reports—would be more efficient, and likely more convincing as to either the presence or absence of this species, because it would also help check on what villagers are calling *makbil sarang*. The detection of this species via camera trap would be significantly improved by using a lure, which could be done by a special permit.

5.5. Identifying Rare Look-alike Species

A number of recent studies have detected rare mammalian carnivores by camera trapping in Meghalaya or other areas in the region [44,45,81–83], including sun bears [84]. There is a difference, though, between detecting a species inadvertently during a biodiversity survey, and searching for a specific species that may or may not exist or may never have occurred there historically. Searching for a rare species is especially difficult when there is another more common look-alike species present. This not only makes eyewitness accounts less reliable, but also may cause errors in camera-trap identifications. Misidentification of camera trap images of black bears and sun bears is not uncommon [19]. Two recent published papers reporting photographic evidence of sun bears in China misidentified black bears [85,86]. Video footage confirmed at least one sun bear in China, although this occurred <1 km from the border with Myanmar, where a known population exists [17].

Historically, sun bears were reported from a number of places where we now know they did not exist. Strikingly, these erroneous reports were based on dead animals that could be examined closely, and included Sri Lanka (called Ceylon at the time; [87]), the terai of Nepal [88,89], and Tibet [90,91]; in this last case, it was noted that, whereas the skull of the specimen matched that of a sun bear, the skin was similar to that of a black bear.

Knowledge of the presence of sun bears in India came relatively late compared to the other bear species. Jerdon's [92] comprehensive *Mammals of India* (first published 1867) listed four species of bears in India, including brown, Asiatic black, sloth, and the "red cat-bear" (red panda, not actually a bear, *Ailurus fulgens*), but did not include sun bears. Blyth's [93] 1863 inventory of specimens in the Asiatic Society's Museum contained sun bears, but not from India. Sterndale's [94] 1884 comprehensive natural history of the mammals of India listed sun bears as stretching westward only to Burma (Myanmar). However, by 1888, Blanford [95] described their western range limit as Chittagong (now Bangladesh) and the Garo Hills (then part of Assam). This is the earliest record that we could find mentioning the presence of this species in the Garo Hills, where we worked. Blanford did not cite a source for his information, but a specimen from the Garo Hills (plus several other specimens from other unknown sites in Assam) was listed in Sclater's [96] 1891 updated catalogue of mammals in Indian museums, meaning that it had arrived since Blyth's inventory. Hinton and Lindsay [97] noted the collection of another sun bear specimen from the Garo Hills during 1919–1920. Finn [98], which was an update of Sterndale's work, repeated the Garo Hills as the western range limit of sun bears. In times when hunting bears for sport was legal in India, sun bears were reported to be far less common than black bears and sloth bears in NE India, and the occasional hunting of a sun bear warranted publication in the *Journal of the Bombay Natural History Society* [99]. As a consequence, the distribution of sun bears in NE India was always rather vague. Gee [100] reviewed a number of general places where sun bears had been reported in the early–mid 1900s, including Assam, and reiterated previous authors' mentions of their occurrence in the Garo Hills, but provided no new information.

It is within this context that we consider the most recent record of a sun bear in western Meghalaya, in what is now BNP. The person who reported this incident from the early1980s (Anne Wright) was an accomplished Indian naturalist who had commonly seen black bears and sloth bears, and observed this one freshly killed individual to be different enough to warrant writing a letter to a local bear expert [12]. We had the occasion to talk to Mrs. Wright, through her daughter, and although she did not recall the incident, she felt that her knowledge of the other bear species supports her previous claim that it must have been a sun bear. An alternate explanation is that it was a black bear that had some characteristics of a sun bear. Unfortunately, there is no photographic corroboration, and we are unaware whether the specimen ended up in a museum. In the most recent case from the area, two cubs that were initially reported to be sun bears turned out to be black bears. However, the characteristics of the two species may be more easily confused in cubs.

Sloth bears are more widespread across India, and western Meghalaya appears well within their historical range [12], although there are no recent confirmed records from the state [101]. Nevertheless, it is important not to dismiss the possibility that the species still exists, but in very low numbers. Choudhury [102] reported a sloth bear that was killed in Assam in 2017, <10 km from the border of Meghalaya (<100 km from BNP), where no recent records of the species had existed, and far from any known population. This report was documented only because the bear had attacked and injured multiple people.

6. Conclusions and Conservation Implications

The assessment of species status is the first step in the "assess-plan-act" conservation cycle of the IUCN Species Survival Commission [103]. On the most basic level, assessment involves document presence. Across Asia, there remain many places where investigators are seeking to ascertain the presence of bears, using methods similar to ours [104,105].

Our aim was more complicated: we were trying to find a place where three bear species still co-occur within their historical range. We concluded that this was not the case in BNP, as there is no evidence of the presence of sloth bears, and we were explicitly told by local people that this species no longer exists there. However, we could not ascertain for certain whether there is just one bear species (black bear), or two. We could not confirm the presence of sun bears, but the data we collected allow for the possibility that they could be present. Determining that a species is absent is far harder than determining its presence, because the latter requires just a single unequivocal documentation. The enigma represented by our study is how to proceed when the documentation is far less certain. We had one eyewitness account by a local person; additionally, we measured claw marks that could have been sun bears, but a more parsimonious explanation (due to their co-occurrence with black bear marks on the same trees) is that they were juvenile black bears.

Where black bears and sun bears co-occur in Southeast Asia, their habitat use, and food habits are very similar. Despite this, the relative abundance of these two species varies widely across the region of their co-occurrence. We assume that differences in the ratio of these two species are mainly habitat- and food-related, even if these factors are not well understood. We did not assess the food resources for bears in BNP, so we do not know whether a low density of fruiting trees could account for the scarcity or absence of sun bears.

The two species are also likely to compete at some level. However, it is not simply that black bears, being larger, always outcompete sun bears. In some areas, even at the northern edge of sun bear range, sun bears are more common than black bears (as mentioned above). In North America, American black bears can outcompete larger brown bears in some circumstances [106]. It may be that whichever bear species initially becomes more common is able to displace the other.

Human influences (e.g., disturbance, poaching, crops) also may favor one species over another. In Mizoram (southeast of Meghalaya), Sethy and Chauhan [52] reported the frequent depredation of crops by sun bears in a similar *jhum* agricultural system, so it is unclear why crop depredation is so low around BNP. It is possible that, despite the indications from park personnel and local people that there were few conflicts and negligible poaching, human-caused mortality was sufficient to depress the abundance of bears. Our camera trap observations of people with guns headed to the interior of the park suggest that hunting occurs more commonly than we were led to believe.

If sun bears do exist at a very low density, then an important conservation action to protect them would be to ensure protection against poaching. That action would be beneficial to a host of species. However, the point here is that a rare species that is very vulnerable to extirpation may provide the extra incentive to take action. In that way, knowing whether sun bears exist in this park is important. In fact, the occurrence of this species, at the most westward extent of its historical range, would also likely generate more conservation attention to this park in general.

Another reason for seeking to know whether sun bears exist in BNP is because such an understanding might help improve the conservation across the region. A recent study in a reserve forest in southern Assam, bordering eastern Meghalaya, reported the previous extirpation of sloth bears and recent extirpation of sun bears, but the continued presence of black bears (with the caveat that this study was based on local interviews, so subject to misclassification of species [107]). In Bangladesh, sloth bears were extirpated, and sun bears nearly extirpated, while black bears persist [15]. In Bhutan, black bears are common and sloth bears were thought to occur, but extensive camera trapping yielded just a single photo of a sloth bear near the Indian border [18]. In Vietnam, sun bears seem to have declined to lower numbers than black bears [108].

Hence, there seem to be a number of parallels with what may have occurred, or is occurring, in our study site. As such, BNP could provide valuable lessons in so far as assessing the presence of a rare species. In closing, we offer the following guidance, based on experiences from this study:

1. Information from local ecological knowledge should be evaluated cautiously when the species look somewhat alike. We obtained intriguing information on the possible existence of sun bears, but none was definitive, and all could have stemmed from stories told within the communities.
2. The sign of ecologically similar species can be misleading. We used a key that worked well to differentiate between the two species when investigating their habitat use in Southeast Asia; however, that key turned out to be less useful for detecting the presence of a potentially rare species whose sign was the same as juveniles of the common species.
3. Camera trap photos would provide the most definitive evidence of presence, but a significant effort may be necessary to obtain a photo of a rare species. Neither our camera trapping effort nor a more extensive effort before ours was sufficient. Directed camera trapping in areas where a person claimed to have seen the rare species would be more practical than a general survey.
4. The absence of proof that a species exists is not necessarily evidence of the species'absence. Our study was largely a documentation of the failure of the methods of detection. We note that sun bears had not been known to exist in Arunachal Pradesh (the northern-most state of NE India) until a single camera trap photo was obtained in Namdapha National Park in 1996–1997 [109]; however, since then it has been realized that a significant population resides there [1].
5. Whereas it can be difficult to assess whether a certain species still exists in any given area, this is just the beginning of the important process of learning what happened to them and why. We hope that sun bears are eventually documented in BNP, but even if they are deemed not to occur there anymore (presuming they once did), the timing and reasons for their disappearance would lend insights into what is occurring across the region.

Acknowledgments

The authors are thankful to the International Association for Bear Research and Management (IBA) for financial support. We are also grateful to the IUCN SSC Bear

Specialist Group (BSG) for additional support for field surveys, and to a number of BSG members who provided useful insights that aided in the collection and interpretation of data. We sincerely acknowledge the Chief Wildlife Warden of Meghalaya and the Field Director of Balpakaram National Park (BNP) for permission and logistical support, and Maureen Sangma, Conservator of Forest, Meghalaya, for her support in getting the proper permissions and assisting us during field surveys. We would like to thank the forest field staff for participating in field work and for translating the interviews from Garo to Hindi. A special thanks goes to the following individuals for assisting us in our field efforts: Arzoo Malik, Nilmani Rabha, Tushar Sangma and Shikha Srikant. Many thanks also to Kashmira Kakati and Shikha Srikant for in-depth discussions about the data from their mammal biodiversity study, and for sharing their experiences about BNP and the surrounding landscape. Their input was critical to our follow-up survey. The designation of geographical boundaries in the maps presented in this paper do not imply the expression of any opinion whatsoever on the part of IUCN or the authors concerning the legal status of any country or delimitation of its frontiers or boundaries.

References

1. Sethy, J.; Chauhan, N.S. Status and distribution of Malayan sun bear in Namdapha Tiger Reserve, Arunachal Pradesh, India. *Internat. J. Conserv. Sci.* 2016, *7*, 533–552.

2. Sethy, J.; Chauhan, N.P.S. Assessing habitat use by sun bears in Namdapha Tiger Reserve, Arunachal Pradesh, India. *Appl. Ecol.Envrion. Res.* 2016, *14*, 215–236. [CrossRef]

3. Gouda, S.; Chauhan, N.S.; Sethy, J. Status and distribution of Malayan sun bear (*Helarctos malayanus*) in Dampa Tiger Reserve, Mizoram, India. *J. Wildl. Biodivers.* 2019, *3*, 45–56. [CrossRef]

4. Gouda, S.; Chauhan, N.S.; Sethy, J.; Sahu, H. Daily activity pattern of Malayan sun bear in Dampa Tiger Reserve, Mizoram, India. *J. Wildl. Biodivers.* 2020, *4*, 56–64. [CrossRef]

5. Steinmetz, R.; Garshelis, D.L.; Choudhury, A. Adaptations and competitive interactions of tropical Asian bear species define their biogeography: Past, present, and future. In *Bears of the World: Ecology, Conservation and Management*; Penteriani, V., Melletti, M., Eds.; Cambridge University Press: Cambridge, UK, 2021; pp. 45–52, ISBN 978-1-108-48352-0.

6. Htun, S. The status and conservation of bears in Myanmar. In *Understanding Asian Bears to Secure Their Future*; Japan Bear Network:Ibaraki, Japan, 2006; pp. 45–49.

7. Vinitpornsawan, S.; Steinmetz, R.; Kanchanasakha, B. The status of bears in Thailand. In *Understanding Asian Bears to Secure Their Future*; Japan Bear Network: Ibaraki, Japan, 2006; pp. 50–56.

8. Steinmetz, R. Ecology and distribution of sympatric Asiatic black bears and sun bears in the seasonally dry forests of Southeast Asia. In *Dry Forests of Asia: Conservation and Ecology*; McShea, W., Davies, S., Bhumpakphan, N., Eds.; Smithsonian Institution Press: Washington, DC, USA, 2011; pp. 249–279.

9. Steinmetz, R.; Garshelis, D.L.; Chutipong, W.; Seuaturien, N. The shared preference niche of sympatric Asiatic black bears and sun bears in a tropical forest mosaic. *PLoS ONE* 2011, *6*, e14509. [CrossRef]

10. Steinmetz, R.; Garshelis, D.L.; Chutipong, W.; Seuaturien, N. Foraging ecology and coexistence of Asiatic black bears and sunbears in a seasonal tropical forest in Southeast Asia. *J. Mammal.* 2013, *94*, 1–18. [CrossRef]

11. Ngoprasert, D.; Steinmetz, R.; Sribuarod, K.; Gale, G.A. The overlap of sympatric sun bears and Asiatic black bears in space and time. *Mamm. Biol.* 2022, *102*, 143–153. [CrossRef]

12. Choudhury, A. *Records of Sloth Bear and Malayan Sun Bear in North East India*; Final Report to International Association for Bear Research and Management; The Rhino Foundation for nature in NE India: Guwahati, Assam, India, 2011; p. 56.

13. Choudhury, A. *Records of Asiatic Black Bear in North East India*; Final Report to

International Association for Bear Research and Management; The Rhino Foundation for nature in NE India: Guwahati, Assam, India, 2013; p. 97.

14. Sethy, J.; Sahu, H.K.; Chauhan, N.P.S. Status of bears and their conservation in North-east India. In *Biodiversity Conservation, Research and Management*; Sahu, H.K., Sethy, J., Mishra, R.K., Eds.; Himalaya Publishing House: Mumbai, Maharashtra, India, 2015; pp. 11–22, ISBN 978-93-5202-849-8.

15. Islam, M.A.; Uddin, M.; Aziz, M.A.; Muzaffar, S.B.; Chakma, S.; Chowdhury, S.U.; Chowdhury, G.W.; Rashid, M.A.; Mohsanin, S.; Jahan, I.; et al. Status of bears in Bangladesh: Going, going, gone? *Ursus* 2013, *24*, 83–90. [CrossRef]

16. Islam, M.A. (University of Dhaka & WildTeam, Dhaka, Bangladesh). Personal communication, 2022.

17. Li, F.; Zheng, X.; Jiang, X.-L.; Chan, B.P.L. Rediscovery of the sun bear (*Helarctos malayanus*) in Yingjiang County, Yunnan Province, China. *Zool. Res.* 2017, *38*, 206–207. [CrossRef]

18. Garshelis, D.L.; Dharaiya, N.A.; Sharp, T.R.; Steinmetz, R.; Wangdi, Y.; Wangchuk, S. *Detecting and Monitoring Sloth Bears at the Northern Edge of Their Range: Status of the Transboundary Population Linking Northeastern India to Bhutan*; Final Report to International Association for Bear Research and Management; 2015; p. 19.

19. Ngoprasert, D.; Steinmetz, R. Differentiating Asiatic black bears and sun bears from camera-trap photographs. *Internat. Bear News* 2012, *21*, 18–19.

20. Sharp, T.; Dharaiya, N.A.; Garshelis, D.L. Differentiating sloth bears and Asiatic black bears in camera-trap photos. *Internat. Bear News* 2016, *25*, 10–12.

21. Sathyakumar, S.; Kaul, R.; Ashraf, N.V.K.; Moorkerjee, A.; Menon, V. *National Bear Conservation and Welfare Action Plan 2012*; Ministry of Environment and Forests: New Delhi, India; Wildlife Institute of India: Dehradun, India; Wildlife Trust of India: Noida, India, 2012; p. 359. Available online: https://www.wti.org.in/ (accessed on 22 July 2022).

22. Talukdar, N.R.; Choudhury, P.; Barbhuiya, R.A.; Ahmad, F.; Daolagupu, D.;

Baishya, J.B. Mammals of Northeastern India: An updated checklist. *J. Threat. Taxa* 2021, *13*, 18059–18098. [CrossRef]

23. Choudhury, A. (The Rhino Foundation for nature in North East India, Guwahati, Assam, India). Personal communication, 2018.

24. Das, P.K.; Ghosh, R.K.; Chakraborty, T.K.; Bhattacharya, T.; Ghosh, M.K. Mammalia. In *Fauna of Meghalaya, Part 1*; Zoological Survey of India: Calcutta, India, 1995; pp. 23–128.

25. Lyngdoh, A.W.; Kumara, H.N.; Karunakaran, P.V.; Babu, S. A review on status of mammals in Meghalaya, India. *J. Threat. Taxa* 2019, *11*, 14955–14970. [CrossRef]

26. Chaudhary, H. (Chief Wildlife Warden of Meghalaya, Shillong, Meghalaya, India). Personal communication. 2017.

27. Guhathakurta, P.; Bhagwat, P.P.; Satpute, U.S.; Menon, P.; Prasad, A.K.; Sable, S.T.; Advani, S.C. *Observed Rainfall Variability and Changes over Meghalaya State*; India Meteorological Department, Ministry of Earth Sciences: Pune, India, 2020; p. 29.

28. ISFR. *India State of Forest Report 2021*; Forest Survey of India: Dehradun, India, 2021; p. 586.

29. Khan, M.L.; Menon, S.; Bawa, K.S. Effectiveness of the protected area network in biodiversity conservation: A case-study of Meghalaya state. *Biodivers. Conserv.* 1997, *6*, 853–868. [CrossRef]

30. Mao, A.; Hynniewta, T.; Sanjappa, M. Plant Wealth of Northeast India with reference to ethnobotany. *Indian J. Tradit. Knowl.* 2009, *8*, 96–103.

31. Goswami, R. Forest Cover, Hunting and Animal Abundances across State and Community Forests of Meghalaya, India. Ph.D. Thesis, Manipal University, Manipal, India, 2015.

32. Jeeva, S.; Mishra, B.; Venugopal, N.; Kharlukhi, L.; Laloo, R. Traditional knowledge and biodiversity conservation in the sacred groves of Meghalaya.

Indian J. Tradit. Knowl.* 2006, *5*, 563–568.

33. Kharkongor, B.M.; Tiwari, B.K. Sacred groves of Meghalaya: A review. *Internat. J. Sci. Res.* 2015, *6*, 346–349.

34. Jeeva, S.R.D.N.; Laloo, R.; Mishra, B. Traditional agricultural practices in Meghalaya, North East India. *Indian J. Tradit. Knowl.* 2006, *5*, 7–18.

35. Aiyadurai, A.; Singh, N.J.; Milner-Gulland, E.J. Wildlife hunting by indigenous tribes: A case study from Arunachal Pradesh,north-east India. *Oryx* 2010, *44*, 564–572. [CrossRef]

36. Aiyadurai, A. Wildlife hunting and conservation in northeast India: A need for an interdisciplinary understanding. *Internat. J.Gall. Conserv.* 2011, *2*, 61–73.

37. Sangma, S.S.N. *Management Plan of Balpakram National Park for 2017-18 to 2021-22*; Balpakram National Park: Baghmara, SouthGaro Hills, Meghalaya, India, 2017.

38. Kumar, A.; Marcot, B.G.; Saxena, A. Tree species diversity and distribution patterns in tropical forests of Garo Hills. *Curr. Sci.* 2006, *91*, 1370–1381.

39. Permanent Delegation of India to UNESCO. *Garo Hills Conservation Area (GHCA)*; UNESCO World Heritage Centre: Paris, France, 2018. Available online: https://whc.unesco.org/en/tentativelists/6356/ (accessed on 30 June 2022).

40. Kumar, A.; Marcot, B.G. Key Tiger habitats in the Garo Hills of Meghalaya. *J. Chemo Biosph.* 2010, *1*, 90–98.

41. Garshelis, D.L.; Joshi, A.R.; Smith, J.L.D. Estimating density and relative abundance of sloth bears. *Ursus* 1999, *11*, 87–98.

42. Steinmetz, R.; Garshelis, D.L. Distinguishing Asiatic black bears and sun bears by claw marks on climbed trees. *J. Wildl. Manag.* 2008, *72*, 814–821. [CrossRef]

43. Steinmetz, R.; Garshelis, D.L. Estimating ages of bear claw marks in Southeast Asian tropical forests as an aid to population monitoring. *Ursus* 2010, *21*, 143–153. [CrossRef]

44. Kakati, K.; Srikant, S. First Camera-trap record of small-toothed palm civet *Arctogalida trivirgata* from India. *Small Carniv. Conserv.* 2014, *50*, 50–53.

45. Kakati, K.; Srikant, S.; Momin, H.G.; Magne, F.; Sangma, P.; Sondhi, S.; Naniwadekar, R.; Borah, J.; Smith, D. Records of ferretbadgers *Melogale* from the states of Meghalaya and Arunachal Pradesh, India. *Small Carniv. Conserv.* 2014, *51*, 4–10.

46. Kakati, K. (USAID, Kathmandu, Nepal); Srikant, S. (Tejpur, Assam, India). Personal communication, 2018–2022.

47. Momin, K.C.; Suresh, C.P.; Momin, B.C.; Singh, Y.S.; Singh, S.K. An ethno-botanical study of wild plants in Garo Hills region of Meghalaya and their usage. *Internat. J. Minor Fruits Med. Aromat. Plants* 2016, *2*, 47–53.

48. Islam, M.S.; Sarwar, A.K.M.G. The Garo tribe's ethnobotanical knowledge about medicinal plants. *Jordan J. Nat. Hist.* 2020, *7*, 93–107.

49. Kumar, A.; Marcot, B.G.; Patel, R. *Wildlife Conservation in Fragmented Tropical Forests. A Case of South Garo Hills, Meghalaya, North East India*; LAP Lambert Academic Publishing: Balti, Moldova, 2017; p. 154.

50. Sethy, J.; Chauhan, N.P.S. Dietary preference of Malayan sun bear *Helarctos malayanus* in Namdapha Tiger Reserve, Arunachal Pradesh, India. *Wildl. Biol.* 2018, *2018*, 1–10. [CrossRef]

51. Ramesh, T.; Sankar, K.; Qureshi, Q. Additional notes on the diet of sloth bear *Melursus ursinus* in Mudumalai Tiger Reserve as shown by scat analysis. *J. Bombay Nat. Hist. Soc.* 2009, *106*, 204–206.

52. Sethy, J.; Chauhan, N.S. Human-sun bears conflict in Mizoram, North East India: Impact and conservation management. *Internat.J. Conserv. Sci.* 2013, *4*, 317–328.

53. Wong, W.-M.; Leader-Williams, N.; Linkie, M. Managing human-sun bear conflict in Sumatran agroforest systems. *Hum. Ecol.* 2015, *43*, 255–266. [CrossRef]

54. Fredriksson, G.M.; Wich, S.A. Trisno Frugivory in sun bears (*Helarctos*

malayanus) is linked to El Niño-related fluctuations in fruiting phenology, East Kalimantan, Indonesia. *Biol. J. Linn. Soc.* 2006, *89*, 489–508. [CrossRef]

55. Clark, D.A.; Brook, R.; Oliphant-Reskanski, C.; Laforge, M.P.; Olson, K.; Rivet, D. Novel range overlap of three ursids in the Canadian subarctic. *Arct. Sci.* 2019, *5*, 62–70. [CrossRef]

56. Clark, D.; Barnas, A.F.; Brook, R.K.; Ellis-Felege, S.N.; Fishback, L.-A.; Higdon, J.W.; Manning, K.; Rivet, D.; Roth, J.D.; Trim, V.; et al. The state of knowledge about grizzly bears (Kakenokuskwe osow Muskwa (Cree), *Ursus arctos*) in northern Manitoba. *Arctic* 2022, *75*, 105–120. [CrossRef]

57. Madhusudan, M.D. *Wildlife Distribution and Hunting South Garo Hills*. Rufford Small Grants Foundation Final Report. 2005, p. 29. Available online: https://apply.ruffordsmallgrants.org/ (accessed on 22 July 2022).

58. Seidensticker, J.; Yoganand, K.; Johnsingh, A.J.T. Sloth bears living in seasonally dry tropical and moist broadleaf forests and their conservation. In *The Ecology and Conservation of Seasonally Dry Forests in Asia*; McShea, W.J., Davies, S., Bhumpakphan, N., Eds.; Smithsonian Institution Scholarly Press: Washington, DC, USA, 2011; pp. 217–236.

59. Garshelis, D.L. Understanding species–habitat associations: A case study with the world's bears. *Land* 2022, *11*, 180. [CrossRef]

60. Sarania, B.; Guttal, V.; Tamma, K. The absence of alternative stable states in vegetation cover of northeastern India. *R. Soc. Open Sci.* 2022, *9*, 211778. [CrossRef] [PubMed]

61. Laurie, A.; Seidensticker, J. Behavioural ecology of the sloth bear (*Melursus ursinus*). *J. Zool.* 1977, *182*, 187–204. [CrossRef]

62. Joshi, A.R.; Garshelis, D.L.; Smith, J.L.D. Seasonal and habitat-related diets of sloth bears in Nepal. *J. Mammal.* 1997, *78*, 584–597. [CrossRef]

63. Rajpurohit, K.S.; Krausman, P.R. Human-sloth bear conflicts in Madhya Pradesh, India. *Wildl. Soc. Bull.* 2000, *28*, 393–399.

64. Meijaard, E.; Groves, C.P. The geography of mammals and rivers in mainland Southeast Asia. In *Primate Biogeography: Progressand Prospects*; Lehman, S.M., Fleagle, J.G., Eds.; Springer: New York, NY, USA, 2006; pp. 305–330, ISBN 978-0-387-29871-9.

65. Grimaud, J.; Grall, C.; Goodbred, S.; Steckler, M.S.; Sincavage, R.; Pickering, J.L.; Paola, C.; Seeber, L.; Hossain, M.S. Flexural deformation controls on Late Quaternary sediment dispersal in the Garo—Rajmahal gap, NW Bengal Basin. *Basin Res.* 2020, *32*, 1242–1260. [CrossRef]

66. Hora, S.L. Hora's Satpura hypothesis: An aspect of Indian biogeography. *Curr. Sci.* 1950, *19*, 364–370.

67. Hora, S.L. The Satpura hypothesis. *Sci. Prog.* 1953, *41*, 245–255.

68. Moore, J.C. Squirrel geography of the Indian subregion. *Syst. Zool.* 1960, *9*, 1–17. [CrossRef]

69. Meijaard, E. Discussion on two Bornean species of bear. *Internat. Bear News* 1996, *5*, 7–8.

70. Crudge, B.; Wilkinson, N.M.; Do, V.T.; Cao, T.D.; Cao, T.T.; Weegenaar, A.; Hunt, M. *Status and Distribution of Bears in Vietnam, 2016.*; Technical Report; Free the Bears/Animals Asia: Vietnam, 2016; p.44. Available online: https://cdn.shopify.com/s/files/1/0015/8135/0959/files/Thuc-trang--Su-phan-bo-cua-loai-Gau-o-Viet-Nam_2016-Status--Distribution-of-Bears-in-Vietnam_Tieng-Viet.pdf?16577557392472449834 (accessed on 22 July 2022).

71. Lozier, J.D.; Aniello, P.; Hickerson, M.J. Predicting the distribution of Sasquatch in western North America: Anything goes with ecological niche modelling. *J. Biogeog.* 2009, *36*, 1623–1627. [CrossRef]

72. Lan, T.; Gill, S.; Bellemain, E.; Bischof, R.; Nawaz, M.A.; Lindqvist, C. Evolutionary history of enigmatic bears in the Tibetan Plateau–Himalaya region and the identity of the yeti. *Proc. R. Soc. B* 2017, *284*, 20171804. [CrossRef]

73. Taylor-Ide, D.C. *Something Hidden Behind the Ranges. A Himalayan Quest*; Mercury House: San Francisco, CA, USA, 1995; ISBN 56279-073-0.

74. Encyclopedia of Cryptozoology. Available online: https://cryptidarchives.fandom.com/wiki/Nepalese_tree_bear (accessed on 17 July 2022).

75. Bista, M.; Panthi, S.; Weiskopf, S.R. Habitat overlap between Asiatic black bear *Ursus thibetanus* and red panda *Ailurus fulgens* in Himalaya. *PLoS ONE* 2018, *13*, e0203697. [CrossRef]

76. Chutipong, W.; Lynam, A.J.; Steinmetz, R.; Savini, T.; Gale, G.A. Sampling mammalian carnivores in western Thailand: Issues of rarity and detectability. *Raffles Bull. Zool.* 2014, *62*, 521–535.

77. Cremonesi, G.; Bisi, F.; Gaffi, L.; Zaw, T.; Naing, H.; Moe, K.; Aung, Z.; Mazzamuto, M.V.; Gagliardi, A.; Wauters, L.A.; et al. Camera trapping to assess status and composition of mammal communities in a biodiversity hotspot in Myanmar. *Animals* 2021, *11*, 880. [CrossRef] [PubMed]

78. Rasphone, A.; Kéry, M.; Kamler, J.F.; Macdonald, D.W. Documenting the demise of tiger and leopard, and the status of other carnivores and prey, in Lao PDR's most prized protected area: Nam Et—Phou Louey. *Glob. Ecol. Conserv.* 2019, *20*, e00766. [CrossRef]

79. Lahkar, D.; Ahmed, M.F.; Begum, R.H.; Das, S.K.; Lahkar, B.P.; Sarma, H.K.; Harihar, A. Camera-trapping survey to assess diversity, distribution and photographic capture rate of terrestrial mammals in the aftermath of the ethnopolitical conflict in Manas National Park, Assam, India. *J. Threat. Taxa* 2018, *10*, 12008. [CrossRef]

80. Borah, J.; Wangchuk, D.; Swargowari, A.; Wangchuk, T.; Sharma, T.; Rabha, N.; Basumatari, A.; Kakati, N.; Ahmed, M.F.; Sharma, A.; et al. *Tigers in the Indo-Bhutan Transboundary Manas Conservation Complex*. Technical Report; Manas National Park, Royal Manas National Park, WWF-India, Aaranyak, ATREE, UWICE and Bhutan Foundation. 2012, p. 27. Available online:

https://www.researchgate.net/publication/346678995_TIGERS_ACROSS_BORDERS_Tigers_in_the_IndoBhutan_Transboundary_Manas_Conservation_Complex_TMCC (accessed on 22 July 2022).

81. Lama, S.T.; Ross, J.G.; Bista, D.; Sherpa, A.P.; Regmi, G.R.; Suwal, M.K.; Sherpa, P.; Weerman, J.; Shrestha Lama, S.; Thapa, M.; et al. First photographic record of marbled cat *Pardofelis marmorata* Martin, 1837 (Mammalia, Carnivora, Felidae) in Nepal. *Nat. Conserv.* 2019, *32*, 19–34. [CrossRef]

82. Khatonier, P.; Lyngdoh, A.W. First record of spotted linsang *Prionodon pardicolor* (Mammalia: Carnivora: Prionodontidae) with photographic evidence in Meghalaya, India. *J. Threat. Taxa* 2021, *13*, 19649–19651. [CrossRef]

83. Borah, P.; Deka, J.R.; Ahamad, M.; Sharma, R.; Badola, R.; Hussain, S.A. First photographic evidence of Asiatic black bear *Ursus thibetanus* in Kaziranga Tiger Reserve, India. *J. Threat. Taxa* 2022, *14*, 20677–20679. [CrossRef]

84. Kakati, K. First Camera trap record of the Malayan sun bear from Assam, Northeast India. *Internat. Bear News* 2012, *21*, 50–51.

85. Zhou, Z.; Huang, Z.; Hu, Y.; Li, J.; Wu, J.; Hu, H. New record of sun bear (*Helarctos malayanus*) in Jilong County, Tibet, China. *Acta Theriol. Sin.* 2017, *37*, 414–416.

86. Bai, D.; Chen, Y.; Li, J.; Tao, Q.; Wang, L.; Piao, Y.; Shi, K. Mammal diversity in Shangyong Nature Reserve, Xishuangbanna,Yunnan Province. *Biodivers. Sci.* 2018, *26*, 75–78. [CrossRef]

87. Deraniyagala, P.E.P. The sun bear of Ceylon. *Spolia Zeylanica. Bull. Nat. Mus. Ceylon* 1955, *29*, 289–290.

88. Hodgson, B.H. On the Mammalia of Nepal. *J. Asiat. Soc. Bengal* 1832, *1*, 335–349.

89. Hodgson, B.H. *Catalogue of the Specimens and Drawings of Mammalia and Birds of Nepal and Thibet*; Order of the Trustees: London,UK, 1846.

90. Lydekker, R. On the occurrence of the Bruang in the Tibetan Province. *Proc. Zool.*

Soc. Lond. 1906, *21*, 997–999.

91. Lydekker, R. *The Game Animals of India, Burma, Malaya, and Tibet*; Rowland Ward, Ltd.: London, UK, 1907.

92. Jerdon, T.C. *The Mammals of India; A Natural History of All the Animals Known to Inhabit Continental India*; Thomason College Press:Roorkee, India, 1867.

93. Blyth, E. *Catalogue of the Mammalia in the Museum Asiatic Society*; Savielle & Cranenburgh: Calcutta, India, 1863.

94. Sterndale, R. *Natural History of the Mammalia of India and Ceylon*; Thacker, Spink & Co.: Calcutta, India, 1884.

95. Blanford, W.T. *The Fauna of British India Including Ceylon and Burma: Mammalia*; Taylor and Francis: London, UK, 1888.

96. Sclater, W.L. *Catalogue of Mammalia in the Indian Museum, Calcutta. Part II*; Government of India: Calcutta, India, 1891.

97. Hinton, M.A.C.; Lindsay, H.M. Report no. 41, Assam and Mishmi Hills. Bombay Natural History Society's Mammal Survey of India, Burma and Ceylon. *J. Bombay Nat. Hist. Soc.* 1926, *31*, 383–403.

98. Finn, F. *Sterndale's Mammalia of India*; Thacker, Spink & Co.: Calcutta and Simla, India, 1929.

99. Higgins, J.C. The Malay bear. *J. Bombay Nat. Hist. Soc.* 1932, *35*, 673–674.

100. Gee, E.P. A Note on the Occurrence of the Malayan sun bear *Helacrtos malayanus* (Raffles) within Indian limits. *J. Bombay Nat. Hist. Soc.* 1967, *64*, 352–354.

101. Choudhury, A.; Budnah, C.; Kumar, S. Bear Conservation Action Plan Meghalaya. In *National Bear Conservation and Welfare Action Plan 2012*; Sathyakumar, S., Kaul, R., Ashraf, N.V.K., Moorkerjee, A., Menon, V., Eds.; Ministry of Environment and Forests: New Delhi, India; Wildlife Institute of India: Dehradun, India; Wildlife Trust of India: Noida, India, 2012; pp. 223–232. Available online: https://fdocuments.net/document/national-bear-conservation-action-plan-for-

india.html?page=2 (accessed on 22 July 2022).

102. Choudhury, A. Recent killing of a sloth bear *Melursus ursinus* in a new locality in Assam. *Internat. Bear News* 2017, *26*, 20–21.

103. Rodríguez, J.P.; Sucre, B.; Mileham, K.; Sánchez-Mercado, A.; De Andrade, N.; Bezeng Bezeng, S.; Croukamp, C.; Falcato, J.; García-Borboroglu, P.; González, S.; et al. Addressing the *Biodiversity Paradox*: Mismatch between the Co-occurrence of biological diversity and the human, financial and institutional resources to address its decline. *Diversity* 2022, *14*, 708. [CrossRef]

104. Garshelis, D.L.; Pigeon, K.; Hwang, M.; Proctor, M.; McShea, W.J.; Fuller, A.K.; Morin, D.J. The need to step-up monitoring of Asian bears. *Glob. Ecol. Conserv.* 2022, *35*, e02087. [CrossRef]

105. Proctor, M.F.; Garshelis, D.L.; Thatte, P.; Steinmetz, R.; Crudge, B.; McLellan, B.N.; McShea, W.J.; Ngoprasert, D.; Nawaz, M.A.; TeWong, S.; et al. Review of field methods for monitoring Asian bears. *Glob. Ecol. Conserv.* 2022, *35*, e02080. [CrossRef]

106. Mattson, D.J.; Herrero, S.; Merrill, T. Are black bears a factor in the restoration of North American grizzly bear populations? *Ursus* 2005, *16*, 11–30. [CrossRef]

107. Talukdar, N.R.; Choudhury, P. Conserving wildlife wealth of Patharia Hills Reserve Forest, Assam, India: A critical analysis. *Glob. Ecol. Conserv.* 2017, *10*, 126–138. [CrossRef]

108. Brian Crudge, Free the Bears, Luang Prabang, Laos. Personal communication. 2022.

109. Karanth, K.U.; Nichols, J.D. *Ecological Status and Conservation of Tigers in India. Final Technical Report (February 1995 to January2000)*; Centre for Wildlife Studies: Bangalore, India, 2000.

Art – 282. TUBERCULOSIS IN FREE-RANGING AND CAPTIVE WILD ANIMALS: PATHOLOGICAL AND MOLECULAR DIAGNOSIS WITH HISTOMORPHOLOGICAL DIFFERENTIATION OF GRANULOMATOUS LESIONS

Megha Sharma, M. Karikalan, P. Dandapat, M. Asok Kumar, V. Beena, S. Chandra Mohan, **S. Ilayaraja**, Arvind Mathur, Abhijit Bhawal, A.M. Pawde and A.K.Sharma

Abstract

Tuberculosis (TB) is a serious zoonotic threat, impacting the human-livestock-wildlife interface globally. Here, we evaluated the status and histomorphological differentiation of TB lesions in 89 morbid cases of wild animals (bovids, cervids, carnivores, non-human primates, and pachyderms) in India. Histomorphological and molecular studies were done using Ziehl-Neelsen (ZN) staining, immunohistochemistry, and polymerase chain reaction (PCR), whereas cultural isolation was performed on selected samples. A total of 32 (35.95%) cases were confirmed as TB, comprising of 12 carnivores, 09 bovids, 06 cervids, 04 non-human primates, and a pachyderm. The TB lesions in the lungs, liver, and lymph nodes varied from the large-sized caseous nodules filled with dry cheesy material in bovids and cervids to variable-sized cavitations containing liquefied caseum in carnivores' lungs. The lungs, livers, and spleens of non-human primates exhibited small to medium-sized nodules. Histologically, lesions were divided into four categories (Types I, II, III, and IV) based on the extent of necrosis, the presence of mineralization, giant cells, and fibrous encapsulation. Extensive caseous necrosis with calcification, abundant giant cells, and thick fibroblastic encapsulation were consistent findings in the lungs, livers, and lymph nodes of bovids and cervids, whereas airway impaction with cellular exudate containing a teeming number of acid-fast bacilli and, at times, alveolar rupture leading to cavity formation was present in the lungs of carnivores. Absence of calcification and fibrous encapsulation was recorded in lungs of non-human primates. Immuno histochemical labelling with anti-early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) antibodies showed mild, moderate, and intense positive reactions in type II and III, type I, and type IV granulomatous lesions, respectively. Molecular detection by PCR revealed *Mycobacterium tuberculosis* (12 carnivores, 02 non-human primates

and 01 pachyderm), *M. bovis* (02 cervids and 01 bovid) and *M. orygis* (02 cervidsand 01 bovid). Cultural isolation confirmed *M. tuberculosis* in 03 carnivores and *M. orygis* in 02 cervids and 01 bovid. Our findings imply that TB is quite prevalent in the wildlife of India and there are considerable differences in the histomorphological lesions induced by distinct Mycobacterium species in different wild animals. The circulation of TB organisms in wild animals warrants a strict surveillance programme to identify the carrier status of these animals so that effective TB control strategies can be formulated to prevent spillover and spillback incidences at the human-livestock- wildlife interface.

Introduction

Tuberculosis (TB), a worldwide problem at the wildlife-livestock human interface, is caused by the genus Mycobacteria, which comprises non-sporulating, non-motile, acid-fast bacilli of 1–4 μm length and 0.3–0.6 μm width [1]. More than 150 species, which are sometimes grouped as Mycobacterium tuberculosis complex (MTBC) and Mycobacteria other than MTBC (MOTT), come under the genus Mycobacterium [2]. MTBC comprises genetically similar pathogens that cause TB in mammals [3] and are broadly classified into human and animal-adapted strains. *M. tuberculosis* and *M. africanum* are the most common human pathogens, with no known animal reservoirs [2]. The animal-adapted strains include M. microti [4], the "Dassie bacillus" [5,6], M. bovis [7], M. caprae [8], M. pinnipedii [9], M. mungi [10], and M. orygis [11]. M. bovis, a globally distributed bacilli and the main cause of bovine TB, also infects humans, domestic animal species, and wildlife [12]. M. tuberculosis is primarily a human pathogen and is isolated only from domestic and wildlife species that have lived in close proximity to humans for an extended period of time [13]. In recent years, M. orygis has emerged as a cause of zoonotic TB, especially in South Asia [14]. Non-tuberculous mycobacteria (NTM) such as M. smegmatis and M. fortuitum, which were once thought to be saprophytes, have since been discovered to cause TB in blue bulls (Boselaphus tragocamelus) and spotted deer (Axis axis) [15,16]. Wildlife species have been identified as potential reservoirs of M. bovis for livestock and humans, posing a threat to the national control and eradication programmes going on in many countries [17]. The host species and mycobacterium implicated can affect the histomorphological aspects of TB lesions in different wild animal species [18]. Comprehensive investigations including various captive and free-ranging wild animals in

India to elucidate the species of mycobacterium involved in wildlife TB are still absent, and the disease is mostly reported as spontaneous cases. Here we studied the status of TB in different wild animal species of various Indian states with the evaluation of differences in histomorphological lesions in various wild animal species.

1. Materials and methods

1.1. Samples used for the study

The Centre for Wildlife Conservation, Management, and Disease Surveillance at the Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India, examined a total of 89 TB suspected morbid cases between the years of 2018-20 based on history, gross, and histological findings of various species of free-ranging and captive wild animals (12 bovids, 16 cervids, 44 carnivores, 07 non-human primates, and 10 pachyderms). Among 89 total cases examined, 19 were archival cases from the Centre for Wildlife Conservation, Management and Disease Surveillance, IVRI, and tissue samples from 70 cases were collected and received during the study period. The scope of the sampling comprises 23 distinct zoological and national parks, safaris, state forest agencies, and other locations across 12 Indian states. (Table 1 and Fig. 1).

Fig. 1. The geographical distribution of different zoological and national parks, safaris, and state forest departments in India from where the morbid samples were collected/received. The stars (★) show the distribution in 12 states of India.

Table 1: Details of the source and habitat of the wild animals included in the study.

S. No.	Source	Habitat	No. of cases
1	Agra Bear Rescue Centre, Uttar Pradesh	Captive	15
2	Bandhavgarh Tiger Reserve, Umaria, Madhya Pradesh	Free ranging	2
3	Deer Park, IVRI, Bareilly, Uttar Pradesh	Captive	2
4	Dudhwa Tiger Reserve, Palia Kheri, Uttar Pradesh	Free ranging	1
5	Etawah Lion Safari, Uttar Pradesh	Free ranging	1
6	Haridwar Forest Division, Uttarakhand	Free ranging	[illegible]
7	Jaipur Zoological Park, Rajasthan	Captive	1
8	Jim Corbett National Park, Ramnagar, Uttarakhand	Free ranging	11
9	Kanpur Zoological Park, Uttar Pradesh	Captive	[illegible]
10	Kanan Pendari Zoological Garden, Bilaspur, Chhattisgarh	Captive	[illegible]
11	Mukundra hills Tiger Reserve, Kota, Rajasthan	Free ranging	1
12	MC Zoological Park, Chhatbir, Punjab	Captive	4
13	Mussoorie Forest Division, Dehradun, Uttarakhand	Free ranging	3
14	National Zoological Park, New Delhi	Captive	9
15	Panna Tiger Reserve, Madhya Pradesh	Free ranging	3
16	Pilikula Biological Park, Mangalore, Karnataka	Captive	1
17	Rajaji Tiger Reserve, Dehradun, Uttarakhand	Free ranging	2
18	Sakkarbaug Zoological Park, Junagadh, Gujarat	Captive	[illegible]
19	Sepahijala Zoological Park, Tripura	Captive	5
20	Shimla Reserve Forest Sanctuary, Himanchal Pradesh	Free ranging	1
21	Social Forestry Division, Bareilly, Uttar Pradesh	Free ranging	1
22	Social Forestry Division, Sambhal, Uttar Pradesh	Free ranging	1
23	Tata Steel Zoological Park, Jamshedpur, Jharkhand	Captive	[illegible]
	Total		89

1.2. Postmortem examination

Necropsy examination of the animals was carried out systematically, and gross lesions were recorded. The size, color, shape, consistency, distribution, and location of the gross lesions in various organs were noted and photographed. For bacterial isolation and molecular research, tissue samples of TB-suspected organs from dead cadavers were collected in 5 mL sterile cryovials. Representative tissue samples were collected in 10% neutral buffered formalin (NBF) for histopathological and immunohistochemical examinations.

1.3. Molecular diagnosis

DNA was isolated from fresh tissue samples using the DNeasy tissue kit and

QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations with appropriate modifications. Isolated DNA samples were subjected to diagnostic TB PCR amplification based on 16S rRNA and MPB70 gene segments [19] which also differentiate MTBC organisms from MOTT organisms (*M. avium* and *M. intracellulare*). All MTBC positive samples were subjected to PCR based on 12.7 kb gene fragment [20] to distinguish *M. tuberculosis* from *M. bovis*. Following that, all of TB positive samples were subjected to PCR based on the regions of difference (RD 4 and 9) [21] in order to distinguish between other MTBC members. The amplified products were confirmed to be of expected size in a 1.5% (w/v) agarose gel in 1X TAE buffer.

1.4. Histopathology

Tissue sections were prepared and stained with hematoxylin and eosin for histopathological examination following standard procedures using 10% NBF fixed tissues. The tissue reaction was histopathologically classified into four categories according to Chakrabarti et al. and Laisse et al. [24], with appropriate modifications.

Type I - Epithelioid granuloma without necrosis: This type of granuloma was characterized by the presence of irregular clusters of large foamy epithelioid macrophages which were surrounded by lymphocytes and sometimes giant cells.

Type II - Epithelioid granuloma with minimal necrosis and calcification: This type of granuloma had small central areas of caseative necrosis with or without calcification, which were surrounded by epithelioid cells, lymphocytes, and, at times, a few multinucleated giant cells and thin fibrous encapsulation.

Type III - Epithelioid granuloma with extensive necrosis and calcification: Large, multicentric granulomas with extensive caseous necrosis and multiple areas of mineralization were present in this type. Granulomas were often thickly encapsulated and surrounded by epithelioid cells, lymphocytes, and a large number of giant cells.

Type IV - Liquefied caseum/cavity formation along with acute or chronic inflammation: In this type, small to large areas of caseo- purulent exudate containing inflammatory cells such as macrophages, neutrophils, and lymphocytes, without any calcification and fibrous encapsulation were present.

1.5. Special staining

Detection of acid-fast mycobacterium and calcification in paraffin embedded tissue sections was done by Ziehl–Neelsen (ZN) and Von Kossa staining, respectively, following standard protocols [25].

1.6. Immunohistochemistry (IHC)

For immunohistochemical staining, 3–4 μm sections were cut from the tissue block and mounted on the Poly L-lysine coated slides, followed by the standard procedure described [26]. Polyclonal antibodies raised in rabbits against ESAT-6 and CFP-10 antigens (dilution 1:100) were used as primary antibodies, while a horseradish peroxidase (HRPO)-- conjugated anti-rabbit IgG antibody raised in goats (Merck-Genei™, India) at dilution 1:50 was used as secondary antibody in this study. Instead of primary antibodies, the sections were treated with PBS as a negative control (antibody control).

1.7. Bacterial isolation

Samples suspected of mycobacterium were individually homogenized after collection. The homogenized samples were mixed with an equal volume of 4% sodium hydrochloride (NaOH) and incubated at 37 °C for about 20 min [27]. This homogenate was centrifuged at 2500rpm for 20 min at room temperature. The supernatant was then dis- carded, and the sediment was neutralized with 2/3 N HCL containing bromocresol purple indicator. The sediment was resuspended with 1 mL of normal physiological saline solution. The homogenized suspension (100 μL) was inoculated in duplicate onto Lowenstein-Jensen (LJ) media slants containing pyruvate and/or glycerol. The inoculated slants were incubated at 37 °C for about 4–6 weeks for Mycobacterium growth.

2. Results

Of the total 89 cases studied, 32 (35.95%) cases were diagnosed with TB. The remaining 57 instances (64.05%) were found to have spontaneous lung affections such as verminous pneumonia (n=09), fungal granuloma (n=06), acute and chronic suppurative bronchopneumonia (n=36), and metastatic tumors (n=06). Based on PCR, histopathology, ZN staining, and IHC, 17 (53.12%) of the total 32 cases were identified as pulmonary TB and 15 (46.88%) cases as generalized TB (Table 2).

Table 2: Wild animal species found TB positive in this study.

S. No.	Species	Total no. of cases	Tuberculosis	
			Generalized	Pulmonary
A.	**BOVIDS**			
1.	Bison (*Bison bison*)	2	1	1
2.	Blue bull (*Boselaphus tragocamelus*)	6	4	1
3.	Blackbuck (*Antilope cervicapra*)	4	-	1
4.	Cape buffalo (*Syncerus caffer*)	3	-	1
5.	Chinkara (*Gazella bennettii*)	1	-	-
	Total	16	5	4
B.	**CERVIDS**			
1.	Spotted deer (*Axis axis*)	7	2	3
2.	Sambar deer (*Rusa unicolor*)	2	1	-
3.	Thamin deer (*Rucervus eldii*)	2	-	-
4.	Hog deer (*Axis porcinus*)	1	-	-
	Total	12	3	3
C.	**CARNIVORES**			
1.	Leopard (*Panthera pardus*)	20	-	-
2.	Sloth bear (*Melursus ursinus*)	15	5	7
3.	Tiger (*Panthera tigris*)	4	-	-
4.	Asiatic lion (*Panthera leo persica*)	1	-	-
5.	Fishing cat (*Prionailurus viverrinus*)	1	-	-
6.	Golden jackal (*Canis aureus*)	1	-	-
7.	Indian wolf (*Canis lupus pallipes*)	1	-	-
8.	Leopard cat (*Prionailurus bengalensis*)	1	-	-
	Total	44	5	7
D.	**NON-HUMAN PRIMATES**			
1.	Rhesus macaque (*Macaca mulatta*)	5	1	2
2.	Grey langur (*Semnopithecus entellus*)	1	1	-
3.	Pig-tailed macaque (*Macaca nemestrina*)	1	-	-
	Total	7	2	2
E.	**PACHYDERMS**			
1.	Asian Elephant (*Elephas maximus*)	9	-	1
2.	Hippopotamus (*Hippopotamus amphibius*)	1	-	-
	Total	10	-	1
	Grand total (A + B − C − D − E)	89	15	17

2.1. Molecular diagnosis

The genomic DNA isolated from tuberculous organs was subjected to PCR for the amplification of 16S rRNA and MPB70 genes, to diagnose TB. Twenty-one DNA samples (12 sloth bears, 04 spotted deer, 02 rhesus macaques, 01 bison, 01 blue bull, and 01 elephant), yielded an amplicon size of 1030 bp and 372 bp (Fig. 2A), which are specific for MTBC organisms. The duplex PCR based on the 12.7 kb gene segment was then employed on 21 earlier positive DNA samples, in which 15 samples (12 sloth bear, 02 rhesus macaques, and 01 elephant) showed an amplicon size of 262 bp, i.e., specific for *M. tuberculosis,* and 8 samples (04 spotted deer, 02 bison, 01 black buck and 01 blue bull) showed an amplicon size of 168 bp, i.e., specific for *M. bovis* (Fig. 2B). For reconfirmation of MTBC organisms, all positive DNA samples were subjected to PCR based on the presence or absence of genomic RD regions (RD 4 and RD 9), which revealed an amplicon size of 235 bp for the presence of RD 9 in 15 samples (12 sloth bears, 02 rhesus macaques, and 01 elephant), and 268 bp for the absence of RD 4 in five samples (02

Spotted deer; blue bull, bison and black buck, 01 each), whereas 03 samples (02 spotted deer and 01 bison) showed the absence of the RD 9 region (108 bp) and the presence of RD 4 region (172 bp) (Supplementary Fig. 1, 2), which indicated the absence of *M. tuberculosis* and *M. bovis*, that was later confirmed as *M. orygis* by whole genome sequencing (data not shown here).

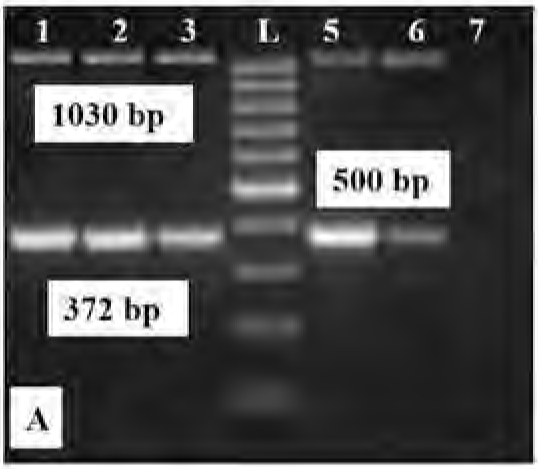


Fig. 2A. Amplification of 16S rRNA and MPB70 gene segment to differentiate MTBC organisms from NTM in tissue samples. Lane L: 100 bp ladder Lane 1–3, 6: Samples showing 372 bp and 1030 bp amplicon specific for MTBC complex Lane 5: Positive control Lane 7: Negative control.

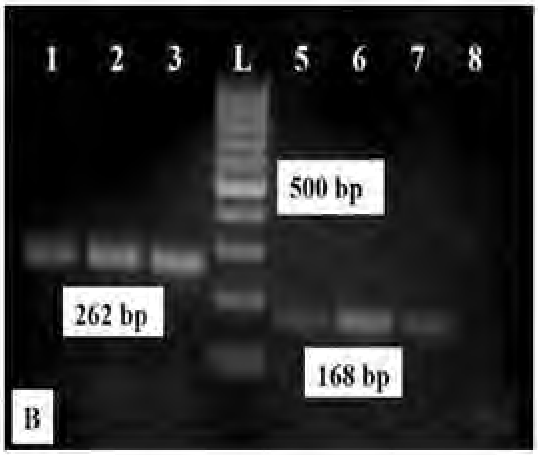


Fig. 2B. Duplex PCR for differentiating M. tuberculosis from M. bovis in tissue samples. Lane L: 100 bp ladder Lane 1,2: Samples showing 262 bp amplicon specific for M.tu berculosis Lane 3: Positive control of M. tuberculosis Lane 5,7: Samples showing16 8bp amplicon specific for M. bovis Lane 6: Positive control of M. bovis Lane 8: Negative control.

Gross pathology

Out of 16 (17.98%) bovid cases, 09 (56.25%) cases were diagnosed as TB, of which 04 (44.44%) (bison, blackbuck, blue bull, and cape buffalo, 01 each) were of pulmonary TB and 05 (55.56%) cases (01 bison and 04 blue bulls) were of generalized TB. Lung lesions in bison and blackbuck revealed diffuse, variable-sized hard nodules containing whitish-brown cheesy material that was cut with a gritty sensation. Large nodules containing pale yellowish caseous material and enclosed by thick connective tissue were found on the lungs of cape buffalo (Fig. 3A) and blue bulls. Apart from the lung involvement, the liver and mediastinal lymph nodes in three blue bulls showed large, encapsulated nodules filled with dry, inspissated, gritty textured caseous material (Fig. 3B).

A total of 12 (13.48%) cervids were screened, out of which 06 (50.00%) were diagnosed as TB, comprising of 03 (50.00%) cases of pulmonary TB (spotted deer) and 03 (50.00%) cases of generalized TB (02 spotted deer and 01 sambar deer). The lung parenchyma was studded with whitish-brown caseous nodules of varying sizes that were embedded as well as projecting over the surface (Fig. 3C). Apart from the lung parenchyma, the bronchial, mediastinal, and mesenteric lymph nodes were all enlarged and filled with caseo-necrotic material in all three cases with generalized TB. Pinpoint miliary nodules were also observed in the liver (n = 3) and kidneys (01 sambar).

Out of 44 (49.44%) sloth bears examined, 12 (27.27%) were found to be TB positive, with 07 (58.33%) cases of pulmonary TB and 05 (41.67%) cases were of generalized TB. Pulmonary lesions in sloth bears showed variable-sized nodules on the lung surface, which on dissection showed cavitary lesions containing liquefied caseum (Fig. 3D). Other organs, such as the liver, revealed miliary nodules embedded in the parenchyma in two cases, while gross abnormalities in the spleen, adrenal glands, mediastinal lymph nodes, and kidneys were apparently absent.

A total of 07 (7.86%) non-human primates were screened, of which 04 (57.14%) cases were classified as TB positive, including 02 (50.00%) instances of pulmonary TB in rhesus macaques and 02 (50.00%) cases of generalized TB in a grey langur and a rhesus macaque. Variable-sized, diffusely dispersed, soft-textured nodules were found on the lungs and thoracic pleurae (Fig. 3E). The spleen was commonly involved in generalized form (grey langur and rhesus macaque, 01 each). The spleen was considerably enlarged, with a hard texture and medium-sized pale nodules filled with caseous material (Fig. 3F).

Miliary nodules on the liver and lymph nodes were also discovered, in addition to the spleen.

One elephant (10.00%) was diagnosed with pulmonary TB out of a total of ten (11.24%) pachyderms. Multiple medium-to-large caseous nodules containing yellow-greyish cheesy material were seen in the lungs.

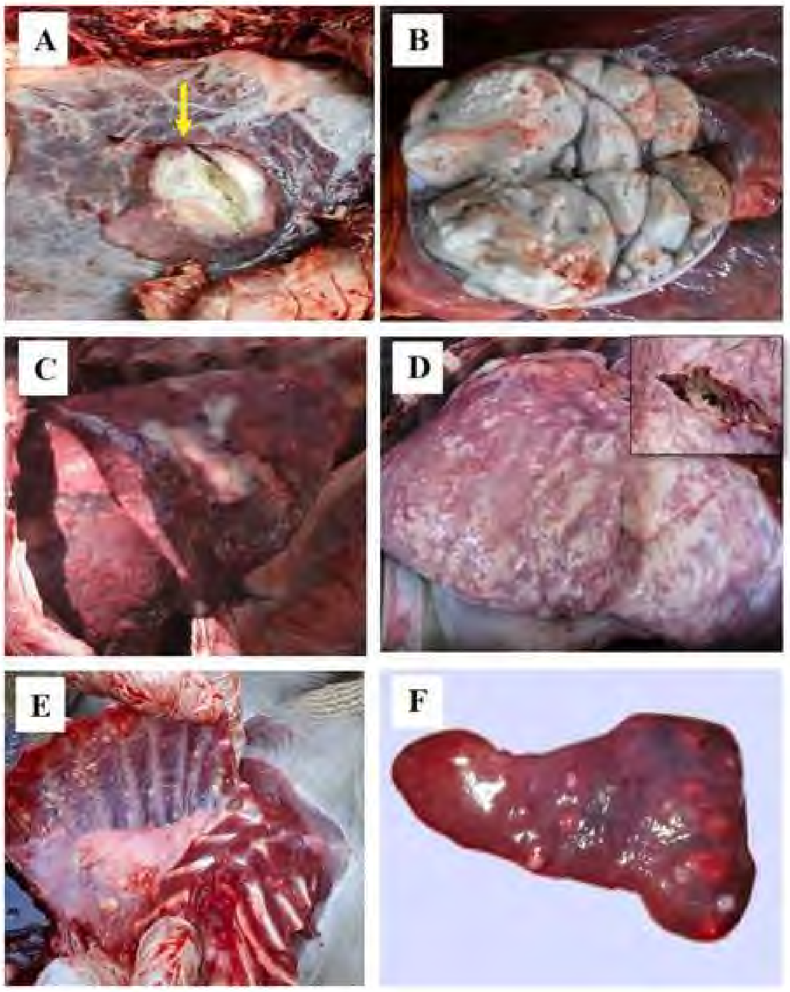


Fig. 3. Gross pathological lesions observed in different wild animals. A. Cape buffalo, lungs: large, focal, well demarcated, round nodule (arrow) filled with yellowish caseous material. B. Blue bull, mediastinal lymph node: enlarged and impacted with white inspissated caseous material. C. Spotted deer, lungs: non-uniform, multifocal to coalescing pale-yellowish nodules embedded in the parenchyma. D. Sloth bear, lungs: widespread pale-yellowish nodules embedded in the parenchyma. Inset: cut surface showing soft nodules containing liquefied caseum. E. Rhesus macaque, lungs: multifocal to coalescing, widespread variable-sized nodules in the pleurae as well as on the lungs. F. Rhesus macaque, spleen: variable-sized multifocal to coalescing pale- white nodules distributed throughout the parenchyma.

2.2. Histomorphology and immunohistochemistry

The histopathological lesions were categorized as types I, II, III, and IV based on the presence or absence of caseous necrosis and mineralization, presence or absence of giant cells and degree of fibrosis. The comparative analysis of gross and histopathological lesions of TB in different wild animals is represented in Table 3. The tuberculous lungs of bovids primarily showed type III granulomatous lesions. It was found in eight cases (04 blue bulls, 02 bison, 01 blackbuck, and 01 cape buffalo), in which lung sections revealed large granulomas with extensive caseous necrosis and multiple calcified areas surrounded by epithelioid cells, lymphocytes, a large number of giant cells, and proliferating fibroblasts, with associated atelectatic changes and abundant acid-fast bacilli (Fig. 4A), (Supplementary Fig. 3A and 3B). The calcification was confirmed by Von Kossa staining. The immunohistochemical staining with anti-ESAT-6 and CFP-10 cocktail antibodies revealed intense to moderate brown granular positive staining inside the epithelioid and giant cells as well as in the caseum against the antibody control (Fig. 5A and B). In one blue bull, the type II granulomatous tissue reaction was seen in the lungs. Three blue bulls and a bison had type III granulomatous lesions in the mediastinal lymph nodes and liver with abundant acid-fast bacilli observed/oil field immersion (o.i.f.) (Fig. 4B, C and 4D), whereas in another blue bull, the liver showed a type II granulomatous reaction.

Multiple regions of caseation and calcification were found in the lungs of four spotted deer and one sambar deer (Type III). Calcification was confirmed by Von Kossa staining. ZN staining revealed 50–75 AFB/o.i.f. extracellularly in the necrotic mass as well as within the giant cells.

During immunohistochemistry, moderate to intense brown granular positive staining inside the caseous material as well as in the inflammatory cells of the granuloma was present (Fig. 5C and D). The lungs of one spotted deer had a type I granulomatous reaction (Fig. 4E). In sambar deer, liver sections and mediastinal lymph nodes exhibited type III and type II granulomatous reactions, respectively, whereas type I tissue reaction was present in two spotted deer (Supplementary Fig. 3C). The lung sections of eight sloth bears revealed widespread areas of caseative necrosis surrounded by epithelioid cells, lymphocytes, and a few multinucleated giant cells (Type IV) but without any fibrous tissue capsule or mineralization. The alveoli were occasionally filled with liquefied caseum containing neutrophils, macrophages, and lymphocytes, and there was alveolar rupture with caseum percolating to the adjacent alveolus. In addition, ZN staining revealed a teeming number (>100/o.i.f) of acid-fast bacilli in the exudate as well as within the macrophages (Fig. 4F). The immunohistochemical staining showed an intense brown granular reaction in the alveolar and bronchiolar exudate (Fig. 5E). Type II granulomas were also found in the lungs of the remaining four sloth bears. The liver, kidneys, spleen (three cases each), lymph nodes, and adrenal glands (two cases each) of the sloth bears indicated type I granuloma, which was formed of focal to diffuse accumulations of large, pale, foamy macrophages, sometimes surrounded by lymphocytes (Supplementary Fig. 3D). The type II granulomas were recorded in the liver sections of two and the lymph node sections of one sloth bear. The bronchial and bronchiolar lumens were filled with mucocellular debris comprising of denuded necrotic epithelium, mononuclear inflammatory cells and abundant acid-fast bacteria (Supplementary Fig. 3E and 3F).

Lung sections of 03 cases of rhesus macaques revealed type II granuloma (Fig. 4G), whereas type I granuloma with a focal concentration of macrophages without caseation was found in the lungs of one grey langur (Supplementary Fig. 3G). A type II granulomatous reaction was found in the spleen sections of three cases (01 grey langur, 02 rhesus monkey) with moderate number of acid-fast bacilli present inside the giant cells. In immunohistochemistry, positive staining reactions in the cytoplasm of giant cells as well as in the peripheral inflammatory cells surrounding caseation in the cytoplasm were observed (Fig. 5F). A type I granuloma was seen in the livers, kidneys, and lymph nodes (02 cases each).

In lungs sections of the elephant, multiple airways were filled with inflammatory exudate with large areas of caseous necrotic mass comprising of epithelioid cells, lymphocytes, and a few cells attempting to form giant cells were seen (Fig. 4H), (Supplementary Fig. 3H). ZN staining revealed abundant acid-fast bacilli in the airways. The immunohistochemical staining showed moderate to intense brownish coloration in the cellular exudate.

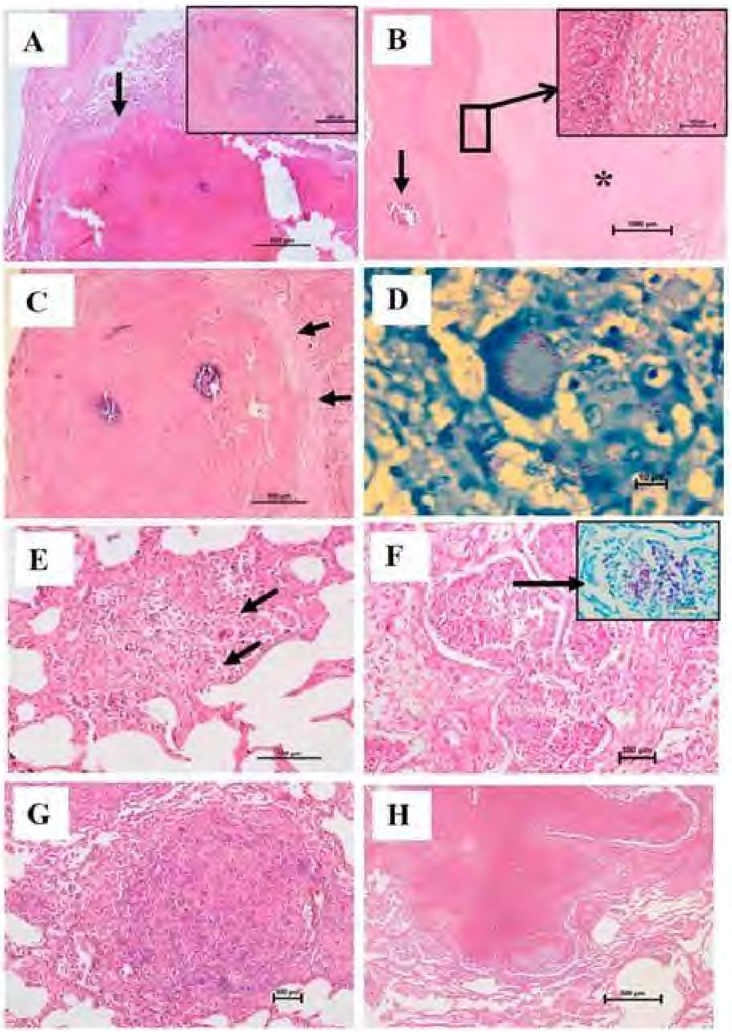


Fig. 4. Histopathological characterization of TB granuloma in different wild animals. A. Blue bull, lung: encapsulated (arrow) caseous granuloma with the foci of calcification and associated atelectatic changes. H&E 4X. Inset: periphery of granuloma showing abundant giant cells and fibroblasts. H&E 4X B. Blue bull, mediastinal lymph node: large areas of caseation with foci of calcification (arrow) sur- rounded by thick fibrous capsule (*). H&E 2X. Inset: higher magnification of the periphery of granuloma showing mononuclear cells and giant cells. H&E 20X. C. Blue bull, liver: caseo-calcifried granuloma with thick connective tissue encapsulation (arrows) and associated hepatocytes degeneration. H&E 4X. D. Blue bull, liver: periphery of the caseous granuloma showing numerous acid-fast bacilli inside giant cell as well as in the macrophages. ZN 100X. E. Spotted deer, lung: tiny granuloma composed of focal accumulation of epithelioid macrophages and giant cells (arrows) in the interstitium with associated emphysema. H&E 20X. F. Sloth bear, lung: alveoli filled with liquefied caseum and at times there was alveolar rupture with the caseum percolating to the adjacent alveolus. H&E 10X. Inset: abundant acid-fast bacilli in alveolar liquefied caseum. ZN 20X. G. Rhesus macaque, lung: caseous necrotic areas admixed with inflammatory cells in the interstitium. H&E 10X. H. Elephant, lung: multiple airways were filled with inflammatory exudate forming large areas of caseation. H&E 4X.

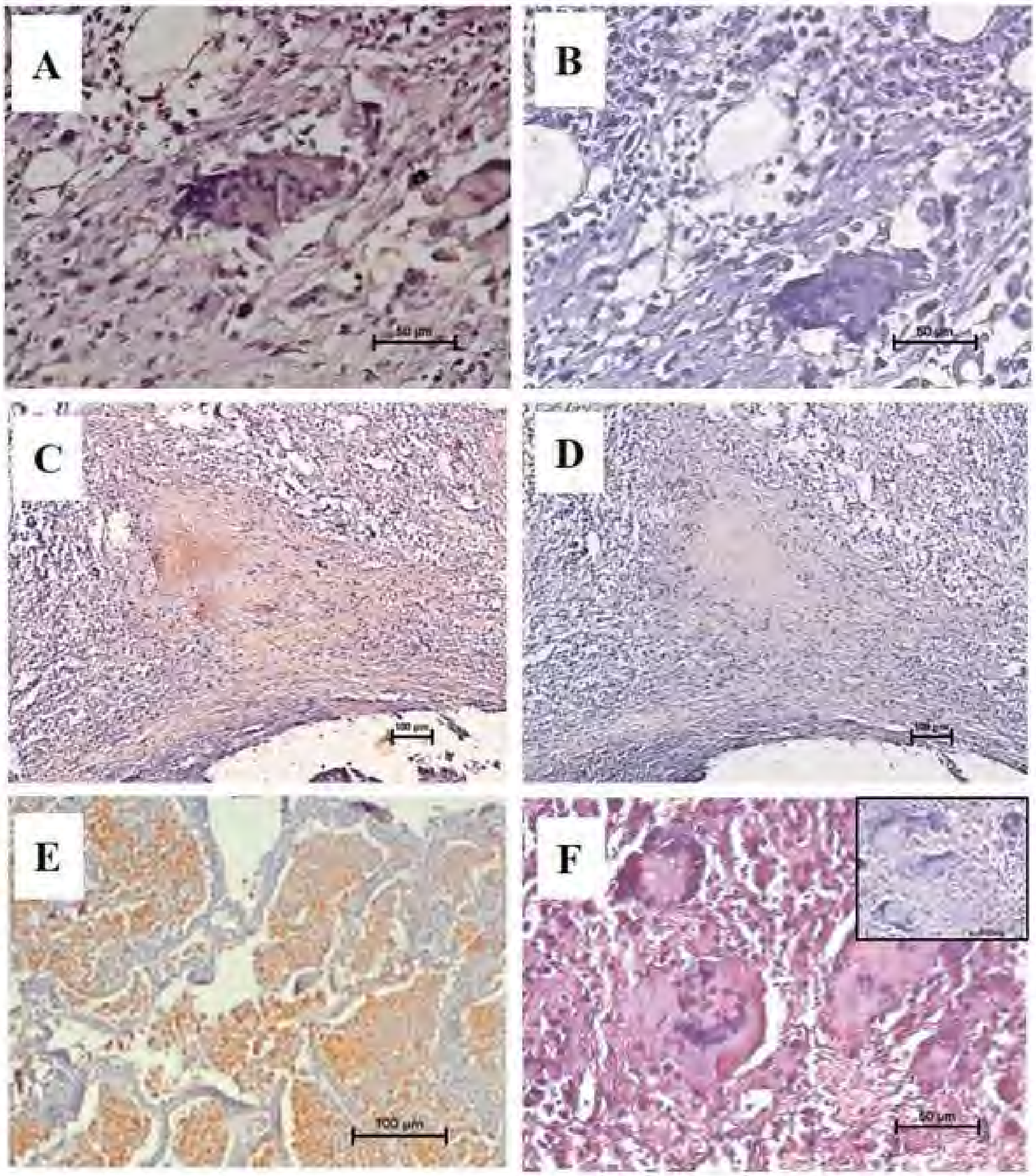


Fig. 5. Immunohistochemical labelling with anti-ESAT-6 and CFP-10 cocktail polyclonal antibodies. A. Blue bull, lung: Moderate positive signals in the giant cells and mononuclear cells, peripheral to granuloma. IHC DAB 40X. B. Blue bull, lung: antibody control. IHC DAB 40X. C. Spotted deer, lung: moderate positive reaction in the fibrocellular areas of granuloma. IHC DAB 10X. D. Spotted deer, lung: antibody control. IHC DAB 10X. E. Sloth bear, lung: Intense positive labelling in liquefied alveolar caseum. IHC DAB 20X. F. Rhesus macaque, spleen: positive signals in the center of granuloma as well as in giant cells. IHC DAB 40X. Inset: antibody control. IHC DAB 40X.

Cultural isolation from tissue samples

Tissues from the lungs and lymph nodes of 06 cases, comprising of 03 sloth bears, 02 spotted deer, and 01 bison, were cultured in duplicate in glycerol and/or sodium pyruvate, containing LJ media. Colonies of three sloth bear cultures were dry, rough, and granular in appearance, whereas colonies of two spotted deer and a bison were moist, smooth, and granular. The cultures demonstrated pure acid-fast rods in clusters by ZN staining. With the help of PCR and whole genome sequencing, we confirmed three isolates (sloth bears) as *M. tuberculosis* and three others (two spotted deer and one bison) as *M. orygis*.

Discussion

TB is an ancient zoonotic disease that affects humans, livestock, and wild animals [28]. According to WHO's global TB report 2019, under-developed and developing countries record nearly 1.4 million human deaths every year due to TB. India has the world's highest bovine population with 21.8 million TB affected cows [29], creating a major wellspring for spill-over to various hosts like humans and wild animals. In this study, we examined the status of TB in wild animals from various states of India along with the histomorphological differentiation of lesions observed in affected animals. The results showed that multiple wildlife species were affected by TB, with variation in histomorphological lesions being produced that depended predominately upon the host species affected. The gross lesions observed in the lungs of wild bovids and cervids typically resemble *M. bovis* and *M. orygis* associated lesions in domestic cattle [12,15,30–33] whereas in carnivores, variable-sized nodules with liquid material (caseum) that often develop into cavitations were noted [34]. The post-primary complex of TB in later stages led to caseation and fragmentation, thus resulting in the formation of cavities [35]. The liver and lymph nodes of bovids andcervids displayed extensive granulomatous lesions with widespread areas of caseation and calcification [31]. Infection of deer with myco- bacteria other than *M. bovis* also results in enlargement and case onecrotic lesions of lymph nodes (mediastinal, mesenteric, and hepatic) [15]. Thapa et al. [36] recorded extrapulmonary lesions in the lymph nodes, gastrointestinal tract, and liver of three spotted deer that died from *M. orygis*-caused TB. As observed in the present study, similar kinds of TB nodules were observed in the lungs, spleen, and lymph nodes of rhesus macaques [37,38]. The coalescing nodular lesions may extend into the thoracic pleura and secondarily to the liver, spleen, kidneys, and various lymph nodes [39]. Elephants affected by *M. tuberculosis* showed nodules with caseous foci, which in advanced stages may progress to cavitating lesions [40,41].

The histopathological examination of suspected TB tissue sections offers great help for TB diagnosis. The histopathological picture varies with the species of mycobacterium involved as well as with the host species affected [18]. Exessive caseous necrosis with calcification and an abundance of giant cells, as well as extensive fibrosis, were seen in the lungs of bovids and cervids [24,31,32,42,43], whereas TB granuloma in carnivores showed widespread areas of liquefied caseum mixed with neutrophils, macrophages, lymphocytes, and a few giant cells but no calcification [34]. Similar types of lesions were reported in post-primary TB in humans caused by *M. tuberculosis* [35]. A cavity was formed due to the communication of liquefied caseum with the airways, which pro- vides more oxgenated air to bacilli. Hence, an increased rate of multiplication of bacilli was observed [44]. Lung lesions with mild in- tensity were observed in some sloth bears, indicating the initial stages of granulomas [35]. Other visceral organs of sloth bears, such as the liver, kidneys, spleen, lymph nodes, and adrenal glands, had miliary lesions, indicating hematogenous seeding [23]. A wide variation in the type of granulomas was seen in the lungs of rhesus macaques. It ranged from coalescing granulomas with central necrosis to non-necrotic granulomas with epithelioid macrophages, giant cells, and lymphocytes but no calcification or fibrous connective tissue proliferation [37,38]. In non-human primates, there appears to be practically no difference in the disease manifestation caused by *M. tuberculosis* and *M. bovis*, and infection with other MTBC members is very rare [38]. Different reports have indicated that rhesus monkeys are more susceptible to TB than other monkeys [45,46]. Histopathology of lung sections of an elephant showed caseous granulomatous lesions comprised of macrophages, epithelioid cells and lymphocytes [40] however in our study, just a single case of elephant was evaluated. ESAT-6, a 6-kDa protein, and CFP-10, a 10-kDa protein, are the two immunodominant proteins most frequently used in skin patch tests, various biosensing systems, and immunohistochemistry assays for the diagnosis of TB [47]. These proteins are most recognized for producing granulomas in the early stages of infection, as well as giant cells, foamy macrophages, epithelioid macrophages, and caseous necrosis. The Mycobacteria genome contains genes aligned in pairs that code for a protein family that includes CFP-10 and ESAT-6. Their secretion is an active process involving a specific transport mechanism, which is made up of multiple flanking genes [48, 49]. Assays using combinations of these proteins are typically more helpful in the diagnosis of TB because about one-third of all TB patients are seronegative for any one antigen [50]. Immunohistochemistry against ESAT-6 antigen was found to be highly sensitive (88.6%) in humans which depicts its potential application for the diagnosis of TB [51]. The causative agent is more likely to be found in immature granulomas with a higher number of inflammatory cells than in mature caseating granulomas with fibrosis due to the degradation process in the granuloma, which is consistent with the findings of moderately intense signals in Type I lesions as opposed to mild signals in Type II and Type III lesions [52]. Previous studies also showed higher intensity of staining in cavitary lesions [53] and in the cytoplasm of macrophages and giant cells [51], which largely supported the present observations. Isolation of mycobacteria is considered the gold standard for the diagnosis of TB [54], in the present study, *M. tuberculosis* was isolated from three sloth bears, and *M. orygis* from 02 spotted deer and 01 bison.

Molecular diagnosis revealed the presence of *M. tuberculosis* in captive sloth bears, rhesus macaques, and an elephant. It might be due to the close association of these animals with infected humans, as taming them as a part of cultural, religious, and entertainment activities which is a common practice in India. Earlier reports also suggested the transmission of *M. tuberculosis* from humans to Asian elephants and a marmoset [40,55]. Moreover, non-human primates (especially macaques) share the greatest physiological similarities with humans and have been used for investigating TB pathogenesis and evaluating responses of drugs and vaccines during pre-clinical trials [56], which gives strong evidence of the transmission of TB from humans by monkeys. Various wild animal species act as maintenance hosts of *M. bovis,* making them a potential source of *M. bovis* for livestock and humans [17]. Wild animals can also be affected by spill-over from domestic livestock living in the periphery of forest reserved lands [17]. Recently, the first cases of TB caused by *M. orygis* in dairy cattle and humans were reported from the country [14,33], thus indicating a strong possibility of *M. orygis* transmission at the livestock-human-wildlife interface.

Thus, it is evident from this study that TB is highly prevalent in free-ranging and captive wildlife in India. *M. bovis* is predominant in free- ranging wild bovids and cervids with histomorphological characteristics that closely resemble those of domestic cattle irrespective of the mycobacterium species involved. Captive wild animals living in close proximity with humans are at high risk of contracting *M. tuberculosis* with lesions that closely resemble those of human TB. Sloth bears with lung cavitations and an abundance of acid-fast bacilli in the airways show high infectivity and may act as super shedders. A thorough understanding of the epidemiology and pathology of TB in various wild animals is a must for the development and implementation of effective control strategies.

Acknowledgments

This work was supported by the research fund of the Indian Council of Agricultural Research-Indian Veterinary Research Institute (ICAR- IVRI), Izatnagar, India. We gratefully acknowledge the Director, ICAR- IVRI, Izatnagar, Bareilly, Uttar Pradesh and the Central Zoo Authority of India for providing the necessary facilities to conduct the present research work. We are thankful to all the Directors and Veterinary officers of different National Parks, Zoos and Rescue centers in India from where samples were collected or received at the Centre for Wildlife, ICAR- IVRI.

References

[1] K. Sakamoto, The pathology of *Mycobacterium tuberculosis* infection, Vet. Pathol. 49 (2012) 423–439, https://doi.org/10.1177/0300985811429313.

[2] N.H. Smith, K. Kremer, J. Inwald, J. Dale, J.R. Driscoll, S.V. Gordon, D. van Soolingen, R.G. Hewinson, J.M. Smith, Ecotypes of the *Mycobacterium tuberculosis* complex, J. Theor. Biol. 239 (2006) 220–225, https://doi.org/10.1016/j. jtbi.2005.08.036.

[3] D. Brites, S. Gagneux, The nature and evolution of genomic diversity in the *Mycobacterium tuberculosis* complex, Adv. Exp. Med. Biol. 1019 (2017) 11–26, https://doi.org/10.1007/978-3-319-64371-7_1.

[4] A.Q. Wells, A.H. Robb-Smith, The Murine Type of Tubercle bacillus (The Vole Acid- Fast bacillus), MRC Special Report Series Medical Research Council, London, 1946.

[5] N. Smith, The 'dassie' bacillus, Tubercle 41 (1960) 203–212.

[6] D.V. Cousins, R.L. Peet, W.T. Gaynor, S.N. Williams, B.L. Gow, Tuberculosis in imported hyrax (*Procavia capensis*) caused by an unusual variant belonging to the *Mycobacterium tuberculosis* complex, Vet. Microbiol. 42 (1994) 135–145, https:// doi.org/10.1016/0378-1135(94)90013-2.

[7] A.G. Karlson, E.F. Lessel, *Mycobacterium bovis* nom. nov, Int. J. Syst. Bacteriol. 20 (1970) 273–282, https://doi.org/10.1099/00207713-20-3-273.

[8] A. Aranaz, E. Liebana, E. Gomez-Mampaso, J.C. Galan, D. Cousins, A. Ortega, J. Blazquez, F. Baquero, A. Mateos, G. Suarez, L. Dominguez, *Mycobacterium tuberculosis* subsp. *caprae* subsp. nov.: a taxonomic study of a new member of the *Mycobacterium tuberculosis* complex isolated from goats in Spain, Int. J. Syst. Bacteriol. 49 (1999) 1263–1273, https://doi.org/10.1099/00207713-49-3-1263.

[9] D.V. Cousins, R. Bastida, A. Cataldi, V. Quse, S. Redrobe, S. Dow, P. Duignan, A. Murray, C. Dupont, N. Ahmed, D.M. Collins, W.R. Butler, D. Dawson,

[10] D. Rodríguez, J. Loureiro, M.I. Romano, A. Alito, M. Zumarraga, A. Bernardelli, Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov, Int. J. Syst. Evol. Microbiol.53 (2003)1305–1314, https://doi.org/10.1099/ijs.0.02401-0.

[11] K.A. Alexander, P.N. Laver, A.L. Michel, et al., Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi,* Emerg, Inf. Disp. 16 (2010) 1296–1299, https://doi. org/10.3201/eid1608.100314.

[12] J. van Ingen, Z. Rahim, A. Mulder, et al., Characterization of *Mycobacterium orygis* as *M. tuberculosis* complex subspecies, Emerg. Infect. Dis. 18 (2012) 653–655, https://doi.org/10.3201/eid1804.110888.

[13] S.D. Fitzgerald, J.B. Kaneene, Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control, Vet. Pathol. 50 (2013) 488–499, https://doi.org/10.1177/0300985812467472.

[14] H.K. Prasad, A. Singhal, A. Mishra, N.P. Shah, V.M. Katoch, S. S Thakral, D.

[15]. V. Singh, S. Chumber, S. Bal, S. Aggarwal, M.V. Padma, S. Kumar, M.K. Singh, S.

[16] K. Acharya, Bovine tuberculosis in India: potential basis for zoonosis, Tuberculosis 85 (2005) 421–428, https://doi.org/10.1016/j.tube.2005.08.005.

[17]S.C. Duffy, S. Srinivasan, M.A. Schilling, T. Stuber, S.N. Danchuk, J.S. Michael, M. Venkatesan, N. Bansal, S. Maan, N. Jindal, D. Chaudhary, P. Dandapat, R. Katani, S. Chothe, M. Veerasami, S. Robbe-Austerman, N. Juleff, V. Kapur, M. A. Behr, Reconsidering *Mycobacterium bovis* as a proXy for zoonotic tuberculosis: a molecular epidemiological surveillance study, The Lancet Microbe 1 (2020) e66–e73, https://doi.org/10.1016/S2666-5247(20)30038-0.

[18]S.U. Channakeshava, R. Singh, R. Verma, K.P. Singh, A.K. Sharma, R.S. Chauhan, Association of *Mycobacterium smegmatis* with chronic granulomatous lesions in captive deer (*Axis axis*), Indian J. Vet. Pathol. 30 (2006) 59–61.

[19]R. Verma, A.K. Sharma, A. Kidangam, T. Mandal, H. Verma, Generalized tuberculosis in nilgai (*Boselaphus tragocamelus*) caused by *Mycobacterium fortuitum*, Indian J. Vet. Pathol. 36 (2012) 33–36.

[20] A.L. Michel, B. Müller, P.D. Helden, *Mycobacterium bovis* at the animal-human interface: a problem, or not, Vet. Microbiol. 140 (2010) 371–381, https://doi.org/ 10.1016/j.vetmic.2009.08.029.

[21] G.W. De Lisle, R.G. Bengis, S.M. Schmitt, D.J. O'Brien, Tuberculosis in free-ranging wildlife: detection, diagnosis and management, Rev. Sci. Tech. 21 (2002) 317–334, https://doi.org/10.20506/rst.21.2.1339.

[22] S. Wilton, D. Cousins, Detection and identification of multiple

mycobacterial pathogens by DNA amplification in a single tube, Genome Res. 1 (1992) 269–273, https://doi.org/10.1101/gr.1.4.269.

[23] C.S. Bakshi, D.H. Shah, R. Verma, R.K. Singh, M. Malik, Rapid differentiation of *Mycobacterium bovis* and *Mycobacterium tuberculosis* based on a 12.7-kb fragment by a single tube multiplex-PCR, Vet. Microbiol. 109 (2005) 211–216, https://doi.org/ 10.1016/j.vetmic.2005.05.015.

[24]R.M. Warren, N.C. Gey van Pittius, M. Barnard, A. Hesseling, E. Engelke, M. De Kock, M.C. Gutierrez, G.K. Chege, T.C. Victor, E.G. Hoal, P.D. van Helden,

[25]Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference, Int. J. Tubercul. Lung Dis. 10 (2006) 818–822.

[26]L.G. Luna, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, third ed., McGraw-Hill, New York, 1968.

[27]A.K. Chakrabarti, K. K Halder, S. Das, S. Chakrabarti, Morphological classification of tuberculous lesions: preliminary observations, Indian J. Tubercul. 41 (1994) 139.

[28] C.J. Laisse, D. Gavier-Wid´en, G. Ramis, C.G. Bila, A. Machado, J.J. Quereda, E. O. Ågren, P.D. van Helden, Characterization of tuberculous lesions in naturally infected African buffalo (*Syncerus caffer*), J. Vet. Diagn. Invest. 23 (2011) 1022–1027, https://doi.org/10.1177/1040638711416967.

[29]D. Sheehan, B. Hrapchak, Theory and Practice of Histotechnology, second ed., Battelle Press, Ohio, 1980, pp. 226–227.

[30] S. Karimi, M. Shamaei, M. Pourabdollah, M. Sadr, M. Karbasi, A. Kiani,

[31] M. Bahadori, Histopathological findings in immunohistological staining of the granulomatous tissue reaction associated with tuberculosis, J. Tubercul. Res. 2014 (2014), 858396, https://doi.org/10.1155/2014/858396.

[32]R. Cruickshank, I.P. Duguid, B.P. Maimion, R.H.A. Swain, Medical Microbiology, twelfth ed., Edward Arnold Publishers, Churchill Livingstone, Edinburgh, 1975.

[33] S. Malama, J.B. Muma, J. Godfroid, A review of tuberculosis at the wildlife- livestock-human interface in Zambia, Infect. Dis. Poverty. 2 (2013) 1–5, https:// doi.org/10.1186/2049-9957-2-13.

[34] S. Srinivasan, L. Easterling, B. Rimal, X.M. Niu, A.J. Conlan, P. Dudas, V. Kapur, Prevalence of Bovine Tuberculosis in India: a systematic review and meta-analysis, Transbound. Emerg. Dis. 65 (2018) 1627–1640, https://doi.org/10.1111/ tbed.12915.

[35]L.A. Corner, Post mortem diagnosis of *Mycobacterium bovis* infection in cattle, Vet. Microbiol. 40 (1994) 53–63, https://doi.org/10.1016/0378-1135(94)90046-9.

[36]J.B. Kaneene, R. Miller, I.N. de Kantor, C.O. Thoen, Tuberculosis in wild animals, Int. J. Tubercul. Lung Dis. 14 (2010) 1508–1512.

[37]D.G. Priya, P. Sunanda, J.H. Khorajiya, D.J. Ghodasara, B.P. Joshi, K.S. Prajapati, A case report of generalized tuberculosis in a captive Nilgai (*Boselaphus tragocamelus*), Health 13 (2014) 15–24.

[38]A.K. Refaya, N. Kumar, D. Raj, M. Veerasamy, S. Balaji, S. Shanmugam, A.Rajendran, S.P. Tripathy, S. Swaminathan, S.J. Peacock, K. Palaniyandi, Whole- genome sequencing of a *Mycobacterium orygis* strain isolated from cattle in Chennai, India, Microbiol. Resour. Announc. 8 (2019), https://doi.org/10.1128/ MRA.01080-19 e01080-19.

[39]M. Sharma, M. Karikalan, P. Dandapat, V. Beena, M.A. Kumar, A. Kumar,

[40]M. Sudhagar, A.M. Pawde, Pulmonary tuberculosis in captive sloth bear (*Melursus ursinus*) due to *Mycobacterium tuberculosis*, Indian J. Vet. Pathol. 46 (2022) 96–99, https://doi.org/10.5958/0973-970X.2022.00016.5.

[41]R.L. Hunter, J.K. Actor, S.A. Hwang, V. Karev, C. Jagannath, Pathogenesis

of post primary tuberculosis: immunity and hypersensitivity in the development of cavities, Ann. Clin. Lab. Sci. 44 (2014) 365–387.

[42]J. Thapa, C. Nakajima, B. Maharjan, A. Poudel, Y. Suzuki, Molecular characterization of *Mycobacterium orygis* isolates from wild animals of Nepal, Jpn. J. Vet. Res.63 (2015) 151–158, https://doi.org/10.14943/jjvr.63.3.151.

[43]F. Min, Y. Zhang, J. Pan, J. Wang, W. Yuan, *Mycobacterium tuberculosis* infection in rhesus monkeys (*Macaca mulatta*) and evaluation of ESAT-6 and CFP10 as immunodiagnostic antigens, EXp. Anim. 62 (2013) 281–293, https://doi.org/ 10.1538/expanim.62.281.

[44]K. Matz-Rensing, T. Hartmann, G.M. Wendel, J.S. Frick, S. Homolka, E. Richter, M. H. Munk, F.J. Kaup, Outbreak of tuberculosis in a colony of rhesus monkeys (*Macaca mulatta*) after possible indirect contact with a human TB patient, J. Comp. Pathol. 153 (2015) 81–91, https://doi.org/10.1016/j.jcpa.2015.05.006.

[45]M.A. Garcia, D.M. Bouley, M.J. Larson, B. Lifland, R. Moorhead, M.D. Simkins, D. C. Borie, R. Tolwani, G. Otto, Outbreak of *Mycobacterium bovis* in a conditioned colony of rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques, Comp. Med. 54 (2004) 578–584.

[46]A. Zachariah, J. Pandiyan, G.K. Madhavilatha, S. Mundayoor, B. Chandramohan, P.K. Sajesh, S. Santhosh, S.K. Mikota, *Mycobacterium tuberculosis* in wild asian elephants, Southern India, emerg, Inf. Disp. 23 (2017) 504–506, https://doi.org/ 10.3201/eid2303.161741.

[47]S.K. Mikota, Tuberculosis in elephants, in: R.E. Miller, M.E. Fowler (Eds.), Zoo and Wild Animal Medicine, WB Saunders, 2008, pp. 355–364.

[48]S.V. Tessaro, L.B. Forbes, C. Turcotte, A survey of brucellosis and tuberculosis in bison in and around Wood buffalo national park, Canada, can, Vet. J. 31 (1990) 174.

[49]A.T. Rao, L.N. Acharjyo, Causes of mortality in captive bovids at Nandankanan Zoo, Indian Vet. J. 73 (1996) 793–795.

[50]K.L. Helke, J.L. Mankowski, Y.C. Manabe, Animal models of cavitation in pulmonary tuberculosis, Tuberculosis 86 (2006) 337–348, https://doi.org/ 10.1016/j.tube.2005.09.001.

[51]B.J. Gormus, J.L. Blanchard, X.H. Alvarez, P.J. Didier, Evidence for a rhesus monkey model of asymptomatic tuberculosis, J. Med. Primatol. 33 (2004) 134–145, https://doi.org/10.1111/j.1600-0684.2004.00062.X.

[52]I. Sugawara, L. Sun, S. Mizuno, T. Taniyama, Protective efficacy of recombinant BCG Tokyo (Ag85A) in rhesus monkeys (*Macaca mulatta*) infected intratracheally with H37Rv *Mycobacterium tuberculosis*, Tuberculosis 89 (2009) 62–67, https://doi. org/10.1016/j.tube.2008.09.008.

[53]P.S. Renshaw, K.L. Lightbody, V. Veverka, F.W. Muskett, G. Kelly, T.A. Frenkiel, S. V. Gordon, R.G. Hewinson, B. Burke, J. Norman, R.A. Williamson, M.D. Carr, Structure and function of the complex formed by the tuberculosis virulence factors CFP-10 and ESAT-6, EMBO J. 24 (2005) 2491e8, https://doi.org/10.1038/sj. emboj.7600732.

[54]K.L. Lightbody, D. Ilghari, L.C. Waters, G. Carey, M.A. Bailey, R.A. Williamson, P. S. Renshaw, M.D. Carr, Molecular features governing the stability and specificity of functional complex formation by *Mycobacterium tuberculosis* CFP-10/ESAT-6 family proteins, J. Biol. Chem. 283 (2008) 17681e90, https://doi.org/10.1074/jbc. M800123200.

[55]L. Majlessi, P. Brodin, R. Brosch, M.J. Rojas, H. Khun, M. Huerre, S.T. Cole,

[56]S. Leclerc, Influence of ESAT-6 secretion system 1 (RD1) of *Mycobacterium tuberculosis* on the interaction between mycobacteria and the host immune system, J. Immunol. 174 (2005) 3570e9, https://doi.org/10.4049/jimmunol.174.6.3570.

[57]A. Raja, U.D. Ranganathan, R. Bethunaickan, Improved diagnosis of pulmonary tuberculosis by detection of antibodies against multiple *Mycobacterium tuberculosis* antigens, Diagn. Microbiol. Infect. Dis. 60 (2008) 361e8, https://doi.org/10.1016/ j.diagmicrobio.2007.11.006.

[58]S. Sumi, V.V. Radhakrishnan, Evaluation of immunohistochemistry with a panel of antibodies against recombinant mycobacterial antigens for the diagnosis of tuberculous lymphadenitis, Int. J. Med. Med. Sci. 1 (2009) 215–219, https://doi. org/10.5897/IJMMS.9000103.

[59]T. Yamaguchi, U. Costabel, A. McDowell, J. Guzman, K. Uchida, K. Ohashi,

[60]Y. Eishi, Immunohistochemical detection of potential microbial antigens in granulomas in the diagnosis of Sarcoidosis, J. Clin. Med. 10 (2021) 983, https:// doi.org/10.3390/jcm10050983.

[61]M.R. Purohit, T. Mustafa, H.G. Wiker, O. Morkve, L. Sviland, Immunohistochemical diagnosis of abdominal and lymph node tuberculosis by detecting *Mycobacterium tuberculosis* complex specific antigen MPT64, Diagn. Pathol. 2 (2007) 36, https://doi.org/10.1186/1746-1596-2-36.

[62]R.C. Kesarwani, P. Anjana, M. Ashutosh, S.A. Kumar, Polymerase chain reaction (PCR): its comparison with conventional techniques for diagnosis of extra- pulmonary tubercular diseases, Indian J. Surg. 66 (2004) 84–88.

[63]A.L. Michel, H.F.A.K. Huchzermeyer, The zoonotic importance of *Mycobacterium tuberculosis*: transmission from human to monkey: case report, J. S. Afr. Vet. Assoc. 69 (1998) 64–65. https://hdl.handle.net/10520/EJC99214.

[64]J.C. Pena, W.Z. Ho, Monkey models of tuberculosis: lessons learned, Infect. Immun. 83 (2015) 852–862, https://doi.org/10.1128/IAI.02850-14.

Art – 283. XENOGRAFTING OF TILAPIA FISH SKIN FOR SNARE WOUND MANAGEMENT IN WILD SLOTH BEAR (*Melursus ursinus*): A NOVEL APPROACH

A. Sha. Arun, S.P. Patil and B. Shrikrishna

Abstract

In a rescue operation, Wildlife SOS Bannerghatta Bear Rescue Centre and the Karnataka Forest Department rescued a wild female sloth bear injured by a poacher's snare. Approximately 2-3 years of age, the bear had suffered a lethal injury on her right foreleg just above the metacarpal region due to the snare. So, the bear was missing the first four digits and claws with gangrenous and septic wounds along the entire paw region. It was estimated that the bear had been injured for 6-10 days prior to her rescue. After a thorough medical examination and stabilization period that lasted for five days, a procedure of skin grafting using fresh tilapia fish skin xenograft was performed to facilitate the healing of the wound. A bandage was applied to cover the graft, that remained intact for two weeks. Fourteen days after the procedure, a close re-examination of the wound bed showed partial absorption of the tilapia skin graft. It diffused a microcapillary network and a thin whitish layer formed over the wound's surface, indicating granulation and formation of the collagen layer. The wild sloth bear was made to undergo training which utilized the principles of positive conditioning wherein the bear was rewarded with a treat every time she performed a desired action such as extending her paw. This allowed wound dressing to happen regularly and smoothly. After six weeks, the wounds completely healed.

Keywords: Leg injury, poachers' snare, sloth bear, tilapia fish skin, xenograft

Introduction

Skin grafting is an ancient technique dating back to 1500 BC once practiced by the Egyptian empire. It is mentioned in Egyptian papyrus role called 'Elbert Papyrus' and one of the Ayurveda's earliest pieces of literature 'Sushruta Samhita' which depicts that this technique was even 3000 years ago (Kohlhauser et al., 2021). The skin grafting procedure involves harvesting skin from the donor and incorporating it into the recipient. It is widely used inhuman medicine. Recent advancements in skin graft procedure in veterinary practice are helpful techniques for rapid healing of wounds that are delayed naturally or wound that cannot be healed by the movement of local skin around the wound (Erwin et al., 2016).

The grafts are generally classified into three categories. The first being autografts (donor site and the recipient site is on the same animal), second being allografts (a tissue graft from a donor of the same species as the recipient, but not genetically identical), and third being xenograft (donor and recipient species are different). Autografts are the most successful grafts as the host and recipient of the graft are immunologically identical. Allografts and xenografts are temporary and used in burns and large denuded wounds (Swaim, 1990). In the case of wild animals, practicing autograft is difficult due to their aggressive behavior and added stress to the animal by harvesting extra tissue from the same individual, which will result in a double wound, and managing both would be an additional challenge for the wildlife veterinarian. On top of it, the expected behavior of any wild animal is self-mutilation, and there are bleak possibilities of applying e-collar or restraining them frequently in a crush cage (Arun etal., 2020, 2022).

Tilapias are freshwater fish and typically have laterally compressed, deep bodies like other cichlids. Tilapia fish skin has non-infectious microbiota even after sterilization of the skin, and it has a high amount of type I collagen and type III collagen (Lima-Junior et al., 2019). Tilapia skin is readily available. It is aqualitative, safe and an inexpensive material that can be easily applied in surgical procedures. Nile tilapia skin also has a high level of biocompatibility in nature. The collagen extract is a biocompatible type I collagen with potential biomedical material for use in clinical regenerative medicine (Alves et al., 2018; Ibrahim et al., 2020). The objective of the experiment was to use fish graft to use in wound healing of bear using standard protocols.

Materials and Methods

Preparation of fish skin

Freshly caught tilapia fish from the local fishermen was procured and euthanized as per the standard protocol. The fish was examined and checked for any skin discontinuations. Usable fish skin was measured (Fig. 1) with the measuring tape and later on it was matched with the dimensions of the wound taken (Fig. 2) to avoid a mismatch of graft to the wound. Scales were brushed and obliterated from the skin. With the help of a sterile surgical blade, incisions were made across the three borders as per the wound dimensions, with one edge attached to the spine of the fish to provide grip and avoid tearing of the skin while debriding. The skin was separated from the underlying muscles, and debridement of underlying tissues was done (Fig. 3).

Fig. 1. Usable fish skin measurement

Fig. 2. Wound dimensions measurement

Fig. 3. Skin removal and debridement of underlying tissues

Sterilization of the fish skin

Sterilization of skin was carried out using chlorhexidine gluconate, povidone-iodine, and metronidazole. The tilapia skin was subjected to successive treatments of each disinfectant for different timing. The skin was dipped in chlorhexidine gluconate (4%) for 5 minutes, followed by 10% povidone-iodine for 10 minutes and metronidazole for 15 minutes, respectively (Fig.4 - 5). After the treatment with the disinfectants, the skin was placed on a sterile surgical cloth over a sterile sponge, and skin was spread over it. The edges of the skin were held with the help of sterile needles to avoid rolling and shrinking of the skin (Alves et al., 2018; Ibrahim et al., 2020) (Fig. 6).

Fig. 4. Process of sterilization of fish skin

Fig. 5. Sterilization of fish skin

Fig. 6. Holding of fish skin

Patient preparation

The sloth bear was chemically immobilized by the combination of drug ketamine hydrochloride and xylazine hydrochloride with a dose rate of 5 mg kg-1 body weight and 2 mg kg-1 body weight, respectively with the aid of Dart gun (Fig. 7). Before the immobilization, the animal was made to fast for 12 hours. During the procedure, the utmost caution was maintained and all the vitals were monitored (Fig. 8). The surrounding area of the wound and the leg fur was shaved up to the elbow and later cleaned with 4% chlorhexidine gluconate,10% povidone-iodine, and metronidazole. The wound was washed with normal saline including cleaning of visible dirt and debris, and disinfection of the wound was undertaken by the same agents as described above (Fig. 9).

Fig. 7. Chemical immobilization using dart gun

Fig. 8. Monitoring of the vital signs

Fig. 9. Wound cleaning and grafting site preparation

Wound preparation

For successful grafting, the vascularity of wound tissue is an essential factor. The excessive fat and unwanted tissues which can hinder the adhesion of the graft were scrapped with the surgical blade, and the wound was freshened across edges along the entire surface (Fig. 10).

Fig. 10. Wound preparation

Graft preparation

The dimensions of the graft were decided asper the dimensions of the wound. With the help of the tip of the 22 number bp blade, tiny 7-8 pores were made on the graft for aeration of the wound (Fig. 11). A thin layer of collagen ointment (silver sulphadiazine USP 1% w w-1 and collagen base) was applied over the surface in contact with the wound (Fig. 12). The graft was placed over the wound and sutured from the edges of the wound with absorbable suture (4-0) by deep, simple interrupted suture pattern. Initially, one border of the wound was sutured to ensure that while suturing opposite edge proper tension is available, and the graft stays in close contact with the underlying injury (Fig. 13). Four sutures in the central part, its adjacent areas were made to hold the graft in contact with the same place (Fig. 14).

Fig. 12. Application of collagen ointment on the contact surface of the graft

Fig. 13. Suturing of the graft

Fig. 14. Central sutures for Holding of the graft

Bandaging of the wound

Aerated gauze bandages were applied over the graft site to facilitate easy airflow to the wound. Apart from the wound bandaging, additional bandages were extended till the shoulder joint as a safety measure to ensure that even if the bear tries to remove the dressing, the bandage will not tear from the main site of the operation (Fig. 15).

Fig. 15. Wound bandaging

Postoperative measures

Before the shifting of bear, one extra room was made available to allow the bear to rest while existing room is washed and cleaned. It was ensured that both the chambers were free from visible dirt and any infective materials. Both rooms were checked for any sharp protruding elevations. Daily examination of the bandages was carried out to avoid infections at the site through soiling with water or urine.

Postoperative treatment regime

Daily supplementation included two capsules of cod liver oil and NASID drug carprofen@4 mg kg-1 body weight for seven days twice a day. No antibiotics were supplemented. Diet Supplementation included 6-7 liters of nutritiously standardized porridge twice a daily, along with ten boiled eggs a day with 2 kg seasonal fruits like watermelon.

Results and Discussion

Fourteen days after the procedure, the bandages were still intact as the bear had no complications like irritation and did not try to remove the bandages. After 14 days, the bandages were removed, and the grafting site was examined (Fig. 16). The graft was absorbed partially, and the wound showed a white layer over it, depicting that the collagen had absorbed. The injury was evident in the presence of microcapillaries at the contact surface. No secondary infections and inflammation were observed over the surface (Fig. 17, Fig. 18).

In the recent past, skin grafting has been a promising development in the veterinary field and can be widely practiced for wound management. Several studies have recorded successful results in cats, dogs, and bears (Aisa and Vernon, 2016). Tilapia skin as a xenograft can be utilized as a biological dressing in the veterinary field. Under wildlife veterinary field, the skin grafting has rarely been practiced and there are least available literatures. Present study with a complete stepwise procedure along with clear documentation will no doubt make the wildlife veterinarians job much easier in future. This will be also tremendously beneficial to the wildlife especially in captivity.

Fig. 17. Post procedure wound healing and changes after14 days

Fig. 18. Pre-operative wound

Fig. 19. Post-operative wound changes

Acknowledgement

The authors greatly appreciate the support of Shri K. Satyanarayan and Smt. Geeta Seshamani of Wildlife SOS, for their continued encouragement for such procedures. Also, they profoundly thank the authorities of Karnataka Forest Department and the Executive Director, Bannerghatta Biological Park for their kind co-operation and opportunities. Thanks to Sri Prashant Kishor Bhangale for assisting us during the preparation of the surgical procedure.

References

Aisa, J. and Vernon, J. 2016. The use of skin grafts in small animal reconstructive surgery. *Companion Animal*, 21(11): 642-648.

Alves, A.P.N.N., Lima Ju´ nior, E.M., Piccolo, N.S., de Miranda, M.J.B., Lima Verde, M.E.Q., Ferreira Ju´ nior, A.E.C., de Barros Silva, P.G., Feitosa, V.P.,de Bandeira, T.J.P.G., Mathor, M.B. and de Moraes, M.O. 2018. Study of tensiometric properties, microbiological and collagen content in nile tilapia skin submitted to different sterilization methods. *Cell Tissue Bank.* 19(3): 373-382.

Arun, A. Sha., Virk, A.S. and Patil, S.P. 2022. Surgical treatment of baculum fracture in sloth bear (*Melursusursinus*): A case report. *e-planet* 20(1): 74-78.

Arun, A.S., Bakde, R. and Adhithyan, N.K. 2020. Successful rescue and rehabilitation of an injured wild sloth bear trapped in a poacher's snare. *e-planet*18 (2): 158-163.

Erwin, G., Handharyani, E. and Noviana, D. 2016. Subjective and objective observation of skin graft recovery on Indonesian local cat with different periods of transplantation time. *Vet. World*9(5): 481-486. http://www.ucdavis.edu/health/news/healing-animals-fish-skin http://www.vice.com/en/article/us_/article/ne4x3k/ burned-bear-paws-fish-skin

Ibrahim, A., Hassan, D., Kelany, N., Kotb, S. and Soliman, M. 2020. Validation of three different sterilization methods of Tilapia skin dressing: impact on microbiological enumeration and collagen content. *Front. Vet. Sci*. 7: 597751.

Kohlhauser, M., Luze, H., Nischwitz, S.P. and Kamolz, L.P. 2021. Historical evolution of skin grafting—a journey through time. *Medicina* 57(4): 348.

Lima-Junior, E.M., de Moraes Filho, M.O., Costa, B.A.,Fechine, F.V., de Moraes, M.E.A., Silva-Junior, F.R., Soares, M.F.A.D.N., Rocha, M.B.S. and Leontsinis, C.M.P., 2019. Innovative treatment using tilapia skin as a xenograft for partial thickness burns after a gunpowder explosion. *J. Surg. Case Rep*. 2019(6): rjz181.

Swaim, S.F. 1990. Skin grafts. *Vet. Clin. North America:Small Anim. Pract.* 20(1): 147-175.

Art – 284. A STUDY ON CLINICAL DIAGNOSIS OF TUBERCULOSIS IN FREE RANGING AND CAPTIVE WILD ANIMALS OF INDIA

Sharma M., Karikalan M., Asok Kumar M., Sree Lakshmi P., Sharma K., **Ilayaraja S.**, Mathur A., and Pawde A. M.

Abstract

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

Keywords: Elephant, Lateral flow assay, *Mycobacterium*, Sloth bear

Introduction

The complex and dynamic interactions between humans, domestic animals, and wildlife lead to an increased chance of the emergence of diseases in new host species (Hassell *et al*., 2017). Tuberculosis (TB), a deadly air-borne disease, has proved to be a worldwide problem at the wildlife-livestock-human interface. It is caused by a group of intimately related bacterial species termed the *Mycobacterium tuberculosis* complex

(MTBC) (Brites and Gagneux, 2017). According to WHO's global TB report 2019, under-developed and developing countries record nearly 1.4 million human deaths every year due to TB. India has the world's highest bovine population with 21.8 million TB affected cows (Srinivasan *et al*., 2018), thus creating a major well spring for spill-over to various hosts like humans and wild animals. Amongst the members of MTBC, *Mycobacterium bovis* is having the widest host range thus infecting many species of domestic animals, wildlife, and also humans (Bernitz *et al*., 2021; Ncube *et al*., 2022). *M. tuberculosis* is majorly a human pathogen, however, causes TB in domestic and wild animals living in close and prolonged contact with humans (Teppawar *et al*., 2018). Ante-mortem diagnosis of TB in wild animals is difficult due to the sub-clinical nature of the disease and the limited application of the standard diagnostic tests employed for domestic animals and humans (Thomas *et al*., 2021). Culture stands as the gold standard, but culture-based approaches to wild animals have several shortcomings, like trouble with sample collection, problems related to sample contamination, and transport (Lekko *et al*., 2021; Thomas *et al*., 2021). The tuberculin skin test (TST) is the most commonly used test in domestic animals, but difficulties in capturing wild animals after 72 h and optimizing tuberculin doses make it unsuitable for free-roaming wild animals (Che-Amat *et al*., 2016). Serological assays, being simple, rapid, and relatively inexpensive, offer a useful alternative. The immunochromatographic test (ICT), viz., lateral-flow assay (LFA), an animal-side test that uses a cocktail of a limited number of antigens, has several advantages for application in wild animals (Bruns *et al*., 2017; Fresco-Taboada *et al*., 2019). In addition, PCR primers designed to target *16S rRNA*, genes coding for MTBC specific proteins and insertion sequences (IS6110) can be used as a rapid identification tool of mycobacterial isolates (Verma *et al*., 2022). The majority of the work on TB in wild animals in India is based on morbid data and there are very few reports on ante-mortem diagnosis of TB in wild animal species that are too limited in terms of identification of the MTBC species involved (Sharma *et al*., 2014). This study documents *M. tuberculosis* infection in seven sloth bears (*Melursus ursinus*) and two elephants (*Elephas maximus*) as well as *M. bovis* in a spotted deer (*Axis axis*), based on LFA, ZN staining, and PCR.

Materials and Methods

Clinical samples and DNA isolation

A total of 207 wild animals belonging to various species of bovids, cervids, carnivores, non-human primates, and pachyderms from various national and zoological parks, rescue centers, etc. across 10 Indian states (Fig. 1; Supplementary Table 1 (ST1)) with or without any apparent disease symptoms were examined clinically from August 2017 to November 2020. Samples included 156 blood samples, 103 faecal swabs, 73 serums, and 64 nasal swabs/trunk wash fluids were collected (Table 1). Total DNA was isolated using the DNeasy blood and tissue kit and the QIAamp DNA StoolMini kit (Qiagen, Germany) as per the recommendations of the manufacturers along with suitable modifications. All samples were properly labeled and stored at -20°C until further use.

Fig. 1: The geographical distribution of different zoological and national parks, safaris, and state forest departments from where the clinical samples were collected/received in India. The stars (★) show the distribution in 10 states of India

Table 1: Species-wise details of different clinical samples

S. No.	Species	Total clinical cases screened	Samples screened				
			Nasal swab/trunk wash fluid[a]	Faecal swab	Blood	Serum	Total
A	**BOVIDS**						
1	Chinkara (*Gazella bennettii*)	6	6	6	3	6	21
2	Wildebeest (*Connochaetes taurinus*)	6	6	-	6	6	18
3	Oryx (*Oryx gazella*)	4	4	4	4	4	16
4	Blackbuck (*Antilope cervicapra*)	3	-	2	2	3	7
5	Giraffe (*Giraffa camelopardalis*)	2	2	2	2	2	8
6	Nilgai (*Boselaphus tragocamelus*)	2	-	2	-	-	2
7	Impala (*Aepyceros melampus*)	1	1	1	1	1	4
8	Indian gaur (*Bos gaurus*)	1	-	1	-	-	1
	Total	25	19	18	18	22	77
B	**CERVIDS**						
1	Spotted deer (*Axis axis*)	13	5	7	3	3	18
2	Sambar deer (*Rusa unicolor*)	5	-	3	2	2	7
3	Thamin deer (*Panolia eldii*)	2	2	2	2	2	8
	Total	20	7	12	7	7	33
C	**CARNIVORES**						
1	Lion (*Panthera leo*)	78	-	9	69	1	79
2	Sloth bear (*Melursus ursinus*)	11	11	10	-	7	28
3	Leopard (*Panthera pardus*)	5	-	5	2	2	9
4	Tiger (*Panthera tigris*)	3	-	3	-	1	4
5	Jackal (*Canis aureus*)	1	-	1	-	-	1
Total		98	11	28	71	11	121
D	**NON-HUMAN PRIMATES**						
1	Bonnet macaque (*Macaca radiata*)	1	-	1	-	-	1
2	Hoolock gibbon (*Hylobates hoolock*)	10	5	10	10	5	30
3	Rhesus macaque (*Macaca mulatta*)	2	-	2	-	-	2
	Total	13	5	13	10	5	33
E	**PACHYDERMS**						
1	Elephant (*Elephas maximus*)	50	22	31	50	28	131
2	Rhinoceros (*Rhinoceros unicornis*)	1	-	1	-	-	1
	Total	51	22	32	50	28	132
	Grand total A+B+C+D+E	207	64	103	156	73	396

Clinical examination

Clinical examination of wild animals under study was done following physical and chemical immobilization. The physical parameters like body condition, color of the mucous membranes, palpation of peripheral lymphnodes, and absence or presence of nasal discharge were recorded.

Lateral flow assay

Serum samples collected during clinical examination were screened for antibodies against MTBC organisms using the MycoPac Dual kit (Cisgen Biotech, Chennai, Tamil Nadu, India). The LFA kits and sera samples were first brought to room temperature, after which about 10 µL of the test serum was added to the two sample wells of the kit, and after a few minutes, 2 drops of sample buffer were added into both the wells. The results were read within 5-10 min. The appearance of the colored line in the control area is a must to validate the results of the kit. The appearance of a colored reaction in either of the test lines (1 and 2) or in both of them was considered as seropositive against TB. The presence of a colored reaction in test lines 2 and/or 4 was an indication of seropositivity against pathogenic MTBC organisms. The development of a colored reaction only in test line 3 was interpreted as sero-reactive against NTM organisms.

Ziehl-Neelsen staining

Ziehl-Neelsen (ZN) staining method was used to detect acid-fast mycobacteria in heat fixed smears prepared from nasal and faecal swabs as described earlier (Sharma *et al*., 2022).

Molecular diagnosis

DNA samples were examined by conventional PCRs using primers IS6110 F: CTC GTC CAG CGC CGC TTC GG, IS6110 R: CCT GCG AGC GTA GGC GTC GG for identification of MTBC organisms (Miller *et al*., 1997) and 12.7 kb F: TTC CGA ATC CCT TGT GA, 12.7 kb R1: GGA GAG CGC CGT TGT A, 12.7 kb R2: AGT CGC CGT GGC TTC TCT TTT A for differentiation of *M. bovis* and *M. tuberculosis* (Bakshi *etal*., 2005). The amplified products obtained were confirmed to be of expected size in 1.5% (w/v) agarose gel (Thermo Scientific, USA) in 1X Tris Acetate EDTA buffer.

Results

The clinical signs of TB were found to be absent in most (n=202) of the examined wild animals. There was no manifestation of any clinical signs, even in 16 sero- positive (LFA) animals. However, four sloth bears exhibited clinical signs like variable degrees of emaciation, dullness, pale mucous membranes, intermittent anorexia, and respiratory distress. Progressive weight loss was also observed in an elephant (Fig. 2). Out of the total 73 serum samples screened by LFA, 21 (28.76%) serum samples, comprising of eight (38.09%) bovids, i.e., three wildebeest (*Connochaetestaurinus*), two blackbuck (*Antilope cervicapra*), two giraffe (*Giraffa camelopardalis*), and one chinkara (*Gazella bennettii*), seven (33.34%) sloth bears, two (9.52%) cervids, i.e., sambar deer (*Rusa unicolor*) and spotted deer (*Axis axis*), and four (19.04%) elephants (*Elephas maximus*) were tested positive for antibodies against MTBC organisms (Fig. 3; Supplementary Tables 2 and 3 (ST2 and ST3)).

Fig. 2: Emaciated and debilitated TB-positive elephant

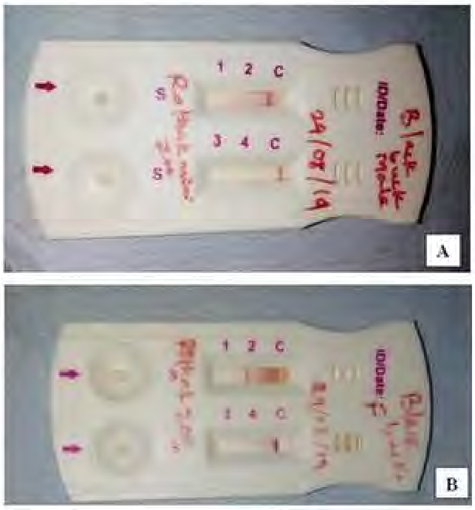


Fig. 3: Blackbuck. Negative result of lateral flow assay (LFA) shows the absence of a colored line in the test area and presence of a colored line in the control area (A), and positive result of LFA shows the presence of colored lines in both the test and control area (B)

A total of 103 faecal smears and 64 nasal or trunk wash fluid smears were subjected to ZN staining. Only nine (8.74%) faecal and nine (14.06%) nasal or trunk wash fluid smears of seven sloth bears and two elephants were found positive for AFB (Fig. 4). Genomic DNA isolated from nasal swabs/trunk wash fluids, faecal swabs and blood samples was screened by the IS6110 insertion sequence and a 12.7 kb fragment-based PCR. In IS6110 insertion sequence-based PCR, out of the 156 blood samples, three samples (0.64%) of a spotted deer and two elephants were found TB positive with an amplicon size of 123 bp. Out of the 103 faecal swabs, 64 nasal swabs or trunk washes were screened of them nine (8.74%) faecal and nine (14.06%) nasal swabs or trunk wash fluids of seven sloth bears and two elephants were found positive (Supplementary Tables 2 and 3 (ST2 and ST3)) These positive samples were further subjected to duplex PCR using 12.7 kb fragment primers in which DNA isolated from nasal and faecal swabs of seven sloth bears and trunk wash fluids, faecal swabs and blood of two elephants revealed an amplicon size of 262 bp, i.e., specific for *M. tuberculosis*, and DNA isolated from the blood of spotted deer revealed an amplicon size of 168, i.e., specific for *M. bovis* (Fig. 5).

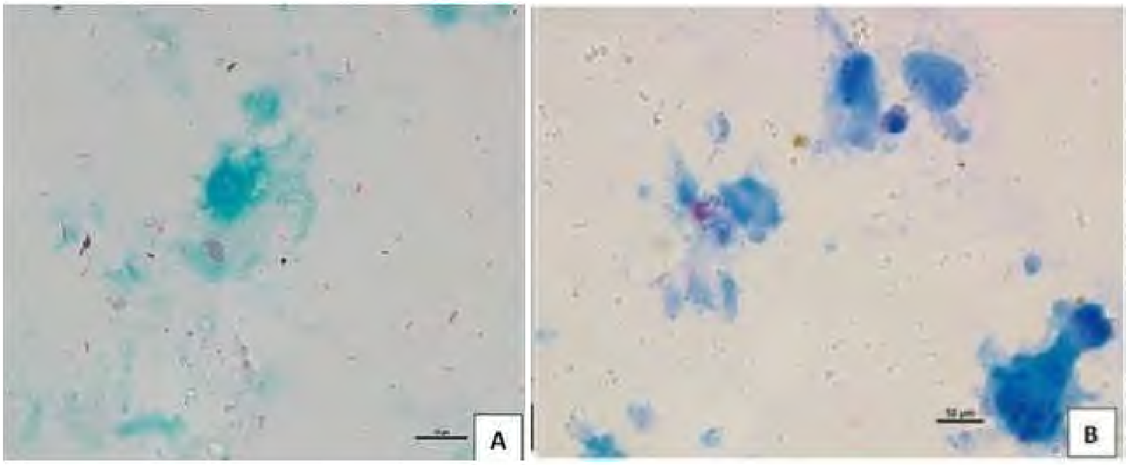


Fig. 4: Ziehl-Neelsen (ZN) staining of nasal (A), and faecal (B)swabs from a sloth bear, revealing pink colored, rod-shaped, and acid-fast bacilli, (ZN, ×1000)

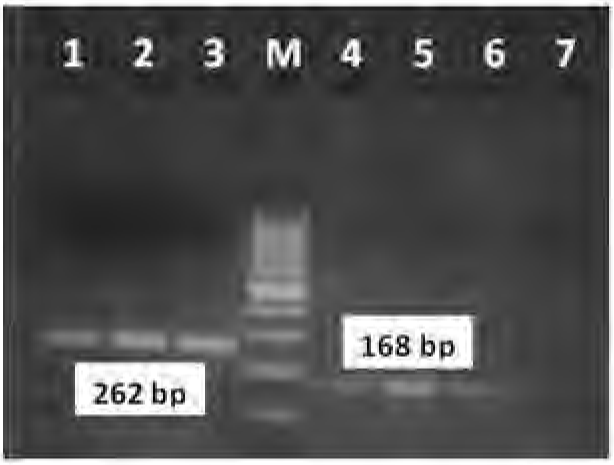


Fig. 5: Agarose gel electrophoresis. Differential amplification of 12.7 kb fragment of MTBC organisms. Lane M: 100 bp ladder. Lane 1: Positive control of M. tuberculosis, Lane 2: Sample from sloth bear showing 262 bp amplicon specific for M. tuberculosis, Lane 3: Sample from elephant showing 262 bpamplicon specific for M. tuberculosis, Lane 4: Positive control of M. bovis, Lanes 5 and 6: Samples from spotted deer showing168 bp amplicon specific for M. bovis, and Lane 7: Negative control

Results of different clinical tests were analyzed by a Chi-square test (χ^2) of independence. Based on the statistical analysis, it was concluded that there was a significant difference in the prevalence of TB among different wild animals. Among the clinically infected animals, seven sloth bears, which showed positive results in LFA and PCR, died within 4-6 months after clinical examination, and all of the cases were confirmed to be suffering from TB on the basis of histopathology, tissue acid-fast staining, and molecular techniques.

Discussion

There is a complex pattern of TB transmission among humans, domesticated animals, and wildlife, and the disease is frequently transmitted from one to another (spill-over and spill-back) (Chugh, 2018; Mohamed, 2020). In India, only a limited number of reports are available on the ante-mortem diagnosis of wildlife TB as well as on the transmission pattern at the wildlife- livestock-human interface. Ante-mortem diagnosis of TB often poses a significant challenge in wildlife species due to rarely evident clinical signs (Lekko *et al.*, 2021; Thomas *et al.*, 2021). In the present study, four sloth bears exhibited clinical signs and symptoms similar to those noticed earlier (Karikalan and Sharma, 2018; Marinaik *et al.*, 2022; Sharma *et al.*, 2022). Clinical signs in cervids and elephants were not apparent in the present study except for weight loss. Similar observations that most of the cervids and elephants seldom show clinical signs until the terminal stage of disease were also made by other workers (Nugent *et al*., 2015; Songthammanuphap *et al*., 2020).

LFA showed seropositivity for MTBC antigens in 21 (28.76%) cases out of which seven sloth bears that died during the study period were confirmed as TB positive by conventional and molecular techniques, thus indicating the importance of LFA in clinical diagnosis of wild animals. Various LFA kits are commercially available for wild animals, such as STAT-PAK™(Chembio Diagnostic Systems, USA), which is validated for use in elephants and non-human primates with sensitivity and specificity of 100%, 95-100% in elephants, 90%, and 99% in non-human primates, respectively (Thomas *et al*., 2021). The MycoPac dual kit, Cisgen Biotech, India, was used in the present study, which claimed to have 95% sensitivity and 98% specificity in sloth bear, elephant, and deer species. This kit uses the cocktail of proteins involved in the pathogenesis of MTBC organisms and other native mycobacterial proteins, which helps in detecting cross-reactivity from NTM (Veerasami *et al*., 2017). ZN staining of smears prepared from clinical samples acts as a quick and economical method that provides a tentative diagnosis for TB. However, it has certain limitations as it cannot distinguish between the members of the *Mycobacterium* genus and other acid-fast organisms like Cryptosporidium and Nocardia (Lawrence *et al*., 2016). Therefore, for confirmation of acid-fast MTBC organisms, concurrent application of other techniques is mandatory. PCR is the most useful molecular technique that helps in species identification of *Mycobacterium*. It is a rapid differentiation technique, hence very useful for routine laboratories (Bakshi *et al*., 2005). TB ante- mortem diagnosis with high sensitivity has been achieved when the combination of techniques is used in wild animals (Thomas *et al*., 2021).

In the present study, *M. tuberculosis* was confirmed as the cause of TB in seven captive sloth bears and two elephants. It might be due to the close association of these bears and elephants with infected humans, as taming the bears for public entertainment was a common practice in India a few years back, along with the major role of elephants in socio-religious activities in the country. This indicates a strong correlation between humans and these captive wild animals with regard to the transmission of *M. tuberculosis*. Detection of *M. bovis* inone spotted deer is indicative of either the role of cervids as the maintenance hosts for *M. bovis* or the case of spillover from domestic livestock living in the periphery of forest-reserved lands.

Thus, from the present study, it is evident that TB is prevalent in free-ranging and captive wildlife in India, encompassing a variety of wild animal species. As supported by the earlier reports, there is a significant spill-over and spill-back of MTBC members at the livestock-human-wildlife interface. The clinical signs are indistinct in wild animals suffering from TB, thus acting as a silent carrier of infection, which poses a serious threat to in-contact humans and animals. Serological assays like LFA were found to be an effective test for screening TB, which when combined with other tests (PCR, ZN staining, etc.), holds good confirmatory diagnostic value in wild animals.

Acknowledgements

The authors are thankful to the Director, ICAR-IVRI, and Directors of all national parks, zoological parks, safaris and rescue centers for providing necessary facilities to conduct the study.

References

Bakshi, CS; Shah, DH; Verma, R; Singh, RK and Malik, M (2005). Rapid differentiation of *Mycobacterium bovis* and *Mycobacterium tuberculosis* based on a 12.7-kb fragment by a single tube multiplex-PCR. Vet. Microbiol., 109: 211- 216.

Bernitz, N; Kerr, TJ; Goosen, WJ; Chileshe, J; Higgitt, RL;Roos, EO; Meiring, C; Gumbo, R; de Waal, C; Clarke, C and Smith, K (2021). Review of diagnostic tests for detection of *Mycobacterium bovis* infection in South African wildlife. Front. Vet. Sci., 8: 588697.

Brites, D and Gagneux, S (2017). The nature and evolution of genomic diversity in the *Mycobacterium tuberculosis* complex. In: Gagneux, S (Ed.), *Strain variation in the Mycobacterium tuberculosis complex: Its role in biology, epidemiology and control. Advances in experimental medicine and biology*. (1st Edn.), Vol. 1019, Springer, Cham. PP: 1-26.

Bruns, AC; Tanner, M; Williams, MC; Botha, L; O'Brien, A; Fosgate, GT; Van Helden, PD; Clarke, J and Michel, AL (2017). Diagnosis and implications of *Mycobacterium bovis* infection in banded mongooses (*Mungos mungo*) in the Kruger National Park, South Africa. J. Wildl. Dis., 53: 19-29.

Che-Amat, A; Risalde, MA; Gonza´lez-Barrio, D; Ortí´z, JA and Gorta´zar, C (2016). Effects of repeated comparative intradermal tuberculin testing on test results: a longitudinal study in TB-free red deer. BMC Vet. Res., 12: 1-9.

Chugh, TD (2018). Human tuberculosis in India: some neglected issues. Curr. Med. Res. Pract., 8: 64-66.

Fresco-Taboada, A; Risalde, MA; Gorta´zar, C; Tapia, I; Gonza´lez, I; Venteo, A' ; Sanz, A and Rueda, P (2019). A lateral flow assay for the rapid diagnosis of *Mycobacterium bovis* infection in wild boar. Transbound. Emerg. Dis., 66: 2175-2179.

Hassell, JM; Begon, M; Ward, MJ and Fe`vre, EM (2017). Urbanization and disease emergence: Dynamics at the wildlife-livestock-human interface. Trends Ecol. Evol., 32: 55-67.

Karikalan, M and Sharma, AK (2018). Studies on pathology of sloth bear diseases with special reference to tuberculosis.Ind. J. Vet. Pathol., 42: 89-92.

Lawrence, DM; Baveja, CP; Kumar, S; Khanna, A and Sapriina, J (2016). Comparative evaluation of fluorescent staining with Ziehl-Neelsen and kinyoun staining in the diagnosis of clinically suspected cases of pulmonary tuberculosis. Int. J. Contemp. Med. Res., 3: 1970-1974.

Lekko, YM; Che-Amat, A; Ooi, PT; Omar, S; Ramanoon, SZ; Mazlan, M; Jesse, FFA; Jasni, S and Ariff Abdul- Razak, MF (2021). *Mycobacterium tuberculosis* and avium complex investigation among Malaysian free- ranging wild boar and wild macaques at wildlife-livestock-human interface. Animals. 11: 3252.

Marinaik, CB; Sha, AA; Manjunatha, V; Shylaja, S; Rathnamma, D; Rizwan, A and Nagaraja, K (2022). Isolation, characterization, and drug sensitivity of *Mycobacterium tuberculosis* in captive Sloth Bears (*Melursus ursinus*): Unnatural habitat with human environment may predispose Sloth Bears to tuberculosis. Front. Vet. Sci., 9: 844208.

Miller, J; Jenny, A; Rhyan, J; Saari, D and Suarez, D (1997). Detection of *Mycobacterium bovis* in formalin- fixed, paraffin-embedded tissues of cattle and elk by PCR amplification of an IS6110 sequence specific for*Mycobacterium tuberculosis* complex organisms. J. Vet. Diagn. Invest., 9: 244-249.

Mohamed, A (2020). Bovine tuberculosis at the human- livestock-wildlife interface and its control through one health approach in the Ethiopian Somali pastoralists: A review. One Health. 9: 100113.

Ncube, P; Bagheri, B; Goosen, WJ; Miller, MA and Sampson, SL (2022). Evidence, challenges, and knowledge gaps regarding latent tuberculosis in animals. Microorganisms. 10: 1845.

Nugent, G; Gortazar, C and Knowles, G (2015). The epidemiology of *Mycobacterium bovis* in wild deer and feral pigs and their roles in the establishment and spread of bovine tuberculosis in New Zealand wildlife. N. Z. Vet. J., 63: 54-67.

Sharma, M; Karikalan, M; Dandapat, P; Beena, V; Kumar, MA; Kumar, A; Sudhagar, M and Pawde, AM (2022). Pulmonary tuberculosis in captive sloth bear (*Melursus ursinus*) due to *Mycobacterium tuberculosis*. Indian J. Vet. Pathol., 46: 96-99.

Sharma, AK; Nayakwadi, S; Chandratre, GA; Saini, M; Das, A; Raut, SS; Swarup, D and Somvanshi, R (2014). Prevalence of pathological conditions in zoo/wild animals in India; a retrospective study based on necropsy. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci., 84: 937-946.

Songthammanuphap, S; Puthong, S; Pongma, C; Buakeaw, A; Prammananan, T; Warit, S; Tipkantha, W; Kaewkhunjob, E; Yindeeyoungyeon, W and Palaga, T (2020). Detection of *Mycobacterium tuberculosis* complex infection in Asian elephants (*Elephas maximus*) using an interferon gamma release assay in a captive elephant herd. Sci. Rep., 10: 1-10.

Srinivasan, S; Easterling, L; Rimal, B; Niu, XM; Conlan, AJ; Dudas, P and Kapur, V (2018). Prevalence of bovine tuberculosis in India: A systematic review and meta- analysis. Transbound. Emerg. Dis., 65: 1627-1640.

Teppawar, RN; Chaudhari, SP; Moon, SL; Shinde, S; Khan, WA and Patil, AR (2018). Zoonotic tuberculosis: A concern and strategies to combat. In: Enanay, S. (Ed.), *Basic biology and applications of actinobacteria*. (1st Edn.), London, UK, IntechOpen. PP: 23-38.

Thomas, J; Balseiro, A; Gorta´zar, C and Risalde, MA (2021). Diagnosis of tuberculosis in wildlife: a systematic review. Vet. Res., 52: 1-23.

Veerasami, M; Venkataraman, K; Karuppannan, C; Shanmugam, AA; Prudhvi, MC; Holder, T; Rathnagiri,P; Arunmozhivarman, K; Raj, GD; Vordermeier, Mand Mohana Subramanian, B (2018). Point of care tuberculosis sero-diagnosis kit for wild animals: combination of proteins for improving the diagnostic sensitivity and specificity. Indian J. Microbiol., 58: 81-92.

Verma, R; Sharma, A and Ramane, S (2022). Comparative evaluation of *pncA* gene, IS6110 and 12.7-Kb fragment- based PCR assays for simultaneous detection of *Mycobacterium tuberculosis* complex (*M. tuberculosis* and *M. bovis*) in cultured strains and clinical specimens. Indian J. Exp. Biol., 60: 192-199.

Art – 285. SLOTH BEAR MATERNAL AND RESTING DEN LOCATIONS IN EASTERN KARNATAKA

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

Abstract

Sloth bears (*Melursus ursinus*) use resting and maternal dens. Resting dens are used by both sexes, usually during the mid-day when sloth bears are least active. Maternal dens are used by female bears to give birth and raise cubs. The purpose of our study was to better understand the denning ecology of sloth bears in eastern Karnataka so that important denning habitat can be delineated and protected. We documented resting and maternal dens from 2014 to 2018 in 5 different study areas surrounded by agriculture and orchards in eastern Karnataka, India. We used landscape and site-specific variables to model the probability of den occurrence. The best fit model for approximating maternal den site selection included distance to forest border, protection level, aspect, slope, and elevation. The best fit model for approximating resting den site selection included distance to forest border, aspect, slope, protection level and ruggedness. Maternal dens were closer to forest borders than resting dens. We postulate that areas near the forest border are chosen for access to resources and that mothers choose dens closer to edges because of the constraints and needs of cubs. In poorly protected, resource-deficient areas, selection of maternal dens near habitat edges presents a tradeoff between higher resource availability closer to human habitation, reduced risk of cub predation, and higher risk of human-caused mortality compared to areas farther from forest borders. Resting and maternal dens were farther from forest borders in better-protected areas, suggesting that if resources are prevalent in the habitat, bears would use locations farther from the forest borders.

Keywords: Bear den, India, *Melursus ursinus*, Sloth bear

Denning plays an important role in Ursid ecology and denning practices of sloth bears (*Melursus ursinus*) vary substantially from those of other bear species, but very little is known about them. For example, sloth bears do not hibernate (Ward and Kynaston 1995, Akhtar et al. 2007), and therefore do not use dens in that capacity; however, sloth bears use resting and maternal dens. Resting dens are used daily by male and female bears when inactive, irrespective of season. Generally, resting dens are used during daylight, as sloth bears are largely crepuscular and nocturnal. Maternal dens are used by female sloth bears to birth and raise cubs.

The structure of resting dens varies depending on what is available in a given habitat. Consequently, resting dens have been documented as naturally occurring caves, crevices between large boulders, the spaces between tree roots, and under fallen trees and shrubs (Eisenberg and Lockhart 1972, Laurie and Seidensticker 1977, Desai et al. 1997, Garshelis et al. 1999, Akhtar et al. 2007, Baskaran et al. 2015). Within the rocky scrub jungles of the Deccan Plateau in eastern Karnataka, India, sloth bears exclusively use naturally occurring caves, likely because of their abundance and because of the protection they afford from weather, disturbance, and predators.

Maternal dens are either excavated or an existing cave, or cave-like structure, is used (Joshi et al. 1999, Arun et al. 2018*a*). Excavated dens can be single or multi-chambered, are often dug into a riverbed bank, and are often obscured by vegetation (Joshi et al. 1999, Arun et al. 2018*a*); however, in the rocky scrub jungle of the Deccan Plateau in eastern Karnataka, all known maternal dens, like resting dens, occur in naturally occurring caves or cave-like structures. Preparturient bears exhibit a preference for caves with ≥2 chambers and some level of complexity, particularly in areas with predators, such as tigers (*Panthera tigris tigris*) and leopards (*Panthera pardus*), that could potentially prey on cubs or adult bears (Seshamani and Satyanarayan 1997). Once sloth bear cubs are born, their mother will stay with them in the den for approximately 1–2 months (Joshi et al. 1999, Arun et al. 2018*a*). Once the mother emerges from her den to eat and drink, she does so independently, leaving the cubs alone for a short time. This pattern of solo foraging outings continues for approximately 2 weeks (Jacobi 1975, Arun et al. 2018*a*). Cubs join their mother for their first trip from the den approximately 2 months after birth. Den structure and location play a role in den site selection for bears. In Denali National Park, Alaska, USA, for example, male grizzly bears (*Ursus arctos*) choose wintering dens in locations where they are not likely to be disturbed and have easy access to food resources in spring (Libal et al. 2011). In many cases, adult female grizzly bears spatially segregate themselves from adult males by denning at higher elevations and at steeper slopes, or temporally segregate themselves by emerging from winter dens at a later date, most likely because of the threat of infanticide (Schoen et al. 1987, Ciarniello et al. 2005, Libal et al. 2011). The majority of sloth bear resting dens in north Bilaspur, Chhattisgarh of central India, were within 1 km of forest edges (Akhtar et al. 2007). Akhtar et al. (2007) theorized that sloth bears chose dens close to forest edges to put themselves close to resources in agricultural fields because forests in this area are highly degraded and do not have an abundance of resources. Our objectives were to describe landscape features associated with sloth bear maternal and resting dens, to compare the landscape features between maternal and resting dens, and to compare the landscape features of maternal and resting dens in areas with different levels of protection to better understand the denning ecology of sloth bears in eastern Karnataka so that important denning habitat can be delineated and protected.

Study Area

Our study took place from 2014 through 2018 in 5 isolated areas located in the rocky scrub jungles of eastern Karnataka including the Deccan Plateau (Figure 1). Karnataka is considered a stronghold of the species (Puri et al.2015). Our study areas have a semi-arid climate characterized by hot summers (24–45°C) during April–June and low rainfall (572–802 mm) from June to November. Temperatures drop when the monsoons occur, July–October, but even during winter, November–May, the area can average roughly 28°C.

Figure 1: Location of sloth bear den study in Karnataka, India, 2014–2018

The 5 study areas were Arisikere (Nagapuri Forest; 13°23′N, 76°17′E) Reserve Forest, Daroji Wildlife Sanctuary (15°36′N, 76°36E), Gudekote Wildlife Sanctuary (14°52′N,76°37′E), Ramnagara Reserved Forest (12°49′N, 77°21′E), and Ramdurga Reserve Forest (15°30′N, 76°20′E; Figure 2). The areas were mostly composed of rocky terrain supporting scrub forest and dry deciduous forest at elevations of 405 m to 1,242 m. These areas have an abundance of naturally occurring caves. Daroji and Gudekote are designated as wildlife sanctuaries and have a higher level of protection with less human activity than the other 3 study areas, which are designated as reserve forest.

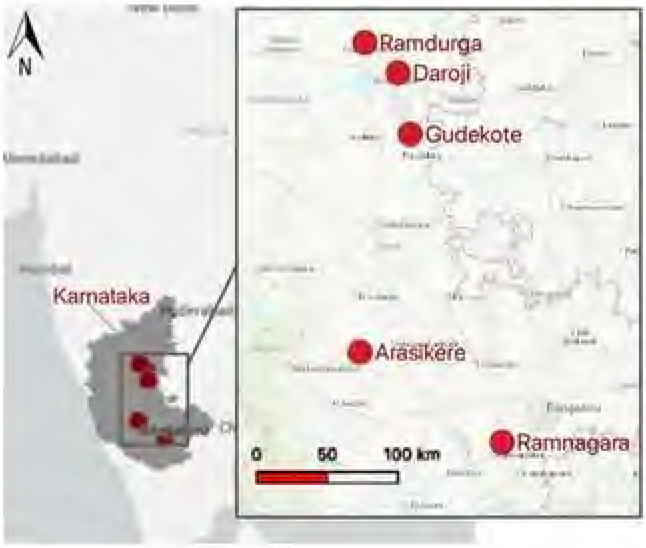


Figure 2: Sloth bear denning sites in eastern Karnataka, India, 2014–2018.

All 5 study areas are relatively small island habitats, ranging from 79.2–326.5 km^2, surrounded by agricultural fields (Table 1). Additionally, smaller habitat patches, ≤2.25 km^2 and located within 1 km of the main (large contiguous) habitat, were associated with and protected as part of the study areas. These natural habitats still exist because they were too rocky and rugged to be converted to farmlands. Each area is very rocky, and large boulders are common, which is also why caves are so prevalent in the area (Figure 3). Major vegetation types included scrub forest, thorn forest, and dry deciduous forest (Champion and Seth 1968), dominated by acacias (*Acacia* spp.), silk plants (*Albizzia* spp.), cassias (*Cassia* spp.), and ziziphus (*Ziziphus* spp.). These areas support a high number of sloth bears, leopards, and other mammals including rusty spotted cat (*Prionailurus rubiginosus*), jungle cat (*Felis chaus*), Indian boar (*Sus scrofa cristatus*), Indian crested porcupine (*Hystrix indica*), and Indian hare (*Lepus nigricollis*).

Figure 3: Sloth bear habitat in the study area in eastern Karnataka, India, 2018.

Table 1: Sloth bear denning study areas that we surveyed in the state of Karnataka on or near the Deccan Plateau, India, 2014–2018.

Study area	Protection level	Area (km^2)	Main land cover
Gudekote	Sanctuary	163.0	Scrub forest
Daroji	Sanctuary	82.2	Scrub forest-dry deciduous
Arasikere	Reserved forest	108.7	Dry deciduous
Ramnagara	Reserved forest	161.1	Scrub forest-dry deciduous
Ramdurga	Reserved forest	79.2	Scrub forest

Methods

Field methods and geospatial analysis

We located sloth bear dens throughout the duration of the study by interviewing local residents and people familiar with the forests in the area and using stratified random grids through each study area, searching for caves. Grid size and the distance between survey transects varied greatly between study areas and within study areas, based on the potential for caves on the landscape. We identified sloth bear dens from the presence of bear sign (e.g., scats, digging, claws marks) located in or around the mouth of the putative den. We set up remote motion-sensitive cameras near caves with sloth bear sign to discern whether the bear was using the cave as a resting or maternal den. We considered only caves documented as having a mother bear with ≥1 cub as maternal dens (Figure 4). We used remote cameras at maternal dens to document activity around the den. We documented all den sites and recorded their global position system locations and the physical dimensions of dens including the slope, the entrance width and height, and the tree and shrub species within a 20-m radius of the den.

Figure 4: Sloth bear mother with 2 cubs returning to their maternal den under a large boulder in the study area in Karnataka, India, 2016.

We imported den locations into ArcGIS Pro (Esri, Redlands, CA, USA) and created feature classes for each den type. We obtained a digital vegetation land cover map of India (Roy et al. 2015) with 60-m resolution to describe the vegetation types in which dens occurred. This map also included a value for the vegetation fraction. Vegetation fraction, or the total areal cover of vegetation within a 1-km area surrounding a given location (e.g., known den site or randomly generated location) ranged from 0.0 to 1.0. A vegetation fraction value of 0.5 indicates that approximately half the land is covered by vegetation in a 1-km square centered on that point. Using digital elevation maps (50-m resolution), we generated slope, aspect, elevation, and ruggedness values for each raster cell. Aspect was reported in values from 0 to 360. We re-sampled aspect into 8 categorical values: N, NE, E, SE, S, SW, W, and NW. We derived slope (range = 0 to 90°) and elevation (m) from digital elevation models. We calculated ruggedness values (Nellemann and Thomsen 1994) for each cell using a 5 × 5 grid centered on the cell containing the den site on the elevation layer. We used the standard deviation of those 25 grid cells around each point to indicate the degree of elevational variation among cells surrounding each den site, providing a general idea of ruggedness in the vicinity of the den. The higher the ruggedness value, the more rugged the landscape (0 = flat). We categorized the level of protection for each of the 5 study areas as a 1 for a wildlife sanctuary or a 2 for a reserved forest. We digitized forest borders around each study area and calculated the distance from dens to these borders in ArcGIS Pro using the distance-to-feature tool. We calculated the distance to agricultural fields and settlements for each den site using the same distance-to-feature tool and the Roy et al. (2015) vegetation map, which also had human settlements as a land classification. Using ArcGIS Pro's random point generation tool, we created 10 times more random points than actual den sites for each class of den within each study area, representing non-selected locations for dens by sloth bears. We used a minimum of200 random points or 10 times the number of dens when the number of dens approximated 200. We excluded random points in areas of non-habitat (e.g., water, agricultural fields, roads, or villages); hence, the number of random point was either <200 or <1,000 depending on the number of dens in the study area and less the non-habitat locations. We used these results for each grouping of maternal, resting, and random points with 9 potential explanatory variables in Program R (R Core Team 2020) to conduct fixed-effects logistic regression and model selection to determine factors related to sloth bear den site selection.

Statistical analysis

We analyzed maternal and resting den data at the population level (i.e., second-order habitat selection; Johnson 1980) in a use-availability analysis and present the relative selection probabilities associated with those variables(Manly et al. 2002). We used fixed-effects models where the response variable was coded as use (den site, 1) or random (0). Our set of *a priori* models were based on a set of working hypotheses as to how sloth bears assess and choose habitats for maternal and resting dens. We used Akaike's Information Criterion adjusted for small sample sizes (AIC_c) and AIC_c weights (ω_i) to rank models and identify a best approximating model (lowest AIC_c value) for maternal and resting dens (Akaike 1973, Burnham and Anderson 2002).

Prior to model development, we evaluated explanatory variables for collinearity and did not include any variables in the same model where $|r| > 0.60$. Our list of explanatory variables included distance to study area (forest) boundaries, elevation, aspect, slope, ruggedness, vegetation fraction, distance to settlements, protection level, and land cover type. In the event of model-selection uncertainty, we obtained model-averaged estimates of β coefficients across all models with $\omega_i \geq 0.05$. We screened final model tables with the R package MuMIn (Bartoń2017) to identify any with uninformative parameters (Arnold 2010). To visualize patterns, we plotted relative selection coefficients against explanatory variables identified as influencing den site selection. We used the MASS (Venables and Ripley 2002) library in version 4.0.0 of program R (R Core Team 2020) and the glm function to perform the analyses.

Finally, we used the 2-sample *t*-test to assess the equality of sample means for site variables from maternal and resting den locations. We also use the 2-sample *t*-test to assess the equality of sample means for site variables for maternal and resting dens in wildlife sanctuaries and reserved forests. We used an alpha level of significance of 0.05.

Results

From 2014 to 2018, field crews located 420 dens in the 5 study areas, including 40 maternal and 380 resting (Table 2). Resting dens were farther from refuge boundaries than maternal dens ($\bar{x}$ = 569 m vs. 364 m, t = 2.741, P < 0.003). Resting dens occurred at higher elevations than maternal dens ($\bar{x}$ = 788 m vs. 707 m; t = 3.195, P < 0.003). The slopes selected by sloth bears for resting ($\bar{x}$ = 14.6°) and maternal ($\bar{x}$ = 13.1°) dens did not differ (t = 1.130, P = 0.130). Similarly, the ruggedness of terrain used for resting dens and maternal dens was not different(t = 0.593, P = 0.277). Den site vegetation fraction differed (t = 2.414, P = 0.008) between resting ($\bar{x}$ = 0.374) and maternal ($\bar{x}$ = 0.327) dens, with resting den site selection showing more diverse sites being chosen than those used for maternity dens. Mean aspects for resting and maternal dens did not vary (183° vs. 193°, t = −0.592, P = 0.277); however, model selection indicated a relationship between aspect and both resting and maternal dens (see below). Resting and maternal den sites were farther from the habitat boundaries in wildlife sanctuaries than they were in reserved forests (568 m vs. 363 m, t = 2.741, P = 0.003).

Table 2: The number of Sloth bear maternal and resting den sites documented in each of the 5 study sites inthe state of Karnataka on or near the Deccan Plateau, India, 2014–2018. We also present the number of random den sites used in logistical regression analyses.

Study area	Number of maternal dens	Number of maternal denrandom points	Number of resting dens	Number of resting denrandom points
Arisikere	4	192	96	978
Ramnagara	10	165	147	873
Gudekote	9	175	36	874
Daroji	4	191	38	962
Ramdurga	13	188	63	919
Totals	40	911	380	4,606

The best fit model approximating maternal den site selection included distance to forest border, protection level, site aspect, slope, and elevation (weight = 49%; Table 3). Elevation, aspect, slope, ruggedness, and vegetation fraction were positively correlated with maternal denning probability, and distance to forest border and level of protection were negatively associated with maternal denning (Figure 5) based on model-averaged estimates. Vegetation fraction and elevation were correlated so we did not include them in the same models (Table 3). Because of the size differences of the study areas, the relationship between probability of a maternal den and distance to forest borders was not apparent when the data from all 5 study sites was pooled; however, when we analyzed them separately there was a negative relationship (Figure 5E).

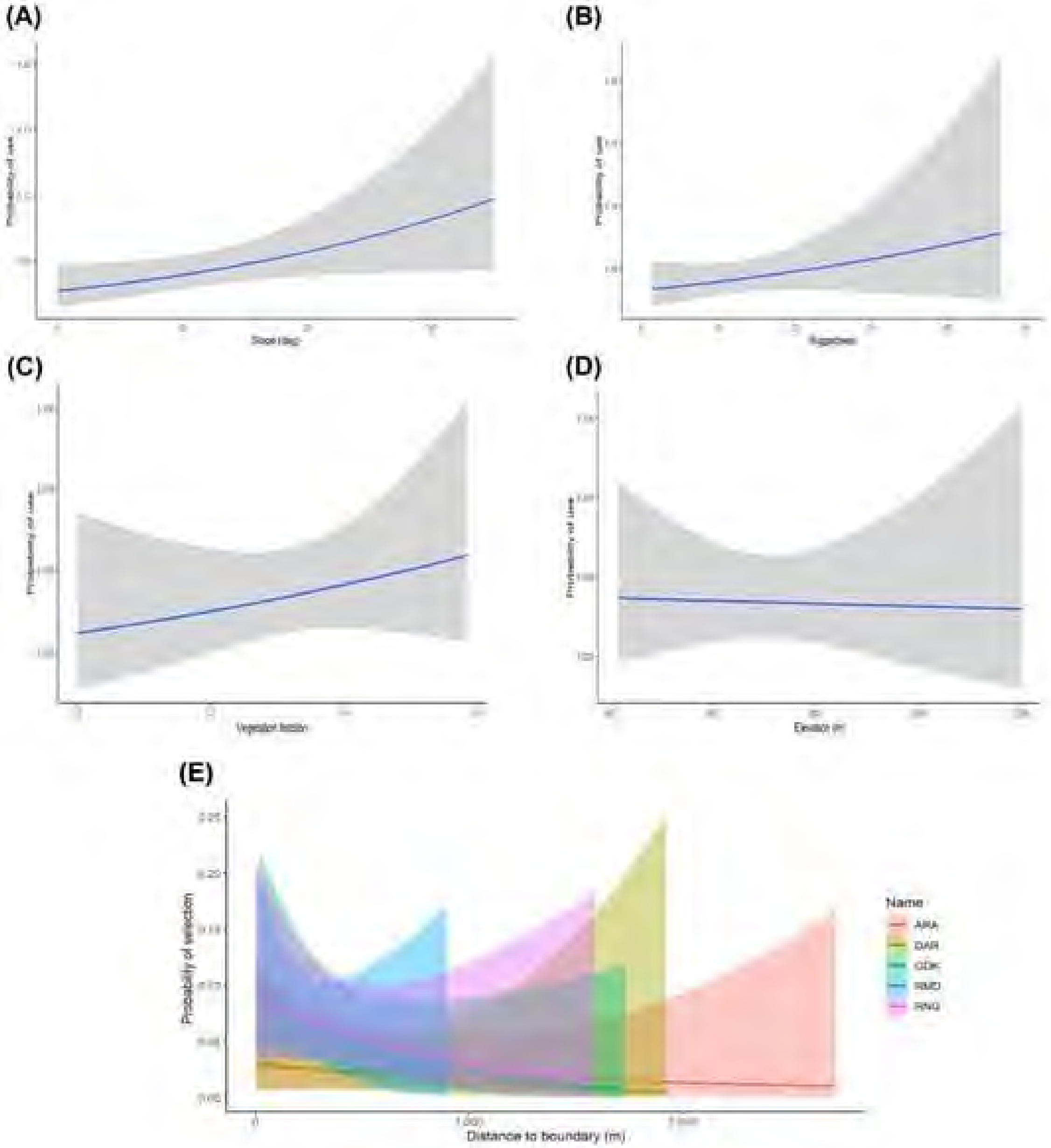


Figure 5: Probability of sloth bear maternal denning in eastern Karnataka, India, 2014–2018, as a function of A) slope, B) ruggedness, C) vegetation fraction, D) distance to settlements, and E) distance to forest border.

Table 3: The *a priori* models used to investigate factors influencing sloth bear maternal den site selection documented between 2014 and 2018 on or near the Deccan Plateau, India. For each model, we present the number of model parameters (*K*), Akaike's Information Criterion adjusted for small sample sizes (AIC_c), AIC_c relative to the most parsimonious model (ΔAIC_c), Akaike weight (w_i), and log-likelihood (L-L).

Model	K	AIC_c	ΔAIC_c	w_i	L-L
Distance to border + protection level + aspect +slope + elevation	6	321.4	0.00	0.490	−145.43
Distance to border + protection level + aspect + slope +vegetation fraction	6	322.6	1.22	0.265	−146.04
Distance to border + protection level + aspect + slope +ruggedness + vegetation fraction	7	324.6	3.29	0.095	−146.03
Distance to border + protection level + aspect + ruggedness +vegetation fraction	6	327.0	5.68	0.029	−148.27
Distance to border + slope	3	327.5	6.18	0.022	−159.75
Distance to border + aspect	3	328.1	6.78	0.017	−153.95
Distance to border + protection level + aspect + slope	5	328.3	6.97	0.015	−149.94
Aspect + slope + vegetation fraction	4	328.7	7.35	0.012	−153.22

The best fit model to approximate resting den site selection included distance to forest border, site aspect, slope, level of protection, and ruggedness (weight = 48%; Table 4). The second-ranked model included the same variables but without distance to forest borders or protection level and accounted for 20% of the Akaike weight. Elevation, ruggedness, slope, and vegetation fraction were positively related to resting den site probability based on model-averaged estimates, whereas the distance to settlements was negatively correlated (Figure 6). We also saw an affinity for the aspects of north and northeast for resting dens, and south for maternal dens (Figure 7). The relationship between probability of a resting den and distance to forest borders, when the data from all 5 study areas was pooled, did not exhibit a clear relationship because of the size differences of the study areas; however, when we split out the 5 study areas, a relationship was clear (Figure 6F). Those resting dens in better protected areas, Daroji and Gudekote, were positively associated with distance from forest border; that is, the farther from the forest border, the more likely a resting den was bound to occur. Vegetation fraction and elevation were correlated so we did not include them in the same models (Table 4).

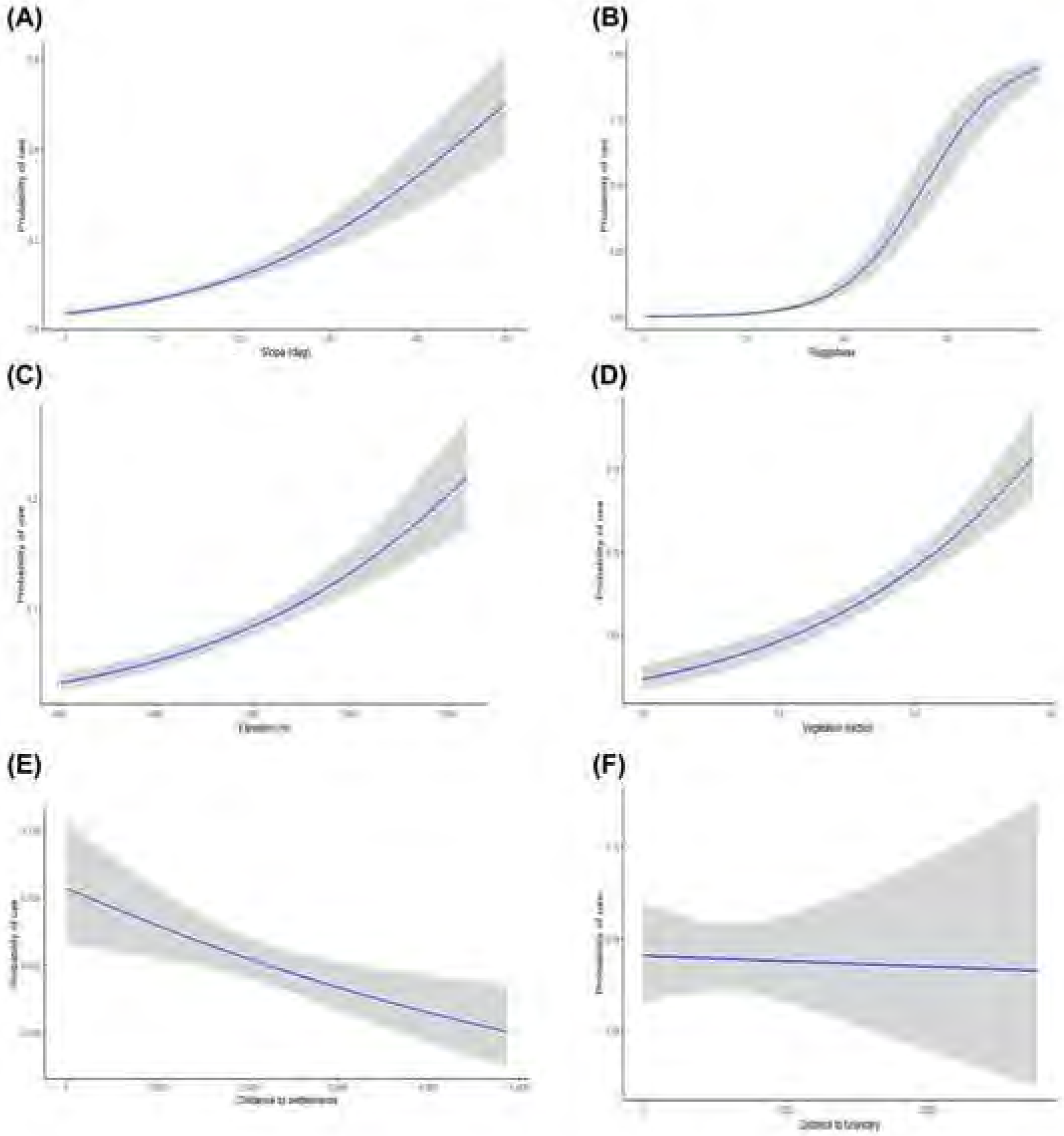


Figure 6: Probability of sloth bear resting den site selection in eastern Karnataka, India, 2014–2018, as a function of A) slope, B) ruggedness, C) elevation, D) vegetation fraction, E) distance to settlements, and F) distance to forest border relative to all.

Table 4: The *a priori* models used to investigate factors influencing sloth bear resting den site selection documented between 2014 and 2018 on or near the Deccan Plateau, India. For each model, we present the number of model parameters (K), Akaike's Information Criterion adjusted for small sample sizes (AIC_c), AIC_c relative to the most parsimonious model (ΔAIC_c), Akaike weight (w_i), and log-likelihood (L-L).

Model	K	AIC_c	ΔAIC_c	w_i	L-L
Distance to border + protection level + ruggedness + aspect + slope +vegetation fraction	7	266.9	0.00	0.476	−117.403
Aspect + slope + ruggedness + vegetation fraction	5	268.6	1.71	0.202	−122.281
Distance to border + protection level + aspect + slope + ruggedness +vegetation fraction + distance to settlements	8	268.8	1.92	0.182	−117.355
Distance to border + protection level + aspect + slope + ruggedness	6	270.3	3.43	0.086	−120.123
Distance to border + protection level + aspect + slope + ruggedness + elevation	7	271.9	4.97	0.040	−119.888
Aspect + ruggedness	3	273.9	6.97	0.015	−119.883

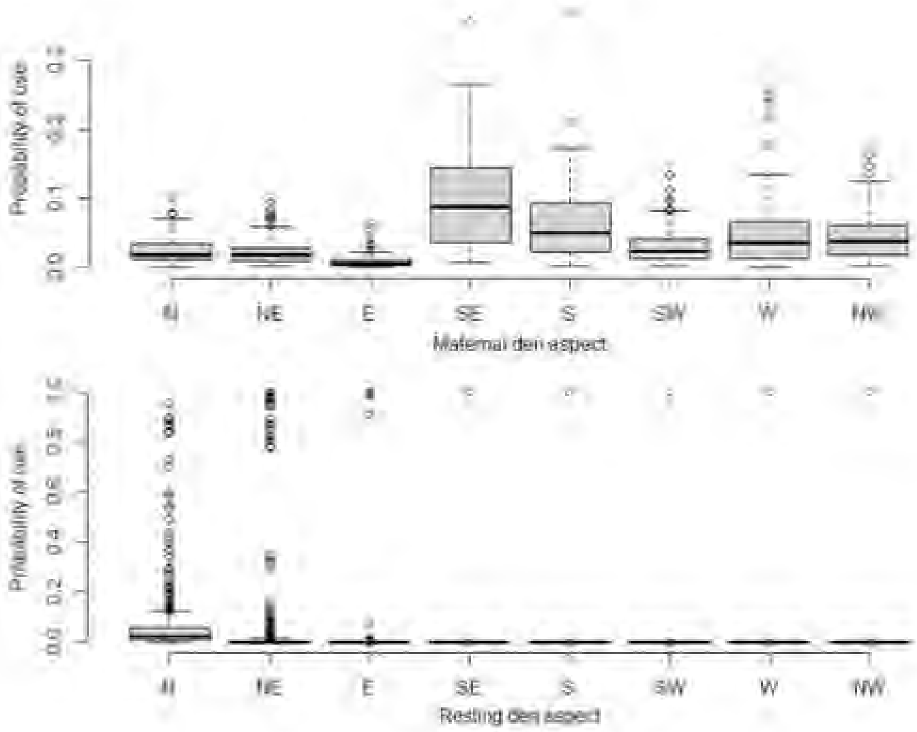


Figure 7: Aspect of slope and probability of sloth bear denning use in eastern Karnataka, India, 2014–2018.

Small habitat patches, ≤2.25 km^2 within 1 km of the main (large contiguous) habitats, were used as locations for maternal and resting dens. A higher percentage of maternal dens, compared to resting dens, were located on these small habitat patches (Table 5).

Table 5: Sloth bear denning selections, resting and maternal, within habitat patches on or near the Deccan Plateau, India, 2014–2018. Small habitat patches are defined as areas ≤2.25 km^2 and within 1 km of a larger contiguous habitat, in eastern Karnataka, India.

Study area	Number of maternal dens	Number of maternal den in small habitat patches	Number of resting dens	Number of resting den in small habitat patches
Arisikere	4	2	96	10
Ramnagara	10	4	147	28
Gudekote	9	0	36	0
Daroji	4	0	38	0
Ramdurga	13	8	63	1
All	40	14	380	39

Discussion

Landscape location key variables

Higher elevations and steeper slopes were positively related to denning probability for resting and maternal dens. Itis probable that these 2 variables offer a level of protection from potential predators and humans, especially when bears den relatively close to human activity. Resting dens occurred at higher elevations than maternal dens. This may be a spurious result in that the distance to the edge of the protected habitat may be the main variable dictating where maternal dens are located; all study areas concurrently increased in elevation as distance to boundaries increased. Den site vegetation fraction varied significantly between resting and maternal dens; resting dens appear to be chosen in areas with more vegetation diversity. The reason for this is unclear, though it could be related to mother bears choosing less desirable areas to avoid male bears.

The apparent predilection for sloth bears, without cubs, to choose north-facing slopes may be related to a decrease in temperature during harsh summers. Temperate bears that hibernate frequently choose south-facing slopes, which may be related to an increase in ambient temperature during harsh winters (Reynolds et al. 1976) or for increased snow load, which helps with insulation (Sorum et al. 2019, Gonzalez-Bernardo et al. 2020). Mother sloth bears may select dens that are less appealing to single bear

(Joshi et al. 1995). Sexual segregation occurs in grizzly bears and is generally associated with the risk of infanticide (Wielgus and Bunnell 1994, Libal et al. 2011,Penteriani et al. 2020).

Distance to habitat edges: maternal dens versus resting dens

Our 5 study areas are each forest patches bordered by farmlands and orchards that host human activity. These protected forested refuges exist as islands in a sea of agriculture. Within these island habitats, maternal dens were significantly closer to forest borders (habitat edges) than resting dens. We postulate 3 theories for this observation. First, by moving near the forest border of the study areas, mother bears reduce the threat of infanticide by large male bears, which are known to avoid human activity (Nevin and Gilbert 2005). Second, by denning near habitat edges, mother bears reduce the threat of predation by leopards and other predators, which also avoid human activity (Naha et al. 2021). Third, areas near the forest border are favored because they are closer to anthropogenic foods and agriculture-associated (i.e., irrigation) water sources.

Infanticide has been reported in many bear species (LeCount 1987, Derocher and Wiig 1999); however, infanticide has not been widely reported in sloth bears and is a rare occurrence (Joshi et al. 1999). Females with cubs have been reported to avoid males by being more active in the late morning rather than the night when adult bears without cubs, male and female, were most active (Joshi et al. 1999). Thus, having some spatial separation from large male bears may also reduce the risk of infanticide. Grizzly bear females with dependent young, avoided adult males through a combination of behavioral strategies, including limiting movements to a few habitat patches after emergence in the spring, and limiting activity between midnight and mid-morning (Gardner et al. 2014). In Slovakia, brown bear family groups used daybeds significantly closer to human settlements than did males or females without cubs, presumably to avoid the risk of infanticide (Skuban et al. 2018). Although the risk of infanticide may be a factor determining habitat selection for forest edges by sloth bear mothers with cubs, it is likely not the primary factor.

Predation, however, is an omnipresent risk for sloth bears. Historically, sloth bears overlapped with Bengal tigers throughout most, if not all, of their range and still do in protected parklands. Tigers prey on sloth bears (Reddyet al. 2004), and are a threat to adult bears, juveniles, and cubs. Leopards, which occur in each of our study areas, though not a threat to adult sloth bears, are a threat to juveniles and young adults. Therefore, it seems plausible that family groups denning at the edges of habitat patches are, at least partly, attempting to avoid predators.

The importance of proximity to food from resting den sites was reported by Akhtar et al. (2004, 2007) in a disturbed forest area in central India; roughly 60% of resting dens occurred within 1 km of human habitation, and 96% of them were within 1.25 km of human habitation, which was predominately agricultural fields. This distribution was assumed to be largely due to degraded habitat within forested areas and the availability of food in agricultural areas. Home ranges of grizzly bears with cubs were smaller than that of female grizzlies without cubs, and more movement is linked to greater risk to cubs (Edwards et al. 2013, Gardner et al. 2014). We suggest sloth bears exhibit the same behavior, partly explaining the selection of maternal den sites near agricultural areas.

Choosing a maternal den site near farmlands, and thus human activity, is a high risk yet high reward venture. There is an abundance of resources close to forest borders, but humans and associated hazards, such as snares, explosive devices hidden in food, and open wells and roads, are likely the most immediate threat to this species, especially where there are no tigers (Arun et al. 2018*b*, 2021, 2022).

We conclude that the advantages of denning near anthropogenic food and water outweighs the risks for many family groups, at least for those in eastern Karnataka (our study areas). Clearly, selecting den sites close to forage reduces a mother's time away from her cubs during those solo forays, and allows the family group to stay relatively close to the safety of the den during the initial cub outings. Grizzly bear cub survival rates in the interior of Alaska increased if the mother bear stayed within 1 km of her den during the den-influenced period, between emergence and the onset of green-up (Gardner et al. 2014). Sloth bears apparently also mitigate the odds of direct conflicts with humans with crepuscular and nocturnal activity patterns, though a study in Royal Chitwan National Park (Joshiet al. 1999) reported that mothers with cubs were more likely to be active in the late morning hours than single bears.

The use of small habitat patches, ≤2.25 km^2 within roughly 1 km of a larger habitat, for resting dens is not as surprising as the relatively high use of them for maternal dens. The use of these small habitat patches for location of maternal denning are for primarily the same reasons that preparturient bears generally chose dens closer to habitat edges than a typical bear would for a resting den. These habitats provide separation from large males, lessens the likelihood of encounters with predators, and provide easier access to food and water; however, the human disturbance risks associated with denning in a small habitat patch are likely still high.

Management Implications

Sloth bears that persist on relatively small, protected habitat patches in eastern Karnataka are generally denning closer to forest edges than expected. Denning near forest edges may be particularly attractive to pregnant sloth bears and family units. Thus, naturally occurring caves and other denning structures near forest edges may have more inherent value to sloth bears than such sites in forest interiors and we recommend they be protected. Bears foraging outside of protected areas are exposed to anthropogenic risks such as snares, explosives, open wells, and even roads. Thus, we recommend those risks should be mitigated through methods such as snare patrols near forest borders or having wildlife crossings where roads occur. Our results suggest that in better protected areas, sloth bears will den farther away from forest edges.

Acknowledgments

We thank anonymous reviewers for their thoughtful comments regarding this manuscript. We are grateful to the Forest Department of the State of Karnataka for their support in our research efforts. We acknowledge R. Puspanathan, B. Govindaswamy, and Y. Pannerselvam for persistent and extensive data gathering to make this publication possible. We also thank S. Stephens for her help in preparing some of the figures. This study was supported by the American Zoological Association (AZA) Bear Taxon Advisory Group and partially financed by Utah's Hogle Zoo, Woodland Park Zoo, Little Rock Zoo, Capron Park Zoo, Cleveland Metroparks Zoo, Fresno Chaffe Zoo, Puget Sound AAZK Chapter, Milwaukee County Zoo AAZK Chapter, Lee Richardson Zoo, San Diego Zoo Global, San Antonio Zoo, and Reid Park Zoo.

References

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pages 267–281 *in* B. N. Petrovand F. Caski, editors. Proceedings of the Second International Symposium on Information Theory. Akademiai Kiado, Budapest, Hungary.

Akhtar, N., H. S. Bargali, and N. P. S. Chauhan. 2004. Sloth bear habitat use in disturbed and unprotected areas of Madhya Pradesh, India. Ursus 15:203–211.

Akhtar, N., H. S. Bargali, and N. P. S. Chauhan. 2007. Characteristics of sloth bear day dens and use in disturbed and unprotected habitat of North Bilaspur Forest Division, Chhattisgarh, Central India. Ursus 18:203–208.

Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's information criterion. Journal of Wildlife Management 74:1175–1178.

Arun, A. S., T. Sharp, H. Pillai, S. Swaminathan, K. Satyanarayan, and G. Seshamani. 2018*a*. Sloth bear (*Melursus ursinus*) maternity denning at the Wildlife SOS Bannerghatta Bear Rescue Centre, India. International Zoo Yearbook 52:1–10. https://doi.org/10.1111/izy.12179

Arun, A. S., T. Sharp, S. Swaminathan, Y. Pannerselvam, K. Satyanarayan, and G. Seshamani. 2022. Sloth bears and anthropogenic risks in Karnataka, India. Ursus 33:1–8. https://doi.org/10.2192/URSUS-D-21-00005.2

Arun, A. S., S. Swaminathan, Y. Panerselvam, T. Sharp, S. Stephens, K. Satyanarayan, and G. Seshamani. 2021. Relocation of a GPS collared conflict sloth bear *Melursus ursinus* (Mammalia: Carnivora) in Karnataka, India. Journal of Threatened Taxa 13:17856–17864.

Arun, A. S., S. Swaminathan, T. Sharp, Y. Panerselvam, K. Satyanarayan, and G. Seshamani. 2018*b*. Use of small explosives are a risk to sloth bears in India. International Bear News 27(2):13–14.

Barton´, K. 2017. MuMIn: multi-model inference. https://CRAN.R-project.org/package=MuMIn

Baskaran, N., S. Venkatesh, S. K. Srivasthava, and A. A. Desai. 2015. On the behavioural ecology of sloth bear (*Melursusursinus* Shaw 1791) in Mudumalai Wildlife Sanctuary, Western Ghats, India. Animal Diversity, Natural History and Conservation 5:313–333.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer, New York, New York, USA.

Champion, H. G., and S. K. Seth. 1968. A revised survey of the forest types of India. Government of India Press, Delhi, India.

Ciarniello, L. M., M. S. Boyce, D. C. Heard, and D. R. Seip. 2005. Denning behavior and den site selection of grizzly bears along the Parsnip River, British Columbia, Canada. Ursus 16:47–58.

Derocher, A. E., and Ø. Wiig. 1999. Infanticide and cannibalism of juvenile polar bears (*Ursus maritimus*) in Svalbard. Arctic52:307–310.

Desai, A. A., N. Bhaskaran, and S. Ventaktesh. 1997. Behavioural ecology of the sloth bear in Mudumalai Wildlife Sanctuary and National Park. Tamil Nadu and Bombay Natural History Society collaborative project, Mumbai, India.

Edwards, M. A., A. E. Derocher, and J. A. Nagy. 2013. Home range size variation in female arctic grizzly bears relative to reproductive status and resource availability. PLoS One 8:e68130.

Eisenberg, J. F., and M. C. Lockhart. 1972. An ecological reconnaissance of Wilpattu National Park, Ceylon. Smithsonian Contributions to Zoology 101:1–119.

Gardner, C. L., N. J. Pamperin, and J. F. Benson. 2014. Movement patterns and space use of maternal grizzly bears influence cub survival in Interior Alaska. Ursus 25:121–138.

Garshelis, D., A. Joshi, and D. Smith. 1999. Estimating density and relative abundance of sloth bears. Ursus 11:87–98. Gonza´lez-Bernardo, E., L. F. Russo, E. Valderra´bano, A. Ferna´ndez, and V. Penteriani. 2020. Denning in brown bears. Ecology and Evolution 10:6844–6862.

Jacobi, E. F. 1975. Breeding sloth bears in Amsterdam Zoo. Pages 351–356 *in* R. D. Martin, editor. Breeding endangered species in captivity. Academic Press, London, United Kingdom.

Johnson, D. H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology61:65–71.

Joshi, A. R., D. L. Garshelis, and J. L. D. Smith. 1995. Home ranges of sloth bears in Nepal: implications for conservation. Journal of Wildlife Management. 59:204–214.

Joshi, A. R., J. L. D. Smith, and D. L. Garshelis. 1999. Sociobiology of the myrmecophagus sloth bear in Nepal. Canadian Journal of Zoology 77:1690–1704.

Laurie, A., and J. Seidensticker. 1977. Behavioural ecology of the sloth bear (*Melursus ursinus*). Journal Zoological Society London 182:187–204.

LeCount, A. L. 1987. Causes of black bear cub mortality. International Conference on Bear Research and Management7:75–82.

Libal, N. S., J. L. Belant, B. D. Leopold, G. Wang, and P. A. Owen. 2011. Despotism and risk of infanticide influence grizzly bear den-site selection. PLoS ONE 6:e24133.

Manly, B. F. J., L. L. McDonald, D. L. Thomas, T. L. McDonald, and W. P. Erickson. 2002. Resource selection by animals: statistical design and analysis for field studies. Kluwer Academic, Boston, Massachusetts, USA.

Naha, D., S. K. Dash, C. Kupferman, J. C. Beasley, and S. Sathyakumar. 2021. Movement behavior of a solitary large carnivore within a hotspot of human-wildlife conflicts in India. Scientific Reports 11:3862.

Nellemann, C., and M. G. Thomsen. 1994. Terrain ruggedness and caribou forage availability during snowmelt on the arctic coastal plain, Alaska. Arctic 47:361–367.

Nevin, O. T., and B. K. Gilbert. 2005. Perceived risk, displacement and refuging in brown bears: positive impacts of ecotourism? Biological Conservation 121:611–622.

Penteriani, V., A. Zarzo-Arias, M. del Mar Delgado, F. Dalerum, E. Gurarie, P. P. Torre, T. S. Corominas, V. M. Vazquez,

P. V. Garcia, and A Ordiz. 2020. Female brown bears use areas with infanticide risk in a spatially confined population. Ursus 31:article e2.

Puri, M. A., A. Srivathsa, K. K. Karanth, N. Kumarand, and K. U. Karanth. 2015. Multiscale distribution models for conserving widespread species: the case of sloth bear *Melursus ursinus* in India. Diversity and Distributions 21:1087–1100.

R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reddy, H. S., C. Srinivasulu, and K. Thulsi. 2004. Prey selection by the Indian tiger *Panthera tigris tigris* in Nagarjunasagar Srisailam Tiger Reserve, India. Mammalian Biology 69:384– 391.

Reynolds, H. V., J. A. Curatolo, and R. Quimby. 1976. Denning ecology of grizzly bears in northeastern Alaska. International Conference on Bear Research and Management 3:403– 409.

Roy, P. S., A. Roy, P. K. Joshi, M. P. Kale, V. K. Srivastava, S. K. Srivastava, R. S. Dwevidi, C. Joshi, M. D. Behera,

P. Meiyappan, et al. 2015. Development of decadal (1985–1995–2005) land use and land cover database for India. Remote Sensing, 7:2401–2430.

Schoen, J. W., L. R. Beier, J. W. Lentfer, and L. J. Johnson. 1987. Denning ecology of brown bears on Admiralty and Chichagof Islands. Bears: Their Biology and Management 7:293–304.

Seshamani, G., and K. Satyanarayan. 1997. The dancing bears of India. World Society for the Protection of Animals, London,United Kingdom.

Skuban, M., S. Find'o, and M. Kajba. 2018. Bears napping nearby: daybed selection by brown bears (*Ursus arctos*) in a human-dominated landscape. Canadian Journal of Zoology 96:1–11.

Sorum, M. S., K. Joly, A. G. Wells, M. D. Cameron, and G. V. Hilderbrand. 2019. Den-site characteristics and selection by brown bears (*Ursus arctos*) in the central Brooks Range of Alaska. Ecosphere 10:1–13.

Venables, W. N., and B. D. Ripley. 2002. Exploratory multivariate analysis. Pages 301–330 *in* Modern applied statistics with S. Springer, New York, New York, USA.

Ward, P., and S. Kynaston. 1995. Bears of the world. Blandford, London, United Kingdom.

Wielgus, R. B., and F. L. Bunnell. 1994. Sexual segregation and female grizzly bear avoidance of males. Journal of Wildlife Management 58:405–413.

Art – 286. ASIATIC BLACK BEAR (*Ursus thibetanus*) ATTACKS IN KASHMIR VALLEY, INDIA

Aaliya Mir, Shanmugavelu Swaminathan, Rashid Y. Naqash, **Thomas Sharp** and **Attur Shanmugam Arun**

Abstract

Asiatic Black Bear attacks are reported rarely throughout the majority of their global range; however, this has not been the case in the Kashmir Valley where over the past 20–30 years attacks have been relatively common. There are several causes for the high number of attacks, though the foremost reason likely stems from the conversion of natural habitat to orchards and agricultural fields. Asiatic Black Bears actively crop raid orchards and agricultural areas putting them into close proximity to humans. The Jammu & Kashmir Wildlife Protection Department has collected data on 2,357 Asiatic Black Bear attacks in the Kashmir Valley between 2000 and 2020. A total of 2,243 (95.2%) resulted in injury and 114 (4.8%) resulted in death. The majority of injuries were reported as minor (57.4%, n=1126), 42.4% (n=832) as grievous, and 1.2% (n=21) caused permanent disability. The highest proportion of attacks occurred from July through November, coinciding with the harvesting season, and the least occurred from December through March, coinciding with when most Asiatic Black Bears are hibernating. Victims of bear attacks were most often working in farms or orchards, and were mostly between the ages of 31–40 years old. Most attacks occurred in the morning hours when people first entered the orchards or agricultural fields to work. The frequency of attacks has declined since 2016, which could be attributed to retaliation killings, better management by the wildlife department, and the engagement of NGO's with local people to create bear awareness and teach safety measures.

Keywords: Bear attacks, bear awareness, crop-raiding, hibernation, Jammu & Kashmir, retaliation-killing, victims, wildlife-conflict, Wildlife Protection Department.

Introduction

Asiatic Black Bear *Ursus thibetanus* though generally cryptic and shy, are involved in crop raiding and to a lesser extent attacks on humans (Chauhan2003; Ali et al. 2018; Jamtsho & Wangchuck 2018; Image 1). Attacks are rare throughout much of their global range which encompasses 18 different countries (Garshelis & Steinmetz 2020), however, this is not the case in India's Kashmir Valley where they are relatively common (Chauhan 2003; Choudhury et al. 2008; Tak et al. 2009; Rasool et al. 2010). Bear attacks in the Kashmir Valley have increased in the last 20–30 years, possibly due to (1) expansion of agricultural practices such as fruit and nut orchards (that are particularly attractive to bears), (2) the lack of fire-arms among farmers, (3) the India-Pakistan border fencing blocking predator movement, continued human encroachment into wild habitat, and (4) a new generation of people not familiar with coexisting with large predators (Choudhury et al. 2008). Installations by security forces may also fragment the habitat and divert the bears into human dominated areas causing human-bear conflicts.

The Asiatic Black Bear is listed as 'Vulnerable' on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species due to habitat loss and the commercial trade for live bears and bear parts (Garshelis & Steinmetz 2020). They are also listed as an Appendix I species under CITES and a Schedule I species in India under the Wildlife Protection Act, 1972.There are few published accounts, or long-term data collections, of Asiatic Black Bear attacks. The majority of scientific literature on the topic are from locations with a relatively healthy number of black bears, namely India, Bhutan, and Japan. Often these accounts are listed alongside crop raiding and livestock depredation (Chauhan 2003; Charoo et al. 2011; Sanwal & Lone 2012; Ali et al. 2018; Zahoor et al. 2020). There is still a great deal that is not understood about Asiatic Black Bear attacks. However, most reported attacks are due to surprise encounters that occur in either the woods or in agricultural areas (Tak et al. 2009; Rasool et al. 2010; Akiyama et al. 2017; Penjor & Dorji 2020). As would be expected, the vast majority of attacks appear to be defensive, however, there have been a few accounts that appear to be more predatory (Yamazaki 2017).

Over the past 20 years, the Kashmir Valley has become a hotspot of Human-Asiatic Black Bear conflicts. This paper chronicles the number of Asiatic Black Bear attacks that occurred in the Kashmir Valley between the years of 2000 and 2020. It also looks at the trends and attempts to discern the causes.

Image 1. Wild Asiatic Black Bear Ursus thibetanus in the Kashmir Valley © Mradul Pathak.

Study Area

The Kashmir Valley is roughly 15,500 km^2 in size (about140 km by 32 km) and is located between 32° & 34°N and74° & 75°E (Figure 1). The average elevation is roughly 1,850 m. The valley is partially surrounded to the north by the Himalayan and Pir Panjal ranges, which have an average elevation of roughly 3,050 m. The climate is mild with precipitation occurring throughout the year, though spring is the wettest season. Summer is usually mild and fairly dry, but the relative humidity is generally high and the nights are cool. July is the warmest month with temperatures averaging around 24.4°C, and January is the coldest with average temperatures around2.7°C. The biggest river in the valley is the Jhelum. Oak- Rhododendron forests (Image 2), cover the valleys and Blue Pine *Pinus excelsa* covers the slopes.

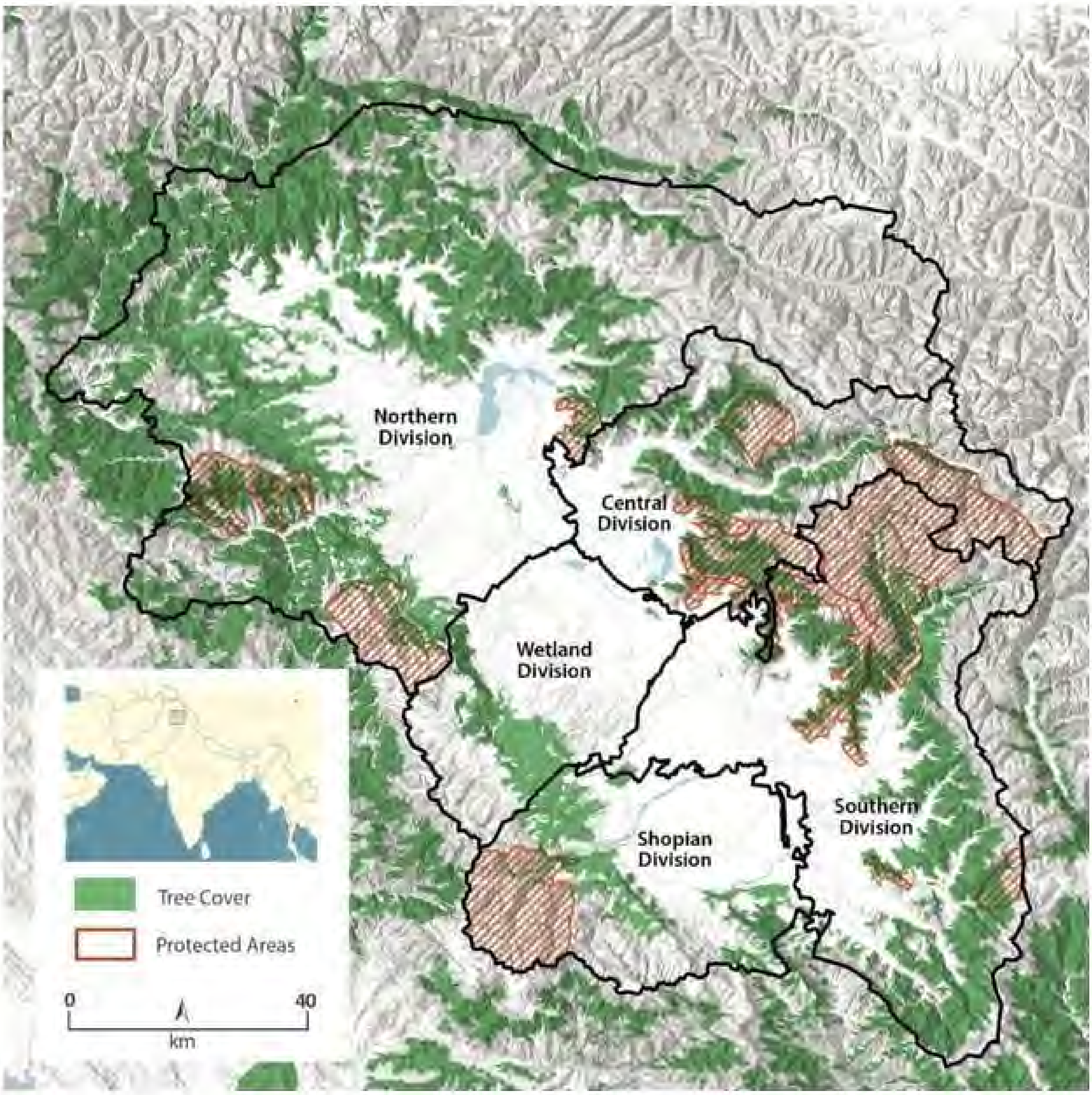


Figure 1. Wildlife divisions in the Kashmir region responsible for handling human-wildlife conflicts including Asiatic Black Bear attacks.

Methods

Asiatic Black Bear attack data was collected by the Jammu & Kashmir Wildlife Protection Department, Kashmir Region, which was established in 1978 and is equivalent to the wildlife wing of the state forest departments in other states. There are five division, namely the Central, South, North, Wetland and Shopian, which maintain data on human-bear conflicts for the purpose of paying compensation for bear attacks. These efforts were intensified, and payment augmented after 2013. We used this data to assess bear attack patterns over time. Ex gratia rates prior to and after 2014 are given in

Table 1. The processing of a case starts with the filing of a police and medical report which is produced to the block level officer of the wildlife department who subsequently forwards it to the higher offices which includes the range officer, wildlife warden, regional wildlife warden, and finally to the chief wildlife warden. The ex-gratia application goes through a lot of scrutiny during processing.

Table 1. Ex gratia paid (in INR) to victims of Asiatic Black Bear attacks prior and post 2014.

Years	Minor injuries	Grievous injuries	Permanent incapacitation	Death
Prior to 2014	5000	Up to 33,000	50,000	100,000
2014-2020	15000	Up to 100,000	Up to 300,000	300,000
* Department of Forest, Ecology and Environment 2014				

Results

Attacks and Deaths by Year

A total of 2,357 bear attacks were reported in the Kashmir Valley between 2000 and 2020, of which 2,243(95.2%) resulted in injury and 114 (4.8%) in death (Table2). The Wildlife Protection Department did not have the resources prior to 2006 to collect detailed bear attack data, and therefore bear attacks prior to 2006 are likely underrepresented in the data set. The maximum number of reported attacks in a single year was 282 in 2010 and included 10 deaths. The number of reported attacks and deaths started diminishing in 2016, and by 2020 the number of attacks was down to 49 with four deaths (Figure 2).

Table 2. Asiatic Black Bear attacks resulting in injury or death between 2006 and 2020 in Kashmir Valley, India.

Year	Number of recorded attacks	% of recorded attacks from the total number of attacks recorded from 2006–2020	Number of recorded deaths	% of recorded attacks that resulted in death the same year
2006	87	3.7	7	8.0
2007	93	4.0	8	8.6
2008	155	6.6	7	4.5

2009	182	7.8	8	4.4
2010	282	12.1	10	3.5
2011	275	11.8	13	4.7
2012	226	9.7	7	3.1
2013	256	10.9	12	4.7
2014	185	7.9	5	2.7
2015	205	8.8.	5	2.4
2016	135	5.8	6	4.4
2017	71	3.0	5	7.0
2018	63	2.7	5	7.9
2019	66	2.8	7	10.6
2020	49	2.1	4	8.2

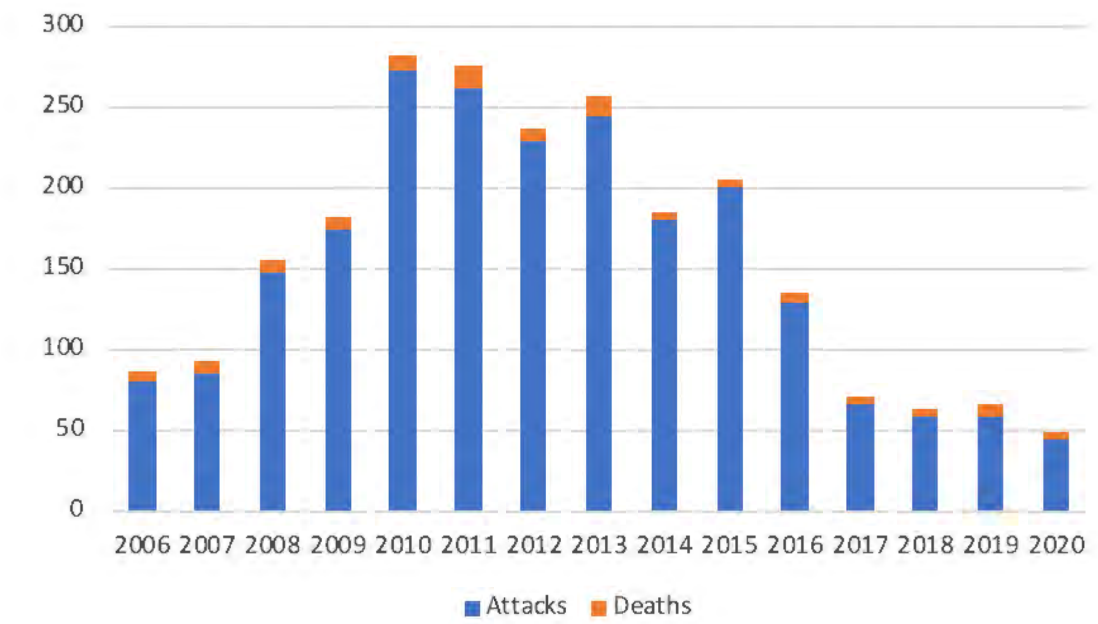


Figure 2. Asiatic Black Bear attacks resulting in injury or death between 2006 and 2020 in and around the Kashmir Valley, India.

Differences Between Districts

The majority of attacks occurred in the South and North divisions. These two divisions are the largest and have the most forest coverage. Additionally, these divisions are undergoing rapid deforestation and urbanization. The Wetland and Shopian divisions used to be part of the North and South divisions, respectively. The Wetland Division does not consist of much prime bear habitat and this results in fewer attacks. The Central Division, which includes Dachigam National Park, has excellent bear habitat. However, the wildlife department in this division is well funded and equipped to deal with human-wildlife interactions and therefore are able to keep bear attacks in check despite the large bear population.

Injuries

The exact type of injuries sustained by the victims were not readily available, however, we were able to classify the injuries in three categories based on reports and the amount of ex gratia paid. The three categories are minor, grievous, and permanent disability. Injuries were considered minor if the victim was treated at a local hospital and did not need to stay in the hospital for more than a day for the treatment (Image 3). Injuries were considered grievous if the victim needed to be referred for special treatment, usually to a specialty hospital, where they can undergo specialized procedures and stay for an extended period (Image 4). Finally, permanent disability when the victims were permanently incapacitated. Overall, the majority of injuries were reported as minor (57.4%, n=1126), 42.4% (n=832) as Grievous, and 1.2% (n=21) resulted in permanent disability.

Image 3. Minor injury due to Asiatic Black Bear. © Wildlife SOS

Image 4. Grievous injury due to Asiatic Black Bear. © Wildlife SOS

Attacks by Month

A total of 1,449 attacks were documented by month (Figure 3). August (n=309, 21.3% of the total attacks) had the most attacks, followed by September (n=203, 14%), October (n=198, 13.7%), and July (n=182, 12.6%). The least number of attacks took place in the month of January (n=26, 1.8%), February (n=32, 2.2%), December (n=44, 3.0%), and March (n=45, 3.1%).

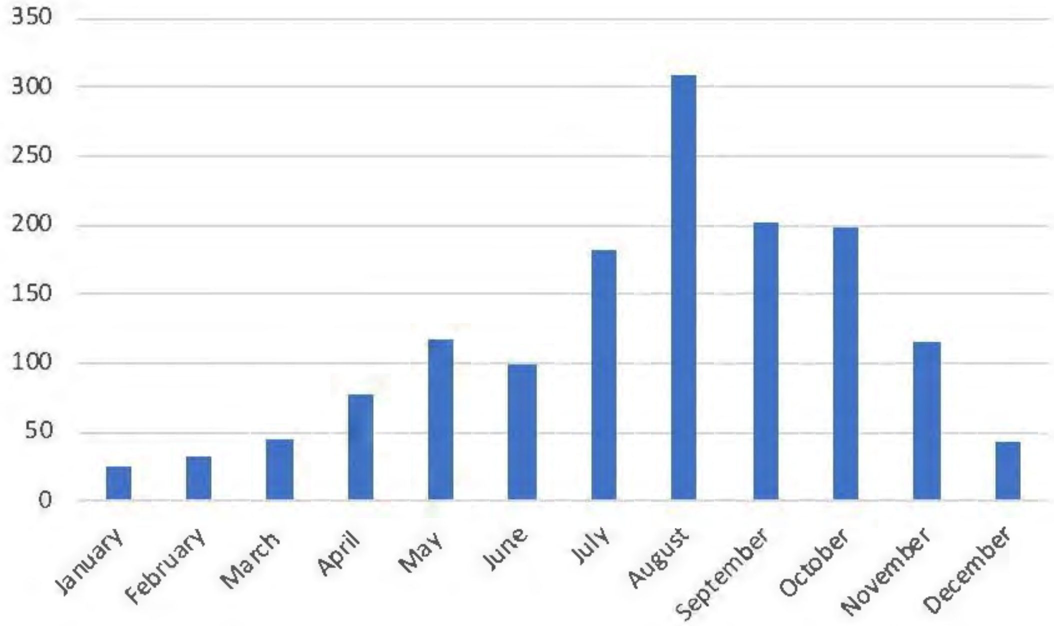


Figure 3. Asiatic Black Bear attacks by month from 2000-2020 in Kashmir, India.

Attacks by Time of Day

A total of 410 attacks were documented by the time of day in which they occurred (Figure 4). The highest number of attacks occurred between the hours of 0901–1000 h (n=75, 18%), and 218 attacks (53%) took place between 0801–1200 h.

Table 3. Asiatic Black Bear attacks by month from 2000–2020 in Kashmir, India.

January	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
26 (1.8%)	32 (2.2%)	45 (3.1%)	78 (5.4%)	118 (8.1%)	99 (6.8%)	182 (12.6%)	309 (21.3%)	203 (14.0%)	198 (13.7%)	115 (7.9%)	44 (3.0%)

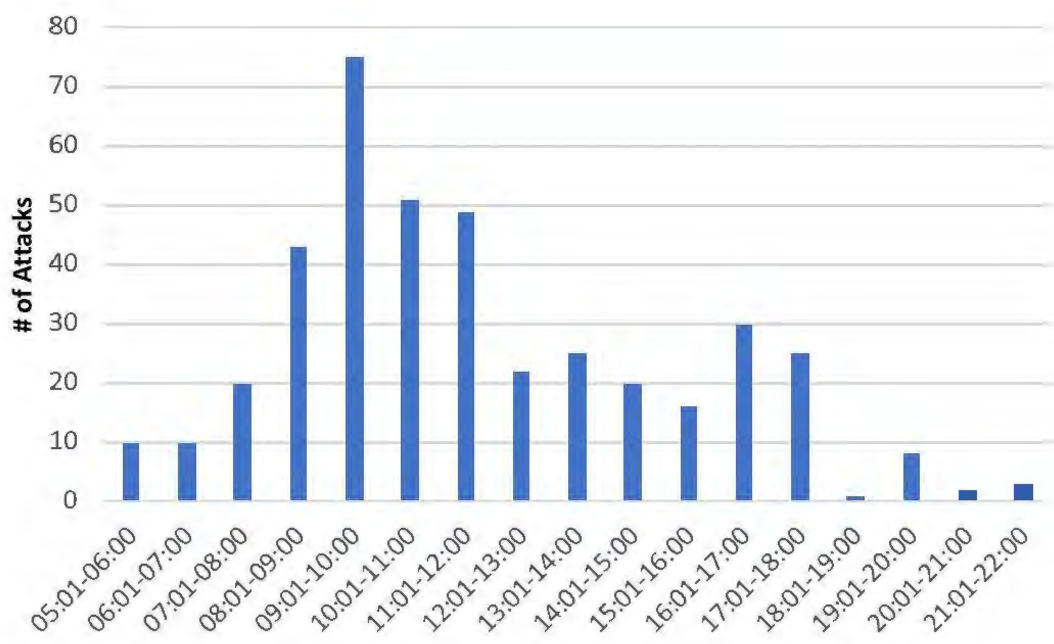


Figure 4. Asiatic Black Bear attacks by time of day from 2000–2020 in Kashmir, India.

Age of People Attacked

A total of 482 attacks were documented by the ageof the victims (Figure 5); 226 of the victims (47%) were between 31 and 50 years of age.

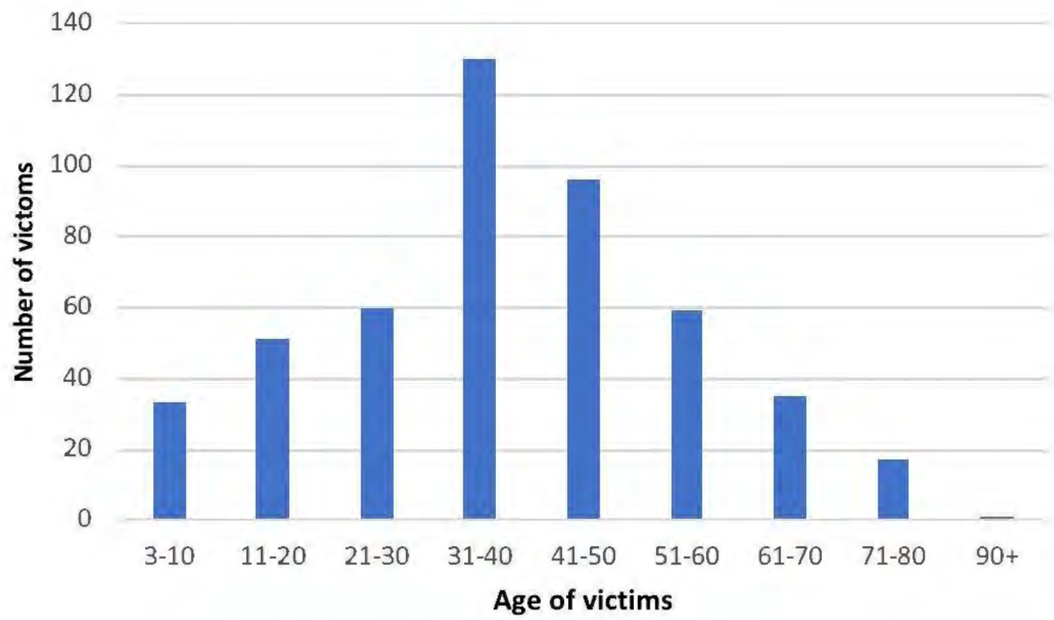


Figure 5. Asiatic Black Bear attacks by age of the victim from 2000–2020 in Kashmir, India.

Activity of People Attacked

The activity of 277 people attacked in the Shopian Division between 2010 and 2019 was documented. People working in, or walking to, fields or orchards made up 176 (63.5%) of the attacks. The second largest group of people attacked were made up of shepherds & herders, and accounted for 33 (11.9%) attacks. People walking toareas not necessarily related to fields or orchards (n=23,8.3%) and people near & around their homes (n=22, 7.9%) also made up relatively high percentages, though many of these people were working in their vegetable gardens. Other activities made up the remainder of the attacks (n=23, 8.3%).

Discussion

General Patterns in Kashmir Valley

There is no data to suggest that the Asiatic Black Bear subspecies, *U. t. laniger* that occurs in northern India, is any more aggressive than other subspecies (Matt Hunt, co-chair IUCN Asiatic Black Bear Expert Team, pers. comm. August 8 2021). It is therefore more likely that the increased number of attacks are related to: 1) bears being in close proximity to humans, 2) a relatively high density of bears in the area, and finally, 3) how humans react to the presence of bears. Along these lines, it is important to note that in orchards, the bears not only eat the fruit and nuts but also potentially do extensive damage to the trees, such as breaking off productive branches. Because of this the bears are often actively chased and shooed away from the orchards. This aggressive interactions between humans and bears could be a contributing factor for the high rate of attacks in the region.

Reasons for the Decreases in Bear Attacks

The decrease in bear attacks since 2016 is likely due to 3 main reasons: 1) a number of bears have been killed in retaliation, 2) proactive work by the wildlife department, and 3) bear awareness programmes conducted by non-governmental organisations (NGOs). The total number of bears killed remains unknown, however, some of these killings have been documented, including incidents when bears have been tied and the tree set on fire. Other bear killings go unnoticed, such as, when bears are secretly poisoned or shot. To date there have been no prosecutions for killing bears.

The wildlife department was able to be much more proactive starting in 2016. The political scenario in Kashmir has been very fragile in recent times, particularly from 2010–2016. Once the wildlife department was up and running, it was still poorly equipped and dealing with frequent closures in the valley. Even communication was hamper as mobile phone connectivity was not steady. These issues paralyzed normal life and resulted in fewer reports of wildlife-human conflicts. Because of this, people often took affairs into their own hands. Since 2016, the wildlife department has had greater man power & the necessary equipment including cages, tranquilizing guns, and vehicles as well as mobile connectivity, to deal with wildlife issues. Presently 42 control rooms work 24hours a day, seven days a week, to attend to the wildlife distress calls. The number of rescue calls to the wildlife department as well as to other NGOs, including Wildlife SOS, has increased, which has led to a more professional handling of human-wildlife conflicts and has reduced the number of bear encounters and injuries to people.

Bear awareness & safety programs are also believed to have played an important role in reducing human- bear conflicts by educating people. These programs are largely being coordinated by NGOs in the region and stress awareness, especially when entering or conducting activities around orchards.

Asiatic Black Bear Attack Overview

The causes and mitigation strategies for Asiatic Black Bear attacks are not well understood, especially in comparison to attacks by other bear species, namely, Grizzly Bears *Ursus arctos*, American Black Bears *Ursus americanus*, Sloth Bears *Melursus ursinus*, and even Polar Bears *Ursus maritimus*. This may be partly due to Asiatic Black Bear attacks being relatively rare. Existing studies tend to agree that the vast majority of Asiatic Black Bear attacks are defensive, most often occurring due to a surprise encounter (Thakur et al. 2007; Taket al. 2009; Rasool et al. 2010; Akiyama et al. 2017). This certainly appears to be the case in Kashmir, India, however, predatory attacks on humans by Asiatic Black Bears have been reported in Japan (Yamazaki 2017;Oshima et al. 2018). This is perhaps not surprising as Asiatic Black Bears are omnivorous and have been reported throughout parts of their range to actively hunt, kill, and eat primates, ungulates, and wild boar (Neas & Hoffman 1987; Hwang 2003; Gursky-Doyen & Nekaris 2007). Predatory attacks on humans appear to be exceptionally rare.

Behavioral approaches to safety in Asiatic Black Bear country should primarily focus on avoiding bear encounters and secondarily surviving defensive attacks with the fewest number of injuries. Making noise while moving into an area that bears may occur, giving the bear a chance to leave the area before the human and bear find themselves at close quarters, is a proven method to avoid attacks by Grizzly & Sloth Bears (Ordiz et al. 2013; Ratnayeke 2014; Sahlén et al. 2015; Sharp et al. 2020). This method would likely be effective in avoiding surprise encounters with Asiatic Black Bears as well.

There are advisories on what to do in case of a defensive attack by a bear. Herrero (2002) advocated falling to the ground and balling up while covering the head and face with your arms for surviving a defensive grizzly bear attack. Asiatic Black Bears, like Grizzly & Sloth Bears, focus on the head and face during an attack (Thakur et al 2007; Rasool et al. 2010). Falling to the ground and covering up allows attack victims to protect themselves from injury while allowing the Asiatic Black Bear to run off which they almost always do after overpowering a person.

Conclusion

The number of Asiatic Black Bear attacks in Kashmir have decreased notably since 2016, probably due to bears being removed from the area as well as government and non-government agencies working to lessen the number of negative encounters. The number of annual attacks should be monitored and tracked to detect future changes. Further studies are required to more fully and accurately understand the best methods to avoid and survive Asiatic Black Bear attacks. It is likely that certain behavioral strategies that work for avoiding or minimizing attacks from other bear species, namely Brown Bears & American Black Bears, will also work for the Asiatic Black Bear. However, this cannot be known with certainty without further research.

References

Akiyama, G., H. Kuwahara, R. Asahi, R. Tosa & H. Yokota (2017). Prompt procedures have a great impact on the consequences of Asiatic Black Bear mauling. *Journal of Nippon Medical School* 84(6): 294–300.

Ali, A., M. Waseem, M. Teng, S. Ali, M. Ishaq, A. Haseeb, A. Aryal& Z. Zhou (2018). Human-Asiatic Black Bear (*Ursus thibetanus*) interactions in the Kaghan Valley, Pakistan. *Ethology Ecology and Evolution* 30(5): 399–415. https://doi.org/10.1080/03949370.2017.1423113

Charoo, S.A., L.K. Sharma & S. Sathya kumar (2011). Asiatic Black Bear- human interactions around Dachigam National Park, Kashmir, India. *Ursus* 22(2): 106–113.

Chauhan, N.P.S. (2003). Human casualties and livestock depredation by black and brown bears in the Indian Himalaya, 1989-98. *Ursus* 14(1): 84-8.

Choudhury, S., M. Ali, T. Mubashir, S.N. Ahmad, M.N. Sofi, I. Mughal, U.K. Sarma, A.K. Srivastava & R. Kaul (2008). Predator Alert - Attacks on humans by leopards and Asiatic Black Bear in the Kashmir valley - Analysis of case studies and spatial patterns of elevated conflict. *Wildlife Trust of India,* 76 pp.

Department of Forest, Ecology and Environment (2014). Payment of ex-gratia relief for deaths/injuries caused to the human life on account of man-animal conflict cases. Government of Jammu and Kashmir, Department of Forest, Ecology and Environment, Secretariat of Srinagar. Government Order No. 244 - FST of 2014

Garshelis, D.L. & R. Steinmetz (2020). *Ursus thibetanus*. IUCN Red List of Threatened Species. Downloaded on January 22, 2022. https:// doi.org/10.2305/IUCN.UK.2008. RLTS.T22824A9391633.en

Gursky-Doyen, S. & K.A.I. Nekaris (2007). Primate anti-predator strategies. Springer Science & Business Media, 404 pp.

Herrero, S. (2002). Bear attacks: their causes and avoidance. Revised edition. Lyons Press, Guilford, Connecticut, USA, 282 pp.

Hwang, M.H. (2003). Ecology of the Asiatic Black Bear and people- bear interactions in Yushan National Park, Taiwan. Ph.D. Thesis, University of Minnesota, 78 pp.

Jamtsho, Y. & S. Wangchuk (2018). Assessing patterns of human- Asiatic Black Bear interaction in and around Wangchuck Centennial National Park, Bhutan. *Global Ecology and Conservation* 8: 183–189. Neas, J.F. & R.S. Hoffmann (1987). "*Burdocas taxicolor*". *Mammalian Species* 277: 1–7. https://doi.org/10.2307/3503907 Ordiz, A., O-G. Støen , S. Sæbø, V. Sahle´n, B.E. Pedersen, J. Kindberg &

J.E. Swenson (2013). Lasting behavioural responses of brown bears to experimental human encounters. *Journal of Applied Ecology* 50: 306–314.

Oshima, T., M. Ohtani & S. Mimasaka (2018). Injury patterns of fatal bear attacks in Japan: A description of seven cases. *Forensic Science International* 286: 14–19.

Penjor, D. & T. Dorji (2020). Circumstances of human conflicts with bears and patterns of bear maul injuries in Bhutan: Review of records 2015–2019. *PLoS One* 15(8): 1–12.

https://doi.org/10.1371/ journal.pone.0237812 Rasool, A., A.H. Wani, M.A. Darzi, M.I. Zaroo, S. Iqbal, S.A. Bashir,

S. Rashid & R.A. Lone (2010). Incidence and pattern of bear maul injuries in Kashmir. *Injury-International Journal of the Care of the Injured* 41: 116–119.

Ratnayeke, S., F.T. Van Manen, R. Pieris & V.S. Pragash (2014). Challenges of large carnivore conservation: sloth bear attacks in Sri Lanka. *Human Ecology* 42(3): 467–479.

Sahle´n V, A. Ordiz, J.E. Swenson & O.-G. Støen (2015) Behavioural differences between single Scandinavian brown bears (Ursus arctos) and females with dependent young when experimentally approached by humans. *PLoS ONE* 10(4): 16. https://doi.org/10.1371/journal.pone.0121576

Sanwal, C.S. & R.A. Lone (2012). An assessment of the Asiatic Black Bear human conflicts in Kupwara District, Jammu & Kashmir, India. *Indian Forester* 138(10): 881–886.

Sharp, T., S. Swaminathan, A.S. Arun, T. Smith, K. Satyanarayan, & G. Seshamani (2020). Sloth bear attacks on the Deccan Plateau of Karnataka, India. *Ursus* 31: 11. https://doi.org/10.2192/ URSUS-D-18-00027.3

Tak, S.R., D.G. Nabi, M.A. Halwai & B.A. Mir (2009). Injuries from bear (Ursus thibetanus) attacks in Kashmir. *Turkish Journal of Trauma & Emergency Surgery* 15: 130–134.

Thakur, J.S., C. Mohan & D.R. Sharma (2007). Himalayan black bear mauling: offense or defense? *American Journal of Otolaryngology* 28(4): 247–250.

Yamazaki, K. (2017). Consecutive fatal attacks by Asiatic Black Bear on humans in Northern Japan. *International Bear News* 26: 16–17.

Zahoor, B., A. Ahmad, R.A. Aziz & M.S. Awan (2020). Damages done by black bear (Ursus thibetanus) in Moji Game Reserve and its surroundings, Leepa Valley, Azad Jammu and Kashmir (Pakistan). *Pakistan Journal of Zoology* 53(1): 217–225. https://doi.org/10.17582/journal.pjz/20170317130336

Report – 287. COLONIZATION OF WILD ELEPHANTS & HUMAN ELEPHANT INTERFACE IN CENTRAL INDIAN LANDSCAPE

Conservation & Conflict mitigation in Forest Divisions of Chhattisgarh state

S. Swaminathan, A. Sha. Arun, S. Ilayaraja, Ashok Kumar, Thomas Sharp, Reegan P, Yogaraj P and **Balasubramaniyan G.**

Complete report was submitted to Chhattisgarh Forest Departmentand and the report can be accessed via email request at research@wildlifesos.org.

Report – 288. DOES SLOTH BEAR (*Melursus ursinus*) **HOLD A UNIQUE INDIVIDUAL IDENTITY? AN EFFORT TO ESTABLISH HUMAN-SLOTH BEAR CONFLICT MITIGATION MEASURES THROUGH INDIVIDUAL RECOGNITION**

Ashish Kumar Jangid

Complete report was submitted to Wildlife SOS by the author and the report can be accessed via email request at research@wildlifesos.org.

Art – 289. ESSENTIALS OF FOOTCARE IN CAPTIVE ELEPHANTS

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

Introduction

Elephants are kept under captive conditions across the world for various purposes. Historical records indicate that peaks in captive elephant management (or more specifically, war elephants) were during the Mauryan period in the 3rd century BC and the Mughal period in the early 17th century, and many elephants were captured for the purpose. For example, during the period 1868-1980, records indicate that 30,000-50,000 wild elephants were captured, especially in the northeastern part of the country. Elephants have been fascinatingly interlaced with Indian culture, tradition, and mythology from time immemorial. Asian elephants are worshiped in India due to their resemblance to Lord Ganesha. They are therefore housed in temples and are utilized in many religious ceremonies. The principal activity of captive elephants depends on the form of management regimen under which they are kept. The forest department maintains elephants mainly for carrying out range of forestry and wildlife activities. The elephants under private ownership are exploited for commercial activities like timber logging, processions and even for begging in the streets. During the year 2009, the Central Zoo Authority placed a ban on use of elephants in zoos and circuses. Indian elephants are placed in Schedule I and Part I of the Indian Wildlife (Protection) Act (1972), conferring it the highest level of protection.

Due to lack of proper elephant husbandry practices and knowledge, non-availability of traditional *mahouts* and experienced veterinarians combined with abuse and over exploitation, the captive elephants suffer from different kinds of health issues that are often fatal. Neglected foot care in captive elephants resulting in traumatic foot conditions remains one of the significant causes of morbidity and mortality. Disseminating necessary foot care and management procedures is the need of the hour.

Elephant foot & limb fact

Thorough knowledge about the unique anatomy of the elephant foot & limb is of paramount importance to recognize the need for foot care in them (Ramsay and Henry, 2001). The foot of an elephant is a masterful piece of evolutionary development, designed with the concept of a graviportality to support the enormous weight of the largest terrestrial mammal. Generally, adult Asian males may weigh around 3.5 to 5.5 tons, and adult females weigh approximately 2.5 to 4.0 tons. Elephants are sub-ungulates; digitigrade on the forefeet (like Hippos and Tapirs) and semi-plantigrade on hind feet. Elephants walk in an ambling way where in the hind foot treads in the print of the forefoot. Elephants cannot trot or gallop due to the almost vertical orientation of their bones. Both forelimbs and hind limbs have slight angulation compared to other mammals. The legs are straight, and the articular surfaces are in line with the axis of the portion. The limbs avoid excess exertion by flexing minimally during locomotion. The bones of the limbs are massive and have a narrow or nil marrow cavity. The radius and ulna are fixed in a prone position. The fibula is separate from the tibia. The elephant leg length increases by lengthening the proximal limb segments but not the distal limb segments. There is a bit of exterior definition of leg segments, and there are no externally identifiable digits. Elephants have nails instead of hooves and most of the elephants have 18 nails, 5 nails on each front foot and 4 in the hindfeet. Twenty nails are very rare (5 nails on each fore and hindfoot), which is considered very auspicious according to Gajasastra. The footpad has a thick fatty cushion that provides a good grip while walking and improves blood circulation throughout the body. The forelimb is longer than the hind leg, and the front feet bear much of the body weight. The hindfoot is smaller than the forefoot and has an oval shape.

The carpal bones are block-like and arranged in two rows of four in the forefoot. The proximal row includes the radial, ulnar, intermediate, and accessory carpal bones. The distal row of bones is referred to as carpal bones one to four (C- 1 to C- 4) . C- 1 to C- 4 articulate with their corresponding metacarpal (MC) bones, with C-4 articulating with MC-5. The elephant carpal bones' position and articulation are different from other ungulates. They permit very little abduction of the carpus. In the Asian Elephant, the three carpal joints (the radioulnar joint, the intercarpal joint, and the carpometacarpal joints) each contain its synovial sac. In the hindfoot, the tarsus is composed of seven bones and arranged in three rows. The talus and calcaneus make up the proximal row, and there are two articular facets between them. The talus is disc-shaped and compressed dorso-plantarly, while the tuber calcaneus points plantarly. The central tarsal bone is the sole bone of the second row of the tarsal bones. The four tarsal bones (T1-4) make up the distal row of tarsal bones and are wedge-shaped. Each tarsal bone articulates the corresponding metatarsal bone, with T-4 also articulating with metatarsal five (MT-5). There are four separate synovial sacs in the tarsus of the African Elephant. The metatarsal bones have an expanded distal extremity for articulation with the proximal phalanx. Metatarsal three (MT-3) is the largest, while MT-1 is the smallest and somewhat triangular. The elephant possesses an unusual structure the prehallux, a cartilaginous segment that extends distally from MT-1 and T1. The prehallux attaches to the sole at a position medial to the midline. The prehallux function is unknown, but it appears to stabilize the tarsus over the digital cushion. The hind foot has five digits that radiate in a craniocaudal orientation.

Digits three and four are more significant than the other digits in both species, and each has three phalanges. In the African elephant, digit one **(D1)** is represented by only a single sesamoid bone (Smuts and Bezuidenhout, 1993, 1994). Still, in the Asian elephant, this digit is described as having one phalanx **(P)** without a sesamoid bone. The other digits have paired sesamoid bones, plantar to the metatarsal-phalangeal articulation. In the African elephant, D-2 has two phalanges, and in the Asian elephant, it has three. Digit five has two phalanges in each species. The proximal and intermediate phalanges are quadrilateral in shape. The third phalanges are slightly spindle-shaped with bilateral transverse processes and a single dorsal process. The third phalanx only loosely articulates with P-2 and appears to be buried in the tissue medial to the corresponding toenail. There is a slight but distinct axial angulation of digits two and four towards the third digit.

Common foot ailments in elephants

Foot problems constitute the single most crucial ailment in captive elephants. Feet disorders can involve the integumentary and the musculoskeletal system. They can be infectious, traumatic, or degenerative. Common foot problems encountered at the nail, skin, sole, or pad include penetrating injuries, trauma, cracks in the sole, nail, or cuticle, overgrown nail, sole, or cuticle, laminitis, foot rots, ingrown nails, pododermatitis, osteomyelitis, arthritis, fractures, dislocations, abscesses, and degenerative joint disease. Neglected chronic foot disease in the advanced stage may become unresponsive to medical and surgical management and may subsequently require euthanasia.

Reasons for foot ailments: Many factors have been suggested to predispose captive elephants to foot disorders, and they are all related to a greater or lesser extent to husbandry. Several factors predisposing the captive elephants to foot disorders have been identified and are strongly associated with the husbandry practices followed:

- Neglected foot care
- Tethering on hard and unhygienic floor
- Forced to stand on the hard floor for prolonged periods
- Overloading and long working hours
- Inappropriate hobbles
- Lack of access to ad libitum water source
- Debility and nutritional deficiencies due to monotonous feeding
- Obesity due to overfeeding, feeding with unconventional food items, and lack of proper exercise.
- Mixed infections due to untreated wounds
- Compensatory weight bearing

Diagnostic approach

Digital radiography and thermography technique are effective diagnostic modalities in the clinical investigation of musculoskeletal problems in elephant feet.

Digital radiography: The elephant foot is a massive structure, and conventional radiographic protocols and equipment do not always result in diagnostic quality images due to inadequate penetration of the x-ray beam. The differences in topography and bone density of various parts of the foot skeleton demand different techniques, therefore, multiple films of the foot. Health and safety as well as welfare reasons. This would demand radiographic protocols to ensure efficiency, speed, and repeatability.

Therefore, upgrading to an appropriate advanced technique with protective gear becomes mandatory to get a better result. Radiography of the feet identifies the extent of an abscess, damage to the phalanges, and osteitis/osteomyelitis lesions in a chronic case of a foot abscess. The portable direct digital radiography with a portable X-ray machine with the capacity of 35 mA, 100 kVp & 100 mAs is the minimum requirement to get a good quality diagnostic radiographic image of the foot and limbs of an elephant. Exposure factors of 55 to 60 mAs and 25 to 30 kVp will provide better image quality of the foot, contributing to the diagnostic value.

For quick and proper radiographic examination, basic training to the elephant for presenting its foot is important. That can be achieved by free contact or positive conditioning through protected contact methods. A minimum of three persons are required in addition to the *mahout* to handle the DR unit, focusing the X-ray beam and positing the flat panel X-ray detector. A small stool/foot rest with a covering case is mandatory to protect the X-ray detector from an elephant's foot pressure. The projection angles that were found to be most useful were 65-70° for the front limb and 55-60° for the hind limb. The beam was centered 10-15 cm proximal to the cuticle in the front and 10-15 cm dorsal to the plantar edge of the sole in the hindfoot, depending on the size of the foot. Since image quality always depends on the factors such as kVp, mAs, time, source image distance (SID), and tissue absorption factor, depending on the x-ray machine capacity and digital radiography units, we must standardize our exposure charts for getting good image quality. The exposure technique guidelines for Asian elephant limbs can be designed by using the equations of kVp = (2 X Tissue thickness [in cm]) + SID (in inches) + 5 and mAs = Tissue thickness (in cm)/2.5.

Thermography: Infrared thermography (IRT) is a safe, modern, noninvasive, non-contact thermal profile and its visualization technique. Elephants are ideal models for thermal imaging studies as their skin is scarcely covered with hair. Thermal or infrared energy is a part of the electromagnetic spectrum with a high wavelength over and above the visible range of the human eye. Instead, we perceive it as heat. Electromagnetic radiation is ubiquitous and may be classified according to its frequency or wavelength. The radiation restricted to the wavelengths from 760 nm to 1 mm is referred to as infrared radiation or "thermal radiation." Unlike visible light, everything with a temperature above absolute zero emits heat. The higher the temperature of an object, the greater the amount of infrared radiation it emits. Even icy objects, such as ice cubes, emit infrared radiation.

Typical exam procedures with a thermal imager for veterinary applications may involve uncontrolled environmental conditions. The imager used should stand up to consistent measurement under extreme environments. Author has used FLIR-E 60 thermal imaging camera for the study and examined 3mts distance. To measure temperature accurately, it is necessary to compensate for the effects of several different radiation sources. This is done automatically by the camera. However, the following object parameters must be supplied for the camera: the object's emissivity, the reflected temperature, the distance between the object, the camera, and relative humidity. Since the skin possesses high emissivity (0.98), the effect of reflected temperature will not affect the thermal measurement. Hence, it can be ignored. We used digital temperature & humidity meters (HTC-2) for recording the environmental temperature & humidity. An important concept is the "color palette." A color palette is the set of colors used in a thermal image, with specific colors varying with temperature. Thermal cameras allow a wide choice of color palettes. It is essential to select a palette that is easy to interpret when examining animals. The instrument used was 'high rainbow' as it has easily distinguishable colors - a palette displaying the coldest areas in blue and the hottest areas in white with red and yellow in between. Factors such as wet skin, skin contamination due to dirt, moisture in the fur, windy locations, direct sunlight, and other heat sources will affect the appearance of thermal images. They can lead to an error in thermal measurements. Thermal imaging cameras are a great tool to determine whether an animal is suffering from pain as in inflammatory conditions. As physiological diagnostic tool, thermography makes it possible 'to see the unseen' before anatomical changes have developed. The diagnosis of localized inflammation would not have been probable without thermography. Since it is portable, easy to use/learn, not stressful to the animal as it is a non-contact, safe remote sensing method, and cheaper when compared to digital radiography, it can be considered an efficient diagnostic tool in the health care of captive elephants.

Foot care tools: All the tools used to maintain foot care in equines can be used in elephants effectively. The electric grinder can be used, but it requires considerable handling experience to avoid severe consequences to the Elephant's feet.

Treatment approaches and standard protocol: The overgrown toenail, cuticle, and sole need to be trimmed by using suitable knives and rasps. If any swelling around the nail beds and discoloration of the skin is noticed, the radiographic examination is mandatory to understand the level of osteomyelitis changes of the underlying bony structure. Always choose the antibiotic based on ABST to avoid inappropriate antibiotics / antibiotic resistance. Meloxicam at 2mg /kg BWT, Combination of trypsin, bromelain, rutoside trihydrate (Rutoheal) 20 tablet can be administered effectively to manage the pain and swelling. Supplementation with vitamin B-complex, vitamin C, E, and A with trace minerals will aid in quick healing. Therapeutic management with oral or injectable antibiotics and anti-inflammatory drugs needs to be continued for a minimum of 10 to 14 days initially. The anti-inflammatory medications can be used as and when required. However, topical dressing, cleaning, and washing the lesions should be done twice daily until complete healing is achieved. The healing period for foot abscesses may be longer based on the stage of infection and the quality of intensive treatment care. This could take a minimum of 45 days to 90 days. Periodical evaluation of kidney and liver functions may also be necessary to overcome drug-induced nephritis and hepatitis, respectively. Any discontinuity in regular dressing and periodic pain management will delay the healing and increase further complications in weight-bearing and locomotion. The inability of the commercially available ointments to percolate the elephant skin and produce the desired effects were observed. Using DMSO and creams such as Soframycin, silver sulphadiazine, and mupirocin, Fusidic acid ointments have been recommended.

Frequent use of irritants (povidone-iodine, Tincture iodine, Copper sulfate) to clean and dress foot abscess should be avoided. Cold fomentation with ice is highly recommended. Don't interchange the foot soaking between formalin and KMNO4 suddenly without proper time intervals to prevent further damage to the soft tissues of the foot. MgSO4 foot soaking (400mg per two litter of Luke warm water) is safe and gives the desired effect. Direct daily application of MgSO4/ Himax ointment on the lesion will also be effective and enhance quick healing.

Preventive foot care

- Since foot care is a major husbandry component for keeping Asian elephants in captivity; every elephant holding facility must have its foot care protocol. It is important to maintain a schedule of foot trimming as a preventive care practice. Adequate and accurate records are needed. There are several ways to keep good records such as the written record-keeping system along with radiograph images, digital still photos, and video clips.
- Offering good quality fodder and nutritional supplements (Biotin, Vitamin-E, C, & A., Zn, Se, and As) for managing the ideal body weight should be of prime significance to support proper foot care.
- Providing adequate exercise is one of the most important aspects of proper elephant husbandry. One to two hours of walking each day should be considered the minimum amount of time an elephant needs for cardiovascular activity without just strolling around the exhibit. Anything less predisposes an elephant to foot problems and obesity, especially later in an elephant's life. Exercise of all joints, tendons, and ligaments is necessary to maintain a healthy foot.
- Proper hygiene practices and minimal time of confinement in stalls must be followed in all captive facilities to avoid constant exposure of the elephants' feet to their faeces and urine. The corrosive nature of urine and the infective components of the faeces sticking to their feet and legs can increase their susceptibility to infection. Regular scrubbing of feet and legs using neem soap and water aided by a hard-bristled brush may ensure better foot hygiene.
- Natural substrates allowing an elephant to dig will exercise and strengthen leg and foot muscles, tendons, and joints. This exercise and activity directly support healthy feet throughout the elephant's life in captivity. Elephants should be housed for much of the day on resilient, interactive, yielding surfaces to enhance their natural behaviors.
- Having the correct equipment, experienced staff, and regular training for the elephants to present their feet without fear is essential for a productive elephant pedicure along with the suitable facility design to implement the foot care protocol.

Art – 290. WELFARE CONCERNS IN MANAGING ELEPHANTS IN CAPTIVITY: A CASE STUDY

Baijuraj M. V. and **Kartick Satyanarayan**

Introduction

Elephant Conservation and Care Center (ECCC) situated in Mathura District of Uttar Pradesh was established as an elephant camp for providing treatment and care to elephants rescued from situations where animals were used for begging on roads, used in processions, temples, and tourism. Additionally, aged animals requiring rest and medical support following active service and those seized by the Forest department requiring shelter also find place for treatment and lifetime care at the centre. These animals are received in compromised health condition and show high degree of stereotypic behavior, have skeletal deformities/changes (ankylosis, lameness, sores), may be obese due altered and unnatural feeding practice (begging on the road), malnourished and even blind. These animals essentially require professional and humane support in management, taking due account of ensuring welfare concerns.

Systematic efforts are put in to ensure that the welfare of the elephants from the time of rescue, during transport, housing, treatment, and daily routine at the center are taken care off. With the help of international collaborations and knowledge exchange programs, positive conditioning to minimize any stress to the elephants and maximize the safety of the keepers have been integral part of management. Studies elsewhere have proved that these basic aspects if managed well, play a crucial role in ensuring welfare of animals in captivity (Greco *et al.,* 2016). Studies have demonstrated that the operant conditioning of an animal can be achieved through positive reinforcement and the primary operant conditioning method to train a captive animal is called shaping.

Besides providing treatment, the center also focusses on addressing the welfare concerns by providing adequate housing facility, chain free environment with innovative nutritional and environmental enrichment. Regular exercise walks and positive training are integral part of overall management.

Positive reinforcement training (PRT) has been adopted for the first time in India at the center and is aimed at facilitating the veterinary procedures besides supporting animal husbandry necessities without restraining or providing stress to the sick and injured elephants. This also contributes to a positive keeper–animal relationships which results in improved animal welfare. An enrichment-based positive reinforcement training has also shown additional benefits to animals under human care (Savastano et al. 2003). Environmental enrichment in captive animals helps to improve the welfare of the animals covering a wide variety of practices like foraging, playing, problem-solving, and exercise (Greco *et al.,* 2016; Meehan *et al.,* 2007; Young, 2003). The enrichments can be permanent or temporary like pools for play and food balls or rewards. There are several studies with different species of animals about the positive impact of enrichment, which reduce stereotypic behaviors and result in good welfare (Shyne, 2006).

The main objectives of environmental enrichment are to minimize stereotypic behavior of elephants under care, manage bull elephants in *musth* without chaining them throughout the *musth* period, unlike the traditional method of tying up and to minimize the stress levels during veterinary procedures and management. Female elephants are allowed to form herds of their choice and it gives them a good opportunity to express and practice their normal behaviors. Well-designed enrichments have also been extremely useful to manage *musth* in male elephants efficiently.

Methods to reduce stereotypies

Different methods to increase activity, avoid boredom and reduce the stereotype behaviors and also ensure maximum enclosure utilization by elephants are provided below.

- Scatter feeding in the enclosure
- Feeding enrichment using puzzle feeder (cages, barrel/cans, pipes)
- Green fodder feed enrichment with vegetation/browse on top of the shed inside, hay nets.
- A slight change in feeding set times for increasing temporal activities.
- Increasing the number of feeding times / day.
- Logs for the bull elephants inside the enclosure and logs outside for cow elephants.
- Tyres / wheels for playing within the enclosures.
- Designing of a large enclosure with adequate precaution for *musth* elephants
- Introduction of enrichment tools to reduce stress levels and divert animals from charging, pacing, hurting himself.
- Temporary covering of the enclosure to avoid the distraction of people and vehicle movements.
- Water availability all the time. (Pond and troughs)

- Operant conditioning using positive reinforcement training can be introduced at different levels which make the treatment procedure stress free for both keepers as well for the elephant
- Long exercise walks for cow elephants daily and for bulls after *musth* period

Conclusion

Ensuring animal welfare is of paramount importance while managing animals in captivity. Operant conditioning of elephants through positive reinforcement to reduce the stress level during treatment has proved as a good option for managing elephants in captivity. To avoid stereotypic behavior, large enclosures as per Central Zoo Authority guidelines with various types of enrichments have been critical in management of captive animals. Authors have experienced that elephants in *musth* can be managed in chain free environment and animals trained through positive reinforcement technique making interventions scientific, ethical and humane. It is prudent that adequately trained human resources and infrastructure are available for managing captive elephants.

Plate 14.1: (a) Elephants rescued from the circus exhibiting high degree of stereotypie behavior; (b) Severe ankylosis and lameness

Plate 14.2: Long exercise walks as a part of daily routine

Plate 14.3: Large enclosure with adequate safety fitments for managing elephants in musth (a) Male makhana in musth in chain free enclosure and (b) Male tusker in musth engaged in feeding activity.

Plate 14.4: Nutritional enrichments (a) Pipe feeder and (b) Cage feeder; Provisioning of water in each enclosure (c) Pond, (d) shower facility

Plate 14.5: Operant conditions using PRT for Makhana in musth at ECCC

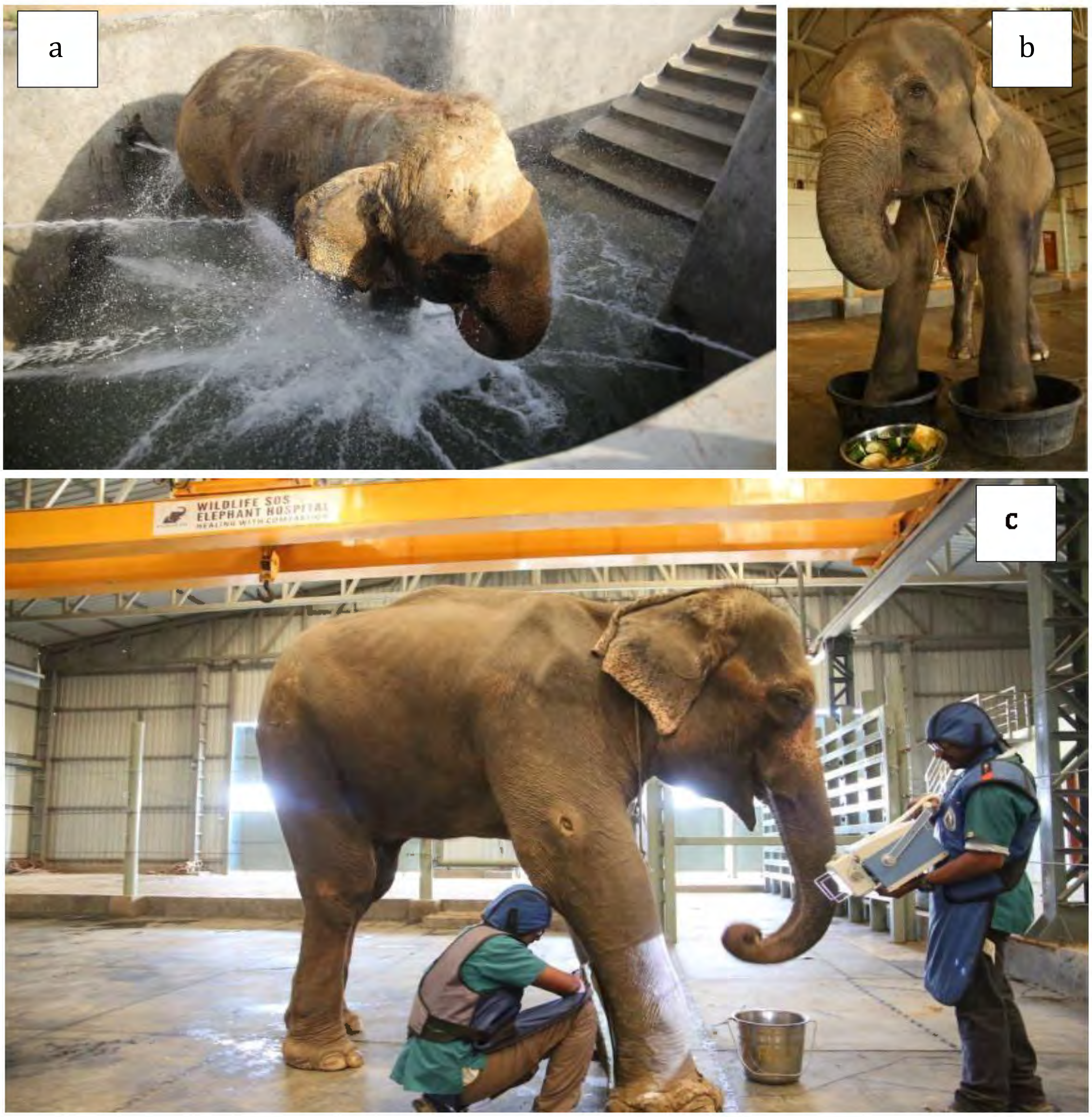


Plate 14.6: (a) Hydro-therapy (b) Foot Dips (c) Infrared therapy as part of veterinary management

Report – 291. POPULATION STATUS OF SLOTH BEARS (*Melursus ursinus*) AND OTHER LARGE MAMMALS IN DAROJI SLOTH BEAR SANCTUARY, KARNATAKA CENSUS REPORT 2022

Swaminathan, S., Arun, A.S., Ashokkumar, M., Sharp, T., Balasubramanian, G., Reegan, P., Yogaraj, P., Richakumar, S. and **Siva Sankari G.**

Complete report was submitted to Karnataka Forest Department
and the report can be accessed via email request at
research@wildlifesos.org.

Report – 292. POPULATION STATUS OF SLOTH BEARS (*Melursus ursinus*) AND OTHER LARGE MAMMALS IN GUDEKOTE SLOTH BEAR SANCTUARY, KARNATAKA CENSUS REPORT 2022

Swaminathan, S., Arun, A.S., Ashokkumar, M., Sharp, T., Balasubramanian, G., Reegan, P., Yogaraj, P., Richakumar, S., and **Siva Sankari G.**

Complete report was submitted to Karnataka Forest Department

and the report can be accessed via email request at

research@wildlifesos.org.

Art – 293. ASIAN ELEPHANT (*Elephas maximus linnaeus*) POPULATION STATUS AND DEMOGRAPHY IN TROPICALFOREST OF SOUTHERN INDIA

M Ashokkumar, C Sakthivel, K Sudhakar, **S. Swaminathan** and Ajay A Desai

Abstract

The assessment of population dynamics and demography of long-lived species can be challenging due to a longer life span and slow reproduction. The population status and demography of the Asian elephant was studied from Dec 2006 to May 2008 at Mudumalai Tiger Reserves and results were compared with earlier studies carried out in the study area in 1985 and 2000 to understand changes in the population size. The population was estimated using distance sampling and mark-recapture methods during wet and dry seasons. In the line-transect sampling, a total distance of 651.5km was walked. To estimate the population based on the capture-recapture method 17 routes were surveyed consisting of 128.5km surveyed twice per month. The estimated elephant density based on capture-recapture and distance sampling was 3.4/km^2 and 3.6/km^2 respectively, which was higher than the earlier estimate (1/km^2 in 1985 and 2.4/km^2 in 2000). Thus, the population of elephants increased significantly when compared to the past. The adult male to adult female ratio was 1:20. Tuskless male Makhnas make up 20% of the male population. Demography data shows that the male population has increased in the population. But lack of older bulls in the population and mortality of males due to retaliatory killing needs further investigation. The major per cent (61%) of mortality was due to natural causes like disease, injuries, and predation by tigers (two calves). There were sex-biased changes in mortalities that have occurred over time; while adult and sub- adult male mortalities were higher (83%) in the earlier study period, adult female mortalities were higher (54.5%) during the present study. Despite the skewed sex ratio elephant population has increased significantly when compared to the past in the study area.

Introduction

The Asian elephant (Elephas maximus) is listed as an endangered species with 60% population occurring in India. The estimated population ranges between 48,323 and 51,680 elephants distributed in 13 range countries across Asia (Williams et al., 2020; Riddle et al. 2010). They have long life spans, slow reproduction rate, and they live in forest habitats, thereby making observations in the wild difficult.

Therefore, demographic status is difficult to obtain, and most populations are not monitored over the long term. The elephant population in in the tropical forest of Southern India were estimated using various methods in the past. The total count method of estimation was used by the Forest Department from 1970 until 1980. Later, the ratio method based on individually identified elephants both herds and solitary males was used by Daniel et al. (1987). Furthermore, the factors such as defecation and decay rate in the line transect indirect method (dung count) were tested in the tropical forest by Dawson (1990). Varman and Sukumar (1995) studied the various parameters of direct count method in the study area. The Tamil Nadu Forest Department has been using the distance sampling methods (both direct and indirect count) and block count to estimate the population of larger mammals especially elephants and other large herbivores since 1995. However, these efforts have been hampered by poor survey design, lack of equipments such as range finder and field compass and trained personnel needed to ensure accuracy of the estimate. Further, the elephant population in India has been estimated using direct block count and indirect count method (Bist 2003; Rangarajan et al. 2010).

In order to investigate the components of population trend such as recruitment, mortality, and reproductive status of the population, it is necessary to know the age structure of the population (Lindeque 1991; Stearns 1992). The first step in this direction is to estimate elephant densities with adequate accuracy and precision. The goal of this research is to understand the changes that have taken place in the elephant population in over the last three decades in the Mudumalai Tiger Reserve. Daniel et al. (1987) used a fairly accurate but time-consuming approach of estimating the population based on the ratio of known to unknown elephants found during the study. Elephants were individually identified based on natural markings such as ear shape, cuts and holes, back shapes, tusk/tush size and tail length and hair pattern in the tail end (Douglas-Hamilton 1972; Daniel et al. 1987). We have used distance sampling, capture-recapture method and ratio method (for comparison with earlier study) to estimate elephant population.

Methods

Study Area

Mudumalai Tiger Reserve (MTR) is in the Nilgiri District of Tamil Nadu (11º 32´

and 11º 42´ N and 76º 20´and 76º 45´ E). It extends over an area of 321 km^2 and forms a part of the Nilgiri Biosphere Reserve (Fig. 1). The sanctuary is in the Western Ghats, which is one of the 35 Biodiversity hotspots of the world (Myers et al. 2000). Altitude in the study area varies from 485 to 1226 m above MSL with a general elevation of about 900 to 1000m. The annual rainfall varies from 1001mm to 1648 mm. This Sanctuary receives rain from both Southwest (May to August) and Northeast (September to December) monsoons. Based on climate, there are three distinct seasons recognized; dry season (January to April), first wet season (southwest monsoon) and second wet season (northeast monsoon). The study area has three major forest types of namely tropical moist forest (MDF), dry deciduous forest (DDF) and tropical thorn forest (TF) (Champion and Seth 1968). The large herbivores include elephant *Elephus maximus*, three species of cervids: chital *Axis axis*, sambar deer *Rusa unicolor* and barking deer *Muntiacus muntjak*, two species of antelopes: the four-horned antelope *Tetracerus quadricornis* and the blackbuck *Antilope cervicapra*. In addition, predators like tiger *Panthera tigiris*, leopard *Panthera pardus* and wild dog *Cuon alpinus* are found. MTR is threatened by habitat degradation from overgrazing, poaching and human disturbance.

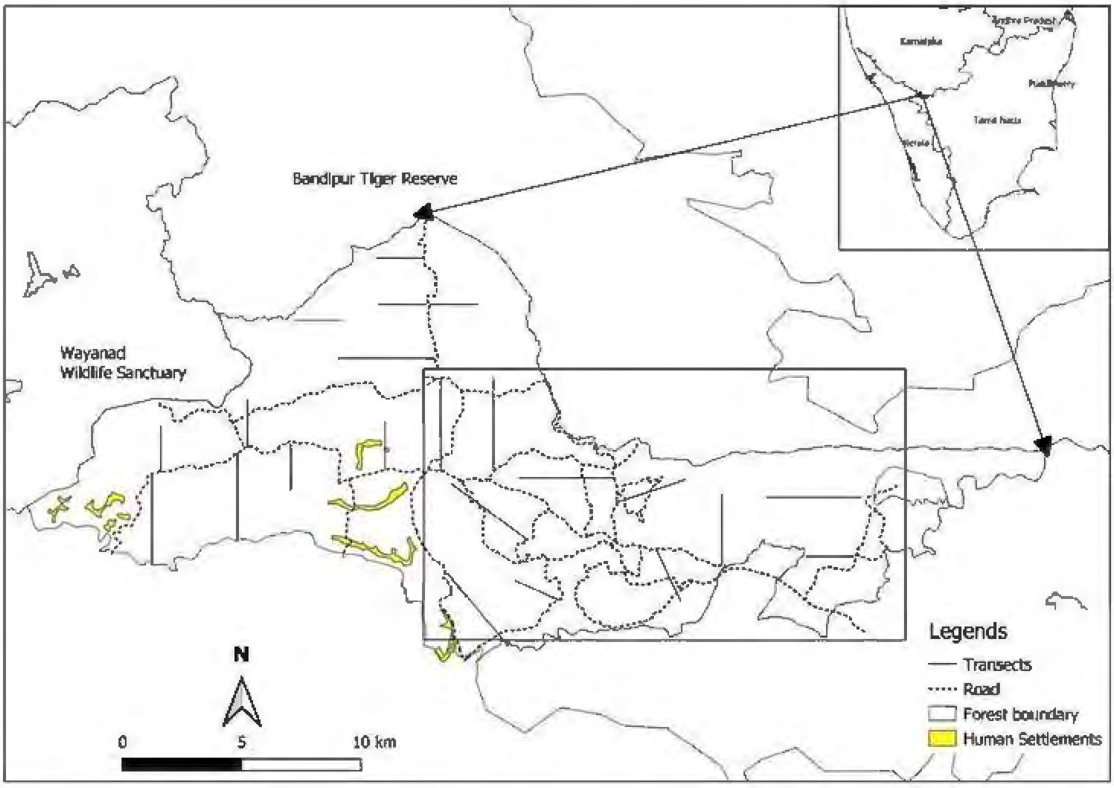


Figure 1: Map of Mudumalai Tiger Reserve showing the locations transect lines and survey routes (Inset rectangle) for mark-recapture sampling.

The study area is a part of Elephant Range-7, which is one of the 11 elephant ranges, declared by the Project Elephant, Govt. of India. This range extends over 12,900 km^2 of forest in the Western and Eastern Ghats. It has a tenuous link with Elephant Range 8, which covers an area of 2385 km^2 and there is a movement of elephants between Elephant Ranges 7 and 8 (Baskaran et al. 1995). These Ranges together cover an area of 15,000 km^2 and support a population of 14,000 elephants (Riddle et al. 2010). The large contiguous area and the large elephant population together represent the largest single Asian elephant population in Asia.

Distance sampling

Elephant density was estimated by line transect direct count method (Buckland et al. 2001) and widely used to assess ungulate population in tropical forests (Karanth & Sunquist 1992; Varman & Sukumar 1995; Baskaran & Desai 2000; Jathanna et al. 2015). The current design of sampling was similar to an earlier study (Baskaran and Desai 2000) by initially stratifying the study area into several strata based on habitat type and also on human disturbance as this influence elephant distribution (Desai & Baskaran 1996).

A grid comprising of 4 km^2 blocks was drawn and overlaid with a stratified vegetation map of the study area. Out of a total of 80 grids squares, 35 grids were randomly selected for sampling. In the selected grids, a transect was laid and the direction of the transect varies according to the topography of the area (Fig. 1). Transects were randomly laid to ensure that spatial variability within these sub-areas is adequately sampled. In total 24 transects of 2 and 4 km length were used for sampling. A total distance of 651.5 km was walked in different seasons (Dry-2007: 225.5km; Wet-2007: 140km; Dry-2008: 286km). Vegetation modification to facilitate movement (while sampling) along these transects was kept to a minimum to avoid biases caused by either attraction or avoidance of animals.

Exploratory analysis of the distance data was carried out as prescribed by Buckland et al. (2001) looking for evidence of evasive movement before detection, rounding and heaping of data to truncate outlier observations to improve subsequent model-fitting. Data were analysed using program Distance 7.2 software (Buckland et al. 2001; Thomas et al. 2010). The fit of possible alternative models to each specific dataset was judged using Akaike's Information Criterion (AIC) values and goodness of fit tests generated by the program as well as by visually judging the fit of the proposed model to observed distance data close to the transect line. The best possible model was selected based on the above considerations. The estimates of the following parameters were generated encounter rate (n/L), cluster density (Ds), cluster size (Y) and animal density (Ď).

Capture-Recapture method

The capture-recapture method was based on individually identified elephants that can be easily identified in the field. All the elephants were photographed systematically and named. We used two different methods to estimate the population. First, we used the simple Lincoln-Peterson estimator (Otis et al., 1978) as this will allow the data to be directly compared with the study done in the mid-1980s which also used this method (Daniel et al. 1987). We also used a more robust sampling design (capture-recapture methods) that allowed using both open (POPAN model Schwaz & Arnason 1996) that do not assume geographic and closed population models (Otis et al., 1978; Pollock 1982) for estimating the male population in the sampling area. Capture-recapture methods, adopted to estimate male Asian elephant population parameters in the adjoining Nagarahole–Bandipur National Parks (Goswami et al. 2007), is widely acknowledged as a robust method to assess Asian elephant populations (de Silva et al. 2011; Gupta et al. 2017; Goswami et al. 2019).

Elephant surveys were carried out for 12 months from May 2007 to April 2008. The sampling was done ina sub-section of MTR using 17 roads in the MTR that covers an effective area of sampling was 160 km^2. All the survey routes consisted of 128.5 km were sampled two times per month. This was based on using the park's boundary on the eastern, western, southeastern and northwestern sides. On these sides their forest contiguity with the Reserve Forests and Bandipur Tiger Reserves. On the northeastern side, the Moyar Gorge acts as a physical barrier for elephants. On the southwestern side, there are settlements. On the western side, there is again forest continuity within the MTR, the sampling road was taken as the boundary in this case.

Demography

The age-sex composition was determined based on the direct sighting of elephants in the field. Data was collected systematically across the entire study area to ensure that all the vegetation strata were well sampled. We classified the elephants into various age-sex categories based on relative height and morphological characteristics (Mckay 1973;

Daniel et al. 1987). Younger elephants (< 15 years) were classified by comparing their height to the oldest adult female in the group. Elephants were placed in broad age groups: Calves (< 1 years), juvenile (1–5 years), sub-adults (5–15 years) and adults (for females ≥ 16 years and males ≥ 20 years). The differentially structured age classification was used for comparison with earlier studies (Daniel et al. 1987; Baskaran and Desai 2000). Inter-calving interval was estimated based on the difference in ages of the last two calves of a female (especially a female with a newborn calf that still has the older calf along).

Results

Population size and Density estimates

Estimated elephant density based on distance sampling methods varied across the season with higher density during the dry season (3.3 elephants/km^2) than wet season (1.8 elephants/km^2; Table 1). The mean group size was higher during the wet season (4.3) than the dry season (3.6–3.8), whereas group density was less in the wet season (0.43) than the dry season (0.8).

Table 1: Estimated elephant population group density, group size and individual density in Mudumalai Tiger Reserve.

Season	Effort (km)	Model	ESW (m)	DS ± SE (95% CI)	E(S) ± SE (95% CI)	D ± SE (95% CI)	% CV
Dry 2007 (n = 20)	225.5	Half Normal + Cosine	51.9 ±7.72	0.85 ±0.32 (0.41–1.77)	3.82 ± 0.77 (2.51–5.82)	3.27 ±1.39 (1.44–7.38)	42.76
Wet 2007 (n = 12)	140.0	Half Normal + Cosine	107.3 ± 26.60	0.43 ±0.196 (0.18–1.04)	4.26 ± 1.16 (2.36–7.72)	1.83 ±0.97 (0.67–4.99)	52.97
Dry 2008 (n = 31)	286.0	Half Normal + Cosine	60.8 ±7.23	0.89 ±0.25 (0.51–1.55)	3.64 ± 0.52 (2.73–4.86)	3.25 ±1.02 (1.76–5.89)	31.42

ESW- Estimated strip width; DS- Mean group density; E(S)- Mean group size; D-Density; Se-Standard error; CI- 95% Confidence Interval, lower and upper limit

A total of 27 male elephants were individually identified in MTR consisted of both adults and sub-adults. These identified elephants were re-sighted on 52 occasions. To determine the population density, we used the method used in the earlier study. The coarse recapture probability was calculated as 1.926 (i.e. 52 recaptures/27 individuals = 1.926) and this was assumed to be the recapture probability for groups also.

The estimated population of the male elephant based on closed capture-recapture is 33.3 ± 3.53, time depended on the model was selected than other models with the lowest AIC values (Table 2). The male elephant population estimated by the POPAN model was 47.04 ± 8.78. The test for population closure indicated a closed population ($z=-999$; $p > 0.05$) based on Otis et al. (1978).

Table 2: Estimated male elephant population based on capture-recapture (closed population estimate).

Model*	Mean ± SE	Lower	Upper	AIC
M_t	33.0 ± 3.53	29.06	44.51	333.5
M_{tb}	28.3 ± 2.25	27.14	39.96	334.6
M_b	27.5 ± 0.84	27.04	31.89	346.5
M_0	34.0 ± 3.91	29.52	46.44	354.4
*-M_o- Null model; M_t-Time variate; M_b- Behavioural model; M_{tb}- Time & Behavioural model				

A total of 187 groups were sighted during the sampling period and this would imply that there are 97.1 individual groups, assuming the same recapture probability for male (i.e. 187 groups/1.926 recapture probability = 97.09). The mean group size was 5.33 elephants and this would imply that the groups account for 518 elephants. So, the total number of elephants for the area would be 545 i.e. 518 elephants in groups and 27 males. This gives a crude density of 3.4 elephants/km^2 (i.e. 545/160 km^2).

Demography and Sex ratio

A total of 516 groups were sighted during the study period of these all individuals in 479 groups were completely classified into different age-sex classes. Adult females constituted 45.5% of observations compared to 2.29% adult males (Fig. 2). Sub-adult, juvenile and calves composed 17.1%, 14.1% and 21%respectively. The adult male to adult female ratio was 1:20. Among adult males, the ratio of Makhnas (tuskless males) to tuskers was 1:4 which implies that Makhnas make up 20% of the male population.

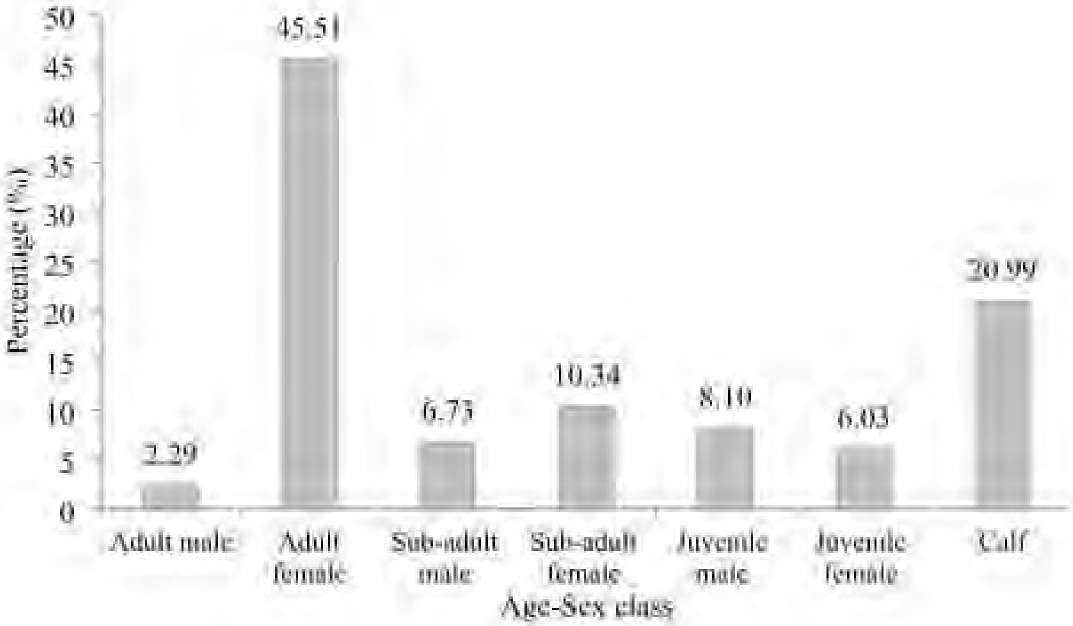


Figure 2: Age-sex structure of the elephant population in Mudumalai Tiger Reserve (n=479 groups).

Comparison of the sex ratios for different age classes in MTR showed improvement in male numbers relative to 1999–2000 period (Table 3). The adult male population shows marginal improvement, but the bulk of the adult class is made up of females, whose population continues to grow rapidly in the absence of any major threat to this age class.

Table 3: Age structure and Sex ratio of the elephant population of Mudumalai Tiger Reserve between 1987 to 2008

Age class	1985-87 (Daniel et al., 1987) (n = 1449)		1999–2000 (Baskaran and Desai, 2000) (n = 586)		Dec 2006 – May 2008 (Present study) (n = 479)	
	Age structure (%)	Sex ratio	Age structure (%)	Sex ratio	Age structure (%)	Sex ratio
Adults	39.3	1:4.9	41.5	1:29.4	48.47	(1:20)
Sub-adults	21.1	1:1.1	24.1	1:2.9	16.56	1:1.5
Juveniles	25.8	1:0.8	24.9	1:1.5	14.07	1:07
Calves	13.81:1*		9.5	1:1*	20.90	1:1*
***-Calves-sex ratio assumed to be equal at birth**						

Mortality

Daniel et al. (1987) had reported that human was responsible for 80.7% of deaths in the present study area. However, in the present study human-related mortality of elephants was 27.8% (Fig. 3). The major per cent (61%) of mortality was due to natural causes like disease, injuries and predation by tiger (two calves). There were sex-based changes in mortalities that have occurred over time; while adult and sub- adult male mortalities were the highest (83%) in the earlier study period adult female mortalities were the highest (54.5%) during the present study.

Association of males with female herds

The association of male elephants in the female herds are shown in Fig. 4. There appears to be no significant change from the earlier study by Daniel et al. (1987). No associations between males and female herds are seen in the 35 years and above age classes, as there are no males in these age classes in Mudumalai Tiger Reserve at present.

Discussion

Population density

Estimated density of elephants based on capture-recapture and distance sampling indicated population density increased in the study area. Baskaran and Desai (2000) had estimated a population density of (± 0.11) for the year 1999–2000. Earlier studies by Daniel et al. (1987) indicated a much lower population density (a little over 1 elephant/km^2) for the same area. But this earlier method used a coarse ratio method (known: unknown) to estimate the population size. However, Baskaran and Desai (2000) argued that despite the differences in methods used and the question ability of the precision involved the differences are significantly large to allow us to assume that there has been a major increase in the population size.

Despite poaching reducing the male numbers, the elephant population in the study area is increasing. Daniel et al. (1987) had pointed to this very fact in their study and stated that despite the high levels of poaching (male-biased) the population was growing, and this was a cause for worry given what then was a density of a little over 1 elephant/km^2. Sivaganesan (1991) and Sivaganesan and Sathyanarayana (1995) had indicated that preferred tree species of elephants were declining rapidly and one of the main causes for the decline of adult trees was feeding by elephants. While this would have been an indicator for the local population other confounding factors like rainfall, fires (Suresh et al. 2010) and other herbivores (deer) suppressing regeneration were not studied and therefore the decline of these tree species could not be attributed entirely to elephants.

Density estimated by capture recapture method

If these densities are extrapolated to the park it gives a population that is three times the size of the population estimated in 1987 (Daniel et al. 1987) using a similar method but covering the entire PA. There can be little doubt that the population has grown significantly in the last two decades, even if we assume that there was some underestimation in the past. What was apparent was that re-sighting (recaptures) was relatively poor in this study relative to that done in Nagarahole (Goswami et al. 2007). This would indicate that some sites are suitable for such studies while others are less so. The effort needed to get good sample sizes in less suitable areas would be high so this method cannot be recommended for all areas even if elephant densities are high. This is slightly higher than the density estimated using other methods but this could be because roads by and large are located in good terrain (also suitable for elephants) and close to water sources (attracts elephants) and some of the roads are especially designed for viewing wildlife (biased towards high-density areas).

Demography

Comparison of the age structure of the MTR with earlier data showed that the adult population has increased significantly due to an increase in adult females as shown by Desai and Baskaran (2000) (Table 1). The decline in the juvenile class could be due to either poor recruitment in the past several years or due to reduced breeding in the past few years. Desai and Baskaran (2000) had recorded only 9.5% calves in the population but cautioned that this could be due to natural reasons as more females are likely to calve in one year and fewer in the subsequent years. Similarly, the high proportion of calves (20.9%) seen during the present study could be an indicator of a normal cycle of high and low births based on the number of adult females that are available for breeding in any given year. However, the data indicates that we need to monitor systematically and regularly to detect any signs of a decline in breeding. The sub-adult class too has declined relative to the other two studies and it could also indicate lower recruitment and more animals moving into the adult age class. However, with a decreased juvenile age class and the sub-adult age class, there is a strong probability that breeding has been affected when the adult male: female sex ratio declined to 1:29 in 2000. It could also imply that the increased population is resulting in increased calf and juvenile mortality due to increased competition for resources and it is affecting recruitment. There is a clear need to monitor the population structure closely to ascertain changes and monitor trends so that we can better manage the population and address long-term conservation needs.

Sex ratio

Comparison of the sex ratios for different age classes in MTR showed improvement in male numbers relative to 1999–2000 period (Table 2). The adult male population shows marginal improvement, but the bulk of the adult class is made up of females whose population continues to grow rapidly in the absence of any major threat to this age class. This was highlighted by Desai and Baskaran (2000). Change in the adult sex ratios of this scale (1:29 to 1:20) can come about by the addition of only a few males being recruited from the sub-adult class. This would be normal given the eight-year gap between the 2000 study and the 2008 (present) study. Given the fact that poaching in the region has not been reduced and there are also deaths of males due to HEC (retaliatory killings) and due to natural mortality, the adult male numbers have not changed significantly, clearly indicating a need for improved vigilance and increased anti-poaching efforts as also efforts to eliminate retaliatory killings. Park managers need to realize that mortality in the non-PA areas also affects their populations. Elephants have large home ranges which extend well outside the PA and into Reserve Forests (RF) areas that have fewer resources for protection and also have different management goals and priorities. This problem was highlighted by Desai (1991) and it needs to be addressed urgently through improved coordination between PAs and RFs and increased support to RF areas. There is a significant improvement in the sex ratio of the sub-adult age class but these changes can be expected to occur fast with improvement in protection as there would be rapid recruitment from the juvenile age class. The juvenile class too shows an improvement in the sex ratio and is comparable to the ratios in 1987.

Impact of poaching

Daniel et al. (1987) had reported that human was responsible for 80.7% of deaths in the present study area. Similarly, 88% of mortality was due to train collision and human-mediated in Rajaji National Park (Williams et al. 2007). However, in the present study, human-related mortality of elephants was 27.8% (Fig. 3). The major percent (61%) of mortality was due to natural causes like disease, injuries and predation by tiger (two calves). Sukumar (1985) estimated that human was responsible for 20% of female and 65% of male elephant death in South India so there has been a general decline in man-mediated mortality of elephants.

There were sex-based changes in mortalities that have occurred over time; while adult and sub-adult male mortalities were the highest (83%) in the earlier study period adult female mortalities were the highest (54.5%) during the present study. This shift is likely due to the reduced male numbers and consequently a reduction in poaching. Poaching has declined over time in terms of the number of males killed. The reason for this is twofold, first, there has been an increased anti-poaching effort that has made it more difficult to poach. And second, there has been a reduction in the number of adult males and also tusk sizes available today (in other age classes) do not make it lucrative to establish and run poaching networks. What poaching takes place is largely opportunistic and not organized as in the past where dedicated poaching gangs operated. However, males are still being killed at higher rates than they are present in the population. Considering this, it then becomes very important that efforts to contain elephant poaching and also retaliatory killings (HEC related) be minimized. Lower male mortality numbers should not create a sense of complacency that could allow organized poaching to return when male numbers are up.

Breeding

Daniel et al. (1987) suggested that disparate sex ratios even in the region of 1:5 would not affect breeding because males were polygamous and not all females come into oestrus in any given year. Given the approximately five years inter-calving interval then it would mean that an average of 20% of the female would breed in any given year. This would mean an effective male: female sex ratio of 1:1 even when the actual sex ratio is 1:5 in the population. Although the study by Daniel et al. (1987) did indicate that there are years with peaks and years with low births, likely, breeding would not get affected at even wider sex ratios. It is difficult to estimate at what ratio breeding would be affected but the present poor representation of sub-adults and juveniles (see Table 4.8) in the population may indicate that breeding has been affected. Adult male: female ratios of 1:29 estimated by Baskaran and Desai (2000) appear to have resulted in poor and the low proportion of calves (9.5%) in their study would indicate that but it could also be due to a naturally low birth year as mentioned by them.

Table 4: Changes in adult and sub-adult male and adult female mortalities in Mudumalai Tiger Reserve and adjoining RF areas.

Age-sex classes	Daniel et al., 1987 (%) (n = 26)	Present study (%) (n = 11)
Adults and sub-adult males	83	45.5
Adult Females	17	54.5

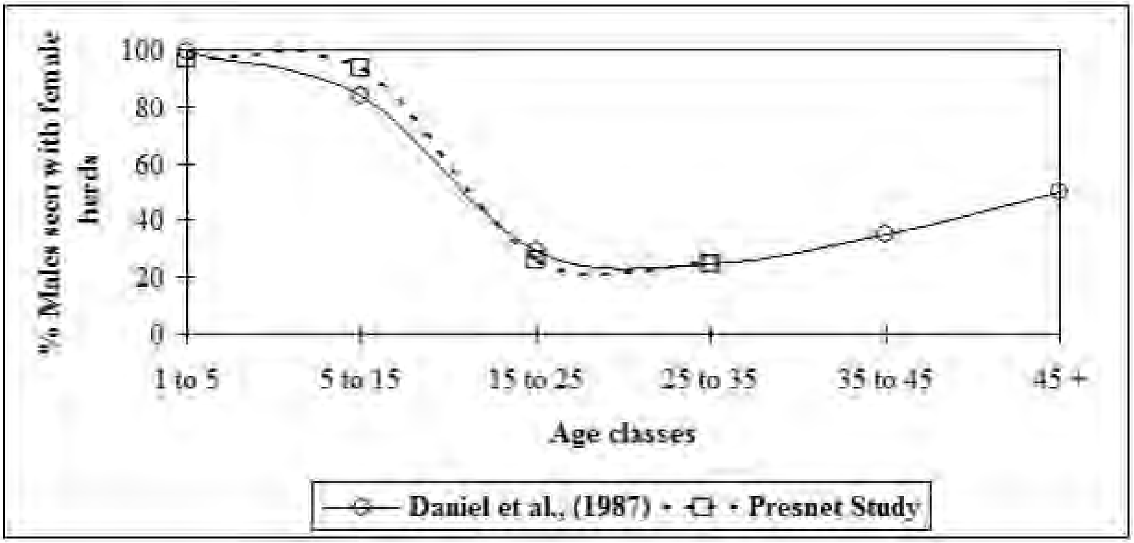


Figure 4: Male association with female herds in Mudumalai Tiger Reserve in the past and present study.

Earlier in the 1980s, most matings were done by the large adult males which were over 30 years (Desai unpublished data) and were the only ones that had extended musth periods. But in the current study males below 20 years showed clear signs of musth (heavy temporal flows and swollen temporal glands) and sustained it for longer. In the 1980's males in this age class only showed mild signs of musth with no temporal swelling and only a small fine small flow of temporal fluid and musth sustained for a week or so. This change shows that younger males that could never mate when the older males were alive were not taking on the role of breeding. Males as young as 10–15 years have been seen in musth. Such a shift in breeding might help overcome the problem of poaching of larger males but now the poaching has shifted to these smaller males and there are no males over 40 years of age in the population. This sustained decline of males would ultimately result in eliminating the genetic diversity of the male population as fewer and fewer males continue to breed.

There appears to be no significant change in the male-female association from the earlier study by Daniel et al. (1987). The decline in male numbers does not appear to have changed their behavioural pattern in terms of their association with females. No associations between males and female herds are seen in the 35 years and above age classes, as there are no males in these age classes in Mudumalai Tiger Reserve at present.

Makhna to tusker ratios

Among adult males, the ratio of Makhnas (tuskless males) to tuskers was 1: 4 which implies that makhnas make up 20% of the male population. Williams et al. (2007) estimated that makhnas constitutes 10% of adult males in Rajaji National Park. Whereas in Sri Lanka 90% of the sub-adult and adult bulls are makhnas (Katugaha et al. 1999). Daniel et al. (1987) had estimated that makhnas made up approximately 13% of the population during their study. In contrast to these habitats, Goswami et al. (2019) estimated a tusker to makhna ratio of 1.2:1 in Kaziranga National Park, suggesting that makhnas comprised ~ 45% of the male population. While the proportion of makhnas in the male population appears to have doubled almost but actual change is significantly greater as the tusker population was already severely depleted by poaching during the study by Daniel et al. (1987). This would mean that the original proportion of makhnas in the population would have been far less than the 13% estimated by them. While the continued decline in the tusker population would account for a significant part of the change in the proportion of makhnas it would not account for all the changes as we are now dealing with a new generation of males. When all the large adult tuskers were poached off in the 1980's and early 1990's, the adult makhnas were also eliminated in the 1990's and in the early part of 2000's due to HEC- related killing and one elephant was even captured due to HEC. Given their very small numbers of HEC related elimination and old age would have eliminated all the older animals. Thus we are dealing with a new generation of males and the increase in the proportion of makhnas would be due to an actual increase in their numbers rather than other factors alone. But when the bulk of the large tuskers were eliminated from the population by the 1990's the large adult makhnas would have monopolized the mating and this could account for some of the increase in the population of makhnas.

Acknowledgements

We thank the TNFD, Tamil Nadu for permitting us to undertake this study. We thank the US Fish and Wildlife Service for providing funding support (AAA No. 98210-6-G113). We thank the former Director and Honorary Secretary of BNHS.

Reference

1. Baskaran N, Desai AA (2000) Elephant population estimation in Mudumalai Wildlife Sanctuary and National Park. Report submitted Tamil Nadu Forest Department, Bombay Natural History Society, Mumbai.

2. Baskaran N, Balasubramanian M, Swaminathan S, Desai AA (1995) Ranging behaviour of elephants and its implications for the management of Nilgiri Biosphere Reserve, India. In: Week with Elephants Danie JC & Datye H (eds). Proceedings of the International Seminar on the Conservation of the Asian elephant. Bombay Natural History Society, Bombay

3. Bist S (2003) An overview of the methods for enumeration of wild elephants in India. Gajah 22:67–70.

4. Buckland ST, Andersen DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2001) Introduction to Distance Sampling: Estimating Abundance of Biological Populations. Oxford University Press Inc., New York, USA.

5. Champion HG, Seth SK (1968) A Revised Survey of the Forest Types of India. Government of India, New Delhi.

6. Daniel JC, Desai AA, Sivaganesan N, Rameshkumar S (1987) The study of some endangered species of wildlife and their habitats. The Asian Elephant Report, October 1985 to September 1987, Bombay Natural History Society, Bombay.

7. Dawson S (1990) A model to estimate the density of Asian elephants (Elephas maximus) in forest habitats. MSc thesis, University of Oxford, Oxford, UK.

8. Desai AA, Baskaran N (1996) Impact of human activities on the ranging behaviour of elephants in the Nilgiri Biosphere Reserve, South India. J Bombay Nat Hist Soc 93(3):145–156.

9. Desai AA (1991) The home range of elephants and its implications for management of the Mudumalai Wildlife Sanctuary, Tamil Nadu. J Bombay nat Hist Soc 88:145–156.

10. de Silva S, Ranjeewa A, Weerakoon D (2011) Demography of Asian elephants from

identified individuals at Uda Walawe National Park, Sri Lanka, Biol Conserv 144(5):1742–1752. DOI: 10.1016/j.biocon.2011.03.011

11. Douglas-Hamilton I (1972) On theecology and behaviour of the African elephant, Ph.D. thesis, University of Oxford, Oxford, UK.

12. Goswami VR, Madhusudan MD, Karanth KU (2007) Application of photographic capture-recapture modelling to estimate demographic parameters of male Asian elephants. Anim Conserv 10:391–399.

13. Goswami VR, Yadava MK, Vasudev D, Prasad PK, Sharma P, Jathanna D (2019) Towards a reliable assessment of Asian elephant population parameters: the application of photographic spatial capture-recapture sampling in a priority floodplain ecosystem. Scientific reports 9(1): 8578. https:/ doi.org/10.1038/s41598-019-44795-y.

14. Gupta M, Joshi A, Vidya TNC (2017). Effects of social organization, trap arrangement and density, sampling scale, and population density on bias in population size estimation using some common mark-recapture estimators. PloS one 12(3):e0173609.

15. Jathanna D, Karanth KU, Samba Kumar N, Goswami VR, Vasudev D & Karanth KK (2015) Reliable monitoring of elephant populations in the forests of India: Analytical and practical considerations. Biol Conserv 187:212–220.

16. Karanth KU, Sunquist ME (1992) Population structure, density and biomass of large herbivores in the tropical forest of Nagarahole, India. J Trop Ecol 8:21–35.

17. Katugaha HIE, de Silva M, Santiapillai C (1999) A long-term study on the dynamics of the elephant (Elephas maximus) in Ruhuna National Park, Srilanka. Biol Conserv 89:51–59.

18. Lindeque M (1991) Age structure of the population in the Etosha National Park, Namibia. Madoqua18:27–32.

19. Mckay GM (1973) Behaviour and ecology of the Asiatic elephant in Southeastern Ceylon, Smithsonian Contribution to Zoology, Vol.125, Smithsonian Institution, Washington, DC.

20. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858.

21. Otis DL, Burnham KP, White GC, Anderson DR (1978). Statistical inference from capture data on closed animal populations. Wildl Monogr 62:1–135.

22. Pollock KH (1982) A capture–recapture design robust to unequal probability of capture. J Wildlife Manag 46:752–757.

23. Rangarajan M, Desai AA, Sukumar R, Easa PS, Menon V, Vincent S, Ganguly S, Talukdar BK, Singh B,Mudappa D, Chowdhary S, Prasad AN (2010) Gajah: Securing the future for elephants in India. Report of the Elephant Task Force, Ministry of Environment and Forests, New Delhi, India.

24. Riddle HS, Schulte BA, Desai AA, Meer LVD (2010) Elephants – a conservation overview. J Threattaxa 2:653–661.

25. Schwarz C, Arnason AN (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics 52:860–873 DOI:10.2307/2533048

26. Sivaganesan N, Sathyanaranaya MC (1995) Tree mortality caused by elephants in Mudumalai Wildlife Sanctuary, South India. In: Daniel JC, Datye HS (eds) A Week with Elephants. Bombay Natural History Society, Mumbai. pp 350–367.

27. Sivaganesan N (1991) The ecology of the Asian elephant in Mudumalai Wildlife Sanctuary with special reference to habitat utilization. Ph.D thesis, Bharathidasan University, Tiruchirapalli, India.

28. Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford.

29. Sukumar R (1985) Ecology of Asian elephant (Elephas maximus) and its interaction with man in South India, Ph.D. thesis. Indian Institute of Science, Bangalore.

30. Suresh, HS, Dattaraja HS, Sukumar R (2010) Relationship between annual rainfall and tree mortality in tropical dry forest: Results of a 19 year study at Mudumalai, southern India, Forest Ecol Manag 259:762–769.

31. Thomas L, Buckland ST, Rexstand EA, Laake JL, Strindberg S, Hedley SL, Bishop JRB, Marques TA,Burnham KP (2010) Distance software: Design and analysis of distance sampling surveys for estimation population size, J Appl Ecol. https:/ doi.org/10.1111/j.1365-2664.2009.01737.x

32. Varman KS, Sukumar R (1995) The line transect method for estimating densities of large mammals in a tropical deciduous forest: An evaluation of models and field experiments. J Biosci 20:273–287.

33. Williams C, Johnsingh AJT, Krausman PR (2007) Population estimation and demography of the Rajaji National Park elephants, North-West India, J Bombay nat Hist Soc 104:145–152.

34. Williams C, Tiwari SK, Goswami VR, de Silva S, et al. (2020). Elephas maximus. The IUCN Red List of Threatened Species 2020: e.T7140A45818198. https:/ dx.doi.org/10.2305/IUCN.UK.2020- 3.RLTS.T7140A45818198.en

Art – 294. INTRA-ABDOMINAL LAPAROSCOPIC STERILIZATION OF MACAQUES: AN ATTEMPT TO MITIGATE HUMAN-PRIMATE CONFLICT THROUGH AN EFFECTIVE ANIMAL BIRTH CONTROL TECHNIQUE

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

Abstract

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

Keywords: Human-primate conflict, sterilization, laparoscopy

1. Introduction

Over the last few decades, human-wildlife conflict (HWC) is escalating worldwide and involving various species (Shaurabh & Radhakrishna, 2017) [10]. The severity of HWC can be attributed to various factors, such as deforestation, habitat loss, large-scale environmental changes, and urbanization (Treves, 2008) [11]. Rhesus macaques (*Macaca mulatta*), being highly adaptable animals, have learned to survive in urban settlements. Easy availability of food sources and rapid reproduction among these urban monkeys has led to a significant increase in their population and life expectancy resulting in human-primate conflicts (Reddy, 2018; Vedula *et al*., 2021) [9, 12]. Due to increased population and nuisance activities, Rhesus macaques are losing conservational support. Based on the cultural role in the Indian community, monkey management is a challenging task. There was an imminent need to counter the growing monkey population and hence, conflict mitigation becomes a huge challenge for the city administration and the forest department as well. Short-term strategies e.g., fencing, caging, guarding, noise-making, repellents, and deterrents whereas sterilization, translocation, and culling are long-term startgies being adopted to mitigate human-primate conflicts (Kansky & T. Knight, 2014; McManus *et al*., 2014; Pebsworth *et al*., 2021) [3, 7, 8]. Considering both diagnostic and therapeutic approaches, the laparoscopy technique holds the most potent and promising aid (Lubell & Frischer, 1976) [5]. The technique involves intervening between intra-abdominal organs with minimal invasiveness, maximum visibility, less postoperative discomfort, and minimal surgical morbidity (Kumar & Kumar, 2013; Maiti *et al*., 2008; Wildt *et al*., 1977) [4, 6, 13]. Wildlife SOS in collaboration with the Agra Development Authority and the Agra District Administration took the initiative of a laparoscopic sterilization drive to mitigate the Human –Primate conflict caused by rhesus macaques in Agra district, Uttar Pradesh; by adopting humane methods of troop capturing, transportation, animal selection, endoscopic surgical procedure, creating permanent identification and re-release. This human-primate conflict mitigation project aimed to combat the increasing monkey menace issue that has been plaguing the city.

2. Materials and Methods

Documentation of the regions having maximum human- primate conflict was carried out and an extensive study of macaque troops was conducted for their behavior, identification, and selection of target macaque troops. Based on the study and population size, suitable-sized conditioning macaque trap cages were deployed to the selected target location. Macaque troops were conditioned to enter the large trap cages through regular feeding. Entire troops were captured after conditioning. After the successful trapping of a troop, individuals were separated by a filter cage and then shifted into the vehicle to avoid any stress or injuries due to infighting during transportation to the hospital site for endoscopic sterilization.

Soon after the animals reached the hospital, an initial examination and selection procedure was carried out to segregate the senile, juvenile, underweight/weak, and possibly pregnant animals for further care and feeding (Figure 3). Only the selected healthy individuals were starved for the sterilization procedure. Xylazine Hydrochloride @ 2 mg/kg bwt & Ketamine Hydrochloride @ 6 mg/kg bwt were used for induction once the animal was placed inside the squeeze cage. The sedated individual was prepared aseptically for the operation by shaving and disinfecting the abdominal region, followed by antibiotic & anti-inflammatory injections. The animal was kept in dorsal recumbency with a slightly inclined position by lifting its hip region with a folded clean cloth- covered cushion. General anesthesia was maintained by applying isoflurane (2%) face mask. 3mm laparoscopic surgical equipment was used to minimize the trauma and the surgical ports were created by adopting three-port technique for vasectomy/ tubectomy. A telescope was inserted through the midline port whereas the cutting and grasping forceps were inserted through the lateral ports (Figure 4). Vas deferens (in males) or fallopian tubes (in females) were located and gently grasped by forceps. A 3-4 cm long fragment of vas deferens/ fallopian tubes were removed by using atraumatic thermo-cautery forceps and scissors connected to an electrocautery unit (Figure 5). The midline port below the umbilicus was closed with 4-0 absorbable suture material using simple interrupted suture and the skin was opposed with 3M Vetbond tissue adhesive glue. The other two lateral incision ports were also opposed to tissue adhesive glue.

After the operative procedure, to ensure identification in case of re-capture, a tattoo artist performed permanent tattooing and tagging of each animal (Figure 6). The macaques were vaccinated against rabies and tetanus toxoid. A day after post-operative care, the entire troop was re-released in the same area it was captured from.

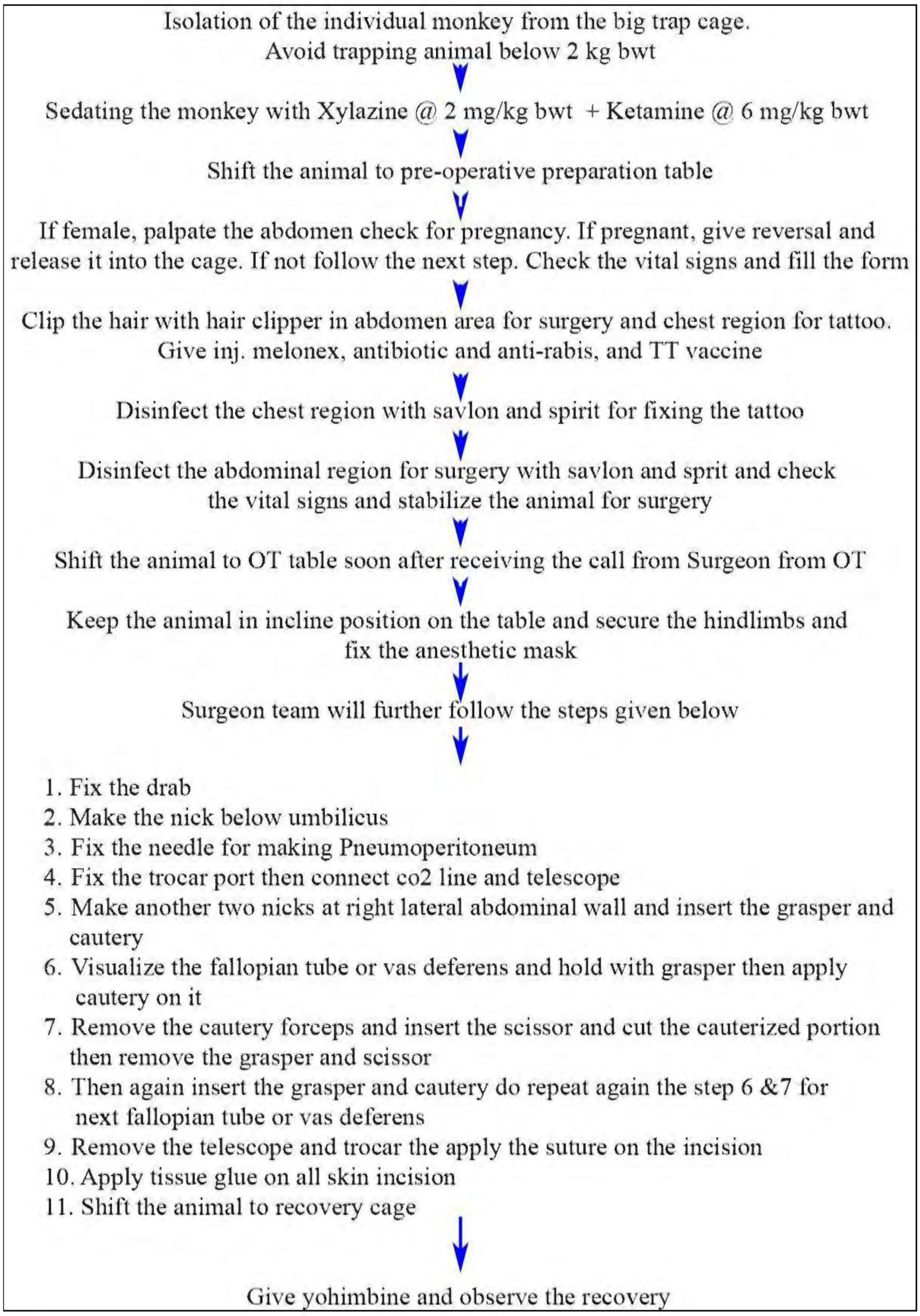


Fig 1: Flowchart showing the procedural sequence for laparoscopic sterilization of Rhesus macaques

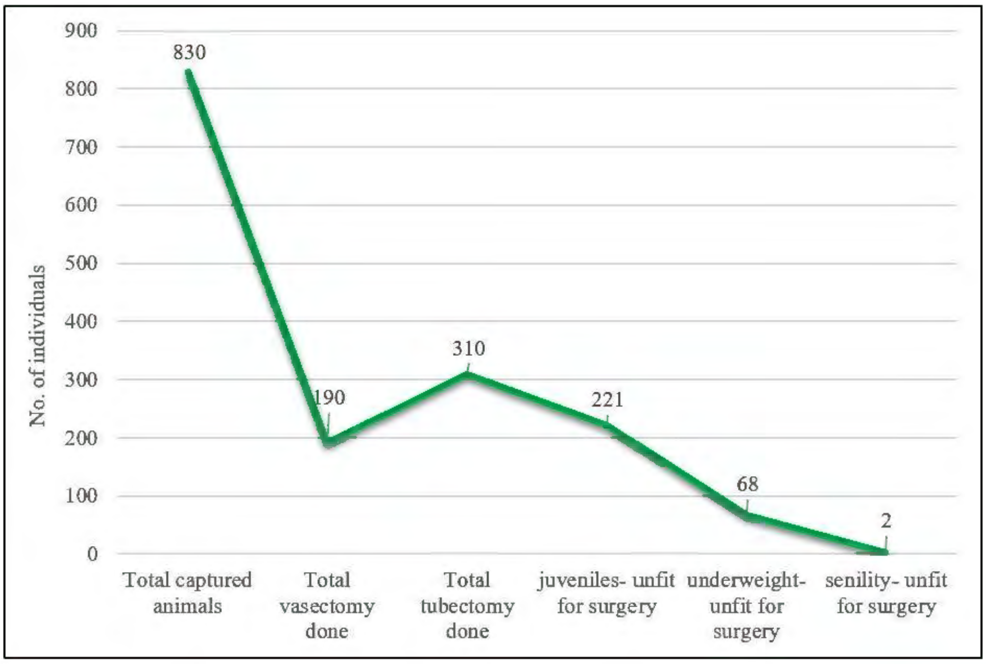


Fig 2: Detailed graphical representation of no. of Rhesus macaques captured, sterilized and unsterilized

Fig 3: Customized operation theatre facility along with laparoscopic unit and instruments

Fig 4: Procedural sequence of patient preparation for endoscopic sterilization

3. Results and Discussions

830 individuals, belonging to 38 troops were captured from 11 different locations out of which 500 individuals were sterilized (190 Vasectomy & 310 Tubectomy). The rest of the animals were not fit for surgery as they were either too young (221 are juveniles), underweight (68 individuals), senile (2 individuals), and 39 pregnant individuals (Figure 2). After surgery, the animals were kept under a day's observation and post-operative care; and re-released along with their troop in the same location from where they were caught. Further one- year continuous monitoring of the sterilized monkeys at the respective released sites revealed that the monkeys had adapted to their routine activity pattern without exhibiting any abnormalities (Figure 7). Out of the 500 sterilization procedures carried out, no mortality was reported. Over other surgical techniques, laparoscopic tubectomy/vasectomy is preferred as it does not affect an individual's social position, and the endocrine axis that drives behavior. In rhesus macaques intra-abdominal laparoscopic sterilization found to be simple, easy, and a faster method of vasectomy/tubectomy (Kumar & Kumar, 2013) [4]. This method requires minimum laparoscopic instrumentation and manpower and thus has the potential for mass sterilization within a minimum amount of time.

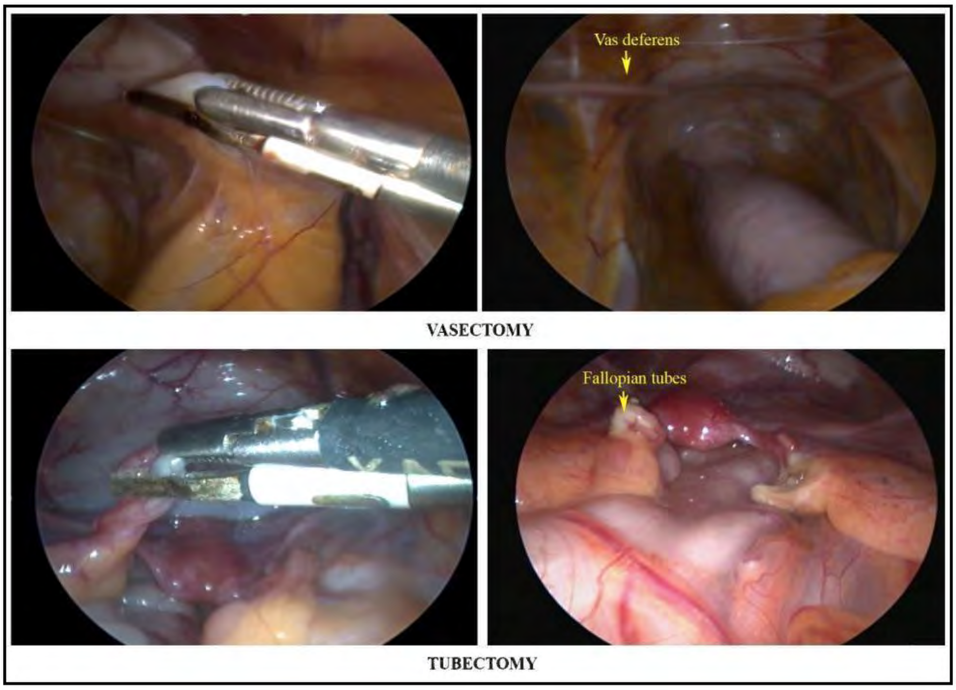


Fig 5: Laparoscopic visualization, grasping and cauterization; vasectomy and tubectomy by electrocautery

Fig 6: Safe permanent tattoo marking for each individual monkey with troop code

Fig 7: A successfully released monkey troop exhibiting natural behaviour after sterilization

The fast-growing human population, improper food waste disposal, and habitat degradation are the major factors that influence the intensity of wildlife conflicts, especially Human- primate conflict in India. Throughout their range in India, rhesus macaques inhabit areas near human beings for fulfilment of their diet. Thus has led to unavoidable competition for space and other resources between man and monkey (Imam, Ekwal *et al*., 2002) [2]. Monkey menace has been reported tot only in India, but also from other parts of the globe. Monkeys population is multiplying every year because of high birth rate and absence of a natural predator (Imam, E. & Yahya, 1995) [1]. The macaque sterilization program initiated by the Agra Development Authority and the District Administration was a successful venture and promises a visible change once sterilization of at least 75% of the population is attained. With this method, the conflict between the Man and Macaques will be reduced to a minimum. When the females do not carry young ones, their aggressiveness will reduce to a significant level as they will only be in search of their food, and not under duress to provide for their young or to protect them. Our studies revealed that macaques are aggressive during the mating season and when they are with the babies.

4. Conclusion

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

5. Acknowledgments

The authors gratefully acknowledge the hon'ble district magistrate, commissioner, Agra, Vice-chairperson, Agra development authority and Uttar Pradesh Forest department for their support. We are grateful to the cofounders of Wildlife SOS, Mr. Kartick Satyanarayan and Ms. Geeta Sheshamani. We extend our heartfelt thanks to Dr. Paolo Martelli & Dr. Karthiyani Martelli, Hong Kong for their technical guidance and support. Special thanks to Mr. Srinu Srikanta Maharana for his valuable input on the manuscript.

6. Reference

1. Imam E, Yahya HSA. Population dynamics and distribution of rhesus monkey in Aligarh district, Uttar Pradesh. Journal of Ecobiology. 1995;7:1-9.

2. Imam Ekwal, Yahya HSA, Malik I. A successful mass translocation of commensal rhesus monkeys *Macaca mulatta* in Vrindaban, India. Oryx. 2002;36(1):87-93.

3. Kansky RT, Knight A. Key factors driving attitudes towards large mammals in conflict with humans. Biological Conservation. 2014;179:93-105.

4. Kumar V, Kumar V. Intra-abdominal laparoscopic vasectomy by electrocauterization in free range Rhesus macaques (*Macaca mulatta*). AVS. 2013;1(5):42.

5. Lubell I, Frischer R. The current status of male and female sterilization procedures. Proceedings of the Royal Society of London. 1976;195:93-114.

6. Maiti SK, Dutta A, Ajith P, Kumar N, Sharma AK, SinghGR. Laparoscopy for direct examination of internal organs in small animals. Journal of Applied Animal Research. 2008;33(2):201-204.

7. McManus J, Dickman A, Gaynor D, Smuts B, Macdonald D. Dead or Alive? Comparing the costs and benefits of lethal and non-lethal human-wildlife conflict mitigation on

livestock farms. Oryx; c2014. p. 1-9.

8. Pebsworth PA, Gawde R, Bardi M, Vijayan BV, Radhakrishna S. To kill or not to kill: factors related to people's support of lethal and non-lethal strategies for managing monkeys in India. Human Dimensions of Wildlife. 2021;26(6):541-58.

9. Reddy MD. Monkey menace, its management. International Journal of Management, Technology and Engineering. 2018;8(12):5904-5908.

10. Shaurabh A, Radhakrishna S. Investigating trends in human-wildlife conflict: Is conflict escalation real or imagined? Journal of Asia-Pacific Biodiversity.2017;10(2):154-161.

11. Treves A. The human dimensions of conflicts with wildlife around protected areas. In: Wildlife and Society: The Science of Human Dimensions. Washington: Island press; c2008, p. 214-228.

12. Vedula P, Karanjkar A, Megha. Pre and post sterilization behaviour studies of Rhesus macaque (*Macaca mulatta*) in Himachal Pradesh, India. IJRASET. 2021;9(8):1263- 1272.

13. Wildt DE, Kinney GM, Seager SWJ. Laparoscopy for direct observation of internal organs of domestic cats and dogs. American Journal of Veterinary Research. 1977;38(9):1429-1432.

Art – 295. CRITICAL INJURIES DUE TO NEGATIVE INTERFACE BETWEEN WILD BOAR AND CAPTIVE SLOTH BEAR IN A FREE RANGING AREA

V.S. Arsh, G. Sriram and **A. Sha. Arun**

Abstract

A rescued dancing female sloth bear (*Melursus ursinus)* at Wildlife SOS; Bannerghatta bear rescue center; aged around 19 years had multiple injuries caused by wild boar attack. The visual examination revealed that the animal had two deep lacerated wounds, one on the myelohyoid region of the mandible jaw and the other on the left thigh region. After induction with ketamine and xylazine in combination at a dose rate of 5 mg/kg and 2 mg/kg, respectively. A surgical procedure involved suturing of the affected area was carried out under gaseous general anesthesia using isoflurane. Radiographic examination revealed no abnormalities. The required post-operative care was provided. After completing the surgical intervention, the bear recovered uneventfully in 20 days.

Keywords: Sloth bear, wild boar, lacerated wound, surgical intervention

Introduction

The sloth bear (*Melursus ursinus)* comes under the family Ursidae along with seven species of bears, including brown bears, polar bears, American black bears, Asian black bears, sun bears, spectacled bears and the giant panda.

The wild boar (*Sus scrofacristatus)* is one of the most numerous and widespread large mammals in the world, belonging to the family Suidae, as reported by Kose*et al.*(2011). The cases of wild boar attacks on sloth bears are under reported. However, the present case involves an attack on a female sloth bear by wild boar. The lower tusks of the wild boar are incredibly sharp. These tusks serve as weapons during the fighting. The animal was immobilized with xylazine hydrochloride and ketamine hydrochloride with the dose rate of 2 mg per kg body weight and 5 mg per kg body weight, respectively (llayaraja *et al.* 2021) and injuries depicts a lacerated wound on the myelohyoid region of the lower jaw (Fig. 1) and left thigh region (Fig. 2).

Fig.1. Fistula on the myelo-hyoid region of the lower jaw

Fig 2. Deep laceration wound on the left thigh region.

Materials and Methods

This procedure was conducted at Wildlife SOS, Bannerghatta bears rescue center, on a rescued dancing sloth bear aged 19 years observed with profuse bleeding from the posterior aspect of the lower jaw and left thigh region. For restraining and in-depth examination of the animal, chemical immobilization was preferred. Sedative and anesthetic agents were selected considering the animal's safety and smooth recovery. The combination of these drugs was found satisfactory for the chemical immobilization of captive sloth bears (Veerasailam *et al.*, 2014). Based on the body weight, the required dose was calculated i.e. for 87 kg body weight, ketamine (100 mg/ml) @ 5 mg/kg b.wt and xylazine (100 mg/ml) @ 2 mg/kg body weight. Hind quarter muscles were the preferred site for administering the anesthetic drugs. Darts were delivered with the aid of a blow pipe.

When the animal was completely immobilized, the vital signs were checked by using multipara monitor, which revealed that heart rate and respiration rate were regular, but there was a slight hyperthermia noticed. The animal was blindfolded and shifted to the Wildlife SOS, Wildlife Veterinary Hospital at Bannerghatta Bear Rescue Center. Radiographic examination of affected areas and whole body revealed no abnormalities. One ml of Tetanus toxoid was given as a routine practice. For the surgical procedure, the sloth bear was intubated with an 18 mm endotracheal tube, and gaseous anesthesia was maintained with 4 liters of oxygen and 2% to 4% isoflurane gas. An intravenous line was started in the saphenous vein.

Injury inflicted fistula formation in the mylo-hyoid region of the mandible jaw and the exposure of the sublingual area results in multiple injuries to the ventral aspect of the tongue (Fig 3).

Fig.3. Puncture wound on the ventral aspect of the tongue region

A lacerated wound of around 6 cm on the left thigh region and an injury on the left elbow region was also found. The wound was thoroughly cleaned with 5% povidone iodine solution and normal saline. Absorbable suture material no.4 was used to suture the tongue, suture material no.1 was used to suture the muscles and suture material no.2 was used to suture the skin edges (Fig 4A, Fig 4B, Fig 4C).

Fig. 4A. Post-Surgical intervention and sutured tongue region

Fig. 4B. Closing of the fistula with absorbable suture material

Fig. 4C. Correction of the lacerated wound on the thigh region

The post-operative medication regimen was adhered to;

1. Oral Antibiotic: Amoxicillin and potassium clavulunate (10mg/kg body weight, twice a day for 10 days; Hatclav, HatvetPharma Pty Ltd)– to avoid the secondary bacterial infections.

2. Oral tramadol (5 mg/kg body weight, twice a day for 7 days: Tramahat, HatvetPharma Pty Ltd.) and combination of enzymatic anti-inflammatory i.e. trypsin, bromelain, rutoside (1 tablet/twice a day for 15 days: Mut TBR, Mut Nature Pty Ltd, Chennai) – to alleviate pain and inflammation.

Results and Discussion

After the surgical procedure the said sloth bear was recovered from anesthesia smoothly and was observed to be alert and active exhibiting normal feeding behavior. The post - operative care was carried out for 15 days and the suture sites were cleaned with 5 % povidone iodine solution and antibiotic fucidic acid cream was applied on daily basis under positive conditioning. The animal was kept in quarantine enclosure and was under continuous observation. The animal showed uneventual recovery after the treatment.

The conflicts between wild boars and sloth bears are either uncommon or not documented in the wild as compared to the leopards and tigers with sloth bears which are mainly occurred as tigers will occasionally prey on sloth bears and leopards are been chased by sloth bears for their kill because of their scavenging behavior. In wild, sloth bears have a large home range and spend most of their time in travelling and foraging for food as they have well developed olfactory system and same for captive bears to engage them with some activities and to keep their mental health in a good state enrichment, training and complexibility in their environment needed to be done as reported by carlstead et al.(1991).

Acknowledgements

The authors are thankful to Executive Director, Bannerghatta Biological Park and Mr. Kartick Satyanarayan and Ms Geeta Seshamani, Co-Founders, Wildlife SOS, for extending their support for the routine activity and veterinary care for the rehabilitated sloth bears at the Bannerghatta Bear Rescue Centre (BBRC). The authors would also like to thank and acknowledge the support of all the bear care staff at BBRC for their valuable field assistance during the upkeep of the injuired rescued sloth bears.

References

Carlstead K, Seidensticker J and Baldwin R (1991) Environmental enrichment for zoo bears. Zoo.boil., 10(1): 3-16.

Ilayaraja S, Palanivelrajan M, Jayathangaraj MG and Arun AS (2021) Surveillance and examination of dental problems in captive sloth bears. Int.J.Anim.Res, 7(10): 141-148.

Kose O, Guler F, Baz AB, Akalýn S and Turan A (2011) Management of a wild boar wound. Wilderness Environ.Med., 22(3): 242- 245.

Veeraselvam M, Sridhar R, Perumal P and Jayathangaraj M G (2014) Chemical immobilization of sloth bears (Melursus ursinus) with ketamine hydrochloride and xylazine hydrochloride: hematology and serum biochemical values. Vet.Med.Int., 1- 5

Art – 296. POACHER'S SNARE AS THREAT TO INDIAN WILDLIFE: A REVIEW

R. Kumar, Y. Pannerselvam, P. Deb, A. Sha. Arun and **T.R. Sharp**

Abstract

A snare is one of the easiest but most destructive hunting methods. In Asia, snares are the most widely used method of hunting because they are cheap to produce and easy to set in large numbers. Wildlife SOS, in collaboration with the Karnataka Forest Department, has attended a total of 42 wildlife rescue calls of sloth bears and leopards entrapped in such illegal snares or traps during the period 2009 to 2019.The snare traps entangled around the hind quarter, leads to internal organ damage and a slow death even after the rescue. Leopards and bears may also suffer serious dental problems from biting the snares, which results in medical problems and the inability to return the rescued animal to the wild. Intensive awareness programs in and around the protected areas regarding this barbaric and primitive trapping tools would help in reducing or avoiding such incidents. Human-animal conflict has been present ever since the fragmentation of forests and increase of biotic pressures have brought wild animals closer to humans in a fierce competition for survival. The present review deals with incidences of sloth bear and leopard mortality or injury due to snare documented across various districts of Karnataka and few other states, this review provides vital information about snares' threat to Indian wildlife. Based on content analysis of newspapers and news portals, we identified 446 incidents of wild animals caught in snare traps from January 2018 to October 2022. Most snare incidents involved wild boars, snakes, nilgai, Indian leopards, jackals and royal Bengal tigers. This review indicates large number of carnivore death as compared to both herbivore and omnivores. We therefore propose a shift in management focus, from current reactive practice to proactive measures that ensure safety of wildlife.

Keywords: Animal welfare, electrocution, extinction, poaching, wildlife trade

Introduction

Globally poaching and illegal wildlife trade is driving many of the world's valuable species into extinction. Elephants, rhinos, and tigers are among the many exotic species that are poached for ivory, horn, and skin in order to make them targets for illegal trade (Kaul et al., 2004; Spillane, 2015). Other animals like wild pigs and deer are hunted as bush meat for protein (Warchol, 2004). Due to the development and emergence of several anti- poaching camps as well as the increased protection inside the protected areas, poachers' motives and hunting techniques have changed. Researchers believe that poachers vary; they may have varied motivations and motives, use different techniques, and use equipment of diverse types (Pires et al., 2016). One of the simple yet deadly methods used by poachers is to set up a snare and the practice of laying snares dates to the early 80s. It is no secret that hunters and poachers use rope, wire, or brake cables for making these simple, low-tech, noose-like traps, which they set in forests in order to capture animals. Snaring is one of the effortless but most effective hunting techniques followed in Asia (Belecky et al., 2020) and other parts of the world. It is becoming increasingly common to use wire snares in Asia due to their ease of construction from readily available materials like bicycle and motorcycle cable wires. Poachers set snares targeted for specific animals. There are tiny, thin, single strand wire snares used to catch small animals like hares at lower levels on the trails, while larger, thicker snares used to trap bigger animals like wild pigs at higher levels.

Snares are essentially long pieces of wire connected at their ends with a loop and attached to stationary objects, such as trees or logs. Using a loop of wire, the snare is suspended from a branch or small tree, catching animals by their necks as they walk through the forest. The snare grips tightly and captures the animal as it continues to move forward. Snare traps are one of the most popular types of traps, not only because they are so easy to use, but also because they are so easy to make. In technical terms, they are wire or cable nooses that are anchored somewhere. It is impossible for the animal to escape the trap once it runs over it, as the noose tightens around the animal's body, neck or limb and it is unable to escape the trap as a result even though it may be simple and effective, it is not at all humane. According to the report by Mongabay on Snare traps decline, but still pose a threat to Leuser's Sumatran rhinos they explained that "Snares are typically made of steel or nylon wire and are easy to build. In addition, they are indiscriminate in what they capture, resulting in non-target species as well as females and juveniles being caught. While most of the trapped animals end up in local wildlife markets or are sold directly to restaurants as bush meat, the high-value species are typically traded in major cities or exported to foreign markets."

Throughout Southeast Asia, snaring is one of the most common types of hunting used to capture animal for human consumption and to stock wildlife farms in order to capture wildlife for human consumption (Becker et al., 2013; Gray et al., 2018). The ungulates are a very common species that is caught in Cambodia, Lao PDR, and Viet Nam, and there is evidence that they are a species that is traded more frequently in Asian countries (Cantlay et al., 2017). A study of wildlife seizures in Cambodia from 2005 to 2017 found that 46% of all wildlife meat seizures (61%) that likely came from snared animals (ungulates, carnivores, lagomorphs) occurred in markets (which were referred to as snared animals), whereas 48% (32% of biomass) occurred in restaurants and resorts. According to the WWF latest analysis report on Snaring crisis, it is concluded that, "There are an estimated 12.3 million snare on the ground in protected areas of Cambodia, Lao PDR and Vietnam." Similarly, according to the statement by Richard Thomas from TRAFFIC's, explained that, over 30,000 snares were removed in Cambodia in 2016 alone; it is likely that many more remain undiscovered. "As snares are a very dangerous device simply because they kill at random, which means all manner of wildlife is at risk. Snares are also very commonly used by poachers to steal tigers from Asia's forests due to their tendency to kill at random. In order to curb this crisis, there is an urgent need for the countries in the Tiger range to intensify their enforcement efforts."

The snaring technique is not only a common method in Asian countries, but all over Africa as well. As a result of the rising global demand for bushmeat in Africa, there is an increase in the silent capturing and poaching of wild animals with the use of snare traps. Since snaring has become a popular method throughout Africa because of the availability of the materials needed (e.g., fence wire, telecommunications or electrical cabling and nylon rope) at an affordable price, this method has become very widespread (Obanda et al., 2008; Mowat et al., 1994). It is widespread throughout Africa for bushmeat to be harvested using snares which is mainly done within the protected areas of forest and savanna as well as in communal or private lands. (Hitchcock, 2000; Poulsen et al., 2009; Lindsey et al., 2013; van Velden et al., 2018;van Velden et al., 2020).

Snares

Of the 18 sloth bears caught in snares that Wildlife SOS attempted to rescue (Table 1 & 2), twelve (67%) were eventually released back to the forest, and in all cases except one, back to the forest they were trapped nearby. Four bears (22%) died in the snare or from the wounds they received while being caught in the snare, and two bears (11%) were put into lifetime care at the Wildlife SOS Bannerghatta Bear Rescue Centre, due to the fact that their injuries were too substantial to release them back to the wild. Eleven of the bears were female, and seven of the bears were male. Half of the bears (n=9) were estimated at 2 years old or younger, the half (n=9) were estimated at 5 years or older.

Ten of the eighteen snares (56%) were found in agricultural areas, three (17%) were found in forest or scrublands and five (28%) did not have a location documented. The average distance of those found in agricultural fields was over 2,000 meters from forest edges. Two of the three snares found in forest or scrublands were less than 300 meters from agricultural fields while one was over 800 meters from agricultural areas.

There was a spike in the number of bears caught in snares between the months of September and December. This is during the harvest time when animals enter the agricultural areas to raid the crops. Six of the bears (33%) caught in snares were caught outside of the harvesting season. However, 3 of these were caught in the scrub areas, not the agricultural areas, and two of them were caught in undocumented locations. Only one bear was caught in the agricultural areas outside of the harvesting time.

Table 1. Sloth bear incidents with human hazards and distance from forest fringes

Type of Hazard	Number of Incidents	Average Distance to Forest Edge (m)	Range of Distances to Forest Edge (m)	Number of Incident in a forest area	Notes
Snares	18	2,117 (n=10)	240 - 8,825	3	5 Locations Unknown

Table 2. Sloth bear incidents by gender with human hazards

Type of Hazard	Number of Incidents	Females (including with cubs)	Males
Snares	18	11	7

Methodology of the study

Apart from the table 1 and 2 data, the rest of the data collection was made through the secondary sources. The relevant information and data were collected by reviewing various website and research articles for content that explains snare wire and traps. In addition, the last five years (January 2018-October 2022) wildlife injury and death data are collected and analyzed using daily newspapers like the Hindu, the Indian Express, as well as articles written by organizations such as WWF, Asia, etc. There is a strong presence of media in India, even in rural areas, and news about wild species is mostly covered by the media, making it a reliable source of information about large mammal conflicts. The study relied completely on information sourced from newspaper media reports, open-source government websites and remotely acquired data. Animal care and use committee approval was not required.

Results and discussion

Snare traps in India

The snare traps are made from materials that can readily be found, including clutch wires, fencing wires, and other materials that can be found around the house. Considering the fact that they are light and easy to carry, they can be used to capture animals without them being aware that they are being caught. Using wire snares and electrocution are the most predominant ways to kill animals. Itis generally the local village communities who set up these traps to be able to catch wild boars, small herbivores, etc. that wander around in the area. It is quite common to set up snares along game trails and near watering holes, (Gubbi et al., 2021) where there is a greater chance of getting caught by the trap. Wildlife killing with snares is illegal in India, but snares remain a popular method for catching wildlife Indian wildlife populations are rarely studied empirically for the effects of snares (Madhusudan and Karanth, 2002; Gurung et al., 2008; Gubbi and Linkie 2012).

Fig. 1. Snare trap/ cable removed by Karnataka Forest department

Cases of snaring in India

According to records, over the course of the last decade, India has witnessed twenty-four tigers and one hundred and fourteen leopards becoming entangled in wire snares. Uttarakhand, Karnataka, and Madhya Pradesh are some of the states that have been high on the radar of snares. Nevertheless, database compiled by Wildlife Protection

Society of India WPSI, a conservation organization fighting poaching and escalating wildlife trade, shows 24 tiger fatalities and 110 leopard deaths in the country in 2010-2018, including five tiger, 14 leopards and 30 other wild animals alone in Maharashtra state. It has also been reported that the greatest number of big cats, 26 leopards and three tigers, have been killed in Uttarakhand and at least 13 leopards have been injured. In Madhya Pradesh, five tigers have been killed by wire snares and one has been injured, the highest number of tigers killed in a state in the last decade.

It is common for people living in close proximity to forests or protected areas to place snare traps either as a means of hunting bushmeat or as a means of defending crops against crop damaging animals. In India, 446 wild animals were strangled in snare traps in between 2018 and 2022 i.e., on an average 89-90 animals per year. During 2018, 99 deaths are reported, followed by 79 in 2019, 84 in 2020, 90 in 2021, and 94 in 2022 (Table. 2). These deaths included 22 cases of Bengal tigers, 32 cases of Indian leopards, 12 cases of Asian elephants (mainly electrocuted snare), and 118 cases of wild boars.

While snare traps are often set for small animals, it appears that large animals are most likely to fall victim to them. In spite of the fact that snare traps are often used to capture small mammal such as Indian hares, and other animals such as wild boars, the majority of the victims were non- targeted species i.e., Bengal tiger, Indian leopard and etc. The occurrence of carnivore cases is 49% (215) while omnivore cases are 33% (149) and herbivore cases are 18% (82) (Fig. 2). The following graphs are an overview of the death of wild animals from snaring from the years 2018 to 2022.

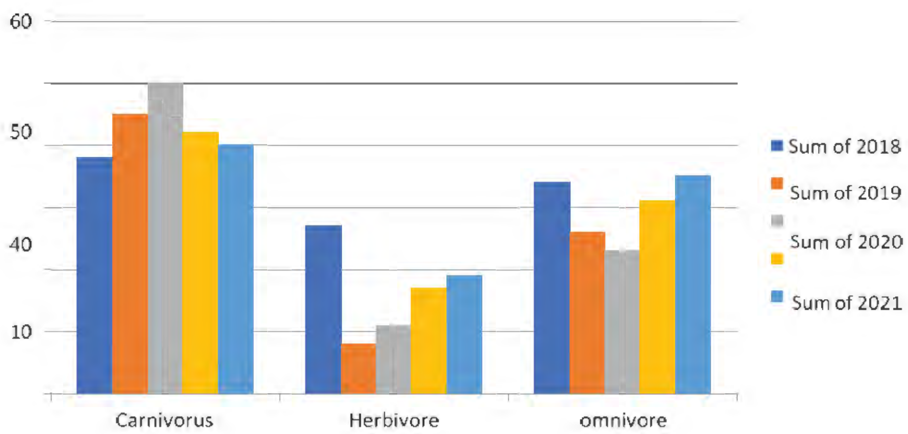


Fig. 2. Snaring victims in India in the past five years, grouped according to their diet

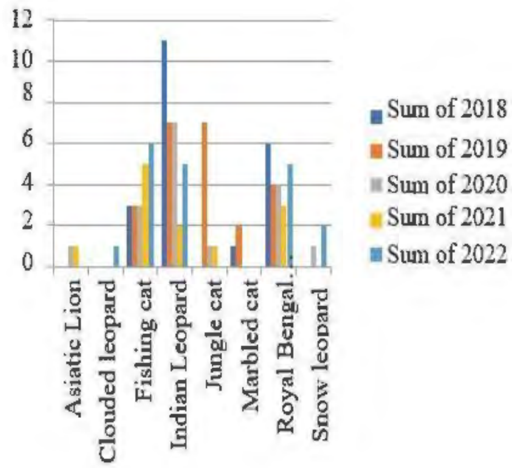


Fig. 3. Felidae

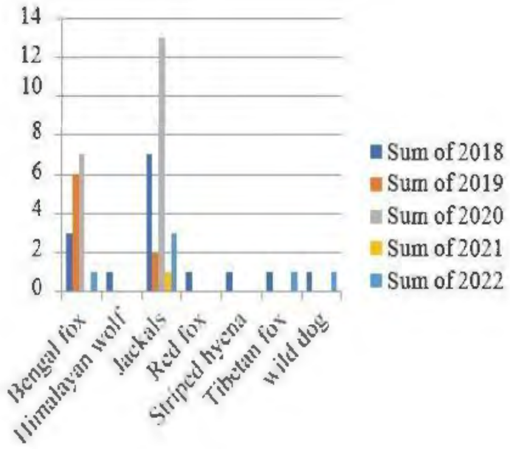


Fig. 4. Canidae

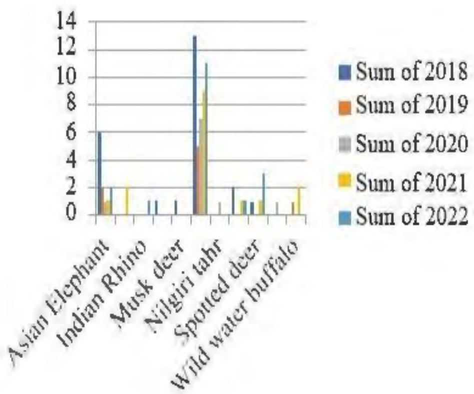


Fig. 5. Herbivores

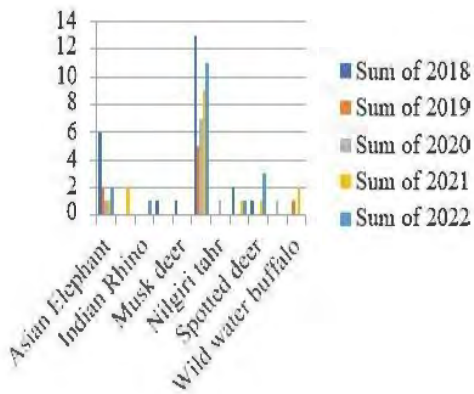


Fig. 6. Aquatic and snake

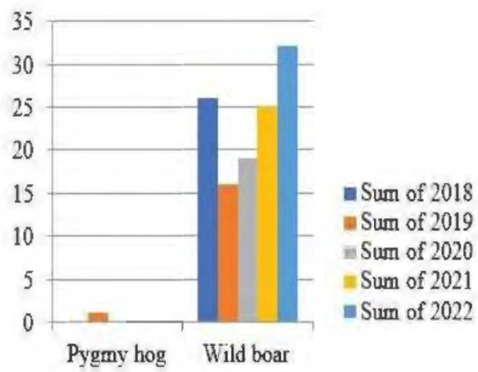


Fig. 7. Suidae

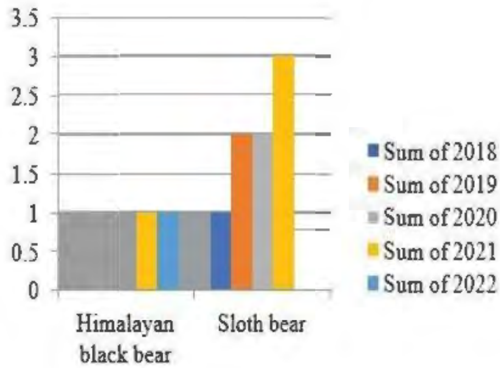


Fig. 8. Ursidae

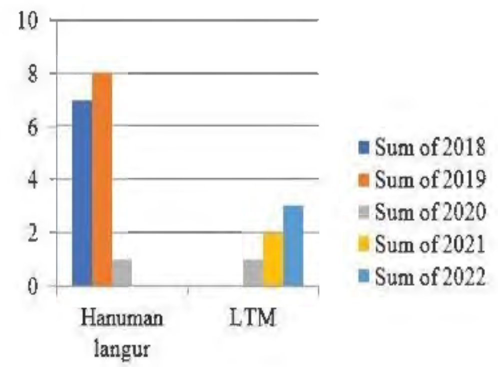


Fig. 9. Primates

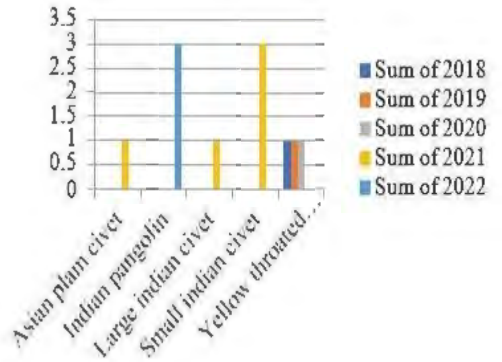


Fig. 10. Other small mammals

Table 3. List of animals died by snare traps from 2018-22 in India

No.	Animal	Type	2018	2019	2020	2021	2022	Total
1	Royal Bengal Tiger	Carnivore	6	4	4	3	5	22
2	Indian Leopard	Carnivore	11	7	7	2	5	32
3	Asiatic Lion	Carnivore	0	0	1	1	0	2
4	Fishing cat	Carnivore	3	3	3	5	6	20
5	Wild boar	Omnivore	26	16	19	25	32	118
6	Snow leopard	Carnivore	0	0	1	0	2	3
7	Clouded leopard	Carnivore	0	0	0	0	1	1
8	Sloth bear	Omnivore	1	2	2	3	0	8
9	Indian Gaur	Herbivore	0	0	0	2	0	2
10	Asian Elephant	Herbivore	6	2	1	1	2	12
11	Lion Tail Macaque	Omnivore	0	0	1	2	3	6
12	Indian Rhino	Herbivore	0	0	0	0	1	1
13	Wild water buffalo	Herbivore	0	1	0	2	0	3
14	Nilgai	Herbivore	13	5	7	9	11	45
15	Bengal fox	Carnivore	3	6	7	0	1	17
16	Striped hyena	Carnivore	1	0	0	0	0	1
17	Spotted deer	Herbivore	1	0	0	1	3	5
18	Swamp deer	Herbivore	0	0	1	0	0	1
19	Kashmir stag	Herbivore	1	0	0	0	0	1

Discussion

Indian wildlife continues to suffer from snare setting, despite a wide range of preventative measures implemented over the years. A number of reasons exist for the prevalence of snare-based poaching in India, including the low costs involved and the low risk of being caught and prosecuted if caught. A cable snare is directly responsible for providing food for the household and indirectly for producing income through the sale of bushmeat, which is sold to the public. Gubbi et al. (2021) explained that the number of snaring incidents were extremely high during the monsoon season which is the peak cropping season when farmers tend to put extra effort into protecting their crops and their livestock, including setting snares to stop herbivores from raiding their crops. There may be a reason for the high number of leopards that get caught in snares during monsoon season.

Finding in this study indicate high number of carnivore death as compared to both herbivore and omnivore animals. Although the traps are mainly set for small mammals like wild pigs, hare’s, mongoose, mouse deer, civets and squirrels etc. (Fig. 15) but the prime victims were the large mammals like Bengal tiger (22 cases), Indian leopard (32 cases), nilgai (45 cases), sloth bear (8 cases), Wild boar (118 cases) and sometime even elephants etc. Apart from that, aquatic species like Gangetic dolphin (1 case), Ganges shark (1 case), red crowned roof turtles (6 cases) and snake species like Indian cobra, etc. (58 cases), have also been victims of the snare traps in India.

When it comes to hunting for human consumption and the stocking of wildlife farms in Southeast Asia, snaring is among the most prevalent methods of hunting for the purpose of capturing animals for human consumption (Harrison etal., 2016; Gray et al., 2018). In Southeast Asia, Cambodia, Lao PDR, and Viet Nam are among the nations most affected by the snaring crisis, with a greater number of snares than anywhere else in the region or in the world (Belecky and Gray, 2020). According to the data collected WWF 2020 Southeast Asia snaring crisis report, it is estimated that between 2005 and 2019 rangers from 11 protected areas in five Southeast Asian countries (Cambodia, Indonesia, Lao People's Democratic Republic, Malaysia, and Viet Nam) removed 371,856 snares (approximately 53,000 snares a year) from 11 protected areas (Belecky andGray, 2020). In Vietnam there is between 60% and80% of the wildlife meat consumed in urban areas that is eaten in restaurants (Nguyen, 2003; Drury, 2011). The most commonly consumed species, which represents almost 75% of all wildlife meat consumption, is wild pig a species that is heavily hunted with snares in mainland Southeast Asia (WWF Vietnam, 2017). Similarly, a study of wildlife seizures collected in Cambodia between 2005 and 2017 revealed that 46% (representing 61% of the seized biomass) of wildlife meat that was likely to have been snared occurred in markets, whereas 48% (42% of biomass) were seized at restaurants and resorts (Heinrich et al., 2020).

Furthermore, a study conducted in Bayanga region of Central African Republic on cable snare hunting, stated that in the Bayanga hunting range, which includes Dzanga-Ndoki National Park, there are on average 4.2 cable snares per square kilometer, with an estimated 9000 total captures per year, or nine captures per square kilometer, which puts the total number of captures around 9 per square kilometer (Noss, 1998). There are, however, studies in South Africa that show that 30-60% of rural households living in communal tenure regions consume bushmeat as a matter of course (Grey-Ross et al., 2010; Martins and Shackleton, 2019).

Snares are cost-efficient, easy to carry and, unlike firearms, easy for poachers to conceal and transport throughout the world. Even though snares are simple in design, they frequently cause severe discomfort and pain to animals in controlled experiments following animal welfare guidelines. This is especially true in remote locations where hunters leave traps unattended for weeks or even months (Mowat et al., 1994; Powell, 2005; Gese et al., 2019). In spite of its indiscriminate nature, snaring has the potential to be very detrimental (i.e., non-target mortalities) (Fig. 14) and extremely wasteful if it is carried out in an irregular manner (Obanda et al., 2008; Lindsey et al., 2011).

Animals captured in such conditions will usually experience prolonged suffering before death. Some animals may be able to escape from the ensnaring trap, either by self-mutilation (such as chewing away at ensnared limbs to free themselves) or by self-harm Noss (1998). In order to survive, these crippled individuals will have to deal with a great deal of hardship. It is well documented that animals suffering from such injuries are likely to have smaller home ranges, to suffer from malnutrition, and to occupy degraded habitats, as they have difficulty defending their territories against healthy animals (Sunquist, 1981; Othman et al., 2019). Animals who suffer from physical ailments are more likely to engage in conflict as their behavior is altered (Becker etal., 2013). For example, it has been reported that physical impairments are the most common factor associated with human-killing tigers in Nepal (Gurung et al., 2008). It has also been reported that elephants wounded in snares pose serious dangers to rural villages, thus escalating conflict with the species that is already prone to antagonistic encounters with humans (Obanda et al., 2008; Becker et al., 2013; Abdullah et al., 2019; Othman et al., 2019).

An animal species with a dominant hunting instinct could be subjected to physical impairments including dental issues, tooth loss, and misaligned canine teeth, which could influence the genetic selection process in terms of breeding. An individual with incomplete or complete ocular or auditory deformities, as well as locomotion defects can alter the individual's behavior, hunting skills, and can even lead to the possibility of a human- wildlife conflict. In terms of physiological impact, cervical bone fractures or defects, dislocated joints, fractured limbs and physiological damages to vital organs are among the most common and leads to mortality such as kidney, spleen, liver, heart. These injuries occur as a result of an excess compression and pressure of the snare which is dependent upon the position of the snare within the animal.

Fig. 11 (a, b, c, d, e, f). Above figures showing different types of snares, i.e., Loop snares mainly used for capturing small animals like Indian hare etc., Net snare traps mainly used for capturing large animals like wild boar, spotted deer etc. and stone traps also used for small mammals.

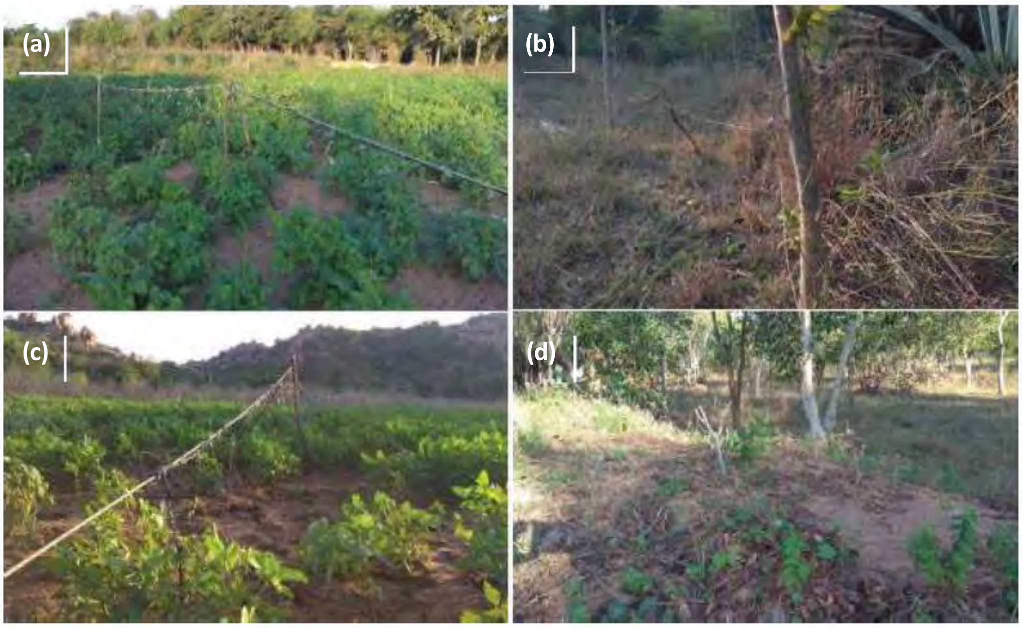


Fig. 12 (a, b, c, d). Wire snares and loops snares placed by people in agricultural fields

Fig. 6. Different types of confiscated snares: net, nylon wire, metal wire and clutch wire

Fig. 13 (a, b, c, d, e). Different types of confiscated snares: Net, nylon wire, metal wire and clutch wire

Fig. 14 (a, b, c, d, e, f). Victims of some non-targeted species

Fig. 15 (a, b, c, d). Some of the most targeted species.

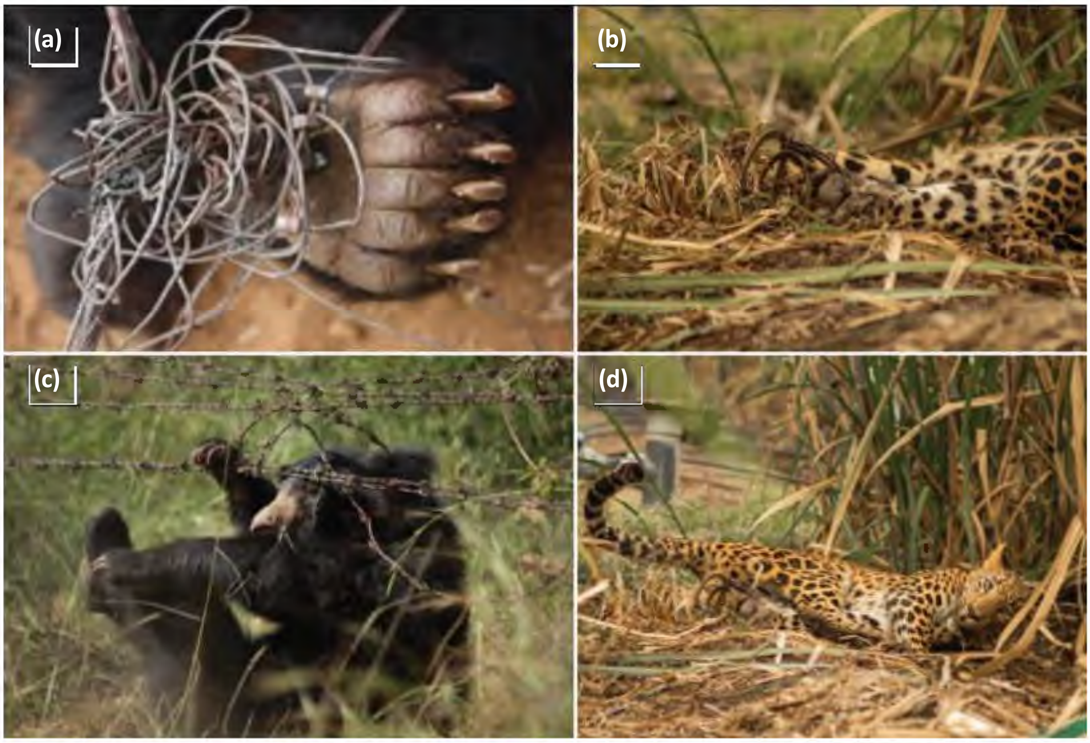


Fig 16 (a, b, c, d). Sloth bear (a and b) struggling after getting trapped in a barbed wire and Leopard (c and d) trapped in a snare trap rescued by Wildlife SOS

Recommendations

A comprehensive review of this article over a wide range of wildlife snaring studies conducted in different countries and the Indian subcontinent led the author to make the following recommendations. Snare patrols should be conducted regularly in and around protected forests to pick up snares that have been setup. As part of its efforts to combat the incidence of snares, the Forest Department should seek help from a wide range of stakeholders and agencies. Poachers who use snares for illegal purposes must be punished and convicted with more severe legal consequences. Besides that, awareness sessions should be conducted continuously all the time, especially when hunting season is believed to be in full swing. Furthermore, educating the people living around forests' fringes that it is a punishable offense under the Indian Wildlife Protection Act 1972.

Acknowledgements

The authors greatly appreciate the support of Shri K. Satyanarayan and Smt. Geeta Seshamani of Wildlife SOS, for their continued encouragement. Also, they profoundly thank the authorities of Karnataka Forest Department, Maharashtra Forest Department for their kind co-operation and opportunities. Photo credits to Mr. Lenu Kannan and Mr. Akash of Wildlife SOS, Documentation Officers. Thanks to Mr. Swaminathan, Dr. Ashok, Mr.Bala, Mr.Reegan, Wildlife Biologists of WildlifeSOS for extending their co-operation for assisting us during the data collection period.

References

Abdullah, A., Sayuti, A., Hasanuddin, H., Affan, M. and Wilson, G. 2019. People's perceptions of elephant conservation and the human-elephant conflict in Aceh Jaya, Sumatra, Indonesia. *Euro. J. Wildlife Res*. 65 (5): 69.

Arun, A.S., Bakde, R. and Adhithyan, N.K. 2020. Successful rescue and rehabilitation of an injured wild sloth bear trapped in a poacher's snare. *e-planet*18 (2): 158-163.

Arun, A.S., Patil, S.P. and Shrikrishna, B. 2022a. Xenografting of tilapia fish skin for snare wound management in wild sloth bear (*Melursus ursinus*): A novel approach. *e-planet* 20 (2): 146-151.

Arun, A.S., Virk, A.S. and Patil, S.P. 2022b. Surgical treatment of baculum fracture in sloth bear (*Melursusursinus*): A case report. *e-planet* 20 (1): 74-78.

Becker, M., McRobb, R., Watson, F., Droge, E., Kanyembo, B., Murdoch, J. and Kakumbi, C. 2013. Evaluating wire-snare poaching trends and the impacts of by-catch on elephants and large carnivores. *Biol. Conserv*. 158: 26-36.

Belecky, M., and Gray, T.N.E. 2020. Silence of the Snares: Southeast Asia's Snaring Crisis, WWF International.

Cantlay, J.C., Ingram, D.J., and Meredith, A.L. 2017. A review of zoonotic infection risks associated with the wild meat trade in Malaysia. *Eco Health* 14 (2):361-388.

Chase, M.J, Schlossberg, S., Griffin, C.R., Bouche´, P.J.,Djene, S.W., Elkan, P.W., Ferreira, S., Grossman, F., Kohi, E.M., Landen, K., Omondi, P., Peltier, A.,Selier, S.A. and Sutcliffe, R. 2016. Continent-wide survey reveals massive decline in African savannah elephants. *Peer J.* 31(4): e2354.

Drury, R. 2011. Hungry for success: urban consumer demand for wild animal products in Vietnam. *Conserv. Soc*. 9 (3): 247-257.

Gese, E.M., Terletzky, P.A., Erb, J.D., Fuller, K.C., Grabarkewitz, J.P., Hart, J.P. and Young, J.K. 2019. Injury scores and spatial responses of wolves following capture: Cable restraints versus foothold traps. *Wildlife Soc. Bull*. 43: 42-52.

Gray, T.N., Hughes, A.C., Laurance, W.F., Long, B., Lynam, A.J., O'Kelly, H., Ripple, W.J., Seng, T., Scotson, L. and Wilkinson, N.M., 2018. The wildlife snaring crisis: an insidious and pervasive threat to biodiversity in Southeast Asia. *Biodiv. Conserv.* 27 (4): 1031-1037.

Grey-Ross, R., Downs, C.T. and Kirkman, K. 2010.An assessment of illegal hunting on farmland in KwaZulu-Natal, South Africa: Implications for oribi (*Ourebia ourebi*) conservation. *South Afr. J. WildlifeRes*. 40 (1): 43-52.

Gubbi, S. and Linkie, M. 2012. Wildlife hunting patterns, techniques, and profile of hunters in and around Periyar Tiger Reserve. *J. Bombay Nat. Hist. Soc.* 109 (3): 165-172.

Gubbi, S., Kolekar, A. and Kumara, V. 2021. Quantifyingwire snares as a threat to leopards in Karnataka, India. *Trop. Conserv. Sci*. 14: 1-8.

Gurung, B., Smith, J.L.D., McDougal, C., Karki, J.B. and Barlow, A. 2008. Factors associated with human- killing tigers in Chitwan National Park, Nepal. *Biol.Conserv.* 141: 3069-3078.

Harrison, R.D., Sreekar, R., Brodie, J.F., Brook, S.,Luskin, M., O'Kelly, H., Rao, M., Scheffers,

B. and Velho, N. 2016. Impacts of hunting on tropical forests in Southeast Asia. *Conserv. Biol*.30 (5): 972-981

Heinrich, S., Ross, J.V., Gray, T.N., Delean, S., Marx, N. and Cassey, P. 2020. Plight of the commons:17 years of wildlife trafficking in Cambodia. *Biol. Conserv*. 241: 108379.

Hitchcock, R.K. 2000. Traditional African wildlife utilization: subsistence hunting, poaching, and sustainable use. *Wildlife Conserv. Sustain. Us*e 12: 389-415.

Kaul, R., Jandrotia, H.J.S., and McGowan, P.J.K. 2004. Hunting of large mammals and pheasants in the Indian western Himalaya. *Oryx* 38 (4): 426-431.

Kendon, T.A., Comley, J., Wilkinson, A., Grobler, M.J., Niemand, W.A., Leslie, A.J., O'Riain, M.J. and Naude, V.N. 2022. Evaluating determinants of wire-snare poaching risk in the Boland Mountain Complex of South Africa*. J. Nat. Conserv.* 70 (2): 126295.

Lindsey, P. A., Romanach, S.S., Tambling, C.J., Chartier, K. and Groom, R. 2011. Ecological and financial impacts of illegal bushmeat trade in Zimbabwe.*Oryx* 45 (1): 96-111.

Lindsey, P.A., Balme, G.A., Becker, M., Begg, C., Bento,C., Bocchino, C. and ZisadzaGandiwa, P. 2013.The bushmeat trade in African savannas: Impacts, drivers, and possible solutions. *Biol. Conserv*. 160 (1): 80-96.

Madhusudan, M.D. and Karanth, K.U. 2002. Local hunting and the conservation of large mammals in India. *Ambio* 31 (1): 49-54.

Martins, V. and Shackleton, C. M. 2019. Bushmeat use is widespread but under-researched in rural communities of South Africa. *Global Ecol. Conserv*.17 (1): e00583.

Mowat, G., Slough, B.G. and Rivard, R. 1994. A comparison of three live capturing devices for lynx: Capture efficiency and injuries. *Wildlife Soc. Bull*. 22: 644-650.

Nguyen, S.V. 2003. *Wildlife Trading in Vietnam: Why it Flourishes*. EEPSEA Research Reports, Singapore, pp. 1-75.

Noss, A.J. 1998. The Impacts of cable snare hunting on wildlife populations in the forests of the Central African Republic. *Conserv. Biol*. 12 (2): 390-398.

Obanda, V., Ndeereh, D., Mijele, D., Lekolool, I., Chege,S., Gakuya, F. and Omondi, P. 2008. Injuries of free ranging African elephants in various ranges of Kenya. *Pachyderm* 44: 54-58.

Othman, N., Goossens, B., Cheah, C.P.I., Nathan,S., Bumpus, R. and Ancrenaz, M. 2019. Shift of paradigm needed towards improving human- elephant coexistence in monoculture landscapes in Sabah. *Int. Zoo Yearbook* 53: 161-173.

Pires, S.F. and Moreto, W.D. 2016. *The Illegal Wildlife Trades*. Oxford Handbooks Online.

Poulsen, J.R., Clark, C.J., Mavah, G. and Elkan, P.W. 2009. Bushmeat supply and consumption in a tropical logging concession in northern Congo. *Conserv. Biol*. 23 (6): 1597-1608.

Powell, R.A. 2005. Evaluating welfare of American black bears captured in foot snares and in winter dens. *J. Mammal*. 86: 1171-1177.

Samantaray, R.K., Roy, P.K., Das, A., Mishra, A.K., Mahapatra, S.N. and Pattanaik, A.K. 2008. Rescue of a gunshot injured sloth bear (*Melursus ursinus*) from Nuapada, Orissa, its successful treatment, translocation and rehabilitation. *e-planet* 6 (2): 27-31

Spillane, J.J. 2015. Africa’s elephant population: Permanently declining or sustainable? *Eastern Afr. J. Hosp. Leis. Tour*. 3 (1): 1-19.

Sunquist, M.E. 1981. The social organization of tigers in Royal Chitwan National Park, Nepal. *Smithsonian Contrib. Zool*. 336: 1-98.

Van Velden, J., Wilson, K. and Biggs, D. 2018. The evidence for bushmeat crisis in African savannas:A systematic quantitative literature review. *Biol. Conserv*. 221 (1): 345-356.

Van Velden, J.L., Travers, H., Moyo, B.H.Z. and Biggs, D. 2020. Using scenarios to understand community- based interventions for bushmeat hunting and consumption in African savannas. *Biol. Conserv*. 248 (1): e108676.

Warchol, G.L. 2004. The transnational illegal wildlife trade. *Crim. Justice Stud*. 17 (1): 57-73.

WWF Vietnam, 2017. *Consumption of wild meat and other Wildlife Products in Hue City*. Internal Report.

Art – 297. COMPARATIVE STUDY ON CHECKLIST OF AEROBIC MICROFLORA OF ORAL AND CLOACAL SAMPLE OF SPECTACLED COBRA; RESCUED FROM SNAKE CHARMERS & WILD COBRA RESCUED FROM THE CONFLICT SITUATIONS IN AGRA, UTTAR PRADESH, INDIA

Ilayaraja S, Arun A Sha, Baijuraj MV and Shiva Prasad Parida

Abstract

Snakebites are a great public health concern in tropical and subtropical countries. It cannot only cause poisoning but also sometimes yield some infections in victims. There are some pathogenic agents in the snake's oral and cloacal cavity. This study was carried out to determine bacterial agents present in the oral and cloacal cavity of Spectacled cobra (*Naja naja*) which were rescued from snake charmers and human-snake conflict situations at Agra, Uttar Pradesh. Oral and cloacal samples were collected in sterile condition from 20 rescued cobras (10 from each group). Later the samples were subjected to microbial culture to understand the aerobic microflora present in oral and cloacal cavity. The results revealed both the oral and cloacal swabs found positive bacterial microflora with a majority of swabs yielding multiple organisms. The most common organisms isolated were *Pseudomonas* Spp., *Staphylococcus aureus*, *E. coli*, *Salmonella, Shigella, Clostridia* Spp*., Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Enterococcus faecium* are known human pathogens and also affected snake health.
Keywords: Human-snake conflict, spectacled cobra, oral & cloacal microflora

1. Introduction

The Spectacled cobra (*Naja naja*), also known as the Asian cobra, or Binocellate cobra, is a species of the genus *Naja* found in India, Pakistan, Bangladesh, Sri Lanka, Nepal, and Bhutan, which a member of the "big four" species that inflict the most snakebites on humans in India. The Spectacled cobra is revered in Indian mythology and culture and is often seen with snake charmers. Based on Indian mythology, Naga Panchami is a day in which traditional worship of snakes especially cobras happens by snake charmers where Hindu adherents live. In India, Spectacled cobras are listed under Schedule II of the Wildlife Protection Act (1972). Fifty percent of snakebite deaths in India is due to cobra bite and has later complication like local necrosis and sloughing of skin which takes several months to recover. This extensive necrosis may be due to both venom and the contaminated microflora (Sujogya Kumar Panda, 2018) [18]. The interaction between microbes and humans can result in the outcomes such as disease, transient colonization, and prolonged colonization. Microorganisms of the healthy flora may aid the host (by competing for microenvironments more effectively than such pathogens as *Salmonella* Spp or by producing nutrients the host can use), may harm the host (by causing dental caries, abscesses, or other infectious diseases), or may exist as commensals (inhabiting the host for long periods without causing detectable harm or benefit). The transient and prolonged colonization implies a distinction based on the duration of the interaction, which may extend to weeks, months, or years. Microbes found in and on the human body can cause serious diseases. Not the recovery of a specific organism, but the recovery of the organism in a normally sterile site is the hallmark of the pathogenesis of microbial infections. For example, *Escherichia coli* is a normal resident confined to the gastrointestinal tract. If it is demonstrated in the stool, it may be considered normal, but is found in the abdominal cavity or the patient's bloodstream, this would be considered abnormal.

Similarly, certain organisms are never present as part of the normal microbial flora in humans; hence their recovery in humans is always associated with clinically significant diseases (e.g., *Bacillus anthracis*, *Brucella* Spp., *Francisella tularensis*, and *Histoplasma capsulatum*, etc.). According to Sahoo, 2018, the buccal swabs from Spectacled cobra were subjected to microbial culture and isolated 74 noticeable colony characters. Snake bite victims especially immune deficiency should be intensively monitored for presence of any potential pathogenic fungal flora as the oral cavities of venomous and nonvenomous snake represent portions of fungi, yeast and penicillium (Dehghani, 2016) [13]. During reptile collections *A. hydrophila*, *E. coli*, *S. marcescens* and *S. maltophilia, C. albicans*, *A. flavus* and *Cladosporium* Spp. found to be secondary causative disease agents (Lukac, 2017) [17]. Considering human pathogens, oral bacterial microflora of free-living Reticulated pythons isolated both gram-positive and gram-negative organisms. Ten snake species comprising the families Boidae, Colubridae, Elapidae, and Viperidae, indicated occurance of Gram- negative bacilli and cocci, gram-positive bacilli and cocci (MG, 2009) [20]. The highest rate of infection belonged to coagulase negative *Staphylococcus* (34.5%) and the lowest rate was for Pseudomonas (3.1%). *Salmonella*

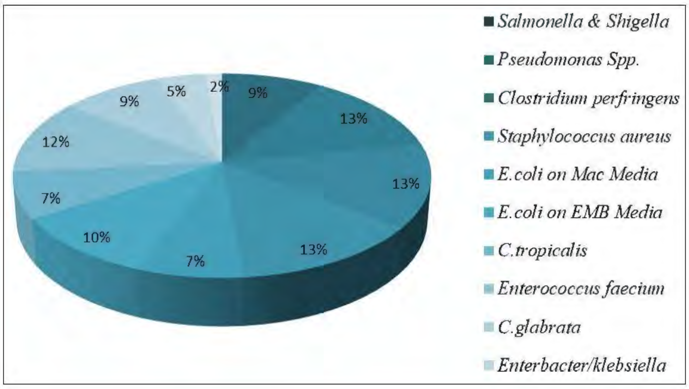
(18.8%); Escherichia and Providencia (each 12.5%); and Proteus, Enterococcus, and *Bacillus* (each 6.2%) were other contributing pathogens found in snakes' oral cavity. Hence, the present study aimed to examine the associated bacteria from the oral and cloacal cavity of Spectacled cobra rescued from human-snake conflict situations (wild) and from snake charmers to provide quality care and management.

2. Material and methods

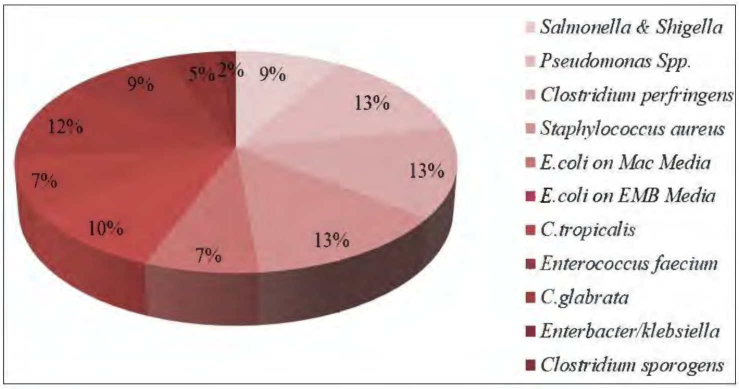
Spectacled cobras were rescued from both human-snake conflict situation and snake charmers at Agra, Uttar Pradesh by rapid response team of Wildlife SOS. During the health evaluation of these snakes by a veterinarian, aseptic samples from oral and cloacal cavity of spectacled cobra was collected by a microbiologist. A total of 20 nos. samples collected in which 10 were from wild and others are from snake charmer. The samples were subjected to microbial culture and IMVIC biochemical test to determine presence of microflora as a part of health evalution to provide quality care and treatment. Staining done by using Lactophenol cotton blue (Hi-media) and the morphological characteristics observed under microscope to identify the fungi. Once the snakes found fit, they were released back to suitable natural habitat.

3. Results and discussion

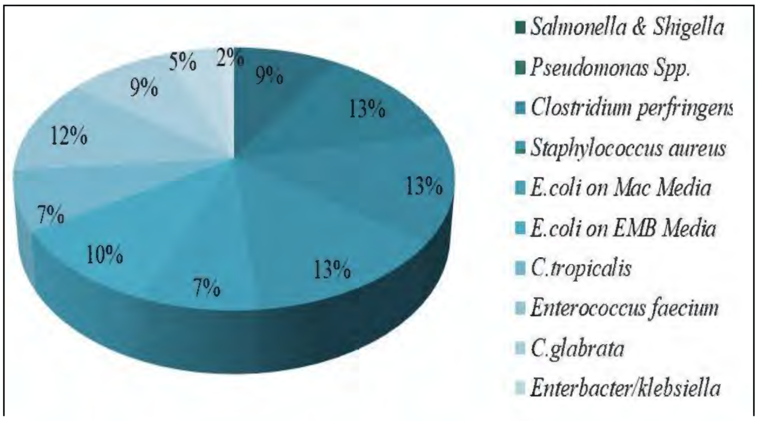
The aerobic microorganisms from oral and cloacal cavities of *N. naja* were successfully isolated (Graph 1 & 2). The cloacal cavity of wild *N. naja* was shown to harbor diverse and abundant microflora. A total of 11 bacterial and 3 fungal strains isolated from oral and cloacal cavity which was known human pathogens. Majorly in wild *N. naja*, *Clostridium perfringens, E. coli and Pseudomonas* Spp. have proportionated distribution whereas, *Enterobacter/ klebsiella and C. glabrata* were the minor components (Graph 1). The culture results of *N. naja* rescued from snake charmers demonstrated abundant diversity of microflora in oral than cloacal cavity may be due to the brutal human handling such as stitching of mouth, destroying of venom gland, fangs, force-feeding contamination, recurrent infection from baskets, etc. The most dominant organisms in *N. Naja* rescued from charmers were *Pseudomonas* Spp.*, Clostridium perfringens, Staphylococcus aureus* followed by *Candida tropicalis, E. coli and Enterococcus faecium* (Graph 3).



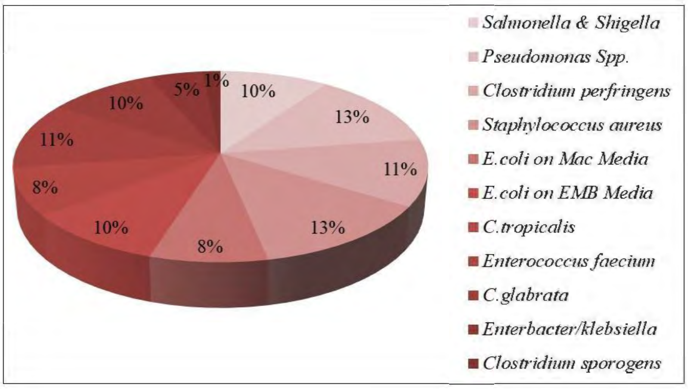
Graph 1: Representation of aerobic microflora found in the oral cavity of rescued wild cobra from conflict situations



Graph 2: Representation of aerobic microflora found in the cloacal cavity of rescued wild cobra from conflict situations

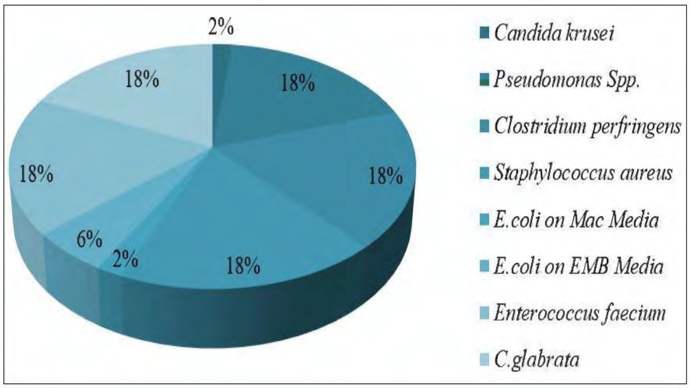


Graph 3: Representation of aerobic microflora found in the oral and cloacal cavity of cobras that were rescued from snake charmers



Graph 4: Representation of aerobic microflora found in the oral and cloacal cavity of cobras that were rescued from snake charmers

In addition to this, the swab samples collected from the snake charmer's wooden box were subjected to microbial culture (Graph 5). The results revealed equivalent diversity of *Pseudomonas* Spp., *Clostridium perfringens, Staphylococcus aureus*, *Candida, glabrata* and *Enterococcus faecium.* Minor diversity of *E. coli* and *C. krusei* were also observed on different media cultures. Differences in habitat, predation strategies, and the type of prey can explain the high variation in bacterial flora. Snakebites have a high rate of infection because of Gram- negative bacteria may be due to their eating habits where the prey head is ingested first, leaving colonization of fecal flora on the oral cavity. This may also explain the higher amount of enterobacterial isolates found in the mouth of the individuals sampled. Bacteria isolated from snakes Oral and Cloacal Cavity, such as *Enterobacter* sp., *Escherichia coli, Clostridial* Spp., *Candida glabrata, Candida krusei, Candida tropicalis,* and *Pseudomonas* sp., could be opportunistic pathogens and generate nosocomial infections. Besides, *Enterobacter* Spp. and Fungal Spp. has been associated with infections of the oral mucosa of humans. On the other hand, pathogens like *Enterococcus faecalis, Salmonella, Shigella,* and *Staphylococcus aureus* can generate zoonosis. Similar bacterial genera were found compared to our results, where predominantly *Staphylococcus, Pseudomonas,* and *Enterobacter* match our findings. Since the microbial diversity associated with snakebite is concerned, handlers should maintain personal hygiene.



Graph 5: Representation of aerobic microflora found in snake charmers' wooden basket

Fig 1: Spectacled cobra (Naja naja) rescued from human-snake conflict by Wildlife SOS

Fig 2: N. naja seized from snake charmers during Naga panchami at Agra, Uttar Pradesh

Fig 3: Wooden basket being used by snake charmer to keep N. naja

Fig 4: Aseptic oral swab sample collection

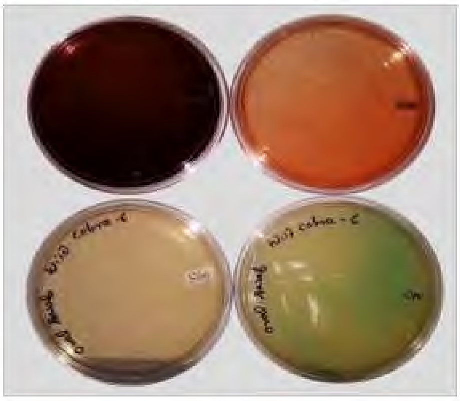


Fig 5: Oral swab culture of wild N. naja showing colonies of different aerobic microbes

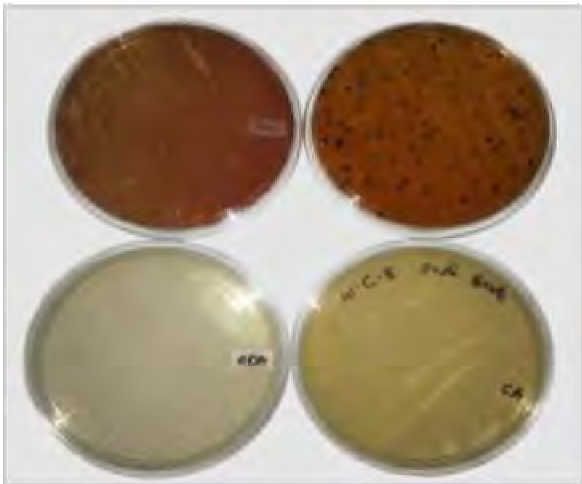


Fig 6: Colonies of different aerobic microbes from the oral swab culture of N. naja rescued from the snake charmers

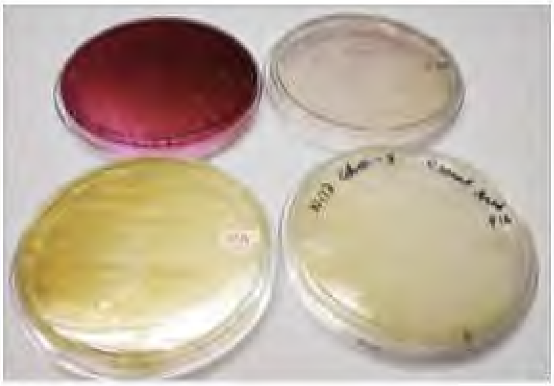


Fig 7: Cloacal swab culture of wild N. naja showing colonies of different aerobic microbes

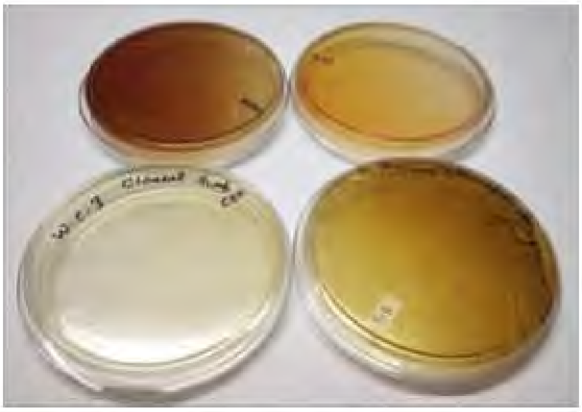


Fig 8: Cloacal swab culture of N. naja rescued from snake charmers showing microbes

Fig 9: Culture results of swab collected from wooden basket of snake charmers showing microbes

Fig 10: IMViC test for identification of bacterial species

4. Acknowledgments

The authors gratefully acknowledge the cofounders of Wildlife SOS, Mr. Kartick Satyanarayan and Ms. Geeta Seshamani. We extend our sincere thanks to Uttar Pradesh Forest Department and rescue unit staffs of Wildlife SOS. Special thanks to Mr. Srinu Srikanta Maharana for his valuable input on the manuscript.

5. References

1. Zancolli G, Mahsberg D, Sickel W, Keller A. Reptiles as reservoirs of bacterial infections: Real threat or methodological bias? Microbial Ecology. 2015;70(3):579–584.
2. Iqbal J, Sagheer M, Tabassum N, Siddiqui R, Khan NA.Culturable aerobic and facultative anaerobic intestinal bacterial flora of black cobra (*Naja naja Karachiensis* in Southern Pakistan. ISRN Veterinary Science.2014; 2014:5.
3. Jho YS, Park DH, Lee JH, Lyoo YS. Aerobic bacteria from oral cavities and cloaca of snakes in a petting zoo. Korean Journal of Veterinary Research. 2011;51(3):243-247.
4. Shek KC, Tsui KL, Lam KK, *et al*. Oral bacterial flora of the Chinese cobra (*Naja atra*) and bamboo pit viper (*Trimeresurus albolabris*) in Hong Kong SAR,China. Hong Kong Medical Journal. 2009;15(3):183- 190.
5. Morgan M. Hospital management of animal and human bites. Journal of Hospital Infection. 2005;61(1):1–10.
6. Arroyo O, Bolaños R, Muñoz G. The bacterial flora of venoms and mouth cavities of Costa Rican snakes. Bulletin of the Pan American Health Organization.

1980;14(3):280-285.

7. Jorge MT, de Mendonça JS, Ribeiro LA, da Silva ML, Kusano EJ, Cordeiro CL. Bacterial flora of the oral cavity, fangs and venom of *Bothrops jararaca*: Possible source of infection at the site of bite. Revista do Instituto de Medicina Tropical de Sao Paulo. 1990;32(1):6-10.
8. Ferreira Junior RS, Siqueira AK, Campagner MV, *et al.*Comparison of wildlife and captivity rattlesnakes (*Crotalus durissus* terrificus) microbiota. Pesquisa Veterinaria Brasileira. 2009;29(12):999–1003.
9. Babalola MO, Balogun JA. The ecology and potential health risk of the oral microflora of python regius and *Clelia scytalina*. International Journal of Microbiology Research. 2013;5(1):349-356.
10. Lukač M, Horvatek Tomić D, Mandac Z, Mihoković S, Prukner-Radovčić E. Oral and cloacal aerobic bacterial and fungal flora of free-living four-lined snakes (*Elaphe quatuorlineata*) from Croatia. Veterinarski Arhiv. 2017;87(3):351-361.
11. Chiang YC, Yang CY, Li C, Ho YC, Lin CK, Tsen HY. Identification of *Bacillus* Spp., *Escherichia coli*, *Salmonella* Spp., *Staphylococcus* Spp. and *Vibrio* Spp. with 16S ribosomal DNA-based oligonucleotide array hybridization. International Journal of Food Microbiology. 2006;107(2):131-137.
12. Jho YS, Park DH, Lee JH, Cha SY, Han JS. Identification of bacteria from the oral cavity and cloaca of snakes imported from Vietnam. Laboratory Animal Research. 2011;27(3):213-217.
13. Dehghani R, Sharif MR, Moniri R, Sharif A, Kashani HH. The identification of bacterial flora in oral cavity of snakes. Comparative Clinical Pathology. 2016;25(2):279–283.
14. Dehghani R, Sharif A, Assadi MA, Haddad Kashani H.,Sharif MR. Fungal flora in the mouth of venomous and non-venomous snakes. Comparative Clinical Pathology. 2016;25(6):1207–1211.
15. Mohapatra DA, Warrell W Suraweera, *et al.*, Snakebite mortality in India: A nationally representative mortality survey, PLoS Negl. Trop. Dis. 2011;5:e1018.
16. Shek K, Tsui K, Lam K, *et al.*, Oral bacterial flora of the Chinese cobra (*Naja atra*) and bamboo pit viper (*Trimeresurus albolabris*) in Hong Kong SAR, China, Hong Kong Med. J. 2009;15:183e190.
17. Shaikh IK, Dixit PP, Pawade BS, *et al.*, Assessment of cultivable oral bacterial flora

from important venomous snakes of India and their antibiotic susceptibilities, Curr. Microbiol. 2017;74(11):1278e1286.

18. Kumar Panda S, Padhi L, Sahoo G. Oral bacterial flora of Spectacled cobra (*Naja naja*) and their antibiotic susceptibilities, Elsevier. Heliyon. 2018;4:e01008.
19. Hor Vate K Tom IC D, Mandac Z, Miho kovic S, Prukner -Rado vcic E, Lukac M, *et al*., Oral and cloacal aerobic bacterial and fungal flora of free-living four- lined snakes (*Elaphe quatuorlineata*) from Croatia, Veterinarski Arhiv. 2017;87(3):351-361.
20. Moreira WMQ, Cunha KC, Ribeiro ACMG, Almeida MTG, Fonseca MG, *et al*., oral microbiota of Brazilian captive snakes, J Venom Anim Toxins Incl Trop Dis. 2009;15(1):54-69.

iv b) 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** **Report: Project Report**

iv c) PUBLICATION BASED INDEX

277	2022	Frontiers in Veterinary Science | Article 844208 | Volume 9 | April 2022	FLA	78
278	2022	e-planet 20 (1): 74-78	FLA	92
279	2022	International Journal of Veterinary Sciences and Animal Husbandry 2022; 7(4): 15-19	FLA	100
280	2022	The Pharma Innovation Journal 2022; SP-11(8): 1854-1856	FLA	109
281	2022	Diversity 2022, 14, 717, pp: 1-20	FLA	117
282	2022	Microbial Pathogenesis 172 (2022) 105752, pp: 1-10	FLA	154
283	2022	e-planet 20 (2): 145-150 (December 2022)	FLA	179
284	2022	IJVR, 2022, Vol. 23, No. 4, Ser. No. 81, Pages 369-374	FLA	190
285	2022	Journal of Wildlife Management 2023;e22360. The Wildlife Society, pp:1-15	FLA	203
286	2022	Journal of Threatened Taxa | 26 January 2023 | 15(1): 22355–22363	FLA	225
287	2022	Wildlife SOS and Chhattisgarh Forest Department	Report	239
288	2022	Wildlife SOS	Report	240
289	2022	Caring for Elephants: Managing Health and Welfare in Captivity, 2022, pp: 129-139	FLA	241
290	2022	Caring for Elephants: Managing Health and Welfare in Captivity, 2022, pp: 162-168	FLA	249
291	2022	Wildlife SOS and Karnataka Forest Department	Report	255

292	2022	Wildlife SOS and Karnataka Forest Department	Report	256
293	2023	Research Square, January 2023, pp:1-17	FLA	257
294	2023	International Journal of Veterinary Sciences and Animal Husbandry 2023; 8(3): 11-15	FLA	276
295	2023	Veterinary Practitioner Vol. 24 No. 1, June 2023, pp: 12-13	FLA	286
296	2023	e-planet 21 (1): 8-20 (June 2023)	FLA	291
297	2023	International Journal of Applied Research 2023; 9(9): 43-49	FLA	310

Editor's Note:

If any of the articles which were published by any of the Wildlife SOS team during their working period with us, is found missing, please intimate the publication department of Wildlife SOS and it will be carried in the next volume.

Follow Us:

@wildlifesosindia @wildlifesos @Wildlifesos Wildlife SOS

Phone: +91 1146601096 | Web: www.wildlifesos.org | Mail: research@wildlifesos.org