IZYB

INDIAN ZOO YEAR BOOK 2021

VOLUME IX

INDIAN ZOO DIRECTORS' ASSOCIATION

Central Zoo Authority
केन्द्रीय चिड़ियाघर प्राधिकरण

Nature Protects if She is Protected

NANDANKANAN

Designed by: Sruthy Boopathy

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Suggested citation: (an article of IZYB as example)
Sahu S. K., Sahoo, N., Panda S. K. & Mohapatra, D. (2021) Cytauxzoonosis in a Bengal tiger (*Panthera tigris* ssp. *tigris*) at Nandankanan Zoological Park, Odisha. Indian Zoo Year Book. Vol. IX: 1-5.

FOREWORD

The Indian Zoo Year Book (IZYB) is a publication of Indian Zoo Directors Association and Central Zoo Authority to showcase and disseminate evidence based research on captive wildlife management in Indian Zoos. The first volume of IZYB was published in 1996. The Odisha State Forest Department and Nandankanan Biological Park are proud to be associated with the publication of Indian Zoo Year Book Volume IX and aid in the spread of scientific inquiry in Indian Zoos.

Established in 1960, Nandankanan Biological Park in Odisha is a premier large Zoo in the country. It has carried out successful breeding of all three Indian crocodilians, Tiger, Indian pangolin etc. The Zoo has taken up the species recovery programme for Gharials in river Mahanadi. The article on successful hand rearing of hyena cub included in the present volume is one of the examples worth mentioning.

Zoos in India and elsewhere shall have a greater role and responsibility in conservation breeding, rescue, rehabilitation, general management of wild animals and above all educating the public on wildlife conservation. The research findings which are compiled in the Indian Zoo Year Book will help the Zoo Managers and Veterinarians to augment their expertise in captive animal management.

I wish the Volume IX of Indian Zoo Year Book a great success.

Sisir Kumar Ratho, IFS
Principal CCF & HoFF, Odisha

सत्यमेव जयते
GOVERNMENT OF INDIA
भारत सरकार
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
पर्यावरण, वन एवं जलवायु परिवर्तन मंत्रालय
Central Zoo Authority
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PREFACE

Zoos, with their living collections, have the potential to significantly contribute to conservation-related research from the perspective of ex-situ management. Zoo management attempts to balance the needs of captive wild animals and the needs of the people (visitors and the community as a whole), using the best available science. Research-oriented work into the biology and behaviour of wild animals is an increasingly co-ordinated effort. The shared results across institutions engaged in similar work and the shared goals of global species conservation are imperative.

The COVID-19 pandemic has led to a dramatic loss of human life worldwide and presented an unprecedented challenge to public health, food systems and work in the world. It has changed the way we look at the world today. Zoos have a lot of significance in today's society. They bring value in terms of conservation of species through scientifically designed programs allowing animals at risk of extinction to live, repopulate research issues and developments in the field of One Health and educate & inform the public about animals in the world.

The Central Zoo Authority (CZA) in collaboration with the Nandankanan Biological Park, Bhubaneshwar, Odisha is pleased to present the Indian Zoo Year Book Vol IX.

The aim is to encourage Indian Zoos to recognise the scientific rigor involved in zoo animal management and to make available scientific and evidence-based research on captive wildlife management in Indian Zoos.

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CYTAUXZOONOSIS IN A BENGAL TIGER (*Panthera tigris ssp. tigris*) AT NANDANKANAN ZOOLOGICAL PARK, ODISHA

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Abstract

An apparently healthy male Bengal tiger aged about 10 years from Nandankanan Zoological Park (NKZP), Odisha, was suddenly found ill with signs of inappetence, depression, lethargy, fever and loss of body condition. Undigested meat and bone pieces were found in the foul-smelling scat. Haemato-biochemical parameters were altered, indicating anemia, leukocytosis, thrombocytopenia, neutrophilia and hepatic dysfunction. Blood sample was found to be positive for *Cytauxzoon felis* by PCR producing a product size of 651bp using ITS1 primers. Clinical signs, haemato-biochemical changes and molecular detection of pathogen confirmed Cytauxzoonosis in the Bengal tiger. Doxycycline and supportive therapy proved efficacious with remission of clinical signs, but relapse occurred two-months post-treatment. Concurrent therapy of doxycycline and imidocarb failed to effect a cure and the tiger succumbed after a chronic illness of six months. Major necropsy findings were hepatomegaly, excess transudates in peritoneal cavity and splenomegaly that corroborated the hemoparasitic disease. *Cytauxzoon felis* in Bengal tiger at NKZP, appears to be the first report among Indian Zoos.

Key Words *Cytauxzoon felis*, cytauxzoonosis, Bengal tiger

Introduction

Feline cytauxzoonosis is an tick-borne haemoprotozoan disease of domestic and wild cats caused by an apicomplexan parasite *Cytauxzoon felis* belonging to Theileriidae family (Lewis *et al.,* 2012; Wang *et al.,* 2017; Nentwig *et al.,* 2018). *Amblyomma americanum* and *Dermacentor variabilis* are the primary tick vectors known to be associated with the transmission and maintenance of this pathogen in its susceptible hosts. Case reports of *C. felis* infection have been documented in wild felids like wild cats, lions, captive bred Asian tigers, and puma (Lloret *et al.,* 2015). The bobcat (*Lynx rufus*) is the natural reservoir host of the parasite. Other closely associated felids like mountain lions, ocelots, spotted cats and jaguars also act as reservoirs or incidental hosts (Haber *et al.,* 2007). Domestic cats can harbour subclinical infections and act as reservoirs (Brown *et al.,* 2008). In some endemic areas, the prevalence of subclinical infection in cats may be as high as 30% (Brown *et al.,*2010). The present case study describes *Cytauxzoon felis* infection in a Bengal tiger at Nandankanan Zoological Park (NKZP), Odisha, India.

Case history and observation

An apparently healthy male Bengal tiger aged about 10 years of NKZP became ill during March 2018 with signs of inappetence, depression, lethargy, fever (103.5°F), loss of body condition and presence of undigested meat and bone pieces in foul smelling scat. The tiger was shifted to the 'In-patient ward' of the zoo hospital for close observation and treatment. Geimsa stained blood smear showed signet ring shaped piroplasms located towards periphery of infected RBCs (Fig. 1) suggestive of *C. felis*. Haemato-biochemical analysis revealed anemia, leukocytosis, thrombocytopenia, neutrophilia and increased level of liver enzymes (Table 1 and 2). Blood sample tested positive for *C. felis* by PCR producing a product size of 651 bp using ITS1 primers. Clinical signs, haemato-biochemical alterations, molecular detection of pathogen and progression of illness lead to the confirmation of Cytauxzoonosis, a haemoprotozoan infection. Accordingly, therapeutic management was initiated with oral Doxycycline @ 10mg/kg b w. In supportive therapy, Udiliv 300 @ 2 tabs, Essential-L[3] @ 4 caps, Cheri @ 2 caps and Becosule @ 2 caps were administered daily with meat to restore hepatobiliary function and improve haemoglobin concentration. After ten days of administration, the tiger responded to treatment with remission of clinical signs. Haemato-biochemical values were found to be improving (Table 1 and 2).

The treatment regimen stretched for a period of 45 days. The tiger was released to a small enclosure in order to provide moderate exercise. However, the above-described clinical signs reappeared in August 2018 with alteration of hemato-biochemical parameters (Table 1 and 2). Earlier therapeutic regimen was reinitiated with concurrent intramuscular administration of a single dose of antiprotozoal drug Imidocarb dipropionate @ 3.5mg/kg. Supportive therapy with oral drugs (as earlier)

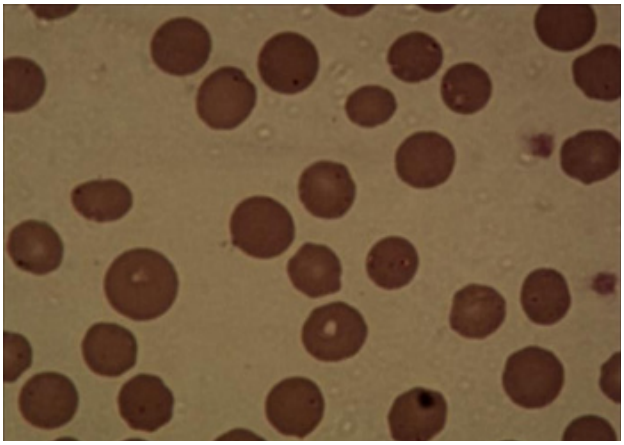

Fig 1 Geimsa stained blood smear showed signet ring shaped piroplasms located towards periphery of infected RBCs.

Fig 2 Light grey colour scat seen after relapse

was also continued. The tiger did not show signs of improvement, the scat was light grey in colour (fig 2) and the animal became debilitated (fig 3) and succumbed on 02.09.2018 with terminal sign of dyspnoea.

Post mortem examination revealed pale yellowish discoloration of visible mucosa and muscles with gum bleeding (Fig 4). There was more than five litres of yellowish red tinged peritoneal fluid. Gall bladder was thickened with stasis of thick dark coloured bile (Fig 5). Liver was enlarged with perihepatitis and multiple necrotic foci throughout its parenchyma (Fig 5). Both lungs were congested and consolidated (Fig 6). The cut surface of lungs revealed yellowish tinged frothy exudates coming out from bronchi and bronchioles (Fig 6). Spleen was enlarged and congested.

Fig 3. Tiger in debilitated condition (after relapse)

Fig 4. Oral mucus membrane pale and yellowish with gum bleeding

Table 1 Haematology of a Bengal tiger suffering from Cytauxzoonosis

Parameters	**Before treatment**	**10 days post-treatment**	**During relapse two months post-treatment**	**Reference range***
Hb (gm%)	7.6	8.0	7.8	7.8-13.8
TLC (cumm)	30,400	14,400	18,050	6,200 -11,050
DLC (%)				
Nutrophils	91	83	87	57-75
Lymphocytes	07	12	10	18-35
Eosinophills	01	03	01	2-6
Monocytes	01	02	02	2-6
Platelet count (lakh/ cumm)	0. 9	2.8	1.4	1.11-4.47

*Srivastav *et al.,* 2012 and Garner *et al.,* 1996

Table 2 Serum Biochemistry of a Bengal tiger suffering from Cytauxzoonosis

Parameters	**Before treatment**	**10 days post-treatment**	**During relapse two months post-treatment**	**Reference range***
ALT (IU/L)	367.1	177.9	487.7	26.01±2.87
AST (IU/L)	228.9	108.0	436.3	16.91±1.60
ALP (IU/L)	151.6	15.3	42.5	19.96±2.27
Glucose (mg/ dl)	87.3	73.5	66.1	81.9-156.1
Total protein (g/dl)	6.3	7.6	6.66	7.18 ± 0.14

*Srivastav *et al.,* 2012 and Garner *et al.,*1996

Fig 5. Liver enlarged (hepatomegaly) with multiple necrotic foci throughout its parenchyma, Gall bladder thickened with stasis of thick dark coloured bile

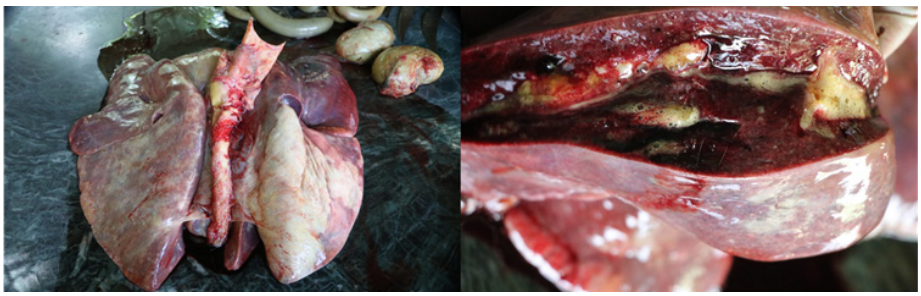

Fig 6. Lungs congested and consolidated with yellowish tinged frothy exudates coming out from bronchi, bronchioles and cut surfaces

Discussion

Whole blood smear examination remains the most common means of confirmatory diagnosis in clinically affected hosts. But, for the differentiation of *C. felis* erythroparasitism from *Babesia spp., Mycoplasma spp* and Howell-jelly bodies (RBC remnants commonly found in felines) PCR assay of whole blood provides the most sensitive result (Wang *et al.,* 2017). In this case, the positive molecular test for *C. felis* confirmed the disease status. These assays are useful for screening cats for identification of potential carriers as well as diagnosis of clinical cases.

Supportive and critical treatment is a mainstay of therapy for feline cytauxzoonosis (Wang *et al.,* 2017). A range of antiprotozoal drugs have been tried across different cases of clinically affected domestic cats viz. Atovaquone, Azithromycin, Imidocarb dipropionate, Doxycycline and Diminazine aceturate along with fluid and hematinic therapy. However, none of the drugs have provided consistent results. Though a study suggests atovaquone with azithromycin as the most preferred regimen (Cohn *et al.,* 2011), there are insufficient case reports substantiating this fact on a consistent basis (Alho *et al.,* 2016). Successful therapy with other drug regimens like Imidocarb dipropionate and Doxycycline have also been infrequently documented (Carli *et al.*, 2014). But the fact remains that despite therapy, the case fatality rate continues to remain over 90% (Lloret et al., 2015). Wang et al., (2017) reported that 60.4% of domestic cats treated with Atovaquone with Azithromycin survived. In contrast, only 25.9% of domestic cats receiving Imidocarb survived. According to Wang *et al.,* (2017), Atovaquone with Azithromycin is considered the most effective treatment for acute infection. The present case was treated with, but the relapse proved to be fatal. The Cytauxzoonosis in the Bengal tiger at Nandankanan Zoological Park appears to be the first report in large cat amongst Indian zoos.

Cytauxzoon felis has yet to be cultured continuously in vitro, which greatly hinders the study of the parasite's biology and vaccine development (Wang *et al.,* 2017). Comprehensive and systematic research is urgently needed to understand the epidemiology, biology,

and possible control. Additionally, limiting contact with free-ranging wild felids and domestic cats seems prudent. Screening via PCR of any captive wild felids coming from or being moved to a region in which *C. felis* is endemic may be incorporated in screening programmes (Lewis *et al.,* , 2012).

Acknowledgement

The authors are very thankful to the Director and other staff of Nandankanan Zoological Park for their support during the period of treatment.

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MANAGEMENT OF CONTRACTED FLEXOR TENDON DEFORMITY IN A SAMBAR DEER (*Rusa unicolor*) FAWN- A CASE REPORT

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Abstract

A day-old fawn of Sambar deer (*Rusa unicolor*) was presented with history of inability to bear weight on both fore limbs since birth. All the physiological parameters were found to be within normal range. Clinical examination revealed congenital deformity in the flexor tendon of both fore limbs with flexed fetlock joints and inability for proprioception flat on ground. Based on history and clinical signs the case was diagnosed as moderate degree of contracted flexor tendon deformity. Application of wooden splint and supporting bandage was carried out. After 10 days, the fawn recovered fully with normal weight bearing. From present case, it was concluded that on early presentation of moderate degree of contracted flexor tendon deformity case could be managed satisfactorily with the proper application of the splints and supporting bandage.

Key Words Congenital deformity, contracted tendon, moderate degree, sambar deer, wooden splint

Introduction

Contraction of the flexor tendons may either be congenital or acquired and may involve deep and/or superficial digital flexor tendons. Aetiology includes arthrogryposis, uterine malpositioning or overcrowding, genetic effects and teratogenic insults during embryonic stage of pregnancy and malnutrition (McIlwraith, 2002 & Nandi *et al.,* 2009). The diagnosis is usually based on the history of being born with the problem and clinical sign (Rashmi *et al.,* 2018). As the tenons are affected, the animal is unable to achieve or maintain the normal extension of the limbs (Anderson *et al.,* 2008). The animals with flexural deformities may be unable to nurse, so failure to acquire passive immunity may be a complicating factor (Weaver *et al.,* 2005). Successful treatment of flexural deformities depends on the site and severity of the deformity, and on the appropriate use of changes in diet and exercise, bandaging and splinting, medical, nonsurgical and surgical therapy (Adams, 2000; Embertson, 1994; McIlwraith, 2002). Moderately affected cases are routinely treated by using bandage, splint, or cast and by providing analgesia using non-steroidal anti-inflammatory drug (Anderson *et al.,* 2008). The present case report describes the successful treatment of a moderate degree of contracted flexor tendon deformity by applying wooden splint with supporting bandage in a Sambar deer (*R. unicolor*) fawn.

Materials and methods

A day-old Sambar deer fawn at the Sardar Patel Zoological Park, Kevadia presented with history of inability to bear weight on both fore limbs since its birth. All physiological parameters were found to be within normal range. Clinical examination revealed contracted flexor tendon of both fore limbs with flexed fetlock joint and inability to keep the limbs flat on ground (Fig.1A). Based on clinical signs the case

Fig. 1 (A) Moderate degree of contracted flexor tendon deformity& (B) Deformity was corrected by application of wooden splint with supporting bandage.

was diagnosed as moderate degree of contracted flexor tendon deformity and it was decided to apply wooden splints with supporting bandages. After injecting Xylazine 0.7 mg/kg, intramuscularly the fawn was restrained in lateral recumbency. An appropriately sized well-padded wooden splint was prepared and applied to the palmar aspect of both fore limbs from elbow joint to foot excluding the hooves (Fig.1B). The fawn was given a single dose of Meloxicam injection @ 0.2mg/kg bwt, intramuscularly as analgesic. Wooden splint was kept in place for 10 days and fawn made an uneventful recovery.

Results and discussion

The fawn started weight bearing on both affected forelimbs after removal of the wooden splint and supportive bandage (Fig. 2). A similar result was found in the study by Shivaprakash and Kumar (2009). Anderson *et al,.* (2008) also reported that the majority of contracted flexor tendon deformities in young cow calves were observed within first few days after birth. Flexural deformity may vary from mild to severe degree. The deformity may be of mild degree where the affected animal is able to walk on the toes, but heels do not touch the ground, moderate degree where the affected animal is able to walk on the dorsal side of the toe instead of heel and to a severe degree when affected animal is forced to walk on the pastern, fetlock or carpus (Weaver *et al.,* 2005).

Fig. 2 Fawn started normal weight bearing on both fore limbs

A complete physical examination of the limb is warranted to rule out other disease conditions before treatment of contracted tendon this may occur with other congenital abnormalities like cleft palate, arthrogryposis and dwarfism (Fazili *et al.,* 2014). In the present case report no other concurrent congenital abnormality was reported. According to Baxter (2011) and (Anderson *et al.,* 2008) treatment of flexural deformity should be initiated soon after recognition of the problem. As the animal gets older the contracted tissues become less responsive to treatment. Most flexural deformities of limbs could be corrected with non-surgical treatment, but surgical method can be used for correction of more severe degree of deformity

of limbs or failure of other methods of treatment (Southwood *et al.,* 1999). The treatment of present case was carried out using a nonsurgical method by application of wooden splint with supporting bandage. Wooden splints, although light in weight, may get dislodged or lead to pressure sores due to their flat contact surface. The hooves were left un-bandaged to support some weight and to further stretch the tendons. It has been advised that the splints should be changed on a daily basis and applied with enough tension to produce a non-contracted limb (Ferguson, 1997). In present case also, the wooden splints and bandages were changed regularly to avoid complications. Careful management of splints in neonates ensures avoidance of skin necrosis at potential points (Weaver *et al.,* 2005).

According to Fazili *et al.,* 2014 proper application and maintenance of the splints for 10 days is sufficient for managing most of the neonatal calves presented with moderate fetlock knuckling. The result of the present case indicates that moderate degree of contracted flexor tendon deformity can be resolved completely by proper application of wooden splint with bandage for 10 days. Non-steroidal anti-inflammatory drugs (NSAIDs) provided analgesia help decrease the pain associated with stretching of contracted soft tissue caused by weight bearing, passive stretching exercise, splints or cast (Anderson *et al.,* 2008). In conclusion, early presentation of moderate degree of contracted flexor tendon deformity can be managed satisfactorily with the proper application of the wooden splint with supporting bandage.

Acknowledgement

We are highly thankful to the senior management of Sardar Patel Zoological Park, for providing the facilities, and cooperation. We are thankful to section keepers for their assistance this case.

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DOCUMENTATION OF CHANGES IN SERUM BIOCHEMISTRIES TO CAPTIVITY IN A FEMALE RESCUED COMMON LEOPARD (*Panthera pardus*) AT PADMAJA NAIDU HIMALAYAN ZOOLOGICAL PARK, DARJEELING.

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Abstract

The present case study documents the effect of captivity on various serum biochemical indices in a rescued wild common leopard (*Panthera pardus*) at Padmaja Naidu Himalayan Zoological Park (PNHZP), Darjeeling. Approximately 5ml of blood samples was extracted through the lateral coccygeal vein, after 19 days (set 1), 43 days (set 2), and 110 days (set 3) in captivity, after physically restraining the animal in a squeeze cage. The samples were centrifuged at 3000 rpm for 15 minutes and analyzed for various serum biochemical parameters in the veterinary hospital of the park using standard commercial kits and a semi-auto analyzer (BTS-320). Kruskal-Wallis test was done to check the significance level. Besides this, 15 blood test reports of clinically healthy captive leopards housed at PNHZP and Bengal Safari from September 2020 to June 2021 were compiled to generate normal biochemical baseline reference values for comparative assessments. On investigation, a significant decline in Alanine amino aspartate (AST) and alkaline phosphate (ALP), and a significant increase in uric acid values were observed ($p \leq 0.05$). Other biochemical parameters, especially cholesterol and triglycerides showed considerable fluctuations. However, the differences were not- statistically significant (p- 0.05). The variation in its biochemical profile has been explained in terms of its habitat, environmental factors, and husbandry practices in the zoo. Furthermore, the results of the present study give an insight into the actual baseline reference range of common leopards that can be helpful to zoo clinicians for diagnostic purposes.

Key Words Zoos, blood serum, biochemical values, and adaptation.

Introduction

Biochemical studies are important in evaluating the health status of animals, and their interpretation signifies the physiological functioning of the organs (Allwin *et al.,* 2019). As far as we inspected, reports on the reference intervals of wild felids are scarce, and the information on the effect of captivity on various serum biochemical parameters was non-existing. Therefore, in this paper, we have documented the variation in the concentration of some basic serum biochemical indices in a rescued wild common leopard, as it progressed to adjust to the captive settings at Padmaja Naidu Himalayan Zoological Park (PNHZP), Darjeeling, apart from evaluating the reference values from 15 healthy captive individuals in the park. The findings in this regard will help develop management measures that are required for successfully managing captive leopards, besides throwing light on baseline biochemical values of their wild counterpart.

Materials and methods

On 3rd January 2021, a female leopard rescued from Balasun Tea Estate, Darjeeling was brought to the veterinary hospital of the park for treatment. Blood samples were collected after 19 days (set 1) during the routine clinical examination. At the time, its wounds around the abdominal area had almost healed. Phlebotomy was done aseptically from the lateral coccygeal vein, after restraining the animal in a squeeze cage. Approximately five ml of blood drawn in a clot activator vile was allowed to stand for 30 minutes. Biochemical values were obtained using Semi-auto Analyzer (BTS-320) and commercial kits from blood serum by centrifuging the coagulated whole blood at 3000rpm for 15 minutes. The analysis was replicated twice by extracting the blood samples after 43 days (set 2) and 110 days (set 3) in captivity. We calculated the mean value of the parameters analyzed more than once for each set and the Kruskal Wallis test was done to find the difference in serum biochemistries with the increasing number of days in captivity using SPSS 16. The significance level was set at $p \leq 0.05$. Here, it

has to be mentioned that the animal was not sampled solely for this study. The analysis was done to check its health status, based on which further decisions regarding shifting of the animal from the hospital were to be made by the management.

Besides this, 15 blood test reports of clinically healthy individuals housed at PNHZP and Bengal Safari, Siliguri, obtained during the routine clinical examination from September 2020 to July 2021 were compiled for comparative assessments. All serum biochemistry test was conducted in the Veterinary hospital of PNHZP.

Table 1: Variation in liver function tests with the increasing number of days in captivity (~ not tested).

	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dl)	total protein (g/dl)
19 days in captivity (set 1)	50.11	47.142	80	3	7.2
	~	41.555	82	2.5	7.3
Mean value (total/N)	**50.11**	**46.85**	**81**	**2.75**	**7.25**
43 days in captivity (set 2)	45.92	~	73	3.6	8.3
	45.22	~	75	3.5	8.5
	41.90	~	~	~	~
	44.52	~	~	~	~
Mean value (total/N)	**44.39**	~	**74**	**3.55**	**8.4**
110 days in captivity (set 3)	40	36.84	31	2.8	8.1
	~	34.05	37	~	~
	~	35.10	30	~	~
Mean value (total/N)	**40**	**35.33**	**32.67**	**2.8**	**8.1**

Table 2: Variation in lipid profile tests and kidney health tests with the increasing number of days in captivity (~ not tested).

N=No of days in captivity	Glucose mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
19 days in captivity	104.8	105	93.59	65	0.42	0.57
	~	103	~	70		0.45
Mean value (total/N)	**104.8**	**104**	**93.59**	**67.5**	**0.42**	**0.51**
43 days in captivity	88.6	145	130.96	99	0.45	0.65
	91.1	142	178.88	103	0.38	0.69
	93.6	~	179.69	83	0.26	0.99
	93.1	~	132.76	~	~	~
Mean value (total/N)	**91.6**	**143.5**	**155.57**	**95**	**0.36**	**0.78**
110 days in captivity	61	175	58.12	68	0.34	1.48
	~	157	~	~	~	1.98
	~	170	~	~	~	~
Mean value (total/N)	**61**	**167.33**	**58.12**	**68**	**0.34**	**1.73**

Table 3: Variation in serum electrolytes with the increasing number of days in captivity.

No of days in captivity	Chloride (mMole/L)	Potassium (mMole/L)	Sodium (mMole/L)	Calcium (mg/dl)	Phosphorous (mg/dl)	Magnesium (mMole/L)
19 days in captivity	93.52	6.55	103.43	7.61	5.16	1.85
	96.93	5.79	134.03	7.4	5.92	1.87
Mean value (total/N)	**95.23**	**6.17**	**118.73**	**7.51**	**5.54**	**1.86**
43 days in captivity	94.71	7.39	132.15	7.64	7.5	2.102
	95.56	7.73	115.75	8.14	6.17	2.041
Mean value (total/N)	**95.14**	**7.56**	**123.95**	**7.89**	**6.84**	**2.07**
43 days in captivity	**87.29**	**5.29**	**183.99**	**7.18**	**4.98**	**2**

Table 4: Baseline serum biochemical reference values of captive leopards (*Panthera pardus*) housed at PNHZP and Bengal Safari.

S.No	Parameters (N=15)	Mean ± Standard error	Standard deviation	Range (Max-Min)
	LIVER FUNCTION TESTS			
1	Alanine amino transferase (ALT) (U/L)	49.48 ± 3.32	12.85	28.63 - 66.87
2	Alanine amino aspartate (AST) (U/L)	36.56 ± 2.53	9.8	20.43 - 55.00
3	Alkaline phoshatase (ALP) (U/L)	38.4 ± 0.63	14.08	25.00 - 78.00
4	Albumin (g/dl)	3.11 ± 0.1	0.39	2.30 - 3.60
5	Total protein (g/dl)	7.11 ± 0.19	0.73	5.00 - 7.90

	LIPID PROFILE			
6	Glucose (mg/dl)	88.03 ±10.12	39.18	49.60 - 172.80
7	Cholesterol (mg/dl)	154.6 ± 7.52	29.11	110.00 - 212.00
8	Triglycerides (mg/dl)	58.19 ± 3.97	15.39	45.13 - 87.35
	KIDNEY FUNCTION TESTS			
9	Blood urea nitrogen (BUN) (mg/dl)	71.13 ± 3.55	13.77	51.00 - 99.00
10	Creatinine (mg/dl)	0.45 ± 0.03	0.13	0.27 - 0.69
11	Uric acid (mg/dl)	1.4 ± 0.11	0.41	3.50 - 8.30
	SERUM ELECTROLYTES			
12	Chloride (mMol/L)	104.73 ± 4.92	19.07	78.23 - 132.48
13	Potassium (mMol/L)	5.66 ± 0.31	1.18	3.50 – 8.30
14	Sodium (mMol/L)	208.84 ± 7.05	26.38	157.00 - 256.51
15	Calcium (mg/dl)	7.94 ± 0.19	0.74	7.21 - 9.48
16	Phosphorous (mg/dl)	5 ± 0.2	0.78	3.57 - 6.85
17	Magnesium (mg/dl)	1.95 ± 0.02	0.08	1.78 - 2.07

Results and Discussions

The results of the present investigation have been summarized in Tables 1-4. For the liver function test, the mean level of ALT recorded for sets 1, 2, and 3 was 50.11, 44.39, and 40 (U/L) whereas the AST value was found as 46.85 and 35.33 U/L respectively for sets 1 and 3. In all cases, the values were comparable to the range of other captive leopards (ALT: 49.48 ± 3.32 U/L and AST: 36.56 ± 2.53 U/L) recorded in the study. Further, a significant decreasing trend ($p=0.05$) in AST and a non-significant decreasing trend in ALT ($p=0.09> 0.05$) with the increasing number of days in captivity were observed. Although these discrepancies are difficult to explain and could be related to individual variation, higher mean values of these liver enzymes have been found in other free-ranging felids such as leopard cats and Iberian lynx (Salakij *et al.,* , 2010) in comparison with captive populations (Teare 2002 and Weaver *et al.,* , 1995), suggesting that its higher concentrations are rather common in the wild, whereas, in captivity, its serum concentration reduces, for an unknown reason, that requires further investigation. The ALP value recorded for sets 1 and 2 were 81 and 74 U/L, which is comparatively higher than the mean values of ALP reported from other captive leopards (38.4 ± 0.63U/L) in our study. However, its value significantly decreased as the animal adapted to the captive settings (ALP = 32.67 U/L; $p= 0.04<0.05$), similar to the observations of Pitorri et al.,2014 in the wild boar. The higher ALP activity may be related to increased bone turnover and greater metabolic rate (Elarabany, 2018) when present in their natural wilderness, however, the causes for its declined values when kept in captivity are unclear.

Although elevated levels of ALP are common in young animals, abnormally inflated values may be indicative of biliary obstruction, hepatic damage, and disease of bone tissues (Hill, 2011). Since no clinical symptoms were observed in the study animal, and the values were always within the reference range given by Shrivastav et al., 2016 in the same species, it is tempting to conclude that the animal was healthy in this regard, and the initial elevated readings can be considered as higher normal range. The albumin (2.75, 3.55, and 2.8 g/dl) and total protein (7.25, 8.4, and 8.1 g/dl) concentrations for sets 1, 2, and 3 were similar to the values observed in other captive leopards housed at PNHZP and Bengal Safari (albumin:

3.11 ± 0.1 g/dl; total protein: 7.11 ± 0.19), but the differences were not significant (p=0.172 >0.05). Age influence on albumin and total protein has been demonstrated by Lowseter *et al.,* 1990 in carnivores, although variation in these parameters may also be due to other factors such as habitat and nutritional intake (Shrivastav *et al.,* 2011). Amongst the lipid profile tests, serum glucose concentration showed a decreasing trend with the highest readings observed for set 1 (140.8 mg/dl), followed by set 2 (91.6mg/dl) and set 3 (61 mg/dl), however, the differences were not statistically significant (p=0.90 >0.05). Stress is the most likely explanation for the initial higher readings due to the release of cortisol because one would anticipate that capturing would be stressful (Kock *et al.,* 1987). The lower values recorded in the following examination may suggest that the study animal was less stressed when it was captured for the second and the third time. Further, an increase in the cholesterol value was observed. This can be explained in light of its feeding habits.

The readily available carbohydrates and lack of exercise may be a cause for the elevated cholesterol concentrations in captive wild animals (Schmidt *et al.,* 2003), however, the differences were not statistically significant (p=0.44<0.05). In all cases, glucose and cholesterol values were comparable to the other captive leopards (glucose 88.03 ±10.12 mg/dl; cholesterol 154.6 ± 7.52mg/dl) sampled in our study. The triglyceride levels were found as 93.59, 155.57, and 58.12 mg/dl which was normally higher than the values observed in captive leopards (58.19 ± 3.97 mg/dl) compiled in our study and the range given by Shanmugam *et al.,* 2017 (4-52 mg/dl). But the differences were not significant (p=0.235> 0.05). Besides oxidative stress, variation in lipid profiles can be attributed to the fact that the study animal was randomly sampled for analysis, without prior fasting. BUN, creatinine, and uric acid were analyzed to evaluate the renal integrity of the animal. A non-significant variation in BUN (67.5, 95, and 68 mg/dl; p=0.102> 0.05), and creatinine values (0.42, 0.36, and 0.34 mg/dl; p=0.538> 0.05) was noted, which were similar to the ranged obtained from other captive leopards in the park (BUN 71.13 ± 3.55 mg/dl; creatinine 0.45 ± 0.03 mg/dl). However, Shanmugam et al., 2017 and Padmanath et al.,2015 have recorded lower values of BUN in their study. Besides this, a significant increase in the uric acid level (0.51, 0.78, and 1.73 mg/dl; p=0.04<0.05) with time was observed. Except for creatinine, BUN and uric acid concentration in the serum are largely influenced by the animal's diet or protein intake (Constable *et al.,* 1998 and Polo, 1995). Therefore, greater values of BUN and increasing levels of uric acid in the study animal may be due to the regular supply of meat, contrary to the wild in which food consumption involves a strenuous process of foraging and hunting.

All serum electrolyte values measured in our study were comparable to resident leopards of PNHZP and Bengal safari. The fluctuation in its values with time has been summarized in table 3, but the differences were not statistically significant (chloride p=0.304; potassium p=0.172; sodium p=0.538; calcium p=0.304; phosphorous p=0.172; magnesium p=0.17). The present case study indicates that environmental factors such as free-ranging, semi-captive and captive settings have a significant effect on various serum biochemical parameters. The paucity of information regarding the effect of captivity on physiological variables for common leopards and other exotic carnivores makes this preliminary study valuable. Furthermore, the results of this study can be helpful for comparative assessments and interpretation of serum biochemical values done for sick animals of the species.

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CHRONIC ABSCESS IN A BURMESE PYTHON: DIAGNOSIS AND SURGICAL MANAGEMENT

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Abstract

A captive Burmese python from Zoological Garden, Alipore that presented with subcutaneous soft tissue mass was diagnosed as chronic calcified abscess by ultrasonography. Surgical management of the abscess under injectable anaesthesia with tiletamine and zolazepam combination was undertaken. Post-operative systemic medications and regular local dressing allowed for healing of the surgical wound uneventfully.

Key Words Reptiles, Mass, Lesion, Ultrasonography, Surgery

Introduction

Abscesses are found in all species, although their presentation may vary. In reptiles, the heterophils possess a different killing mechanism than that seen in mammals. As a result, the reptilian abscess is usually not liquefied. Reptilian pus is caseous, forming hard, "cheese-like" plugs that are nearly impenetrable to antimicrobial therapy. Most reptilian abscesses not only are solid but also are well encapsulated commonly appearing as raised, hard, and well-circumscribed subcutaneous swellings resembling neoplasms, so sometimes termed as "pseudo-tumours". Reptiles are exposed to pathogens on a regular basis. Bites, scratches, environmental trauma, and foreign body can all predispose these animals to abscess formation. Husbandry factors seem to play a significant role in the development of abscesses. Poor lighting, inadequate temperature regulation, nutritional deficiencies, and overcrowding are common predisposing factors (Mader, 2006).

Materials and methods

A captive Burmese python (*Python bivittatus*) weighing 15 kg, was demonstrated a sub-cutaneous soft tissue swelling over the vertebral column with a "mass" like feel on palpation. It was during winter and the reptile did not start feeding or show eccdysis even after cessation of the cold season. Ultrasonographic evaluation, carried out with physical restraint (Fig 1) revealed presence of a subcutaneous circumscribed roundish mass with a hyperechoic wall, which happens to be a characteristic feature of chronic abscess (Penninck & Anjou, 2015). Dorsal portion of the mass showed cavitations with presence of hypoechoic bloody fluid which was aspirated through ultrasound guidance and sent for routine culture. Ventral to the cavitation, an area of diffused hyperechoic zone was noticed (Fig: 2&3). The python was treated with intramuscular injections Enrofloxacin @ 5mg/kg b wt and Meloxicam @ 0.1 mg/kg b wt for 3 days. Thereafter the animal started feeding

and ecdysis also took place. Following this surgical management of the abscess was planned. Injection Zoletil 50 (containing tiletamine hydrochloride 125 mg and zolazepam hydrochloride 125 mg in every 5 ml) was injected intramuscularly @ 3 mg/kg b wt (total dose 1.8ml) and rapid induction was noticed. The surgical site was prepared, and incision was made over the abscess (Fig: 4). Firm calcified materials (Fig:6) with capsule were evacuated using blunt dissection and area was irrigated with normal saline, povidone-iodine and topical Gentamicin (Fig:5). Subcutaneous sutures were placed with 'Vicryl no 1' and everted suture were placed on the skin with nylon (Fig:7). Perioperatively the animal was given 50ml normal saline subcutaneously, and injections Enrofloxacin (100 mg/ml) 1ml and Meloxicam (5mg/ml) 0.6 ml intramuscularly.

Post-operatively, Enrofloxacin was continued for 10 more days and non-steroidal anti-inflammatory drug Meloxicam for two more days at the same dose rate. Regular dressing of the surgical site was carried out. But after two weeks wound dehiscence was noticed at some portion of the skin suture line. These portions were kept open for second intention healing with regular dressing using Povidine iodine and topical Gentamicin. The skin sutures that had healed were removed after six weeks.

Results and Discussion

Radiography, ultrasonography, and advanced imaging techniques such as magnetic resonance imaging (MRI), \computed tomography (CT) can all be used for evaluating the presence of internal abscesses. In this case ultrasonography was used as a useful non-invasive tool for diagnosis of the chronic abscess. For reduction of aesthetic risk and stabilization of the patient's condition, supportive fluid therapy and nutritional support may be initiated before anaesthesia. Many injectable agents

Fig 1: Ultrasonography of subcutaneous swelling in a Burmese python

have been used and investigated for induction and maintenance of anaesthesia in reptiles. Most agents, especially when used alone at high dosages, are associated with pronounced cardiopulmonary depressant effects, prolonged induction and recovery times, and poor muscle relaxation and analgesia. Ketamine HCl is most commonly used in reptiles to produce immobilization and induce anaesthesia. Ketamine HCl alone results in inadequate muscle relaxation, minimal analgesia, and, if used at high dosages, prolonged recovery times. A combination of tiletamine and zolazepam, has been used in this case for immobilization and induction of anaesthesia. At a dose of 2 to 4 mg/kg IM, tiletamine/zolazepam is useful to facilitate handling of large chelonians, lizards, and snakes (Schumacher & Yelen, 2006). In this case this combination drug @ 3mg/kg was adequate for the surgical procedure with rapid induction and smooth recovery.

Fig:2 Irrigation of surgical site.

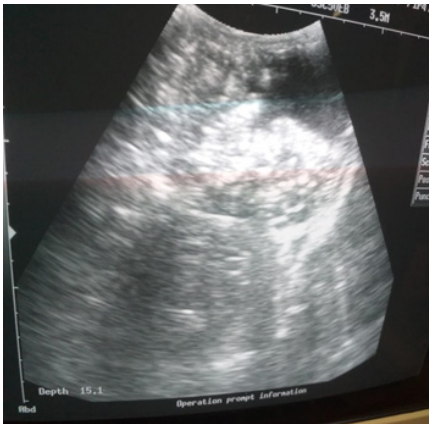

Fig:3 Ultrasonographic photomicrograph shows dorsal caviations and ventral diffuse hyperechoic area.

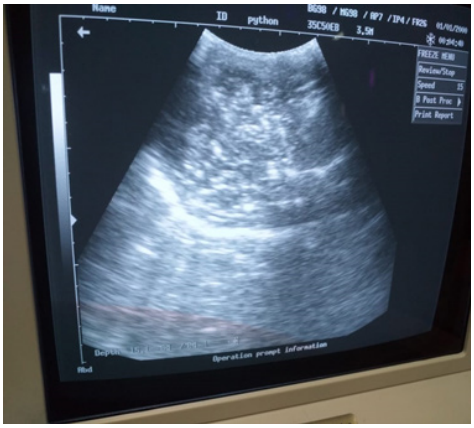

Fig: 4 Ultrasonographic image shows hyperechoic wall of the mass with diffuse hyperechoic zone within the mass.

Abscesses are best excised completely and, this procedure must include removal of the fibrous capsule and calcified material. Once the abscess capsule has been removed, the wound may be left open to heal by secondary intention and granulation. Healing times vary depending on many factors but in general can take anywhere from four to six weeks. Daily debridement and application of an iodine-based solution addresses bacteria that remains on the surface. Recurrence is discouraged by the use of an appropriate systemic antibacterial agent and local application of antiseptics, coupled with high standards of hygiene (Mader, 2006).

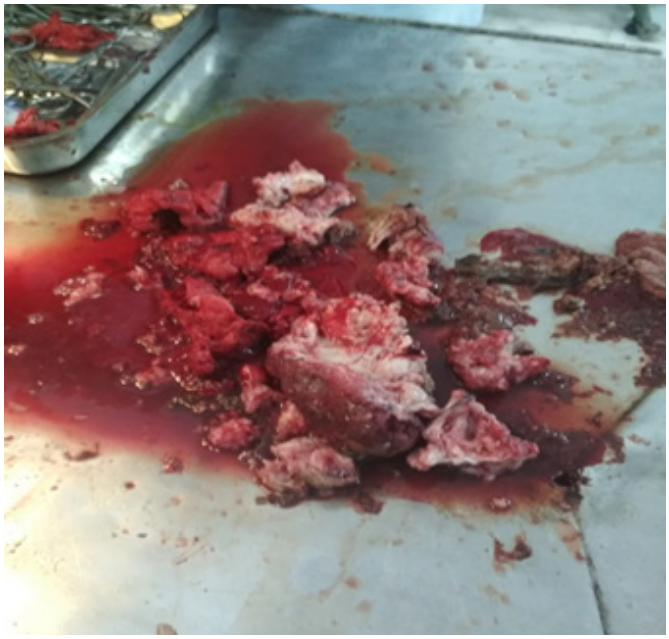

Fig:5 Excised abscess mass.

Acknowledgment

The authors are thankful to Mr Saurabh Chaudhuri, IFS, Addl PCCF and Member Secretary, West Bengal Zoo Authority for his continuous encouragement for modern scientific work and Mr Asis Kumar Samanta, IFS, CCF, Director, Zoological backyard, Alipore for providing necessary facilities for conducting our imaging and surgery.

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INFRARED THERMOGRAPHY A SIGNIFICANT REMOTE EXAMINATION TOOL IN CAPTIVE ELEPHANT'S HEALTH CARE

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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 to be detected by the human eye; instead, we perceive it 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, toe nail 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 noncontact safe remote sensing method and less in coast when compare 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 also thankful to the animal care staff at Wildlife SOS for their sincere assistance.

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MOLECULAR DETECTION OF BACTERIA OF MYCOBACTERIUM AVIUM COMPLEX (MAC) IN INDIAN GREY MONGOOSE (*Herpestes edwardsii*) IN THIRUVANANTHAPURAM ZOO, KERALA

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Abstract

In India, tuberculosis is a highly prevalent disease, which causes high mortality among humans. This study was undertaken to detect the presence of MTBC and MAC organisms in free ranging Indian grey mongoose (*Herpestes edwardsii*) by Polymerase Chain Reaction (PCR). Twenty-one samples were collected from free-ranging mongoose in Zoological Garden, Thiruvananthapuram. The DNA from the faecal samples was extracted by Qiagen stool minikit. The extracted DNA was further subjected to PCR to detect the presence of any mycobacterium (PCR targeting 16SrRNA gene), MTBC (PCR targeting IS6110 and MAC (PCR targeting IS1311). Out of 21 samples, 16 (76.19 percent) were found to be positive for mycobacteria. None of the samples were positive for bacteria belonging to MTBC and MAC. The results of the study indicate that the mongooses in the area are free from mycobacteria of MAC. The positive result obtained in the 16S rRNA PCR could be due to the presence of saprophytic non-pathogenic mycobacteria seen in the soil.

Key Words Mongoose, Mycobacteria, 16S rRNA, IS1311, Polymerase Chain Reaction, Qiagen stool minikit.

Introduction

Pathogens that are inherited between environment, animals (wild or domestic) and humans introduce major challenges in animal and human health (Verma *et al.,* 2013). Tuberculosis (TB) is an ancient as well as emerging disease affecting both human and animal populations. It is a cryptic disease which takes many years to manifest clinical signs in animals.

Infected animals can shed bacteria for long periods. Transmission occurs through direct and indirect routes of infection (Miller *et al.,* 2015). According to the World Health Organisation, in 2010, one-third of the human population was infected by TB (Verma *et al.,* 2014). Tuberculosis is caused by acid-fast, gram-positive bacteria known as the Mycobacterium tuberculosis complex (MTBC). The MTBC consists of genetically closely related pathogens that can cause TB in humans and animals. The MTBC encompasses the human modified pathogens *M. tuberculosis*, *M. africanum* and other members of TB pathogen such as *M. canettii, M. bovis, M. caprae, M. pinnipedii, M. microti, M. orygis, M. suricattae, Dassie bacillus* and *M. mungi* that have been reported in a wide range of mammalian species. The MTB complex

can infect captive wildlife species, whereas the susceptibility, pathogenicity and immune response towards mycobacterial infection vary widely between mycobacteria and host animal species. The diagnostic tools used in domestic animals exhibit moderate performance (Lecu *et al.,* 2011). The most studied TB pathogen is *M. bovis*, which causes bovine tuberculosis in domestic and wild animals.

Very little is known about the MTBC diversity in wildlife (Coscolla *et al.,* 2013). The Mycobacterium avium complex (MAC) consists of *M. avium* (with subspecies *M. avium* subspecies *avium* (MAA), *M. avium* subspecies paratuberculosis (MAP), *M. avium* subspecies silvaticum (MAS) and *M. avium* subspecies hominisuis (MAH) (Turenne *et al.,* 2008). It is a very slow growing, acid fast, non-pigmented bacteria with lipid-rich cell wall. So, MAC has the ability to resist adverse environmental conditions. *Mycobacterium avium* subspecies paratuberculosis causes Johne's disease in most of the ruminant species. They are transmitted through faecal oral routes and cause Crohn's disease in humans (Erume *et al.,* 2001). Indian Grey Mongoose belongs to the family Herpestidae under the order Carnivora. Diseases like Hepatitis E (Li *et al.,* 2006), leptospirosis (Alexander et al., 2010) and salmonellosis (Miller *et al.,* 2015) have already been reported in mongoose. Mycobacterium tuberculosis complex infection in Banded Mongoose (*Mungos mungo*) in Africa has been reported (Alexander *et al.,* 2002 and Bruns *et al.,* 2017) whereas the transmission of disease among the species still remains a hypothesis. With the exception of a report of rabies in Kannur District of Kerala (Jayson and Govind, 2014), reports on the detection of infectious agents in mongoose in India is scarce.

There are no reports on TB among mongoose in India. There have been earlier cases of tuberculosis among captive wild animals at the Zoological Garden, Thiruvananthapuram. The Indian grey mongoose has the habit of scavenging and could be observed to move from one cage to the other feeding on leftover meat in various enclosures, they could have acted as carriers of diseases like tuberculosis from one animal homed in the zoo to the other. The mongoose also could go outside the zoo to nearby residential areas and the premises of the zoo frequented by visitors making them potential carriers of infection for people also. The present study was attempted to test for the presence of MTBC and MAC in Indian Grey Mongoose dwelling in the premises of Zoological Garden, Thiruvananthapuram.

Materials and methods

For the study a total of 21 faecal samples were collected from free ranging Indian grey mongoose in and around the Zoological Garden, Thiruvananthapuram. Total DNA was extracted from the faecal sample by using a QIAamp DNA Stool Mini Kit (Qiagen, Germany) as per the manufactures' protocol.

Extracted DNA was subjected to 16S rRNA-based PCR (Shin *et al.* 2010) for the detection of mycobacteria and to IS1311 based PCR (Shin e*t al.* 2010) for the detection of bacteria of MAC. Mycobacterium phlei and M. paratuberculosis were used as positive controls in the 16S rRNA and IS1311 PCR, respectively. The PCR products were resolved in 1.5 percent agarose gels containing ethidium bromide and documented in a gel documentation system under UV illumination.

Result and discussion

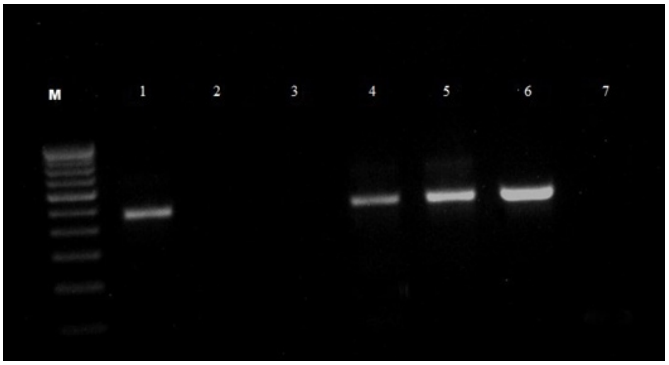

Figure 1: Agarose gel showing 484 bp amplicons generated by targeting 16S rRNA gene of mycobacteria.

Lane M: 100 bp DNA ladder
Lane 1 to 5: Samples
Lane 6: Positive control (*M. phlei*)
Lane 7: Negative control

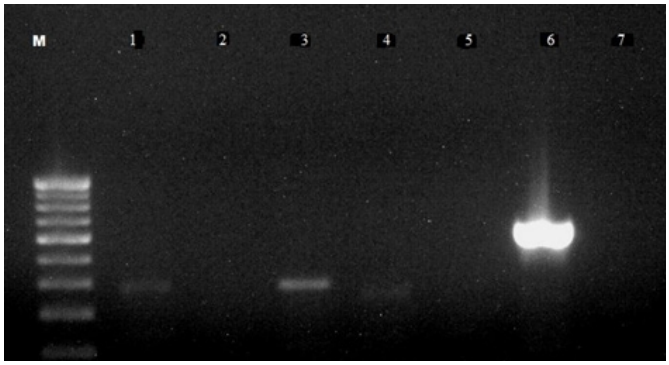

Figure 2: Agarose gel showing non-specific amplicons generated in samples tested by PCR targeting IS1311 gene of MAC.

Lane M: 100 bp DNA ladder
Lane 1 to 5: Samples
Lane 6: Positive control (*M. paratuberculosis*)
Lane 7: Negative control

Of the 21 samples tested, 16 were found to be positive in the PCR for 16S rRNA gene of mycobacteria. Amplicons of 484 bp generated by the PCR reaction were obtained on agarose gel electrophoresis (Figure 1). The positive control, *M. phlei*, also exhibited a single band of 484 bp in the gel. However, in the PCR to detect MAC bacteria, none of the samples yielded positive results. Specific amplicon of 608 bp was obtained in *M. paratuberculosis* which was used as the positive control (Figure 2). In this study, PCR targeting 16S rRNA gene of mycobacteria was conducted to detect mycobacteria in the faecal samples. Twenty-one samples were tested and 16 (76.19 per cent) were found positive. Boddinghaus *et al.* (1990) developed 16S rRNA-based PCR for the rapid detection of mycobacteria. Shin *et al.* (2010) reported that PCR based on primers targeting 16S rRNA gene were suitable for the detection of mycobacteria in faecal samples. All 21 samples were also tested for MAC by conventional PCR targeting IS1311. None of the samples were found to be positive. Specific amplicons of 608 bp in size were obtained only in the positive control. In the study, 16 samples (76.19 percent) were found to harbour DNA for mycobacteria and none of the samples were positive for bacteria of MAC. This may be due to the presence of saprophytic non-pathogenic mycobacteria seen in the soil such as *M. fortuitum*, *M. tokaiense*, or *M. austroafricanum* and *M. heidelbergense* which is ingested during foraging.

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BAMBOO PLANTATION AS A VALUE-ADDED ENRICHMENT FOR LEOPARD (*Panthera pardus*) ENCLOSURES IN CAPTIVITY

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Abstract

Leopards are managed in captivity due to many underlying reasons like conflict, permanent disability, zoo stock etc. Confinement and lack of enrichment has behavioural impact on captive animals. Confining leopards to limited areas may trigger stereotypic behaviour which is unacceptable. It is necessary to provide enrichment that can engage the leopards and provide naturalistic environment to fulfil the natural instincts of the species. Based on the observation in their natural habitat, bamboo was used in leopard enclosures as an enrichment. Two varieties of bamboo, viz *Dendrocalamus strictus* and *Bambusa vulgaris vittate* were planted in the enclosures of leopards to consider their suitability and utility. The *Dendrocalamus strictus* variety was found better suited for leopard enclosures owing to the dense canopy, insulation in summers and fast growth by end of two years. This further provided protection from harsh climatic conditions and mimicked the natural habitat of the leopards. Bamboo can be adopted as a cost-effective enrichment for leopard enclosures in zoos and rescue centres in tropical climate.

Key Words Leopard, Enrichment, Captivity, Bamboo, *Dendrocalamus strictus* and *Bambusa vulgaris*

Introduction

India is one of the 17 mega bio diverse hotpots across the world. Many species of flora and fauna are distributed in diverse habitats of the country. Central India is home to many species of large felids including the tigers (*Panthera tigris ssp. tigris*) and leopards (*Panthera pardus*). In the wild the species may share the same habitat; however, there is competition for prey and territory. The leopards are highly elusive and have an exceptional ability to camouflage in natural habitats (Bailey, 1993). Due to the growing anthropogenic pressure the species has been in constant conflict with humans (Seidensticker, 1990). Episodes of leopards entering human settlements both in rural and urban areas has seen a steep rise. Habitat destruction, lack of prey and water and lack of corridors have rendered the species susceptible to exploitation (Santia Pillai, 1992).

Many leopards are also managed in captivity. The greatest challenge in maintaining the leopards in captivity is to meet the habitat requirements. Enrichment of leopard enclosures is a vital part of captive management and mainly aims to mimic the natural environment and provide avenues to stimulate the natural instincts of the elusive species (Kessler and Turner, 1997). In the wild, leopards are known

to spend a considerable amount of time on tree tops, while a few others hide among thick bushes or dens depending on the landscape and vegetation. With the diverse distribution of the leopards in central India, it becomes very important to consider the origin and habitat of the leopard before opting for enrichment of the enclosures.

The enrichment of a typical leopard enclosure is carried out by providing furniture such as wooden platforms, wallowing ponds, scratch poles, vegetation and grass etc. (Lyons *et al.,* 1997). The utility of the enrichment is subject to individual variation; however, it engages the leopards in captivity and prevents behavioural alterations due to boredom (Markowitz, 1982; Hutchins *et al.,* 1984). Many substrates have been identified for the enrichment of Leopard enclosures. In the current study, the utility of bamboo was studied for enrichment of leopard enclosures in captivity.

Materials and methods

The study was carried out from January 2019 to December 2019 at Wildlife Rescue Centre, Gorewada, Nagpur. It is located 10 km from Zero Milestone of Nagpur city which is also considered as the geographical centre of India. The geographical location of the study area is 21°11'N 79°2'E located 336 m above sea level. Four adult leopard enclosures were identified and were planted with bamboo. Two variety of bamboo, Painted bamboo (*Bambusa vulgaris vittate*) and Calcutta bamboo (*Dendrocalamus strictus*) were planted for comparison of the utility and efficiency. The leopards were introduced in the enclosures and were observed every two hours during the day and every four hours during the night. Their position and activity were noted during the study period. The temperature, sunshine hours, precipitation and wind velocity were recorded.

Leopard	Sitting	Rolling	Scratching	Sun Bask-ing	Napping	Feeding	Hiding	Other Observation
Leopard 1	Yes	Yes	Yes	-	Yes	-	Yes	-
Leopard 2	Yes	Yes	Yes	-	Yes	-	Yes	-
Leopard 3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Scat samples revealed presence of leaves of bamboo
Leopard 4	Yes	Yes	Yes	-	Yes	-	Yes	-
Leopard 5	Yes	Yes	Yes	-	Yes	-	Yes	Spent > 8 hours in summers
Leopard 6	Yes	Yes	Yes	-	Yes	Yes	Yes	-
Leopard 7	Yes	Yes	Yes	-	Yes	-	Yes	Scratching of the bamboo trunks

Sr. No.	Bamboo Variety	Inter nodal length (cms)	Circumference (cms)	Leaf length (cms)	Leaf Breadth (cms)	Average Height (2 years) (feet)	Leaf shedding	No. of shoots (af-ter 2 years)	Canopy Density
1	*Bambusa vulgaris vit-tate* (Painted Bamboo)	26±3.16	12.48±0.66	30.03±4.23	6.23±0.69	17.43±1.33	+/+++	29.60±4.15	++/+++
2	*Dendrocalamus stric-tus* (Calcutta Bamboo)	12.71±1.88	5.70±0.46	12.90±1.75	2.30±0.41	12.80±2.01	+++/+++	35.44±4.78	+++/+++

Fig:1 Leopard enclosure prior to enrichment

Fig:2 Leopard enclosure prior to enrichment

Fig:3 Bamboo Plantations in leopard enclosure

Fig:4 Growth after one year time

Fig:5 Leopard seeking shelter from summer heat under thick bamboo canopy

Fig:6 Leopard hiding among the bamboo plantation in response to visitation by care takers

Discussion

In the wild leopards are highly elusive and utilise the unique camouflage to their advantage to hunt and escape competitors. The greatest challenge is providing enrichment that can mimic the natural habitat (Carlstead and Shepherdson, 1994; Mallapur and Chellam, 2002). In the wild, depending on the prey density, a typical leopard territory on an average is 11 km^2 (Bertram, 1982). An enclosure or exhibit area for captive leopard can range upto 500 m^2. Thus, it is essential to accommodate features that can mimic the natural landscape at the same time providing ample opportunity to monitor and manage the leopards (Markowitz, 1982). In the current study, bamboo was

Fig:7 Comparison of the leaf of *Bambusa vulgaris vittate* (Painted Bamboo) (Top) with *Dendrocalamus strictus* (Calcutta Bamboo)

found to be an excellent substrate for the enrichment of leopard enclosures. In the current study, two varieties of bamboo were utilised for the enrichment of the enclosures. The first *Dendrocalamus strictus* (Calcutta Bamboo) is a variety with small leaves and grows as a thick congregate and is a fast-growing

variety. Second, *Bambusa vulgaris vittate* (Painted Bamboo) has broad leaves and is characterised with the presence of yellow coloured bamboo.

The major advantage that bamboo plantation provided was a thick canopy during the peak summers when temperatures in Nagpur spike to 46ºC. The fallen leaves provide a soft bed for the leopards to rest on. The leaves of the *Dendrocalamus strictus* (Calcutta Bamboo) were found in the scat samples of leopards, highlighting the natural tendency of leopards to ingest bamboo leaves during indigestion.

Certain observations made during the study were very encouraging in recommending bamboo plantation as enrichment of leopard enclosures. The leopards were found to bring meat to bamboo shelters and eat it while hiding in the thick canopy (Shepherdson *et al.*, 1993). The behaviour mimicked the natural tendency of solitary feeding exhibited by the species. The bamboo den also was utilised to escape the visiting handlers and veterinarians. In case of an unexpected events (eg. Sudden appearance of an observer) the leopards would retract to the bamboo den and hide. Thus, bamboo plantation in leopard enclosures not only provided for cover but also helped in fulfilling the natural instinct.

Conclusion

Dendrocalamus strictus (Calcutta bamboo) variety of bamboo was found to provide a great advantage as a naturalistic substrate when used for the enrichment of leopard enclosure. The variety provides dense canopy that is utilised preferentially by captive leopards for routine activities. The study highlights the utility of the substrate in the enrichment of enclosures of captive leopards in particular.

Acknowledgement

The authors acknowledge the permission granted by Hon'ble Principal Chief Conservator of Forest & Chief Wildlife Warden, Maharashtra State to conduct the study. The authors thank Forest Development Corporation of Maharashtra Ltd., Nagpur and Office of the Divisional Manager, Gorewada Project for the facilitation of this study. The authors acknowledge Shri. Nandkishor Kale, Deputy Director, TATR, Maharashtra for identification and selection of bamboo varieties for the study.

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MANAGEMENT OF PARTIAL PARESIS IN GREEN IGUANA (*Iguana iguana*) – A CASE STUDY

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Abstract

In captive conditions, musculo-skeletal injuries are one of the common causes of presentations of reptiles to a veterinarian. The injuries can be due to trauma, fall, nutritional and metabolic deficiencies, infighting etc. The right clinical approach leading to an accurate diagnosis starts with a complete history, review of husbandry and management and finally, identification of the cause. Paresis or paralysis may often be encountered due to trauma, especially in lizards. A juvenile female Green iguana (*Iguana iguana*) was observed dragging its hind limbs resulting from a fall. On examination of the limbs, it was found that the motor reflexes were absent. The radiological (X-ray) examination did not reveal any fracture and hence the condition was tentatively diagnosed as partial paresis due to nerve compression. In order to treat the condition, the iguana was subjected to infra – red therapy for 1 minute b.i.d for three days. In addition to this, it was given multivitamin syrup, nerve and calcium supplements and was advised basking in sunlight for 6 hrs. every day. The reptile showed complete recovery in four days of therapy.

Key Words Green iguana, Partial paresis, Infrared therapy

Introduction

Green iguanas (*Iguana iguana*) (Linnaeus, 1758) are one the largest lizards; adults can reach 50 cm in snout vent length (SVL) and approximately 200 cm in head to tail length (Schwartz and Henderson, 1991; Rivero, 1998; Falcón, 2013). They are extensively found in the pet trade and have been introduced to many locations due to non-judicious release of captive animals (Falcón, 2013). The species is susceptible to metabolic bone disease and various other degenerative health issues due to nutritional deficiencies in captivity (Zotti, 2004). Various therapies have been used to treat these conditions. Disorders of the central nervous system, macular degeneration and stroke have been treated with the use of irradiation in the red/near-infrared spectrum (R/NIR, 630–1000 nm) (Fitzgerald, 2013). However, partial paresis due to trauma and compression has not been recorded comprehensively and the mechanism/implications of the therapy for the same have not been addressed thoroughly (Fitzgerald, 2013)

Case Description

Three juvenile iguanas (2 males, 1 female) are housed at The Madras Crocodile Bank Trust. These Iguanas were part of a shipment seized by the Wildlife Crime Control Bureau and have been under care at the zoo since. They were transferred to a new enclosure on December 8th, 2020. On 12th December, a juvenile female climbed a wooden plank (close to 5 feet above ground) and jumped and landed awkwardly. She was then observed to be dragging her right hind limb at first. After few minutes the animal was dragging both the hind limbs. On closer inspection, no swelling or injury was noticed on either limb. Further, when the reflexes were checked, it was noticed that the animal was lacking pain, pinch and perching reflexes. Radiological examination showed no evidence of fracture and hence the condition was tentatively diagnosed as nerve compression (Fig. 1). In order to treat this condition, the female juvenile iguana was subjected to infra – red therapy for 1 minute (distance of bulb from skin was ~ 30 cm) twice a day for three days and was allowed to bask in sunlight for 6 hrs. (Fig. 2). Along with the infra – red therapy, multivitamin syrup (Verol), nerve (Neurobion forte) and calcium (Exoterra powder Ca + D3) supplements, were administered orally s.i.d for three days (Divers and Mader, 2018; Girling and Raiti, 2019). When restrained, the ventral aspect of hind limbs and the area around pelvist showed bluish – purple discoloration, suspected to be a bruise caused by impact of the fall. Once a day topical application of Bethamethasone valerate and neomycin skin cream (Betnovate – N) was advised for three days to reduce the inflammation and bruising. The animal showed tremendous improvement each day and after four days of therapy, she stopped dragging the limb and had all the reflexes present (Fig. 3) indicating a complete recovery.

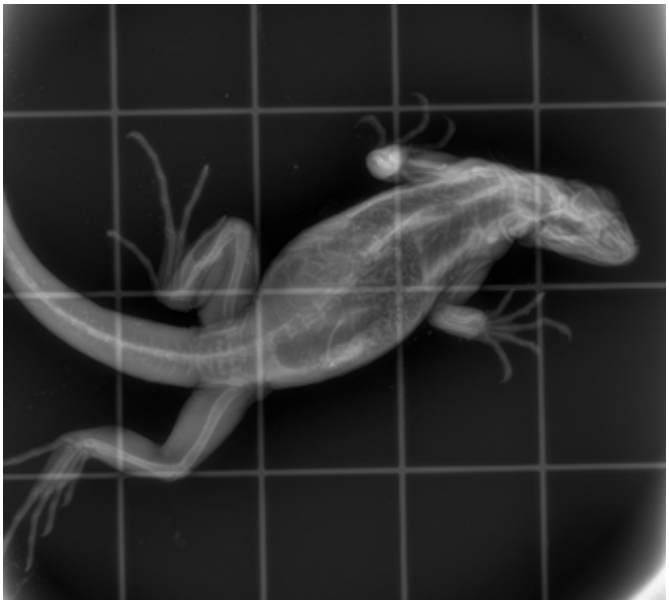

Fig. 1: Radiographic examination

Fig. 2: Infra-red therapy

Fig.3: After Recovery

Conclusion

In reptiles, especially in lizards and chelonians, paresis/paralysis of hind limbs is a common clinical presentation (Divers & Mader, 2018). Though spinal cord trauma is the predominant cause of paresis/paralysis, a complete history and physical examination is essential to rule out other possible causes like nutritional deficiencies, neoplasia, metabolic abnormalities, etc. Definitive diagnosis of the condition is essentially required to formulate treatment plan which may vary as per the case presentation. It is essential diagnose the possible causes of paresis/paralysis, a thorough a physical (orthopedic), and neurologic examination (Divers & Mader, 2018). Thus, early intervention with appropriate diagnosis helps to prevent any further musculo-skeletal degenerative changes and helps to address similar conditions.

Acknowledgement

We sincerely thank the Trustees of the Madras Crocodile Bank Trust for their continued support and encouragement. We thank Ms. J. Shanti and all the animal keepers to be the first responder in case of emergency/incident and especially for their tireless efforts and commitment to animals and our environment.

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ONSET OF CEPHALIC PIGMENTATION IN MALES IN THE RED CROWNED ROOF TURTLE, EMYDIDAE, *Batagur kachuga* (GREY, 1887)

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Abstract

Red-crowned roof turtles (*Batagur kachuga*) have been bred at the Madras Crocodile Bank Trust since 2003. Observations in a three-year-old group, comprising four males and six females, found the onset of cephalic pigmentation in males became visible at three years of age. This coincided with the development of the mental glands in males of the species, and noticeably more bulbous tails in males. This is the first report on the maturity of males in this species, with males averaging 23 cm carapace length, and females 22.7 cm.

Key Words Sexual dichromatism, Tail length, Mental glands, Maturity, Red crowned roof turtle

Introduction

The Chelonia are well known for their differences in adult sizes (sexual size dimorphism), concavities in males in the case of the Testudinidae, longer claw lengths in males (*Trachemys*; Leger), and longer and more bulbous tails in males. In addition, seasonal colour changes occur, sexual dichromatism particularly in males, i.e., *Callagur borneoensis*, *Batagur baska* (Moll, 1980; Moll et. al., 1981), and *Batagur kachuga* (Das, 1991). Male red crowned roofed turtles were previously known to mature at the age of five years (Whitaker, 2010), whilst females are assumed to take a further 15 years. Multiple clutches within a year were first reported from the Madras Crocodile Bank Trust (MCBT), with five females producing six clutches in 2009 (Whitaker, 2009). Males can be told apart from females, with longer tails extending beyond the rim of the plastron, in addition to cephalic pigmentation (Moll, 1986). Adult males have a blue-black head, a broad red patch from the tip of the snout to occiput, two yellow stripes on the side of the head, and six red stripes on the neck (Das, 1991). Rao & Singh (1987) appear to be the first to mention mental glands in males of this species, describing them as "oblong, yellow spots on the throat". Here we report on the onset of sexual maturity in Batagur kachuga males, observed in animals raised in captivity at MCBT at a record age of three years.

Materials and methods

Turtles chosen for this note are from a group of ten *Batagur kachuga* hatched in March 2009, from two clutches of eggs. They were initially raised in aquariums measuring 2.7 sq meters. On 18th October 2010, they were transferred to a larger pen measuring 49 sq meters. Diet included a variety of seasonally

available greens, *Oreochromis sp*, and grasses grown around the edge of the pond. The pond was drained on 12th August 2012, and all animals were measured and weighed. Carapace length (CL), carapace width (CW), plastron length (PL), were measured with tree callipers (±1 cm), and weight (in grams) was measured with a Pesola ™ 5-kilogram spring weighing scale. A Shapiro-Wilk Wilkinson's test indicated all parameters to be normal. A comparison of mean sizes was analyzed using a one-way ANOVA, using the Microsoft Excel™ add-in Statplus Pro 7.3 ™. Alpha is maintained <0.05.

Result

At this age of three years old, turtles displayed marked differentiation between male and female cephalic pigmentation. No differences in size were noted at this early age (Table 1).

Whilst not measured, tail size was longer, but especially more bulbous in males. The chin glands were absent in female *B. kachuga* but were a prominent orange–yellow in males (Plate 3). Cephalic pigmentation in males illustrated the sexual dichromatism in this species. Given that oviposition occurs in February – May at MCBT, males at the age of three years may be mature. This relates to the courtship and mating period, larger *B. kachuga* males have been seen with breeding colours in the adult captive breeding enclosure (Whitaker, 2009). Winokur & Legler (1975), noted that in Emydids, the emerging of mental glands probably occurs at sexual maturity, associated with the appearance of secondary sexual characters (cephalic pigmentation).

Fig. 1: Comparison of lateral head and neck pigmentation in male (Plate 1) *Batagur kachuga*.

Fig. 2: Comparison of lateral head and neck pigmentation in female (Plate 2)

Morphological parameter	Gender	X (S.D)	Range	N	P
CL	M	23.03 (±0.83)	22 – 24.1	4	0.74
CL	F	22.73 (±1.5)	20.1 – 24.5	6	
CW	M	17.86 (±0.67)	17.2 – 18.7	4	0.78
CW	F	17.67 (±1.23)	15.7 – 19.2	6	
PL	M	20.53 (±0.81)	19.7 – 21.4	4	0.62
PL	F	20.92 (±1.36)	18.7 – 22.4	6	
WT	M	1773 (±160)	1636 – 1962	4	0.91
WT	F	1798 (±414)	1216-2145	6	

Table 1. Gender, size, and significance of differences between three-year-old *Batagur kachuga*.CL=carapace length, CW=carapace width, PL=plastron length, WT=weight.

Fig. 3: Comparison of the mental glands, present in male (left), and they're absence in female (right) *Batagur kachuga*.

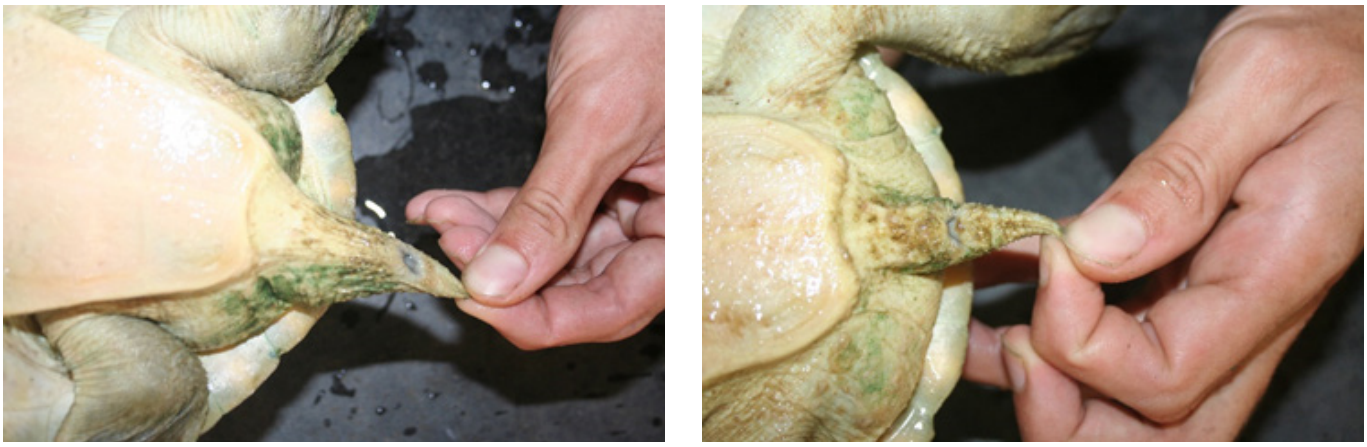

Fig. 4: Comparison of the tails in male (left), and female (right) *Batagur kachuga*.

Discussion

Colouration is primarily due to an interaction between pigments and structural components of an animal. This has been extensively studied in reptiles, concerning genetics, colour expression, function, and selection of these traits (Olson, 2013). The expression of colour in reptiles is mainly produced by the reflection and scattering of light and the animal structure. The interaction between the three types of chromatophore cells, present within dermal tissues, and structures such as collagen fibers and connective tissue are responsible for the colouration of the animal (Bagnara, 1973). The top layer consists of xanthophores (yellow red) which is followed by iridophores (colourless crystals) and lastly by melanophores (black) (Olson 2013).

The patterns and colours displayed by reptiles strongly influences numerous aspects of their social and predatory behaviour as well as affect thermoregulation (Cooper 1992). They also play a major role in mate selection and evaluation of mate quality. In multiple taxa, males are more vividly and conspicuously coloured than females. Differences in colouration between sexes (sexual – dichromatism; as is known to occur in *Batagur kachuga*; Das, 1991) is generally traced to sexual selection. Females tend to choose

mates based on their patterns and colours (Bulté, 2013).

Here we reported on the apparent rapid maturity of male Batagur kachuga, expressed by changes in cephalic pigmentation, tail size, and the prominent mental glands. Females are expected to take up to and over ten years to mature. They need to attain large body sizes to produce large clutch sizes (as in Trachemys scripta, Wilbur, 1975), of a reported 6 – 31 eggs (Sirsi *et al.,* 2017). Oviposition in *B. kachuga* from observations in captivity at the MCBT, indicates two clutches of eggs may be laid in a year.

Acknowledgement

We thank the staff at the Madras Crocodile Bank Trust, for their dedication to preparing food, cleaning enclosures, assisting with measurements, and many other contributions. The Trustees of the Madras Crocodile Bank Trust are acknowledged for their support and encouragement.

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THERAPEUTIC MANAGEMENT OF STOMATITIS AND ROSTRAL ABRASIONS IN RETICULATED PYTHON (*Malayopython reticulatus*)

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Abstract

In terrarium conditions, infections can occur by direct or indirect contact of mucous membranes with infectious agents. As the snakes do not have lymph nodes like mammals, there is no way that the pathogens can be vulnerable locally and therefore, it is easier for pathogens to get into the bloodstream and cause generalized septicaemia with lesions in the internal organs. The most common infection which captive snakes incur is stomatitis characterized by inflammation of the oral cavity. It can be either a primary or secondary type of infection. A 24 yr. old female reticulated python was anorexic for two months and showed extreme weight loss, a general weakening of the body and lethargy. On detailed physical examination, the oral cavity of the python showed inflammatory changes, ulcerations and abrasions. The ventral aspect of the lower jaw was observed to have wounds, which were suspected to be due to inflammation inside the oral cavity. Thus, the condition was tentatively diagnosed as stomatitis with abrasions. Further, antibiotic sensitivity tests revealed sensitivity of the bacteria towards the third-generation cephalosporins. Therefore, the python was treated with inj. Ceftiofur @2.2 mg/kg bd. wt intramuscularly and Silver Sulphadiazine (Silverex) ointment was topically applied over the lesions on the lower jaw. In addition to this, temperature and humidity gradients were worked on to improve the prognosis. The reptile showed complete recovery in one and half months.

Key Words Reticulated python, stomatitis, cephalosporins

Introduction

The longest snake in the world, the Reticulated Python (*Malayopython reticulatus*) is regulated under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Auliya, 2000; Auliya *et. al.,* 2002). One of the most common infections that affect captive snakes is stomatitis which causes infection of the oral mucosa and surrounding tissue characterized by inflammation, ulceration and abrasions in the oral cavity. Bacterial contamination is the primary cause of stomatitis (Grego, 2017). Though stomatitis can be caused by several pathogens, it generally is a sign of systemic disease or secondary to presumed predisposing factors like immunosuppression, poor husbandry conditions, malnutrition, or trauma. In all cases, there is an underlying process acting as a stressor. Bitesfrom prey and abrasive surfaces are also factors. Identification of the bacteria, and correction by treatment is key to improving the health of the

snake. Stomatitis can be cured by administering topical and systemic antibacterials as well as providing supportive care (Rowland, 2016).

Case Description

A 24 yr. old female reticulated python has been housed at MCBT since 1997. The animal was provided with a naturalistic environment and her diet included field rats and whole chicken (weekly). The animal rejected food for two months. Initially, it was assumed that due to change in weather in the winter months, the python had stopped feeding. However, after routine monitoring, it was noted that the python was excessively losing weight.

On close inspection, it was observed that the oral cavity of the python had ulcerations and abrasions on the ventral surface of the lower jaw (Fig. 1) and was diagnosed with stomatitis. The lower jaw groove showed wounds which was assumed to be a sequel to the stomatitis (Fig. 2). Oral swabs were taken for Antibiotic Sensitivity Test which confirmed the presence of bacteria such as Streptococcus, Staphylococcus and Pseudomonas (Fig. 3). These bacteria showed sensitivity towards third generation Cephalosporins. The python was first treated with topical application of Silver Sulphadiazine ointment. For the therapeutic management, inj. Ceftiofur (third-generation cephalosporins) was used @2.2 mg/ kg. b.wt, every 48 hrs. (Carpenter, 2017). Three doses of inj. Ceftiofur were administered. The python started showing improvement and became alert and active. The oral cavity showed no signs of inflammation and became clear (Fig. 4). Modification of the environment; keeping the micro-environment warmer and less humid helped in the healing process (Divers & Mader, 2018; Girling and Raiti, 2019). The python started accepting food 15 days after the last dose of treatment was given and has completely recovered (Fig. 5).

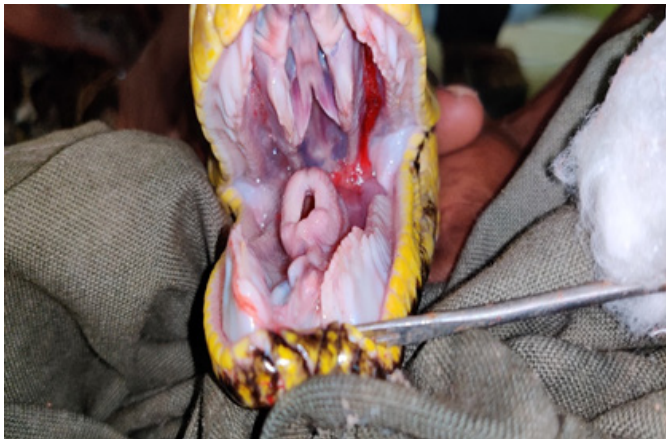

Fig 1: Stomatitis in Reticulated python

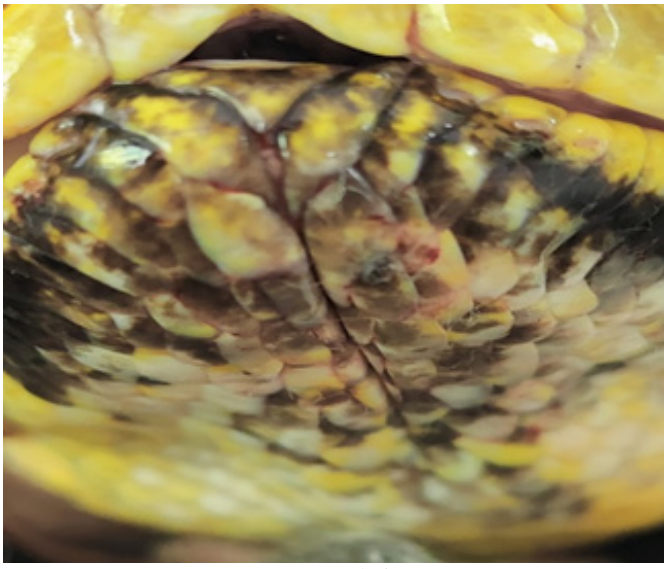

Fig 2: Lesion over the lower jaw

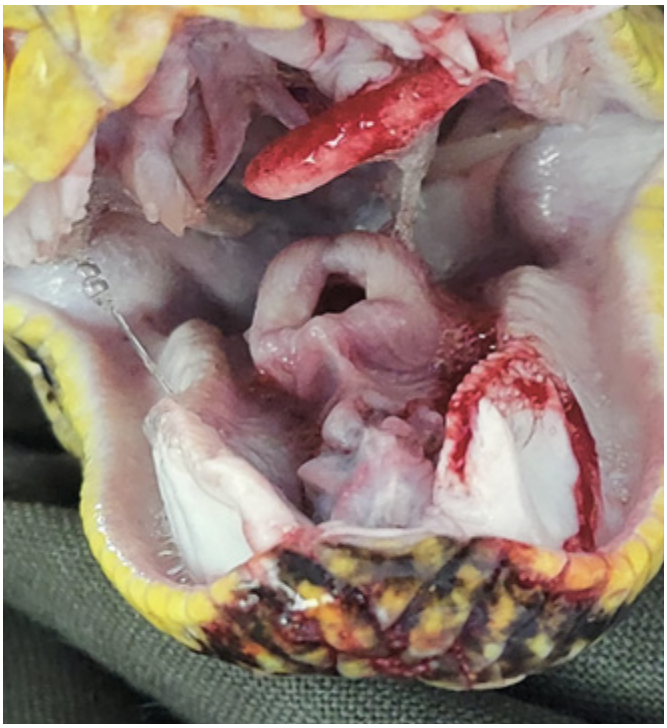

Fig 3: Oral swabs take for ABST

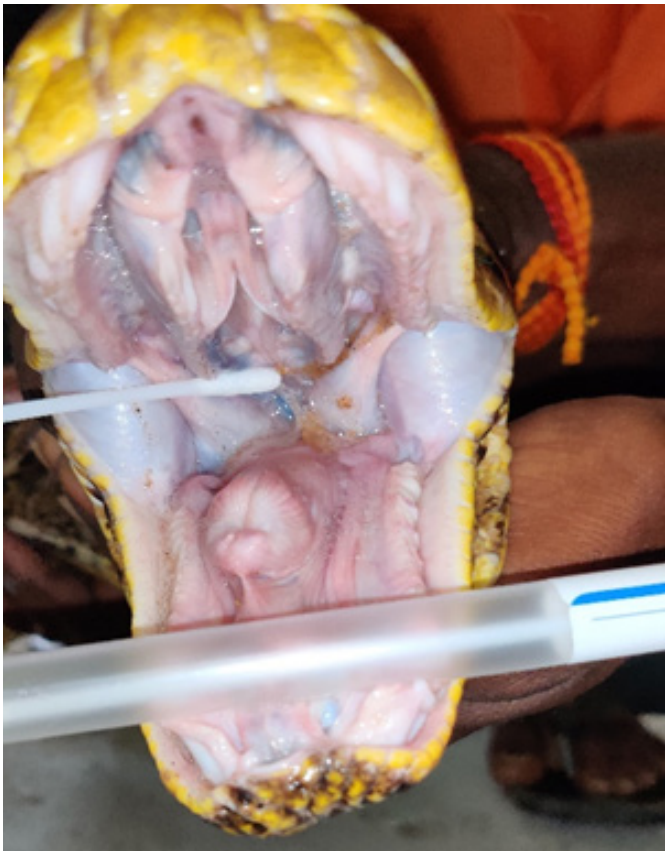

Fig 4: Oral cavity showing no lesions (After treatment)

Fig 5: Python recovered

Conclusion

Investigation of reptile stomatitis should focus on defining the pathology in the oral cavity, evaluating overall health, and identifying a predisposing factor or etiology (Divers & Mader, 2018). In the case of captive reptiles, early diagnosis of the primary cause or any other definitive diagnosis helps the reptile recover at a faster rate (Girling and Raiti, 2019). Appropriate husbandry management like thermal and humidity gradients within their habitat, comfortable hide areas, adequate lighting, biosecurity and diet (Mullineaux and Keeble, 2016) are all important in supporting a convalescing reptile thereby aiding it to get cured at the earliest.

Acknowledgement

We sincerely thank the Trustees of the Madras Crocodile Bank Trust for their continued support and encouragement. We thank all the animal keepers to be the first responder in case of emergency/incident and especially for their tireless efforts and commitment to animals and our environment.

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CASE STUDY ON ECOLOGY AND ETHOLOGY OF TRANSPORTED SPOTTED DEER (*Axis axis*) AT NORTH BENGAL WILD ANIMALS PARK (BENGAL SAFARI), WEST BENGAL

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Abstract

The spotted deer *Axis axis* is India's most common, most visible and predominantly sighted deer in the country. It occurs across India (including Sikkim), Nepal, Bhutan, Bangladesh and Sri Lanka. Habitat use varies seasonally, reflecting food availability. The study was conducted in the North Bengal Wild Animals Park (Bengal Safari). The Park lies at the foothills of the Eastern Himalayas, geographically 28°47′27″ to 26°48′36″N and 88°25′58″ to 88°27′01″E, and covering an area of 297 hectares of Darjeeling district of West Bengal. This paper deals with spotted deer in captivity, herd movements, intra and inter mutual association factors enforcing changes in the hierarchy and breeding cycle.

Key Words Spotted deer, Stressors, Association, Herd composition, Feeding pattern

Introduction

Variation in behaviour in ungulate populations is an area of research, which could provide insights not only into the evolution of ungulate behaviour, but also more generally, into the evolution of the process in individual decision-making (Isvaran, 2005). One such behaviour is related to the ethology of spotted deer in captivity, particularly in the context of the dominance hierarchy, herd composition, herd movements, intra and inter mutual association, factors enforcing changes in the hierarchy (Geist, 1998), the breeding cycle and maintenance of healthy populations in captive conditions.

Distribution: The Spotted deer, Axis axis is India's most common, most visible and the predominantly sighted deer in the country. The Spotted deer occurs over 8–30°N in India (including Sikkim), Nepal, Bhutan, Bangladesh and Sri Lanka (Grubb, 2005).

Fig-1: Native range of Spotted Deer (http//www.ultimateangulate.com)

Habitat and Ecology: Spotted deer thrive in a variety of habitats (Mishra *et.al.,* 1987). Moist and dry deciduous forest areas, especially adjoining dry thorn scrub or grasslands, appear to be optimal. Habitat use varies seasonally, reflecting food availability.

Fig-2. Male Spotted Deer in the kraal

Reproduction and Social behaviour: Axis axis are gregarious by nature; the herd composition may vary from 20-30 to up to 100 (in rare cases) individuals. They have a gestation period of about 7.5 months. The number of fawns produced as well as the mating season may vary from deer in captivity; only one fawn is usually produced, twins are very rare (Ables, 1974). There are usually two major resting periods, before dawn and mid-day (Sankar and Acharya, 2004).

The males participate in a dominance based hierarchical system (Groves *et.al.,* 1982). There are four different aggressive displays among males: Head-down or scare threat, present threat, head-up, and antler threat. Females also partake in aggressive behaviour mostly at overcrowded female sites. Biting, striking, chasing are the commonly seen behaviours in females (de Silva *et.al.,* 1993). Spotted deer have several vocalizations besides the bellowing in mating season (Müller-Schwarze, 1982).

Our study examined the ecology and behaviour of transported captive Spotted Deer (*Axis axis*) during the quarantine period (about 3 weeks between the month of November-December 2016) in the kraal of North Bengal Wild Animals Park. During the study the changes in the feeding pattern, environmental enrichment, stress management post transportation, loading and unloading, weather and climate changes, adaptation towards new habitat was analysed. The social behaviour and association of the spotted deer with the existing fauna of the park was also studied. The study also examined the effect of auditory and visual disturbances on the behaviour of spotted deer. Other parameters such as herd composition, herd movements, food and feeding habits, the daily schedule of activities and reproductive behaviour was studied.

Study Area

The North Bengal Wild Animals Park (Bengal Safari) lies at the foothills of Eastern Himalayas, geographically 28°47′27″ to 26°48′36″N and 88°25′58″ to 88°27′01″E and covering an area of 297 hectares of Darjeeling district of West Bengal. This area extends between an elevation of 350-1500 msl with mixed and deciduous forest cover on the steep northern side, dominated by *Shorea robusta* and grassy alluvial plains which helps in existence of a large variety of flora and fauna (Annual Administrative Report, 2018). The soil cover is sandy loam and the water table is about 15-25m. Summer, monsoon and winter, three seasons are very distinct with distinct 36°C temperature in May–June to 5°C in January-February. Monsoon from early April to early May and annual average rainfall ranges from 2000 mm to 4000mm.

The study was conducted focused on one of the kraals where the deer were kept after transportation from the Hijli Eco Park, Kharagpur. The observations were made during the quarantine period. The total area of the kraal is 1 Ha, covered by chain link fence all around. The vegetation of this area comprised of *Tectona grandis, Shorea robusta, Terminalia belliricia, Litsea glutinosa, Sterculia vilosa, Lagerstomeia spaciosa, Gmelina arborea, Dalbergia stipulacea, Chromolena odorata, Axonopus compressus, Litsea*

monopetala, Clerodendrum infortunatum, Mikania micrantha, Abrus pulchelus, Stephania hernandifolia etc.

Methodology

For this study, the 'focal animal sampling technique' was used. A single individual or group composition was observed for a specified period of time and all the instances of different categories of its behavior were duly recorded (Martin & Bateson, 1993; Altmann, 1973). The technique of "direct observation" was also used (Tak & Lamba, 1984). Observations were made for a continuous period of 20 days, i.e., from 22.11.2016 to 11.12.2016. Observations were made on a daily basis for six days a week. Observation on diurnal rhythm of activities, movements, food and feeding, and behaviour, etc. were made on foot. The observation hours ranged from morning till dawn. Location and daily routes of movements of herd and sub-herd were noted down in writings.

Study of herd composition and H\herd movements – Sex and age classes were categorised as, adult males, adult females and fawns (Ramesh *et.al.,* 2010). Drive nets made of nylon with 10cm mesh size were set in triangular pattern (V-shaped) for capture for determining the sex. The herd movements were recorded from the Day-1 by videos.

Food and feeding – The study on food and feeding was carried out by collecting and visually observing each food plant inside the kraal area and visual observations were made on plants where an individual or herd was feeding. The food preference was determined on the basis of the number of instances on which the spotted deer, individuals or herd were observed to feed and also the length of time for which such feeding was continued. Records from the zookeepers also helped in studying the rate of feeding intensity and change in feeding pattern (de Silva *et.al.,* 1993). The was analyzed visually by selecting specific individuals from herd or sometimes herd as a whole. Tree cover was used as hideouts to analyze the feeding, bedding, resting and aggressive displays. Response to threat was also observed among the deer herd.

Association with the existing fauna - Association of newly arrived spotted deer with the existing fauna of the park was studied by introducing a male from the existing deer population of the park. The association of newly arrived individuals with the primates and other animals in and around the fence line was also studied. To support this study, photographs were taken by Nikon Coolpix P900 and calls were recorded using the same device.

Result and discussion

Observations recorded during the study revealed the following:

Herd composition and movement:- the herd comprised of 19 adult males, 32 adult females and 5 fawns. The herd composition was studied under the following heads:-

- Small aggregations
- Formation of sub herds
- Mother-fawn association
- -• All male association

From Day-1 to Day-3 of the introduction of the deer population in kraal, the herd movement was restricted and was mostly observed along the fence line. No formation of sub herds was observed and no small aggregation was visible during observation hours. Fawns were kept in hidings close to female deer, which made their sightings difficult during these days. From Day-3 onwards, dispersed movements in the herd were observed. Sub herds comprising 2 males and 3 females along with 1 or 2 fawns was observed in dispersed areas for some time and again they intermingled with the prime herd when confronted with any alarm or threat. On Day-6, free mobility among the fawns a

little distance away from their mother was observed. After 14 days, it was observed that the restricted herd movement was scarce and instead scattered aggregations were noted in sitting, resting, feeding positions.

The herd population was female biased, and the herd movement was mostly observed along the fence line and in this case the lead mostly taken up by the female deer and sometimes by the hind. The detachment of the mother- fawn was not observed till the day -6 as the fawns preferred a close association with the mother and afterwards the same association was noticed with little increased mobility of fawn. The composition of groups has been observed to change frequently during feeding periods, when males frequently join groups of females or while fleeing from human observer. Thus, the social groupings of spotted deer were not found to remain permanent.

Food and feeding pattern: The animals were fed Chick pea and wheat bran at Hijli Eco Park, Kharagpur. The feeding time at Hijli Eco Park was 5 PM. Hence an experiment was done to encourage their adaptation towards mixed feeding. On the Day-1, they were supplied with the same feed as they were provided in their earlier location, ie, chick pea and wheat bran.

From Day 2 the mixed feeding was tried by introducing natural fodder such as *Litsea monopetala, Litsea glutinosa, Streblus asper, Malotus nudiflora, Axonopus compresus, Terminalia myriocarpa, Terminalia belrica, Gmelina arborea, Dalbergia stipulacea, Chromolena odorata, Axonopus compressus, Litsea monopetala, Clerodendrum infortunatum, Mikania micrantha, Abrus pulchelus, Stephania hernandifolia* in their feed during their feeding time in the evening.

Fig-3. Herd composition of transported Deer in the kraal

Fig-4: Spotted deer foraging (green fodder) at daylight in kraal

On the Day-3 it was observed that the feeding intensity and feed preference towards the green leaves was very low. From the day-5 onwards, some of the individuals of the herd were observed feeding upon the green foliage supplied. At about 12 days, the feeding intensity and feed preference towards green fodder (*Litsea monopetala, Litsea glutinosa, Streblus asper, Axonopus compresus, Terminalia myriocarpa, Terminalia belrica, Malotus nudiflora, Gmelina arborea, Chromolena odorata, Axonopus compressus, Litsea monopetala, Mikania micrantha*,) was positive. At about three weeks, the development of mixed feeding habit among the herd members was noticed positively. Grazing pattern was also assessed, most of the grazing occurred at dawn, declining throughout the day and then increased strongly at dusk. In poor weather, grazing is reduced and sitting periods are increased at feeding times.

Pacing along fence-lines was more in poor weather.

Fawns were more active, investigating, and tasting possible food resources, including soil, grass, seeds and water, without swallowing. There was a change in the coat colour during the period of 1 to 15 days. This may be due to the introduction of mixed feed comprising of green foliage. The coat was darker, deep brownish in colour noticeably among stags at earlier stages which at later period become lighter in shade. From Day 8 onwards the deer were observed standing on their hind legs to approach the overhead branches. Deer were seen foraging at all hours of daylight; yet when mid-day hours were sunny and hot, they tended to seek tree shade and were not out in the open foraging on grasses. On cloudy days, the deer remained more visible. During the feeding time, Rhesus macaques were observed to visit the feeding location specially when the feed comprised of wheat bran and grams.

Defecation and urination were not often seen and did not occur at specific sites. Concentrations of pellets seemed to be related to time spent at a site; frequently-used loafing areas had more pellets than sites merely traversed by deer.

Mutualistic association with existing fauna of the park: A mutualistic association was observed between the spotted deer and the primates (*Rhesus macaques*). As their presence was positively welcomed by the deer within their home range. During the feeding time, the *Rhesus macaques* were observed to be feeding from the same containers in which the deer were feeding, the presence of *Rhesus macaques* was sometimes disturbing to the deer population as they raised their legs and wagged their tails in response to the defensive attacks of *Rhesus macaques*. The herd was at times observed following the Rhesus macaque that positioned itself on the fence bars. Here the *Rhesus macaque* was found taking the lead and the herd following it. The presence of a newly introduced spotted deer from the old stock was initially not welcomed by the herd from Day1 to Day 5. The isolation was visible. From Day 6 onwards the introduced male Spotted deer mixed with the herd and the association between females of the herd and the introduced male deer was observed.

Response to threat or Call: The Herd and sub herds also responded to various calls and appearances of the existing fauna of the park. The deer were found alarmed at the call of various birds, Sambar, Rhesus macaques and One Horned Rhinoceros in the nearby enclosure. In presence of human observer, they maintained a flight distance of approximately 50-75 m. When confronted with threat or danger of any kind, they stomped their forelegs and emitted a shrill bark while wagging their tails and displaying the white underside. If alarmed by the presence of human observer, the dispersed deer gathered to form the herd and remained in close proximity with each other and a frightened response was noted among the herd members and a sense of panic which made them run around the fence line together keeping the fawns in the middle. When Safari vehicles drove around the nearby area, the deer appeared alert only momentarily before returning to their previous activity. The stress in relation to the feeding pattern was also overcome by the introduction of mixed feed comprising green fodder, towards which a positive response was noted. Hence a significant improvement in the health condition of the deer was also observed.

Aggressive displays: Deer tend to become aggressive to each other in confinement (Pollard and Littlejohn, 1999). Confinement of social groups may be stressful, particularly for low ranking individuals (Pollard and Littlejohn, 1999). Confinement of males during transport might lead to aggressive behaviour, especially when space allowances are greater (Jago *et al.,* 1997). In this study Aggression

among males as well as females was noted. The various types of aggressive behaviour observed were: dashing, biting, nudging and kicking in the case of females, and striking, pawing, preaching, head-down display or threatening posture, actual combats and head-up display in males. Under normal conditions the female deer was observed to lead the herd but under condition of stress and strain, discovery of a stalking predator, the member first to notice the danger made a move, whether it was a female or a male. Under normal circumstances herds or sub herds stuck together, but under unusual conditions, like the presence of a human observer or availability of food, different individuals intermixed freely without showing any aggression.

Conclusion

During the case study the various aspects of ecology and ethology of transported spotted deer (*Axis axis*) were studied and analysed. The transported deer overcame stressors during the observation period and the introduction of mixed feeding in their daily regime proved beneficial and the deer adapted to it very well. Significant changes in coat colouration were observed due to the introduction of green fodder in their diet. Overall, the adaptation rate of the transported spotted deer in the new habitat of the North Bengal Wild Animals Park (Bengal Safari) was positive.

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A CASE STUDY ON KETAMINE-XYLAZINE ANAESTHESIA IN GREY LANGURS (*Semnopithecus entellus*) AT PADMAJA NAIDU HIMALAYAN ZOOLOGICAL PARK, DARJEELING.

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Abstract

The present study was attempted to standardize the dose of anaesthetics in Grey langurs (*Semnopithecus entellus*) at Padmaja Naidu Himalayan Zoological Park, Darjeeling. The animals were chemically immobilized using a ketamine-xylazine mixture and reversal Yohimbine hydrochloride was used, during the blood sample collection for routine clinical examination. Our study suggests that sex and age significantly affect the anaesthetic induction and duration in langurs. It was noted that the prescribed dose of ketamine (0.5-1 mg/kg) and xylazine (0.2mg/kg) was safe and effective in capturing male langurs, with a recovery time of approximately 33 minutes, which was sufficient to carry out small procedures. However, the same dose was insufficient for adult females, in which the anaesthetic effect lasted for less than five minutes. An increase in the dose of xylazine in adult females and the consideration of other biological and environmental factors such as size and body weight, species, pregnancy, disease conditions, and seasons, while the dose of during chemical immobilization of these primate species have been suggested by the authors.

Key Words Spotted deer, Stressors, Association, Herd composition, Feeding pattern

Introduction

Grey langurs (*Semnopithecus entellus*) are widely distributed non-human primates in India and are listed as least concern in IUCN red data book (Fooden, 1980 and Prater, 1993). Despite its distribution, there are limited reports of occurrence in Darjeeling hills. Padmaja Naidu Himalayan Zoological Park (PNHZP) presently houses eight healthy individuals in a troop breeding population of grey langurs on display. While in captivity, langurs require routine clinical check-ups and treatments, for which the animals may be chemically restrained. Reports on chemical immobilization of langurs are scarce. At present, the most commonly used chemical agents are ketamine, xylazine, and medetomidine.

Through the present study, we have reported the drugs and doses used during the chemical restraint process of captive grey langurs in the park for routine physical examination and blood sample collection. The study may be used as a reference by the zoo clinician while handling the same or closely related species.

Materials and methods

Eight apparently healthy grey langurs (six males and two females) were chemically immobilized for blood sample collection for routine clinical examination from February to April 2021. The animals were immobilised with a combination of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride

(100mg/ml), Yohimbine hydrochloride (10mg/ml) was used as a reversal agent. The drugs were delivered using a three ml syringe and blowpipes, intramuscularly. The preferable site for injection was gluteal muscles in the hindquarter. The animals were observed continuously after dosing and assessed for side effects such as ataxia, drowsiness, salivation, and excessive licking. The first sign of the effect of the drugs and the recovery time characterized by the first voluntary movement was recorded. The food and water were withheld for 12 hours prior to the immobilisation to prevent aspiration of the regurgitate and the animals were closely monitored during the entire procedure. Non-parametric Kruskal Wallis test for gender-related differences and co-relation analysis was carried out using SPSS version 16.

Results and discussion

The details of the treatments and the results of the present investigation have been given in Tables 1 and 2. The optimum recovery time recorded was 33.25 ± 12.77 (Standard error) minutes, which is sufficient for small procedures such as blood sample collection, dressing of the injuries, weighting the animals, etc. Throughout the study, the selected dose of ketamine and xylazine was between 0.3-1 and 0.2-0.3 mg/kg which is similar to the dose recommended for monkeys (ketamine 1ml+ xylazine 0.25ml) by Vayas, 2017.

In our study, it was observed that gender significantly affected the induction time of the drugs ($p=0.043 <0.05$) in addition to the recovery time ($p=0.044 <0.05$). Both the females selected in the study were less induced by the anaesthetics with only a mild effect that lasted for less than five minutes. Although this short anaesthetic duration was enough for the veterinarians to collect the blood samples, this duration is not suitable for long procedures. Contrary to this, the given dose effectively induced anaesthesia in males within three to seven minutes with a longer anaesthetic period or recovery time, ranging from 10-96 minutes (Table 1). A similar observation of a shorter anaesthetic period in females has been reported by Sontakke *et al.,* 2007 in Axis deer.

Besides this, a significantly strong negative correlation was noted between age and recovery time, with older animals exhibiting a shorter duration of anaesthesia compared to young adults. Further, the results of our study suggested that a higher dose of xylazine and a lower dose of ketamine is required to immobilize younger individuals. Our observation contradicts the opinion of Sontakke *et al.,* 2017 and Giroux *et al.,* 2015 who suggested that the younger animals require a higher dose than older animals, as the recovery time increased with age. The faster recovery time in adults over the age of eight years in our study may be due to the difference in biomass or the possible subcutaneous injection of the anaesthetics.

Besides this, a high positive correlation between xylazine and induction time whereas a low negative co-relation between xylazine and anaesthetic duration was noted. Ketamine showed a small positive correlation with induction and recovery time. However, in both cases, the correlation was non-significant. Kanu *et al.,* 2018 advocated that the ketamine-xylazine mixture was best suited for long procedures in Rhesus monkeys whereas xylazine alone could be used for short, minor procedures only. However, the doses used in their study were far greater than ours (ketamine: xylazine = 5mg/kg:1mg/kg).

Throughout the study period, the longest recovery time noted was 96 minutes in a healthy young male langur. This can be attributed to the fact that the reversal agent Yohimbine hydrochloride was not administered on completion of the desired procedure and the actual duration of anaesthesia

Table 1: Details of treatments and doses of Ketamine, Xylazine, and Reversal agent administered in captive grey langurs at PNHZP.

S.no	Age (Years/ months)	Sex	Ketamine (mg/kg)	Xylazine (mg/kg)	Induction time (In minutes)	Reversal agent (mg/kg)	Recovery time or duration of anesthesia (in minutes)	Remarks
1	2Y 2M	M	0.5	0.2	3	0.2	16	The animal did fine from the start till the head up
2	3Y 2M	M	0.3	0.2	4		96	The animal did fine but the recovery time was delayed
3	4Y 2M	M	0.5	0.2	5	0.2	63	The animal did fine from the start till the head up
4	3Y 11M	M	1	0.2	2	0.5	66	The animal did fine from the start till the head up
5	11Y 2M	M	0.3	0.2	5	0.2	7	The animal did fine from the start till the head up
6	8Y 2M	F	0.3	0.2	Mild effect		4	Mild effect.
7	9Y 4M	F	0.3	0.2	Mild effect		4	The mild effect may be to subcutaneous injection
8	below 5 years	M	0.5	0.3	7	0.5	10	The animal did fine from the start till the head up
MEAN ± SE			0.46 ± 0.08	0.21 ± 0.01	3.25 ± 0.88	0.32 ± 0.07	33.25 ± 12.77	

Table 2: Pearson correlation analysis on chemical immobilization of captive grey langurs at PNHZP

	age	xylazine	ketamine	effect	recovery time
age	1	0.38	-0.50	-0.11	-0.80**
xylazine		1	0.06	0.61	-0.26
ketamine			1	0.04	0.34
effect				1	0.19
recovery time					1

** Co-relation is significant at the 0.05 level (2-tailed)

without the interference of antagonists could be recorded. Antagonists help the animal to recover from anaesthesia by reversing the effect of xylazine (Sontakke *et al.,* 2017). Further, Kreeger *et al.,* 2002 suggested that a minimum of 30 minutes of anesthetic duration was required to metabolize ketamine residue, despite early administration of an antagonist.
Based on the observations of the case study reported here, administration of a combination of ketamine (0.5-1 mg/kg) and xylazine (0.2mg/kg) to male grey langurs induced safe and effective anaesthesia with sufficient recovery time for small procedures. However, similar doses in females (ketamine/xylazine= 0.3/0.2 mg/kg) were insufficient, although initial signs of anaesthesia were attainted within three to four minutes. But the animals rapidly gained consciousness and were alert to even the slightest disturbances. This can be avoided by increasing the dose of xylazine (Kanu *et al.,* 2018), an observation also noted in other animals like the Himalayan wolf in the park. Since our study was restricted to the captive population of PNHZP, the results might differ from other authors as anaesthesia depends on biological and environmental factors like size and body weight, species, pregnancy, disease conditions, and seasons (Sontakke *et al.,* 2017). Similar information from other zoos is required to generate intraspecific and interspecific comparative databases.

Acknowledgement

The authors are thankful to the director, PNHZP for granting permission to undertake the work, and the West Bengal Zoo Authority (WBZA) for providing financial aid. The support of Mr. Pradeep Kumar Singh, Veterinary compounder, Mr. Vikash Chhetri, Laboratory Assistant, and zookeepers are sincerely acknowledged. Special thanks to Dr. Barkha Subba, Zoo Biologist, PNHZP, for her encouraging words to the scholar.

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SUCCESSFUL BREEDING OF SOUTH AMERICAN COATI *Nasua nasua* (LINNAEUS, 1766) IN SARDAR PATEL ZOOLOGICAL PARK, KEVADIYA, GUJARAT, INDIA

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Introduction

The South American Coati (*Nasua nasua*) are medium-sized typically pale to dark olive-brown. Their colour may vary but from bright ginger to almost black. They have long, mobile snouts that protrude past their lower jaws with strongly curved claws for digging, short, rounded ears, and sharp canine teeth for ripping or slashing of flesh (Caras, 1967; Hunter, 2019). They are omnivorous, gregarious procyonids, common throughout most neotropical forests (Gompper and Decker, 1998; Schaller, 1983). They predominantly feed on invertebrates such as beetles, ants, millipedes, and arachnids, as well as fruits (Hunter, 2019). Adult males are solitary while females and immature males forage in groups (Schaller, 1983). They are mainly terrestrial and diurnal (Hunter, 2019). They are seasonal breeders; mating occurs in August to October and young ones are born in October and November in Iguazu National Park. The gestation period is about 65-77 days (Brown, 1936; Standley, 1992) with a litter size of 1-7 offspring (Ben Shaul, 1962; Findlay *et al.,* 1971; McToldridge, 1969) and a lifespan of 17.7 years in captivity (Hunter, 2019).

Nasua nasua is listed as Least Concern by the IUCN Red list database, but the current population trend is decreasing, and the major threat is hunting and habitat destruction (Emmons & Helgen, 2016). Currently, there are no reports of the breeding of South American Coati in zoos in India and we report the successful breeding from the Sardar Patel Zoological Park, Kevadiya in Gujarat, India.

Materials and methods

Sardar Patel Zoological Park acquired a pair of South American Coati and they were quarantined for one month in a room of 10x10x10 feet. The room had two wooden logs and a cage for roosting. Dry grass was used as a bedding material above the cage. Both the male and female used to roost and sleep on the cage. Daytime was spent feeding, grooming, and playing with wooden logs. In Gujarat, the average temperature ranges over from a minimum of 12°C in winter to a maximum of 42°C in summer and the average humidity ranges from 39% to 61%.

Breeding enclosure

The Coatis were kept in a glass-fronted enclosure with a breeding box of 1.5x1.5x1.5 feet, which was set at 2 feet above the ground with a ladder of 3 feet.

Captive diet

As Coatis are omnivores, and were provided with fruits such as Banana, Watermelon, Muskmelon, Papaya, Grapes and Apple, which makes up to 50-60% of its daily diet. In Addition, Me-o-cat dry cat food biscuits (10-13%), boiled chicken (20-25%) and boiled eggs (6-8) were given (Figure 1). To meet

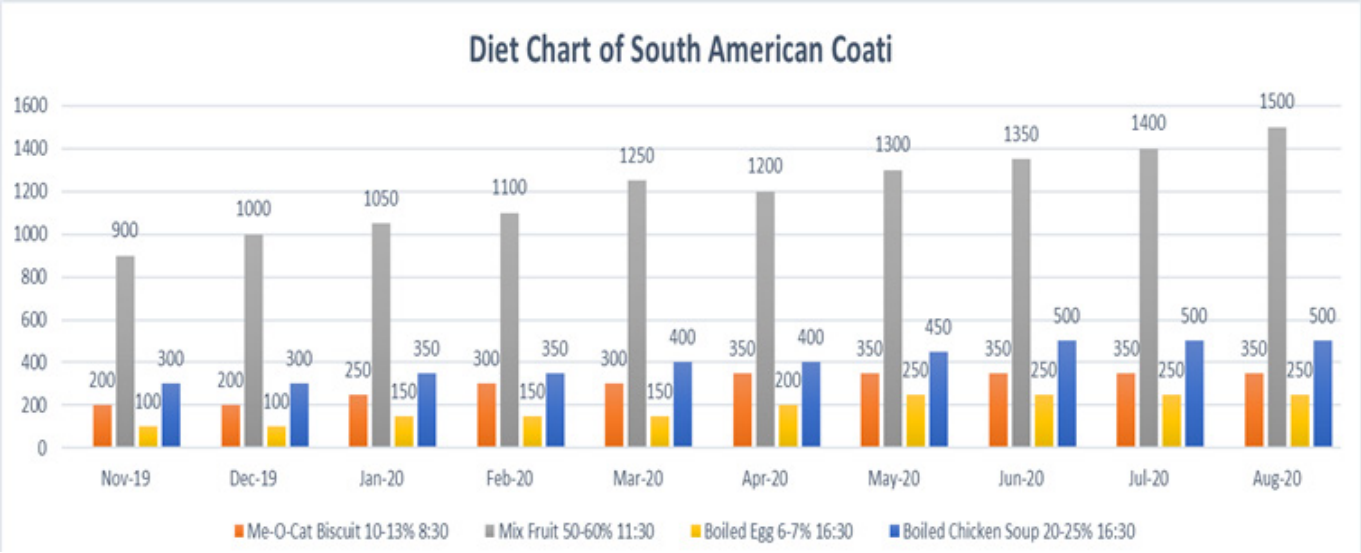

Figure 1. Monthly diet chart of South American Coati with their respected time and quantity.

the requirement of drinking water throughout the day, a cement tank of 2x1x0.5 feet with 20 litres of capacity was kept inside the enclosure. Twice a month, a wooden block of termites was provided to retain maintain their natural feeding habits.

Observation

On 17 December 2019, at 23:20 hrs unusual call was heard. On closer observation it was noted that the animals were mating (Figure 2). Mating occurred for about 25-30 min and on the next day for 50-60 min. The mating continues for three days for a period of 30 minutes in the day as well as at night. After that, both the coatis exhibited show normal behaviour.

On 21 January 2020, both the coatis were shifted to their main glass enclosure. On 25 January 2020, after 40-42 days from mating, the female started showing aggressive behaviour towards male and exhibited a voracious appetite. Moreover, the bodyweight of the female started increasing and that of males seems to decrease (Table 1). As in the wild the female coatis build the nest and give birth inside them, on 30 January 2020, dry grass as a bedding material in the nest box was provided. On 4 February 2020, an increase in the size of six nipples was noted in the female. As soon as the dry grass was provided, the female makes small chunks of it and arranged them inside the nest box. By 22 February 2020, the nest was ready. After a gestation period of 68 days, on 23 February 2020, between 03:00 to 06:00 hrs, the female gave birth to five young ones (Figure 3). The female exhibited parental care (Figure 4) and was aggressive towards others. The eyes of the young ones were closed during birth and it opened after seven days i.e., on 29 February 2020 (Figure 5).

Figure 2. Mating of South American Coati *Nasua nasua*. (Photo by Ravi Patel)

Figure 3. Young ones with closed eyes

Figure 4. Parental care and milk feeding

Figure 5. Young ones with open eyes after seven days. (Photos by Ravi Patel)

Figure 6. Young one feeding on fruits and eggs (Photo by Ravi Patel)

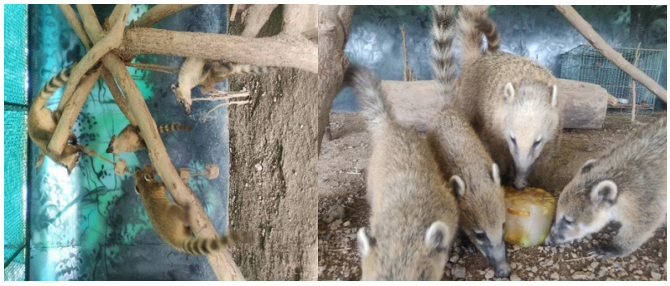

Figure 7. Physical and food based enrichment for young ones (Photos by Ravi Patel)

The weight of the young ones on the 10th day was between 250 to 260gm (Table 1). In the first one month, the female used to spend most of the time inside the box and was seen outside only while feeding and defecating. The male used to check the young while the female was outside the nest box. On the night of 22 March 2020, between 20:00 to 23:00 hrs, the two young ones were seen peeking outside the nest box for the first time. After that all the young ones tried to come out of the nest box and on 27 March 2020, all young ones successfully came out of the nest box but they sleep inside the nest box. In the second week of April 2020, most of the young ones were trying to feed on fruits, boiled chicken, and eggs (Figure 6). After two months, the enclosure was enriched with some wooden playing materials and frozen fruits block for the young ones (Figure 7).

Juvenile coatis were vaccinated against with canine adenovirus-2, canine distemper and parvovirus at 6, 9 and 12 weeks by using Canigen DHP (1ml/animal). They were also vaccinated against rabies at 16 weeks by using Rabigen Mono (1ml/animal). Both the vaccination was given through a subcutaneous route (AZA Small Carnivore TAG 2010). we semiannually examined for prevention of external and internal parasites of coatis as per the recommendation of the AZA Small Carnivore TAG (2010) was done. As suggested by Earle (1942), to promote body growth and prevention of nutritional diseases, coats were supplied with multi-mineral and multi-vitamin supplements in their food. Moreover, scraping of the top soil substrate was done quarterly; Kohrsolin Th 3% was sprayed once a week and burning was done once a month (Central Zoo Authority 2018).

Table 1. Variation in Body weight of Male, female and young-ones

Sr. No.	Date	Weight in Kilogram		
		Male	Female	Young ones
1	20/01/2020	6.3	5.9	
2	07/02/2020	5.9	6.1	
3	21/02/2020	5.4	6.5	
4	27/02/2020	5.5	5.7	0.25-0.26
5	07/03/2020	5.5	5.7	0.38-0.47
6	23/03/2020	6.1	5.7	0.51-0.7
7	07/04/2020	6.07	5.7	0.61-0.83
8	23/04/2020	6.08	6.11	0.89-1.17
9	07/05/2020	6.17	6.19	1.31-1.49
10	23/05/2020	6.27	6.3	1.63-1.81
11	07/06/2020	6.29	6.55	1.9-2.34
12	23/06/2020	6.49	6.72	2.019-2.71
13	07/07/2020	6.52	6.85	2.14-2.97
14	23/07/2020	6. 55	6.86	2.44-3.36

Carnivores are rare and their positions at the top of the food pyramid dictate that they are naturally far less common than the species on which they prey (Hunter 2019). Also, the species is hunted by local people for its meat and fur, which is a major threat to the species (Emmons & Helgen 2016). Thus, breeding of South American Coati in captivity is vital for the conservation of the species.

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MANAGEMENT & CONTROL OF NEWCASTLE DISEASE IN FREE RANGING PEAFOWL (*Pavo cristatus*) - A CASE STUDY

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Abstract

This article discusses the management of Newcastle Disease & vaccination in free-ranging Peafowl (*Pavo cristatus*). Peafowls in the rural areas of Rohat (Dist. Pali, Rajasthan) & adjoining area of Jodhpur district were reported to have neurological signs, loss of ability to fly, paralysis of wings, legs, neck muscles & death. Several affected birds were brought to the Wildlife Rescue & Rehabilitation Center, Jodhpur. Necropsies were conducted to diagnose the possible cause. One carcass was sent to RAJUVAS Bikaner, two were sent to NHISAD, Bhopal, two were sent to the Center for Wildlife, IVRI, Bareilly & visceral organs from two carcasses were sent to the Regional Forensic Science Laboratory, Jodhpur for the toxicology investigations. Necropsy done at IVRI declared the causative agent to be Newcastle Disease Virus. RT-PCR test confirmed the presence of ND Viral genome. Epidemiological surveys in the affected areas were conducted to plan strategies for vaccination against the NCD. After several rounds of vaccination, no birds were found to be have signs described above. Total 115 birds died in Rohat area of Pali, 176 birds were rescued and brought to the Wildlife Rescue & Rehabilitation center, Jodhpur out of which 97 birds could be saved and 55 birds were able to fly again, 11 such birds have been rehabilitated into their natural habitat, remaining birds having persistent neurological signs were not found fit for rehabilitation.

Introduction

Newcastle Disease (*Avian Pneumoencephalitis*) is primarily found in the domestic poultry i.e. domestic fowl (*Gallus gallus*), domestic ducks, Turkeys, Guinea fowl, domestic quails etc. The disease is occasionally reported in the free ranging migratory wild birds as well i.e. water fowl, wild pheasants, Cormorants, Psittacines, crows, pigeons etc. Causative agent of that disease is an RNA virus of Paramyxoviridae family. In domestic poultry the virus is found in three forms i.e. velogenic, mesogenic & lentogenic, which are classified on the basis of virulence of the strain. Newcastle disease in the wild birds is referred as non-poultry infection.

Materials and Methods

Clinical investigations:

First case was reported on 02.05. 2021 from Bhakri (25°58'19.10"N, 73°17'46.21"E) near Rohat of District Pali, Rajasthan. Forest department of district Pali sent two male & one female Indian Peafowl to the Wildlife Rescue & Rehabilitation Center, Jodhpur. Those birds exhibited neurological signs, respiratory distress & dehydration. Later more birds were sent

with similar signs. On 28.05.2021 the first case from Jodhpur district was reported from Bhatinda village (26° 2'27.64"N, 73°16'41.00"E). The Peacock was brought to the rescue center for treatment for a dog bite wound but also have similar signs. By 12.07. 2021, a total 176 affected birds, from Pali & Jodhpur, were presented at the rescue centre.

Epidemiological Surveys:
Considering the large number of affected birds, a survey was planned with DCF Pali and a locally appointed Veterinary officer. As per Regional Forest Officer, Rohat 115 Peafowl died during the entire outbreak.

On 28.05.2021the first case was reported in Jodhpur, and the veterinary officer of the rescue center conducted a survey along with rescue center & wildlife flying squad's rescue team in Village Bhatinda. This area was adjacent to the affected area of district Pali and was separated only by barbed wire fencing. 5 birds had died two days earlier and the villagers had buried the dead birds. Next day a survey was conducted in the Pabupura Bhatan & Mori Joshiyan. There were not affected birds in these areas. The estimated population of free ranging Peafowls was 2000 in affected area of Jodhpur.

Diagnosis of the disease:

Clinical Signs:
Common clinical signs seen in the sick birds at the rescue center were circling, torticollis, paralysis of wings, clonic spasms, tremors, and respiratory distress. A few birds also had green pasty diarrhea.

Post Mortem & Laboratory Findings:
The necropsies conducted initially did not reveal pathognomonic lesions of any specific diseases. Common lesions note was congestion in the trachea and lungs, faint petechial hemorrhages in the proventriculus, inflamed ceca and congestion of brain. Many bird carcasses were sent to different places (Table No 1) for the specific diagnosis. Two bird carcasses were sent to the Center for Wildlife at IVRI, Bareilly. After RT-PCR the presence of Newcastle Viral genome was confirmed. Further a toxicological investigation was conducted at IVRI, but no toxic content was found in the crop & gizzard or in the visceral organs. These results were corroborated as samples from two dead birds were also sent to the Regional Forensic Science Laboratory, Jodhpur for the toxicological investigation, those test results were also negative.

Management & Treatment of the sick birds:
There is no specific treatment available for the birds affected with Newcastle Disease Virus. All rescued birds were kept in the quarantine and treated symptomatically. All safety protocols were followed to prevent spread of the infection to the other birds of rescue center and the biological park. Only birds with mild symptoms recovered after implementation of the following treatment protocol.

1.Fluid therapy either parenteral or the oral
2.Multivitamin Suspensions: oral
    a.Suspension of Vitamin B Complex
    b.Suspension of Vitamin A, D, E.
    c.Suspension of Vitamin E with Selenium
3.Inj. Methycolbalamin (1200 mcg/ml) intra muscular
4.Drinking water pots were removed every night, cleaned & disinfected for the next day use.
5.Every day early in the morning a mixture of above-mentioned oral medicines were given to the birds with small piece of jaggery (approximately 100 g/ 3 litre water) or 100ml honey.
6.Feed include a mixture of multiple grains (barley, millet, maize, rice, wheat) and green fodder (spinach or lucerne)

7.A mixture of oral rehydration salt with dextrose was provided in drinking water during daytime.

Vaccination:

After confirmed diagnosis a mass vaccination program was implemented. Live lentogenic vaccine LaSota strain was used. Route of vaccination was either intra-ocular or with the drinking water.

Vaccination strategy & Plan

After several surveys it was decided to create several small points (Hotspots) for vaccination in affected areas where Peafowls foraged early in the morning. Every day before sunrise cold drinking water with the vaccine was provided along with the feed (grains). A 'Ring-Vaccination' plan was used. First day vaccination was done in the Bhatinda village & immunity booster medicine was given in other areas of adjacent to the Pali district. Five round of vaccination was done in the Bhatinda & four round of vaccination was done at other areas of Jodhpur. PPE kits were used and all safety protocols were followed to prevent cross infection. Every day proper cold chain was maintained during the transport of the vaccine. All other birds of rescue center and Machia Biological Park, Jodhpur were vaccinated. (Table 2)

Release and Post Vaccination Surveys:

After supportive and symptomatic treatment 97 birds were saved but only 55 were found to be able to fly. 11 out of 55 birds were rehabilitated. Several surveys conducted post vaccination and for post release monitoring, did not reveal any evidence of sick bird with those Last survey was conducted on 16.09.2021.

Table No. 1. Diagnostic Investigations

S. No.	Date	Place of Diagnosis	Tests done / Findings	Results
1.	11.05.2021	NHISAD, Bhopal	RT-PCR for Avian Influenza	Negative
2.	13.05.2021	College of Veterinary & Animal Science, Bikaner (RAJUVAS)	Necropsy - Petechial hemorrhages on the mucosa of crop, proventriculus and intestines	Any specific causative agent was not diagnosed due to purification of carcass.
3.	02.06.2021	Regional Forensic Science Laboratory, Jodhpur	Chemical Examination of portion of visceral organs for metallic poisons, cyanide, alkaloids, barbiturates, tranquilizers and pesticides	Negative
4	24.05.2021	Center for Wildlife Conservation, Management and Disease Surveillance, IVRI, Bareilly	Necropsy & RT-PCR of brain sample for NDV and cloacal swab for COVID-19	Positive for NDV genome Negative for Covid-19
5.	07.06.2021	Center for Wildlife Conservation, Management and Disease Surveillance, IVRI, Bareilly	Toxicological examination of crop, proventriculus, gizzard and intestine contents	Negative for heavy metals and commonly used pesticides.

Table No. 2 Ring Vaccination in Jodhpur

S. No.	Date	Name of Village	No. of Hotspot Created	No of rounds done in each area	Total Dose of LaSota Vaccine used
1.	29.05.2021 to 02.06.2021	Bhatinda (Ratna Ram ki Dhani)	8	5	1000
2.	30.05.2021 to 02.06.2021	Village Bhatinda	3	4	500
3.	31.05.2021 to 03.06.2021	Pabupura Bhatan	3	4	500
4.	31.05.2021 to 03.06.2021	Mori Joshiyan	4	4	1000
5.	31.05.2021 to s03.06.2021	Mori Sothran	5	4	1000

Results and discussion

After several rounds of vaccination in affected areas of Jodhpur no bird with clinical signs has been reported in affected areas. The intensive vaccination along with monitoring of the area for affected and symptomatic birds has ensured containment of the spread of the infection in wild peafowls.

Acknowledgements

Dr. S. Shivraj, Deputy Conservator of Forest, & Wildlife Rescue Team - Pali, Rajasthan., Dr. Vijay Borana, Deputy Conservator of Forest Wildlife – Jodhpur, Rajasthan., Wildlife Flying Squad & Rescue Team – Forest Department Jodhpur. Additional Director, Animal Husbandry Range – Jodhpur. Dr. Karikalan Mathesh, Veterinary Scientist, Center for Wildlife Conservation, Management and Disease Surveillance, IVRI, Bareilly, Uttar Pradesh. Dr. Baldev Singh, Veterinary officer, Sanwalta Khurd, (Dept. of Animal Husbandry - Pali), Joint Director Animal Husbandry Pali, Rajasthan., Deputy Director, Regional Disease Diagnostic Center (Animal Husbandry dept.) Jodhpur.

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MANAGEMENT AND BREEDING HISTORY OF NILGIRI LANGUR (*Trachypithecus johnii*) AT ARIGNAR ANNA ZOOLOGICAL PARK, CHENNAI

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Introduction

The Nilgiri langur (*Trachypithecus johnii*) is an Old-World monkey found in the Nilgiri hills of the Western ghats in Southern India. Its range also includes Kodagu in Karnataka, Kodayar hills in Tamil Nadu, and many other hilly areas in Kerala and Tamil Nadu. This primate has glossy black fur on its body and golden-brown fur on its head. It is similar in size and has a long tail like the common langur. Females have a white patch of fur on the inside of the thigh. They typically live-in troops of nine to ten (Singh *et al.,* 2021). The animal is often seen encroaching into agricultural lands. Its diet consists of fruits, shoots and leaves. The species is endangered due to deforestation and poaching for its fur and flesh, the latter believed to have aphrodisiac properties (Malviya *et al.,* 2011).

The species has been listed under Appendix II of CITES. They are also protected under the Schedule I, Part I of Indian Wild life (Protection) Act, 1972 and are listed as Vulnerable C2a (i) under IUCN Red data list. The key to conservation of this species lies in reducing poaching, protecting their habitat and education. The Arignar Anna Zoological Park (AAZP) has been selected by Central Zoo Authority, New Delhi as a co-ordinating zoo for conservation breeding of this endangered species. The Nilgiri langur is endemic to Western Ghats and three tiger reserves having Nilgiri langur are in the state. There is ample scope for reintroduction of Nilgiri langur into their wild habitats. Hence a dedicated recovery plan is required.

The zoo has been maintaining Nilgiri langur in a wet moated enclosure since its inception. The zoo had acquired one male and one female from the Anaimalai wildlife sanctuary and two females from Kalakad wildlife sanctuary of Western Ghats in the1980s and 1990s respectively. The first birth was on 16th July 1991. Since then, the species has been breeding regularly in the zoo (Manimozhi and Kalyanasundaram, 1992). This paper is aimed to share experiences in managing and breeding this unique species in captivity.

Population

The Arignar Anna Zoological Park (AAZP) initially brought in rescued animals from Anaimalai Wildlife Sanctuary and has a record of maintaining this species since 11th June 1982. The first breeding was noticed in the year 1991 with of one male and three females as the founders. So far 45 (26M:17F:2U) births have been recorded. Currently the AAZP is housing a total of 20 animals of which there are eleven males, seven females and two unsexed (11M:07F:02U) animals in one enclosure (Table 1). A total of 24 rescued and captive born animals (11M:13F) have been used for animal exchange programme.

Animal Housing

The Nilgiri langurs are exhibited in two near natural wet moated enclosures. The first enclosure has a total circumference of 196 meters. On one side of the enclosure a cave model animal house is constructed with 3 retiring and feeding cubicles

Table 1. Details of Nilgiri langurs housed in Arignar Anna Zoological Park, Chennai (as on 06.10.2021)

S. No	Local zoo identification house name	Transponder No./ ARKS ID No	Date of Birth/ approximate age at entry if wild	Sex M/F	Parentage		Source of animal captive bred/wild/ Rescued	events trans-fer/ Birth/ Death	Current status
					Sire/Father	Dame/ Mother			
1.	AAZP25	6233265/100199	04.01.2003	F	Kannan	Kavitha	Captive bred	Birth	Living
2.	AAZP34	6233288/100207	3 years old	M	UNK	UNK	Rescued wild on 18.07.2009	Transfer	Living
3.	AAZP39	100355/6233262	25.04.2012	F	UNK	UNK	Captive bred	Birth	Living
4.	AAZP41	6233284/100523	31.03.2014	F	UNK	UNK	Captive bred	Birth	Living
5.	AAZP42	6233281/100524	18.04.2014	F	UNK	UNK	Captive bred	Birth	Living
6.	AAZP43	/6233263/100525	23.04.2014	M	UNK	UNK	Captive bred	Birth	Living
7.	AAZP44	6233282/ 100559	12.04.2015	M	UNK	UNK	Captive bred	Birth	Living
8.	AAZP45	6233287/ 100560	29.05.2015	M	UNK	UNK	Captive bred	Birth	Living
9.	AAZP46	6233280/ 100561	03.06.2015	M	UNK	UNK	Captive bred	Birth	Living
10.	AAZP47	6233266 100590	18.11.2015	M	UNK	UNK	Captive bred	Birth	Living
11.	AAZP48	6233261/ 100607	05.07.2016	M	UNK	UNK	Captive bred	Birth	Living
12.	AAZP49	6233260/ 100613	15.12.2016	M	UNK	UNK	Captive bred	Birth	Living
13.	AAZP50	6233268/ 100627	28.04.2017	M	UNK	UNK	Captive bred	Birth	Living
14.	AAZP51	6233283/ 100628	13.06.2017	M	UNK	UNK	Captive bred	Birth	Living
15.	AAZP52	6233267/ 100695	02.04.2018	M	UNK	UNK	Captive bred	Birth	Living
16.	AAZP53	6233269/ 100669	05.05.2018	F	UNK	UNK	Captive bred	Birth	Living
17	AAZP54	6233264/ 100696	18.01.2019	F	UNK	UNK	Captive bred	Birth	Living
18	AAZP55	6233286/ 100697	18.05.2019	F	UNK	UNK	Captive bred	Birth	Living
19	AAZP56		11.08.2020	U	UNK	UNK	Captive bred	Birth	Living
20	AAZP57		21.02.2021	U	UNK	UNK	Captive bred	Birth	Living

measuring 2.20x2.50x1.80 meters. It houses 4 males, 4 females, and 2 unsexed young langurs. The second enclosure has a circumference of 115 meters with three feeding and retiring cubicles measuring 3.30x3.30x2.50 meters. This houses I male, 4 females and 2 unsexed infants. Both the enclosures have natural vegetation like *Azadirachta indica*, *Pithecellobium dulce* and other herbs and shrubs. Both enclosures have predominantly *Pithecellobium dulce* trees. *Pithecellobium dulce* is a species of flowering plant in the pea family, Fabaceae, that is native to the Pacific Coast and adjacent highlands of Mexico, Central America and northern South America. It is an introduced species and extensively naturalized in the Caribbean, Florida, Guam as well as in India and Philippines. The tree is drought resistant and can survive in dry lands from sea level to an elevation of 1,500 m (4,900ft), making it suitable for cultivation as a street tree. This species provides climbing, perching and natural feeding other than the zoo diet. The seed pods contain a sweet and sour pulp which is eaten raw. The seeds are also edible and contain 28% protein.

Zoo diet chart

The diet chart for the species was prepared after consulting various national and international captive animal diet charts of primates, especially Nilgiri langur. The daily ration for individual Nilgiri langur is as follows: 1) Rice - 30grams, 2) Ground nut (without shell)- 15 grams, 3) Banana – 4 nos., 4) Guava – 1no., 5) Bengal gram – 15grams, 6) Cabbage – 30 grams, 7) Greens – 100 grams, 8) Bread slices – 3 nos., 9) Soya bean– 20 grams, 10) Carrot – 25 grams, 11) Grapes – 20 grams, 12) Honey – 10 ml, 13) Sathugudi /Orange – 1no., 14) Boiled egg – 1no.

The rice and soya bean are cooked and given to animals. The Bengal gram is soaked overnight and offered to animals along with other food stuff daily. The water-melon and cucumber are also added as summer special (April to June).

Feeding:

All food items are checked in the store by the veterinarians and concerned Range Officers for quality and quantity. According to the prescribed diet chart, food items are weighed, stored and segregated in separate bins and distributed to the respective enclosures. The animals are fed once daily between 11:00 am to 12 noon. The animals are fed in groups and in isolation to sick langurs.

Animal hygiene and health:

Every day, all the left-over food is removed, and the animal house is thoroughly cleaned with fresh water. Finally turmeric powder is applied on the floor as an antibacterial agent. Similarly, the yard (enclosure) is also cleaned daily of any unwanted material. Deworming and health tonics are given to animals as per the health status of animals. Deworming is done every six months. The keepers are also checked for any contagious disease through a common health camp once or twice in a year. The water moat and enclosure are cleaned regularly.

Environmental enrichment programme:

Enrichment is a concept which describes how the environment of captive animals can be changed for the benefit of the inhabitants. Behavioural opportunities that may arise or increase as a result of environmental enrichment can be appropriately described as behavioural enrichment. Alternatively, environmental enrichment is 'a process for improving or enhancing zoo animal environment and care within the context of their inhabitants' behavioural biology and natural history. It is a dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioural choices to animals and drawing out their species appropriate behaviours and abilities, thus enhancing animal welfare. Keeping the above concept in mind, AAZP provides swings, ropes, hammocks, perches, plot forms, basket feeding etc.

Breeding:

The AAZP is one of the captive breeding centres in India. The zoo thus far has recorded 45 (26M:17F:2U) young ones (Table 2). The maximum percentage of males are (57%77), the females (37.77%) and unsexed were 4.25%.

Breeding season:

The birth seasons of mammals in AAZP have been studied by Manimozhi *et al.,* 2006. Lack of parental care in Nilgiri langur was reported by Manimozhi and Kalyanasundaram, 1992 in Arignar Anna Zoological Park. The 30 years long record of breeding revealed minimum one and maximum nine young per month except during the month of October. The maximum birth of 9 (20.00%) individuals occurred in the month of July and 7 (15.55%) in the month of April respectively, followed by 6 (13.33%) births in the month of March. In the month of May and December

Table 2. Natality of Nilgiri langur in Arignar Anna Zoological Park, Chennai (1991-2021)

Sl. No.	Date of Birth	Sex	Sl. No.	Date of Birth	Sex
1.	16.07.1991	F	23.	05.02.2009	F
2.	06.07.1993	M	24.	18.07.2009	M
3.	22.12.1993	M	25.	25.05.2011	M
4.	26.12.1994	M	26.	10.06.2011	M
5.	03.09.1995	M	27.	16.02.2012	F
6.	01.06.1995	F	28.	25.04.2012	M
7.	17.05.1996	M	29.	31.03.2014	F
8.	15.07.1996	F	30.	18.04.2014	F
9.	15.07.1996	F	31.	23.04.2014	M
10.	21.11.1997	M	32.	12.04.2015	M
11.	13.03.1998	F	33.	29.05.2015	M
12.	31.03.2000	M	34.	13.06.2015	M
13.	02.03.2002	M	35.	18.11.2015	M
14.	31.07.2002	M	36.	08.07.2016	M
15.	12.12.2002	F	37.	15.12.2016	M
16.	04.01.2003	F	38.	28.04.2017	M
17.	11.02.2004	M	39.	13.06.2017	M
18.	11.03.2004	F	40.	02.04.2018	M
19.	13.07.2004	M	41.	105.05.2018	F
20.	10.03.2007	F	42.	18.01.2019	F
21.	05.04.2007	F	43.	18.05.2019	F
22.	25.12.2008	M	44.	11.08.2020	U
			45.	21.02.2021	U

M-male, F-female, U-unsexed

5 (11.11%) births were recorded. In the month of June 3(6.67%) births were noticed (Table 3). During the month of January and November two (4.44%) births were recorded respectively. In the months of August and September single births were recorded.

Mortality:

The zoo has recorded 21 deaths from 1990 to 2021 (Table 4), of which 15 are males and 6 females. Higher mortality was recorded in males (71.43%) than females (28.57%). Among 21 deaths 4 rescued animals died due to infighting while mixing with resident animals. 3 other animals also died of infighting (migratory male killed). Three deaths of young ones were due to trauma. The other causes of death were shock, impaction, cystic pneumonia and hepatitis, but senility was observed in a single case.

Table 4. Mortality of Nilgiri langur in AAZP, Chennai (1990-2014).

Sl. No.	Date of Death	Sex	Cause of death
1.	06.02.1990	M	Shock
2.	13.01.1991	F	----
3.	07.05.1994	M	Trauma
4.	12.06.1994	M	----
5.	24.08.1995	M	Trauma
6.	16.10.1995	M	Trauma
7.	20.05.1998	M	__
8.	25.05.1998	M	Shock
9.	12.04.2000	M	Impaction
10.	26.05.2001	F	__
11.	15.03.2005	F	Shock
12.	25.05.2005	F	Hepatitis
13.	16.07.2005	M	Senility
14.	05.03.2009	M	Hepatitis
15.	25.07.2009	M	Haemorrhagic shock
16.	25.06.2010	M	Septicaemia
17.	02.05.2012	M	Infighting
18.	15.08.2012	F	Infighting
19.	10.08.2013	M	Infighting
20	16.09.2016	M	Pneumonia (infant)
21	02.10.2017	F	Cystic (Uterus)

Migration within zoo:

During oestrous period of female, male – male aggression was unavoidable if mature males are more in the troop. In these circumstances the dominant male chased away the subordinate male out of enclosure. This was noticed by the animal keeper the next day and the animal was captured and kept in isolation for few weeks before mixing with other Nilgiri langurs. During 2012, a male was chased away/ migrated from first enclosure and entered into the second enclosure which is 100 meters away from the first enclosure and killed a dominant male and two

Table 3. Month wise birth of Nilgiri langur at AAZP

Months	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
Total Births	2	3	6	7	5	4	9	1	1	0	2	5

infants. Another female infant was heavily injured and could be saved due to proper treatment.

Infusion of new genes:

New gene infusion into the captive population of any species serves an integral part to minimize the inbreeding coefficient. Several attempts to introduce a new adult male into the existing population of AAZP have failed. A sub adult male of about 3-year-old rescued from Mudumalai wildlife sanctuary on 18.07.2009 slowly was introduced into the second enclosure of AAZP. He was kept in the cell of the second enclosure for a period of a month and introduced into a group of one male and three females. The group immediately accepted him and now he has sired two infants. The existing dominant male was killed by an intruder male from the first enclosure in the year 2012.

Nilgiri langur status in Indian Zoos (2019-2020):

As per the inventory of Nilgiri langur (2019-2020) a maximum of 74.07% of animals are found in AAZP followed by Sri Chamarajendra Zoological Garden, Mysuru and Trivandrum Zoo 11.11% respectively. The Nehru Zoological Park, Hyderabad has a population of 3.70% (Table 5).

Acknowledgements

I thank the then Additional Principal Chief Conservator of Forests and Director, Thiru. Jana, I.F.S., and Deputy Director and Thirumathi Sudha Ramen, I.F.S of Arignar Anna Zoological Park AAZP), Vandalur for their constant encouragement towards zoo animal research. I also thank Thirumathi V. Karunapriya, I.F.S., the present Additional Principal Chief Conservator of Forests and Director, and Thirumathi R.Kanchana, I.F.S., Deputy Director(A/C), AAZP, Vandalur for their encouragement.

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Table 5. Status of Nilgiri langur in Indian zoos (2019-2020)

Sl. No.	Zoo name	Male	Female	Unsexed	Total
1.	Nehru Zoological Park, Hyderabad	1	0	0	1
2.	Thiruvananthapuram Zoo, Kerala	1	2	0	3
3.	Arignar Anna Zoological Park, Vandalur, Tamil Nadu	11	7	2	20
4.	Sri Chamarajendra Zoological Garden, Mysuru, Karnataka	1	2	0	3
	Total	14	11	2	27

FIBROSARCOMA IN A LEUCISTIC ROYAL BENGAL TIGER (*Panthera tigris ssp. tigris*)

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Abstract

A 17-year-old female Royal Bengal Tiger (White) at Rajiv Gandhi Zoological Park and Wildlife Research Center having a complaint of partial loss of vision was examined for ocular ailment. On clinical examination, a growth was noted on the lower left eyelid, measuring 4 cm long, 2 cm wide and having conical shape at base. The growth increased in size in a couple of months affecting the vision. It was decided to surgically remove the growth. Animal was sedated using Xylazine and Ketamine combination. The entire tissue growth was removed from the base and was histopathologically confirmed as Fibrosarcoma.

Key Words Fibrosarcoma, Royal Bengal Tigress (White), Eyelids

Introduction

Fibrosarcoma is a common tumour in the cat (Morris and Dobson, 2001). Tumors of the eyes and adnexal structures are important in cats and are reported to impair the patient's vision, quality of life and survival. Chakaravarthy *et.al.,* 1991 stated that Fibrosarcoma is a malignant tumour which arises from fibrous connective tissue. A 17-year-old female Royal Bengal Tiger (White) weighing around 110 kg at Rajiv Gandhi Zoological Park and Research Center, Katraj, Pune presented with partial loss of vision. On ocular examination, a growth was noted on the lower left eyelid, and it measured about 4 cm long, 2 cm wide and had a conical shape at base.

Materials and Methods

Clinical examination of the animal on 19th April 2016, revealed a growth on the lower left eyelid, which had increased in size in a couple of months (Fig 1). Animal was sedated on 22nd June 2016 with xylazine (Inj. Ilium Xylazil -100 1) 180 mg and ketamine (Inj.Ketamil 2) 360 mg by intramuscular route. Complete immobilization was achieved after 15 minutes. Atropine sulphate (Inj. Blistropin 3) 1.5 mg and Dexamethasone (Inj.Alkodex 4) 10 mg were used as preoperative medications. Surgery was performed aseptically to remove the growth from lower left eyelid. The mass was removed from the base along with some healthy tissue. The incised part was cauterized with a crystal of Potassium permanganate. Affected part of the left eye was cleaned with Normal Saline (Fig. 2) and eye drops Gatifloxacin 3% (Zymer Eye drop 5) 4 drops b.i.d. were applied for five consecutive days. To avoid secondary bacterial infections, parental antibiotic Cefotaxime (Inj.Taxim

Fig 1. Growth on lower eye lid.

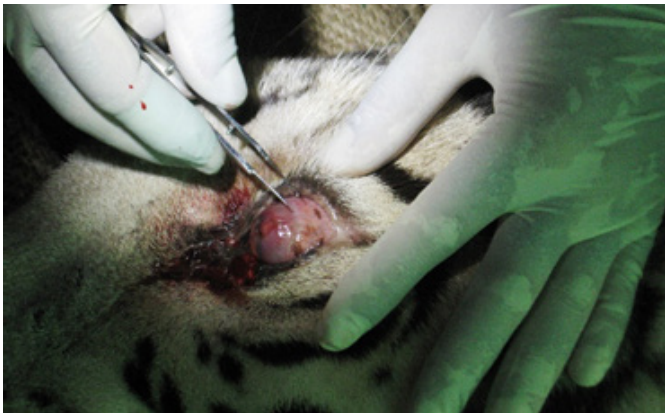

Fig 2. After removing of growth

6) 2.5 gm was given for 3 consecutive days. The complete resection of the growth was completed in about one and half hours. The animal recovered after two hours.

2 cm x 1 cm piece of the incised mass was fixed in 10 % formalin on the same day of operative procedure was further processed at Veterinary Pathology Department, K.N.P. College of Veterinary Science, Shirwal for routine histopathological examination using automatic tissue processors with alcohol-xylene protocol. The prepared paraffin tissue block was sectioned on automated tissue microtome at 5 µ size and the tissue sections were stained with hematoxylene and eosin protocol.

Results and Discussion

The mass was histopathologically confirmed as Fibrosarcoma (Fig 3 and 4). The microscopic

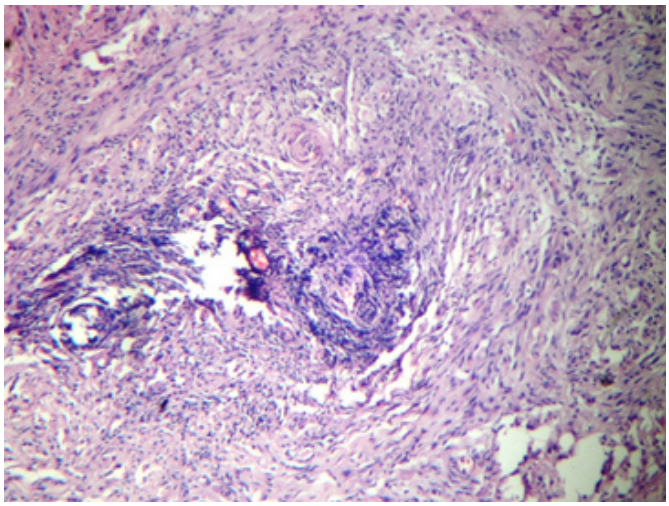

Fig 3. Spindle shape tumor cells arranged in interwoven pattern with elongated to oval shaped nuclei with hyperchromicity.

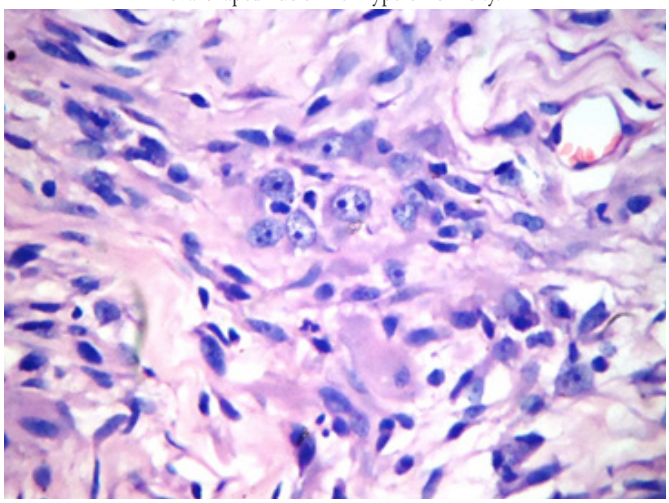

Fig 4. Atypical neoplastic fibroblasts with variable shaped nucleus and mitotic figures are present within a solid area of the neoplasm.

examination of tissue mass revealed solid appearance consisting of proliferated fibrous connective tissue (and fibroblasts) throughout the section. The epidermis was intact with focal inflammatory and ulcerative changes with presence of polymorphonuclear cells and neutrophils with RBCs. The spindle shaped cells in interwoven pattern with dense packing (Fig 3), moreover there were multiple areas having loosely spaced spindle shaped tumor cells separated by eosinophilic collagenous matrix throughout the section. The dense fibrous connective tissue (FCT) appeared elongated and arranged in typical groups/whirls. The fibroblastic stromal cells showed elongated nuclei, with little cytoplasm and indistinct cell margins. Few areas of fibrous tissue showed karyomegaly, enlarged nuclei with few mitotic

changes suggestive of malignancy. Typical whorl like pattern of fibrous tissue was noted focally in the tissue, however, with presence of pleomorphic and elongated fibrocytes and fibroblasts (Fig 4) suggested cancerous nature. Few fibroblasts showed large sized nuclei with mitotic changes and nuclear basophilia. The mitotic index ranged from 0 to 4 per high power field. Cellular pleomorphism with enlarged nucleus of few cells was observed suggestive of malignancy of initial stage. Focal areas of degenerative and necrosis were evident at the periphery of tissue along with hemorrhages.

The cellular features of the growth of biopsy tissue showed proliferative changes of fibrous connective tissue with abundance of collagen deposits. The histologic findings were supportive for fibrosarcoma. Based on histological characteristics, the tumor was diagnosed to be fibrosarcoma which was in agreement with the Goldschmidt and Shofer (1992), Doddy *et al.,* (1996) and Gross *et al.,* (2005).

Squamous cell carcinoma of eyelid in a white tiger at Van Vihar National Park, Bhopal (Gupta *et al.,* 2013), Epidemoid carcinoma of eyelid of a tiger at Nandankanan Zoological Park, Bhubaneswar (Bose *et al.,* 2002) and Carcinoma of sweat gland of lower eyelid of a tigress at Alipore zoo, Calcutta (now Kolkata) (Basak *et al.,* 1976) have been reported earlier from Indian zoos.

Conclusion

Incised growth on the lower left eyelid of the female white tiger was diagnosed as fibrosarcoma.

Acknowledgments

Authors are thankful to Dr. Phiroz Khambatta, Equine Practitioner for help during surgery.

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C- SECTION OF A TRANSVAAL LIONESS - A CASE STUDY AT TATA STEEL ZOOLOGICAL PARK, JAMSHEDPUR

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Abstract

A 3-year-old South African lioness in the terminal stage of pregnancy at Tata Steel Zoological Park, Jamshedpur, Jharkhand, India, was subjected to cesarean section, when the animal failed to deliver a foetus normally. This paper describes surgical procedures including anesthetic protocol carried out to remove dead fetuses followed by successful recovery of the lioness.

Key Words Dystocia, C Section, Pelvic outlet

Introduction

Tata Steel Zoological Park was successful in importing five lion cubs (two males and three females) of 6 months age from National Zoological Gardens at South Africa, Pretoria on 20th June 2012. The lion (*Panthera leo*) is a member of the family Felidae and one of four big cats in the genus Panthera. The lifespan of a lion is approximately 10-14 years in the wild, while in captivity they can live up to 20 years or more (Simuts, 1982). Lions are seasonally polyestrous, with heat lasting 4-6 days. Average litter size is 3-4. Gestation period lasts from 100 to 119 days and weight of the newborn is about 1300 gm. Lions become sexually mature at 3-4 years. Surgical intervention is required in approximately 60-80% of dystocia cases in female dogs and cats (Gilson, 2003). Timely and appropriate intervention in cases of dystocia, either medical or surgical, are crucial for both maternal and foetal survival. Determination of surgical necessity is based primarily on the condition of the dam, progression of labor, and foetal heart rate. This paper described the cesarean section, anesthetic procedure and preoperative management for the lioness with dystocia.

Surgical procedure and discussion

A 3-year-old, African lioness (*Panthera leo crugeri*) in the terminal stage of pregnancy showed signs of inappetence, restlessness, frequent straining, reddish mucus discharge from vagina, straining and cyclic abdominal pain.

Attempts were made to assist sufficient cervical opening so that foetus could be pushed out by the mother while straining. Injection Epidocine (Valenthamate bromide 10 mg/ml) 2 ampoule I/M was given at 11.00 pm on 08.12.14 followed by Inj. Oxytocin – 2 amp I/M after 10 minutes of first injection.

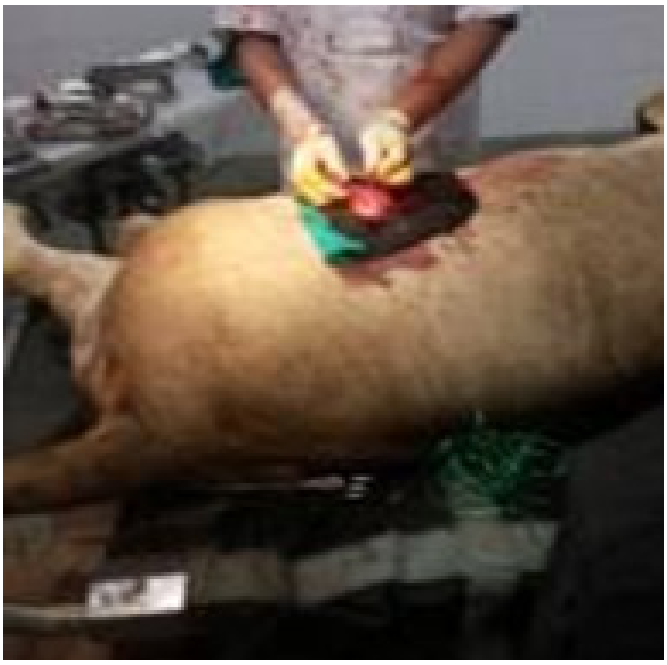
The next day (9.12.14) at 10.00 am 2nd dose of both injections were administered, but no progress was noticed and hence Inj. Xylazine Hcl 2.00 ml + Ketamine Hcl 2.00 ml (100 mg/ml concentration, each) given I/M by darting. Animal underwent sedation smoothly after12 minutes of darting and was shifted to Zoo Vet Hospital for proper examinations and further treatment. On per vaginal examination an oversize of foetal head was palpated near the

pelvic opening. (Picture-1). The foetus was dead in the canal and here was no space for manipulation Only a portion of torn tissue of foetus came out when attempts at extraction were made. It was thus decided to undertake a Cesarean section at 1.30pm on 09.12.14.

Further, to perform surgery and to achieve desired level of sedation additional dose of Xylazine HCl and Ketamine HCl 1.00 ml each were given along with normal saline through slow I/V.

The site was prepared on the left flank parallel to the mid ventral line about 15 cm apart. After skin incision, the layers of muscles and fascia were incised, and the gravid horn of the uterus was exposed (Picture-6). An incision was made on the body of the uterus and the first foetus was removed. The 2nd foetus was inside the pelvis area and away from the surgical opening. (Picture-2). The uterus was first sutured with catgut (Size-1) (Picture-7), Nebasulf powder (Sulfacetamide + Neomycin + Bacitracin, of Pfizer limited) was sprinkled over the suture site. The peritoneum and muscles were sutured separately with catgut (size 2), skin was sutured with silk. Broad spectrum antibiotics Inj. Ceflactum (Ceftriaxone + Sulbactam, of Concept pharma) - 3.00 gm (2.00 gm I/V, 1.00 gm I/M), anti-inflammatory and analgesic formulation, Injection Magludyne (Flunixine maglumine, of Virbac Lab)- 20 ml I/M, Inj. Tetvac (tetanus toxoid) - 2 amp I/M, Inj. Dexona (Dexamethasone) – 2.00 ml I/M, Inj. Neomec LA (Ivermectin long acting of Intas Pharma) 3 .00 ml S/C were administered.

Animal was shifted to a squeeze cage and then revived using Yohimbine HCl – 2.00 ml I/V. Animal responded, raised head, and stood up after 10 minutes. The surgery took about 3.00 hours. Animal was drowsy till 9.00 pm, and started licking milk at

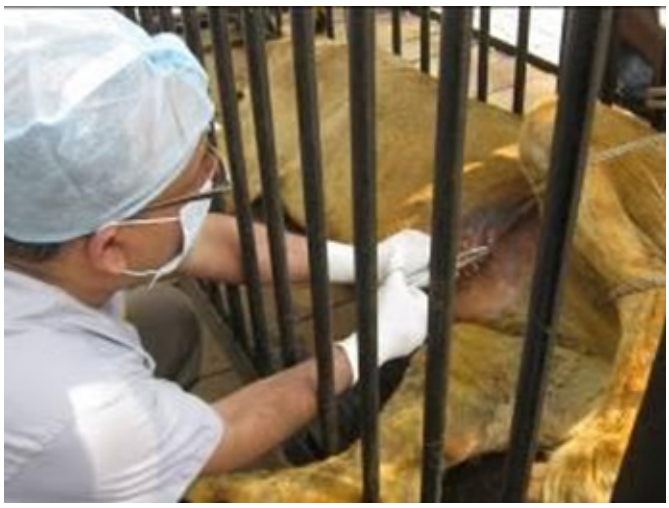


9.15 pm.

On the next day, the patient appeared normal, consumed warm water and milk, and one kg boneless meat. Antibiotic and other supporting medicines were continued along with Serratiopeptidase tab.
On 16.12.14, the lioness removed 80% sutures by

self-mutilation and the abdominal muscle layers were visible. Animal was immobilized by Xylazine and Ketamine Hcl mixture 2.00 ml each, the wound was re-sutured. This time additional support was provided over the site by putting Stainless steel staples and Nylon thread to close the skin (Picture-8).

Inj. Vetum forte (Cefoperazone and Sulbactum) 3.00 gm daily for five days, Inj. Tikola (Isofluperidone Acetate 2 mg /ml) 2.5 ml I/M alt day (tapering dose) for 3 injections. External application of Hydrogen peroxide solution (diluted with water), Betadine solution and Bactroban ointment (Mupirocine) were applied on a regular basis as a follow up therapy. This process proved useful, and sutures were retained for a long time and the wound healed in 15 days (Picture-5). On 02.02.15, the wound had healed completely, and it was decided to the animal back to the enclosure. To remove the different suture material, the animal was immobilized, and suture materials removed under anesthesia (Picture-4). Inj. Ivermectin 3.00 ml S/C injected to prevent maggot infestation. Animal shifted to enclosure (Picture-3).

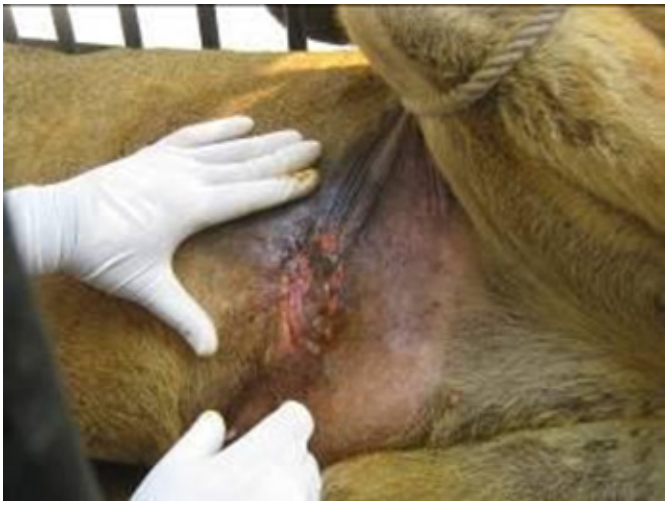


Conclusion

Dystocia is commonly handled by caesarean section in small cats. However, caesarean section in large cats is difficult and uncommon practice in Indian zoos. Prime age, good physical condition of the animal, timely intervention, post-operative care allowed for a successful outcome.

Acknowledgments

The author likes to acknowledge sincere thanks to Mr. Bipul Chakraborty, Director, Zoo for providing permission to perform surgery and all necessary facilities, knowing the narrow chances of success and high risk of life of the patient. Author likes to extend sincere gratitude and thanks to private veterinary practitioners Drs. D. K. Mahato and V.K. Singh for assisting during the surgery and follow up treatment. Author likes to put on record to acknowledge to all animal keepers and zoo hospital staff specially Mr. S.C. Mahato, Veterinary Supervisor & Mr. R. Srinivas, Field Assistant, who were involved for their whole hearted support during the entire episode of the case.



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A STUDY ON MATING BEHAVIOUR OF GREAT INDIAN ONE-HORNED RHINOCEROS IN CAPTIVITY

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Abstract

The Great Indian one-horned rhinoceros (*Rhinoceros unicornis*), the second largest land animal, is one of the most threatened species. Kanpur Zoological Park, Kanpur presently houses four rhinoceros in its collection (3:1). The current study was carried out on a female and a male rhino which are housed in rhino area of Kanpur Zoo. Behavioural observations were recorded using simple observation method and data was collected and analysed. Observations revealed that the female takes initiative in pre-mating play by producing whistling sounds and frequent micturition. She was even found running towards the male to stimulate him for courtship. Interpretations indicate that the desire of courtship and mating in Indian rhinoceroses does not always synchronize. Males experience a period of rut as does the female in oestrus and these episodes of rut and heat must correspond before mating takes place. The oestrous signs in female such as whistling, frequent micturition, moving towards male were observed; while repeated urination, penis protrusion and Flehmens reactions were important signs exhibited by the male. For mating, the female may be first introduced to the enclosure followed by the male so that female gets familiar with the enclosure. Duration of mating was found to be as long as 70 minutes.

Key Words Mating behaviour, Rhinoceros, Conservation breeding, Captive breeding, Courtship

Introduction

The Great Indian one-horned rhinoceros (*Rhinoceros unicornis*) is the second largest land animal. At present living rhinoceros are represented by five species and four genera (Fowler and Miller, 2015). Poaching for their horns (for medicinal use) and loss of habitat makes them one of the most threatened species. These threats are the major drivers of their declining population in wild that need to be managed (in-situ measures) as well supplemented (ex-situ conservation techniques). Conservation breeding is the key to reintroduce the captive bred species into the wild, therefore it is essential to understand the reproductive behaviour of the species, especially in captive facilities. Our study focuses on the concept of understanding the reproductive behaviour of Indian one horned rhinoceros in zoos for future conservation programmes.

The basic anatomy of the female reproductive system is similar in all species, with a bicornuate uterus, which has a short body and long tubular horns (Fowler and Miller, 2015) to accommodate a large foetus. In the male rhinoceros, the testicles are held close to the body along the perpetual fold and are

positioned horizontally. The relaxed penis is curved caudally, a position that results in the characteristic backward directed urination in male. (Fowler and Miller, 2003). They normally produce one calf after completion of around 480 days of gestation (Fowler and Miller, 2015).

Materials and Methods

The study was conducted using simple visual observation technique with the help of binoculars, stop watch and a camera. Study further involved thorough discussion with the experienced keepers, veterinarians and wildlife managers and the history and treatment sheets of the both the animals were studied and each event since 2011 was noted in a chronological order.

The Female rhinoceros named Manu coded in Indian National Stud Book as No.118 was born on 22.06.2002 in Kanpur Zoological Park, Kanpur, India. Usually, female rhinoceros attain sexual maturity at the age of 4.5 years (Fowler and Miller, 2015). To breed rhinoceros in Indian zoo conditions has always been a challenging task considering receptivity of both sexes, safety of animals and resources available. The first attempt to mate Manu was made when she was six years old. Of the seven attempts to breed this female only two attempts were found to be (including present study) successful. Selected male, Rohit identified as No. 85 in Indian National Stud Book of Rhinoceros was born on 20.06.1989 in Kanpur Zoological Park, Kanpur, India. He had experienced two matings with another female before being introduced to Manu.

Observations

For the convenience of the study and to better understand the breeding behaviour, the observations were divided into two parts i.e., pre-mating behaviour and mating behaviour. Pre-mating behaviour involved courtship behaviour (pre-copulation) displayed by both the sexes during their sexual excitement i.e., oestrus in female and rut in male in their respective enclosures. In case of female, first signs of heat were observed with inconsistency in feeding and as the left-over feed increased along with movement towards male exhibit followed by eye contact. As the intensity of heat increased, feed consumption further decreased. This indicated that routine diet is affected during courtship. Another important behaviour observed was the frequency of urine discharge which was found proportional to the heat. The female was also observed producing low whistling sound to attract male. The female moved to-and-fro and spent most of the time close to the common dividing wall (low height approx. 1.5 meters) that had male standing on other side.

Fig 1. Male and female Greater Indian one-horned rhinoceros.

Fig 2. Mating behaviour of Greater Indian one-horned rhinoceros.

The male demonstrated repeated penis protrusion when in rut and constant looking in the direction of the female. Recurring micturition in his exhibit & Flehmen response were also documented during the study.

After 16 hours of observation, female was introduced to the male. Before mating, both the animals were kept separately and were closed in the different houses of the mating enclosure. The female was introduced to common area an hour before male to get acquainted with the enclosure. The enclosure had a large open area with a dry moat and a wallowing pool. Both sexes showed repeated urine discharge over the ground. On observation in context of frequency and volume of urine output, female showed more frequent but less urine discharge in contrast to male who had more discharge but was less frequent. The male exhibited Flehmen on smelling the female urine, while the female produced whistling sound throughout this period. After which, the animals were noted chasing each other, initially male was chased by female and was later reversed. Both the sexes used moat during chase. The chase was recorded to last for 60 minutes.

Later both the sexes were seen standing face to face with their snouts touching each other for three minutes. Soon the male mounted the female and mating started. On being mounted, the female was observed initially moving back for few meters followed by very slow forward movement. After ten minutes while already in copulation both the sexes went into the wallowing pool slowly and remained in it till the end of mating. The total mating time recorded by the team was 70 minutes. The whole event was recorded as a single effort and was found to be non-violent. Both the sexes remained in same enclosure for next 24 hours and were separated the next day.

Results and Discussion

The results of the study were noted and were compared with available references. Few of our results were at par with the results of earlier researchers; however, the study also revealed new findings and few of results deviated from earlier records.

Results suggest that the male and female should be well acquainted with each other and the male should be in proper receptivity before being introduced to the female, as earlier attempt of the mating had gone into vain. It is therefore important to have both sexes in acceptance. According to Laurie, 1982 male rhinoceros may get aggressive and can cause serious injuries to female whereas female may also repel the male advances by simply turning and snorting.

According to Dutta, 1991 during mating, males may become very aggressive and may kill female and moreover rhinoceros females may become aggressive too, especially during courtship chases in wild, which may result in scrapes, cuts or deeper wounds. Best time to mate the female is minimum 16 hours after showing the first sign of oestrus. As up to this period the female peaks in the oestrus cycle and the male following her due to rut also begins to show sexual urge and chances of infighting become less. In captivity the female should be introduced first inside the mating enclosure, at least in an hour in advance to make her familiar with the enclosure so that even if infighting breaks out she can defend herself by running to a safer place (like moats etc).

The study revealed some signs of the oestrous among rhino females such as inappetance or less consumption of feed in small frequencies due to ongoing hormonal fluctuation. Guldenschuh and Houwald, 2002 also indicated that as the intensity of heat increases the female gradually decreases the diet due to increased

level of hormones. Frequent movement of female towards male and repeated micturition all over ground to distribute pheromones on a larger area, so that number of males may approach her, was one of the most significant signs. Under natural circumstances this gives her added advantage to select best male to mate. Snorting or whistling sound made by females to attract the male was another important sign.

The male showed repeated penis protrusions during rut, staring at the female to show dominance, frequent micturition to send signal to the female regarding being receptive to mate and Flehmen response. Similarly, Laurie, 1982 found that Bulls test the female's reproductive status by tasting her urine, which is then followed by a pronounced curling of the upper lip, known as 'Flehmen response'.

In our study the only female of Kanpur Zoo was mated with the only breedable male who happened to be her father. According to the study of Zschokke and Baur (2002) neither gestation period nor birth mass was affected by inbreeding. However, inbred calves grew slower and had a lower mortality rate. They concluded by suggesting the lack of negative effects of inbreeding of mother and offspring suggests that inbreeding avoidance in the Indian rhinoceros may be not as important as it is in other species. The day time was selected for mating due to convenience of the availability of the man power to control the possible infighting among male and female. Our decision of mating during day time followed Tripathi, 2013 who suggests that courtship and mating behaviour can occur at any time of the day or night among Indian rhinos.

Observations also revealed that the female takes initiative in pre-mating play by producing whistling sound and by frequent micturition, even chasing the male was recorded. This act of courtship was observed for 60 minutes and was followed by actual mating (copulation) which lasted for more than an hour. It was also observed that mating began at the ground of the enclosure followed by movement of both the sexes inside the wallowing pool while male remaining mounted over female. The results of our observations are similar to Srivastava and Nigam, 2010, who opine that mating in rhino is initiated by female who runs around the potential breeding male by making loud sounds and frequently squirting urine and occasionally pushing the male. The male rhino then chases the female for hours till the female rhino gets exhausted and remain in same place and then mating initiates.

In a similar study by Laurie, 1982 it was found that if the female turned and ran and the male chased her, sometimes over several kilometres. The male generally squeak-panted during such chases while the female honked or bleated very loudly. After such a chase the male usually caught up with the female again by following her scent. Similarly in our study it was found that until the female is receptive, she may repeatedly drive the male away with mock charges and other defensive behaviour. Loud whistling by Indian Rhinos announces reproductive condition and location, which typically occurs 6-10 hours prior to courtship or breeding activity. The whistling attracts bulls that respond with pre-copulative behaviour, such as prolonged chases. Such behaviour ensures that females ultimately mate with the strongest (most dominant) male in the vicinity. It was also found that the female suffered mating stress for three days but returned to normal behaviour after three days. The reason may be exhaustive pre-mating run and long duration mating and due to bearing weight of a mounted rhino bull over her back for seventy minutes.

Conclusion

This study is especially helpful in captive breeding as it defines that both the rhinos should only be allowed for mating when both the sexes are socialized to promote successful mating and to also prevent infighting in captivity. This study also revealed the signs of receptiveness in both sexes.

Acknowledgement

Authors are thankful to Mr Deepak Kumar, IFS, Director, Kanpur zoological park, Kanpur, India for providing all facilities to conduct the study. Thanks are also due to Mr K Praveen Rao, IFS, chief conservator of forest, wildlife, western region, Kanpur for providing photographs.

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TREATMENT OF IDIOPATHIC VENTRAL OEDEMA IN A FEMALE ASIAN ELEPHANT (*Elephas maximus*)

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Introduction

Ventral oedema is noticed in a number of diseases in elephants like Herpes, Iron deficiency, Liver fluke infestation, Tuberculosis, Renal diseases, Salmonellosis, Trypanosomiasis (Fowler and Miller 2003).

Case Study

V.J.B.U. and Zoo has two female elephants named Laxmi (age 58 yrs) and Anarkali (age 52 yrs). Elephant Anarkali was observed to have ventral abdominal oedema and diarrhoea, although her feed intake was normal. The ventral oedema was of a sudden onset and it increased over a period of time. The ventral oedema was measured using a tape and the measurements were length- 54", width- 38", height- 18" (Fig 1).

Diagnosis

The faecal sample test came negative for Fasciola eggs. A blood test to check the level of proteins and haemoglobin was carried out. Hypoproteinemia was observed. The blood test readings along with the reference range (Fowler and Miller 2003) are given in Table no.1.

Treatment

The elephants were dewormed with Ivermectin bolus suspecting it to be a case of endoparasitism. However, since Ivermectin alone has no effect on Trematode infestation a repeat deworming was carried out with oral Triclabendazole and Ivermectin @ 9mg/ kg b.wg. not exceeding 7200mg/ animal and 0.2 mg/ kg b.wt. respectively (Islam,1997) and repeated after a week. Inj. Furosemide @ 500mg/ animal i/m was given for three days to reduce ventral oedema. Oral multivitamin syrup fortified with amino acids and

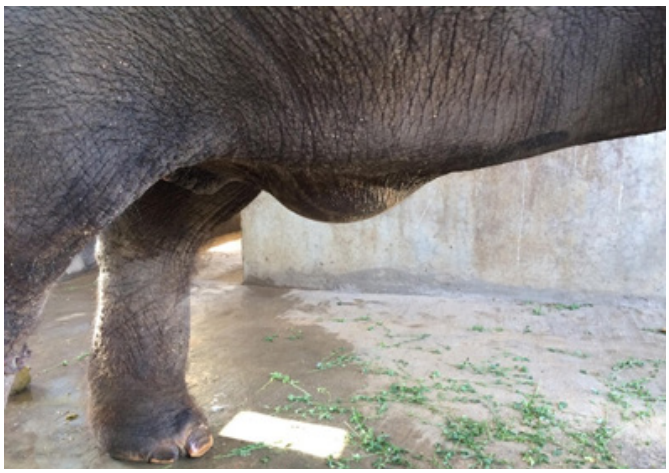

Fig 1: Ventral edema before treatment

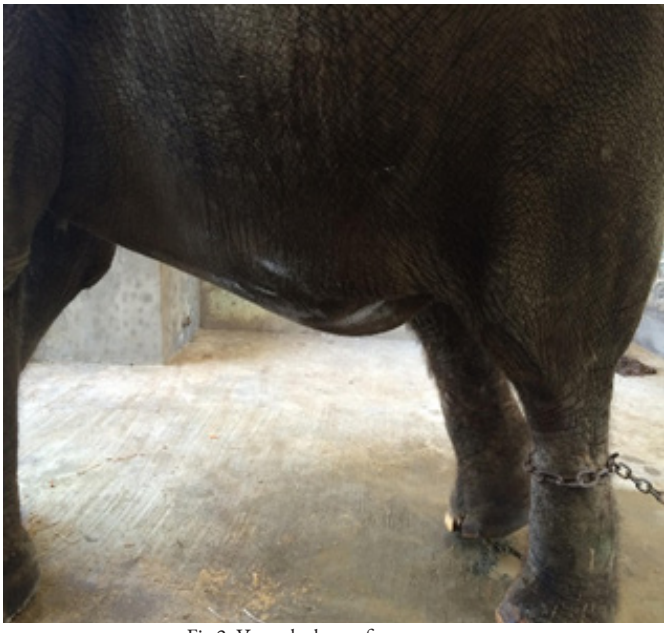

Fig 2: Ventral edema after treatment

Table 1: Blood parameters

Sl. no	Parameters	Result	Reference
1.	TEC	4.01 x 10^6/cmm	3.11 x 10^6/cmm
2.	Hb	17.2 gm %	13.3 gm%
3.	Bilirubin	0.5 mg/dl	0.2 mg/dl
4.	SGPT	35 IU/L	8 IU/L
5.	SGOT	28 IU/L	22 IU/L
6.	Serum protein	6.8 g/dl	8 g/dl
7.	Serum albumin	2.3 g/dl	3.2 g/dl
8.	Serum globulin	4.5 g/dl	4.9 g/dl
9.	Serum Alkaline Phosphatase	235 IU/L	145 IU/L
10.	BUN	19.8 mg/dl	12 mg/dl
11.	Serum Creatinine	1.3 mg/dl	1.6 mg/dl
12.	Uric acid	4.7 mg/dl	0.2 mg/dl
13.	Na+ K+ Cl-	124.9 mEq/L 5.1 mEq/ L 93.7 mEq/L	130 mEq/L 4.6 mEq/L 89 mEq/L
14.	CPK	218 IU/L	227 IU/L

liver tonic were continued for fifteen days to correct the elevated liver values and hypoproteinemia. Oral electral water was administered daily for preventing dehydration due to diarrhoea. The oedema was reduced as shown in figure 2. Hot fomentation using jute bags soaked in warm water was applied on the ventral aspect of the abdomen for a period of one week.

Discussion

Stool sample examination was done by Floatation technique which showed no presence of trematode eggs as Zinc sulphate floatation technique is suggested as a diagnostic test for stool sample examination for detection of *Fasciola sp.* eggs. (Islam, 1997). Haematology and biochemistry revealed elevated Alkaline phosphatase which indicated an underlying hepatic condition with low albumin levels which indicated hypoproteinaemia. The signs of ventral oedema, elevated liver value, low protein levels, in this case, were similar to the signs shown in trematode infestation and hence the Elephant was treated accordingly. Oral suspension of Triclabendazole and Ivermectin was administered through feed which was readily accepted and repeated after a week. Liver tonics were given throughout the treatment period through concentrate feed mixture. The ventral oedema and diarrhoea reduced over a period of 15 days with the use of anthelmentics, liver supplements, multivitamin syrup fortified with amino acids and electral water. No antibiotics were administered during the treatment. Symptoms of liver fluke infestation include colic, diarrhoea, constipation, depression, icterus, hypoproteinaemia, dependant oedema, anemia and death (Fowler and Miller, 2003). There are reports of death due to fasciolosis in young elephants in Assam in India whereas no clinical signs were found in adult elephants (Islam, 1997). The grazing of elephants on wet and marshy grounds should be avoided as a control measure (McGaughey, 1962).

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MANAGEMENT AND BREEDING OF OSTRICH (*Struthio camelus*) IN BHAGWAN BIRSA BIOLOGICAL PARK, RANCHI, JHARKHAND

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Abstract

Ostrich, an exotic large flightless bird is housed at Bhagwan Birsa Biological Park, Ranchi, Jharkhand. They were brought from PGRI, Kattupakkam, Chennai, Tamilnadu. The average age of sexual maturity observed in the zoo is 3 years. The number of eggs laid in one clutch is noted to be 8 to 11. The average incubation period and hatchability % recorded in Bhagwan Birsa Biological Park is 64 days and 44% respectively.

Introduction

Ostrich (*Struthio camelus*), the large flightless birds are native to more than 25 African countries. Adult males range from 2.1 to 2.75 metres (about 9ft) in height, and female Ostriches range from 1.7 to 2.0 metres (about 5.7 to 6.7 ft). They weigh more than 100 kg (average 115kg) (Davies, 2003). Ostrich eggs, world's largest egg, average about 150mm (6 inches) in length, 125mm (5 inches) in diameter and weigh about 1.35 kg. Males are mostly black but have white plumage on the wings and tail whereas the females and young ones are mostly brown in colour. The head and most of the neck of both the sexes is reddish to bluish in colour. The legs have powerful thighs and are bare (i.e., featherless). The lifespan is recorded upto 40-45 years (Davies and Bertram, 2003).

Historical Distribution

Ostriches are native to the north and south equatorial forest of Africa. Research conducted by Birbal Sahini Institute of Palaeobotany has found molecular evidence that Ostriches lived in India 25,000 years ago. DNA tests on fossilized eggshells recovered from eight archaeological sites in the state of Rajasthan, Gujarat & Madhya Pradesh found 92% genetic similarity between the egg shells and the North African Ostrich. This suggests that Ostriches travelled between India and Africa before the two land masses drifted apart (Anonymous, 2017 and Prasad, 2017).

Diet in nature

They mainly feed on seeds, shrubs, grass, fruit and flowers. Occasionally they also eat insects such as locusts. Like other birds Ostriches lack teeth. They swallow pebbles that act as gastrulates to grind food in gizzard.

Diet in nature

Poultry Feed (18% protein)	-	2 kg/adult bird/day
Chopped Spinach	-	1 kg/adult bird/day
Chopped Cabbage	-	1kg/adult bird/day

Breeding Behaviour of Ostrich

Based on literature, Ostrich mature sexually at the age of 02-04 years. Females mature about six months earlier than males. The female lays fertilized egg (after mating with males) in a single communal nest, a simple pit of 12" to 24" deep and 3m (9.8ft) wide is dug in the ground by the male. Incubation of eggs is generally done by the dominant female during the day and by males at night. Incubation period is 35 to 45 days[1].

Captive population and breeding behaviour in BBB Park

In September 2014 the BBB Park, Ranchi procured 4 (2:2:0) Ostrich from PGRI at Kattupakkam, Chennai, Tamil Nadu and housed them in a 6000 square meter rectangular enclosure. One pair was 1 year old & the other pair was 2 years old. At the zoo, one 2-year-old male died due to pseudomonas infection in October 2014. After a year the surviving male reached two years and attained sexual maturity. The featherless part of the lower leg and the beak becomes pink/red which is indicative of sexual maturity and is due to hormonal implications. The birds started courtship and mating in October. 2015. Simutaneously a shallow pit of 8 feet diameter and 1.5 feet deep was dug. The pit was filled with mixture of sand, soil and pebbles. Paddy straws, twigs and dried leaves were made available nearby. The female used this as a nesting material and laid her first egg in Jan 2016. The dominant female laid 11 eggs over a period of 2-3 days.The dominant female started incubation and the 1st hatch was noticed in 1st of April 2016. After 63 days of incubation 03 chicks hatched.

The dominant female laid two more clutches, the 2nd clutch hatched after 70 days of incubation in the month of December 2016 and a total of 06 (Six) chicks hatched out of 11 eggs. In the third clutch, 4 chicks out of 9 eggs, after 61 days of incubation. Whenever the incubating female left the nest for food the second female or sometimes the male took over the incubation.

Observation and Discussion

At BBB Park breeding of Ostrich was observed and recorded in the 2016 (Two clutches) and 2018 (one clutch).

1st hatch in April 2016 – 3 chicks
2nd hatch in Dec. 2016 – 6 chicks
3rd hatch in March 2018 – 4 Chicks

Fig. 1 Male and Female Ostrich.

Fig 2. Adult Ostrich with chicks

Table 1: Breeding details of Ostrich recorded in BBB Park:

Sl. No.	Number of eggs	Incubating period	Date of hatch	No. of chicks	Hatching %
1	8	63	9.4.2016	3	37.5
2	11	67	01.12.2016	6	57.5
3	11	62	23.03.2018	4	36.3

Incubation period was calculated from the date of last egg to the date of hatching. Average incubation period recorded at BBB Park is 64 days.

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ASSESSING BEHAVIOURS AND ENCLOSURE USE OF CAPTIVE SNAKES AT BONDLA ZOO, USGAO, GOA

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Abstract

The zoological community is constantly on the lookout for methods to improve management and care of captive animals. Indicators of a well-cared for and thriving animal are found primarily through expressions of contentment, play and ability to cope with reasonable stressors; all considered as positive indicators of welfare. Identifying these measures for reptiles can be challenging, and welfare assessment methods in reptiles are under-investigated. A preliminary study was undertaken at Bondla Zoo, Usgao, Goa, to assess behaviours and enclosure use of select species of captive snakes. The study indicated that enclosures, while meeting spatial requirements must also be include enrichment in the form of appropriate thermal gradients, basking zones, specific retreats or hides, water and other furnishings to promote welfare and behavioural diversity of its occupants. Enclosures housing multiple snakes must further account for overcrowding and crypto-overcrowding principles, that is, they should allow for both free movement as well as possess furnishings provisioned such that all animals can use them at any one time. A study is proposed to assess this theory further, as well as to gain more insight into snake cognition and behaviour.

Key Words Snakes, Zoo, reptiles, Husbandry, Welfare

Introduction

Zoos are continuously exploring ways to improve welfare and management of animals in their care. Previously, a lack of negative indicators in welfare practices was taken as evidence of well-being. An animal free of stereotypies, abnormal behaviour and anomalous feeding habits was considered to be in good health (Manteca *et al.* 2016). These measures, however, proved to be insufficient to demonstrate optimal condition, and it is now necessary to include positive indicators of welfare in assessments (Miller *et al.* 2020). Broadly, "these can be found in indications of contentment/pleasure, luxury behaviours, and behaviours that support ability to cope with challenge" (Mccormick, 2012, p.1). This sort of behavioural diversity is encouraged when the spatial requirements for individual species are met (Warwick *et al.,* 2021). Additionally, these spaces must be enriched consistent with natural history and allow for expression of species-specific behaviours. Wild snakes from established ecotypes have exhibited behavioural differences depending on the pace of life, physiology, personality axes and the ecological niches they occupy (Gangloff *et al.,* 2017). If designing of enclosures is approached by assessing the precise resources or conditions the animal seeks, it has the potential to make a more meaningful impact on welfare, over the approach of making small increments above baseline standards (Hoehfurtner *et al,* 2021; Warwick *et al.,* 2018; Mellor, 2015). However, more research into natural history, ecology and ethology of individual

species of snakes is required to make informed decisions.

The aim of this preliminary study was to evaluate enclosure use and behaviours exhibited by select species of captive snakes at Bondla Zoo, Usgao, Goa. All observations took place on site. Temperatures were recorded within all enclosures using a digital thermo-hygrometer with probe. Data was recorded by two observers and inter-observer reliability was tested for agreement on identification of ethogram behaviours with a success rate of 99.2% {number of behaviours per individual observed by both observers (139)/ number of observations made (140)}. Data was collected using open-source software Open Data Kit (ODK) (https://getodk.org/). Sampling was done using the one-zero recording rule of focal sampling.

Analysis and graphs were obtained through Excel. Nine adult snakes - four Russell's Vipers (*Daboia russelii*), three Indian Rock Pythons (*Python molurus molurus*) and two King Cobras (*Ophiophagus hannah*) - were selected for the study, considering the diversity in individual natural histories and scientific data available on their ecology. Since each enclosure housed multiple individuals of the same species, markers for identification based on pattern, colour and size were used. All snakes were observed individually for a total of 130 minutes each over the course of eight days. The snakes were assessed broadly for spatial use, enclosure furniture use and behavioural diversity.
The four *D. russelii* are housed together in a glass fronted enclosure, with sand substrate, a branch, a water bowl and a light source. The three *P. molurus molurus* are housed together in a walled circular pit, enclosed entirely by a wire mesh and with a network of branches and a water body. The two O. hannah are housed together in a glass fronted enclosure, with leaf litter, rock surfaces, a water body, branch and a light source. A feeding room is attached at the far end, and shielded from visitor view. All enclosures allow for snakes to adopt rectilinear postures (Warwick *et al,* 2021).

D. russelii (Fig. 2), were found to favour regular substrate (RS) over any enclosure furniture, which is expected of a typically terrestrial species. Higher occurrence of resting behaviours is likely due to it being an ambush predator of a slow-living ecotype, therefore maintaining low levels of activity (Glaudas, 2021; Gangloff *et al.,* 2017). Behavioural diversity other than resting was low. Average temperature recorded in the chosen Back Left of the enclosure was 23.2°C. The Preferred Optimal Temperature Zone (POTZ) value for *D. russelii* is unknown, however, a POTZ of 26–28°C is recommended for tropical species (Karthik, *et al.,* 2013). This suggests thermoregulatory behaviour in individuals aggregated together (Contact) within the enclosure, despite them anecdotally being observed as a solitary species (Graves and Duvall, 1987). Aggregation may also function to conserve body water and maintain humidity, as well as provide protection from external threats. (Costanzo, 2013; Noble and Clausen, 1936, Grave *et al.,* 1991).

Table 1: Recorded average temperatures from either end of *D. russelii*, *P. molurus molurus* and *O. hannah* enclosures at Bondla Zoo, Goa.

Species	Avg. LH Temp (°C)	Avg. RH Temp (°C)	Avg. Addnl. Temp (if any) (°C)
D. russelii	23.2	23.5	
P. molurus molurus	26.1	25.6	
O. hannah	25.4	25.0	Water body – 24.3

Fig. 1: Grid created for assessing enclosure use of *Daboia russelii*

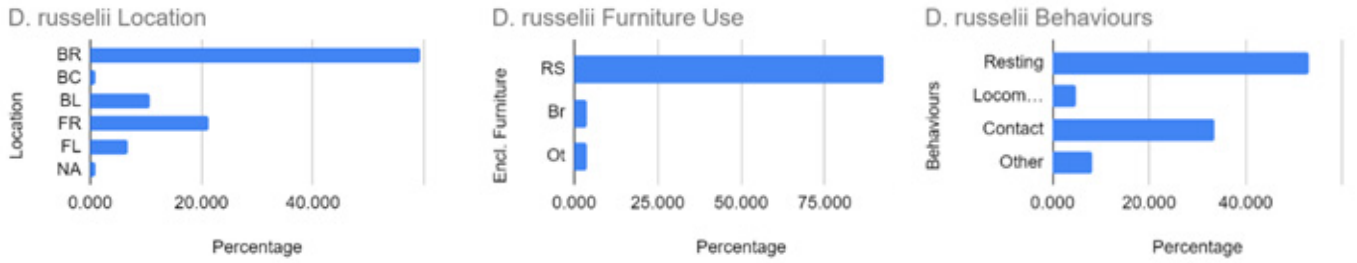

Fig. 2: Left to right – Enclosure use, furniture use and behaviours recorded in *D. russelii*. The back right (BR) of the enclosure was utilized the most (59.223%). Regular substrate (RS) was preferred (92.727%) and individuals were mainly recorded exhibiting resting (R) behaviours (53.104%) in contact with one other (33.592%).

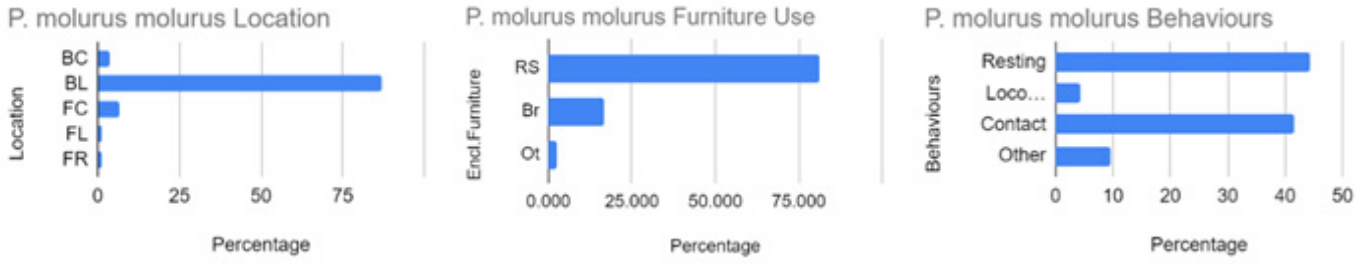

Fig. 3: Left to right – Enclosure use, furniture use and behaviours recorded in *P. molurus molurus*. The back left (BL) of the enclosure was utilized the most (87.179%). Regular substrate (RS) was preferred (81.176%) and individuals were mainly recorded exhibiting resting (R) behaviours (44.471%) in contact with one another (41.555%).

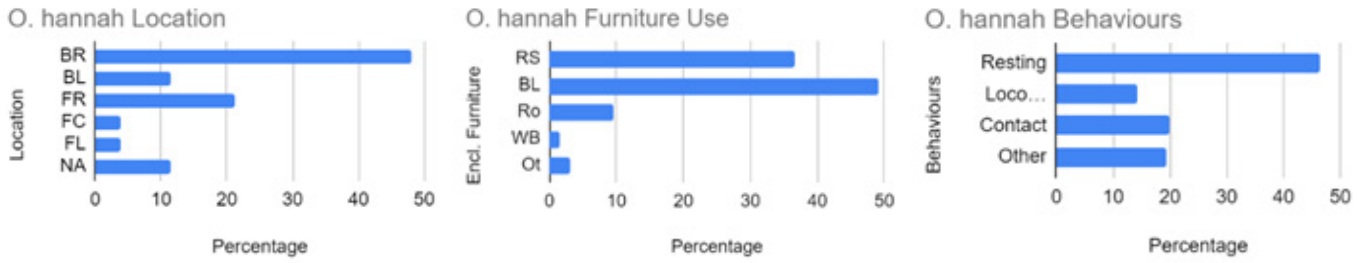

Fig. 4: Left to right – Enclosure use, furniture use and behaviours recorded in *O. hannah*. The back right (BR) of the enclosure was utilized the most (43.077%). Bamboo leaf litter (BL) was preferred (49.206%) and individuals were mainly recorded exhibiting resting (R) behaviours (46.362%) in contact with one another (19.958%).

Table 2: Ethogram Created for Behavioural Observations for Snakes At Bondla Zoo, Usgao, Goa

Sr.No.		Behaviours	State/Event	Abbreviations	Description
	GAPE	**Gape Smell**		**GS**	Gaping similar to a yawn to pick up chemical cues, drawing in scents or information from the air or from another object/ conspecific
		Gape Mouth Rub		**GM**	Occasional gaping, observed along with rubbing of mouth against different surfaces (may be a sign of mouth rot)
		Gape Flex		**GF**	Stretching – wide gape, snake flexes fang and/or jaw musculature individually, in quick succession (a snake may gape/ yawn after a long rest period)
	MOVEMENT	**Periscoping**		**P**	Raising the front part of the body (head and neck) vertically upwards, with head leaning forward slightly
		Tail Movement a		**TM - a**	Slow swishing of tail, with tail in contact with the ground throughout
		Tail Movement b		**TM - b**	Uplifted rapid movement of tail in the air
		Exploratory		**ME**	Continuous regulated movement in no fixed path or pattern, chin slightly raised off ground
		Climb		**MC**	Moving over or climbing up cage furniture. Specify if rock, branch or water bowl
		Flee		**MF**	Quick, reactive movement away from aversive source (person, object, conspecific, feed item)
	AGONISTIC	**Hiss Short**		**HS**	Producing a short, sharp hiss, sounds like a prolonged 's'
		Hiss Long		**HL**	Producing a prolonged hissing noise, accompanied by visible inflating and deflating of the body (inhaling and exhaling)
		Strike Defensive		**SD**	Rapid forward movement of head and neck towards an aversive source. This may not always result in a bite. Make a note if the mouth is -Closed -Partially open -Wide open
		Strike predatory		**SP**	Rapid forward movement of head and neck towards a prey item, with intention to bite. Make a note if the snake -Strikes and holds -Strikes and releases -Strikes and misses
		Contact Twitching		**CT**	Nudging/pushing with the body against a conspecific moving over the individual
		Combat		**CB**	Two individuals wrapping around each other with the upper bodies intertwined, each attempting to subdue the other
	MAINTENANCE	**Basking** **Fully exposed**		**B - a**	Basking under a heat/light source with entire body exposed to light, sometimes accompanied by flattening of the body
		Basking Partially Exposed		**B - b**	Basking under a heat/light source with body partially exposed to light, sometimes accompanied by flattening of the body
		Soaking		**So**	Staying in water bowl for a long time

	RESTING	**Resting alert**		**RA**	Snake is stationary, body loosely coiled, with neck and chin relatively straight, head resting on ground OR slightly raised. Responsive to movement inside and outside the enclosure
				RA - a	Snake is alert, body coiled, head resting on coil
				RA - b	Snake is alert, neck forms a characteristic "S" shape, chin resting on ground
		Resting Sleep		**RS - a**	Snake is stationary, body loosely coiled, chin resting on ground. Not responsive to any movement inside or outside the enclosure
				RS - b	Snake is stationary, body coiled, chin resting on or within coils. Head may or may not be visible
	INTERACTION WITH BOUNDARIES	**Moving on**		**IB - On**	Moving on glass ledge or wooden ledge of enclosure boundaries
		Vertical		**IB -V**	Moving upwards against enclosure boundaries
		Horizontal		**IB -H**	Moving along the sides of the enclosure with body in contact with the edges
		Snout touching		**IB - STB**	Pushing/ nudging at boundaries/ substrate/ furniture with snout
	DEFECATING			**DF**	Passing stools
	INGESTION	**Drinking**		**D**	Drinking water from bowl, characteristic jaw movement will be visible
		Eating		**E**	Consuming prey item
	CONTACT	**Coil Together**		**Co - T**	Occupying the same space, spread over a small area. Define using enclosure grid
		Spread together		**Co - S**	Seeming like a single unit, occupying a small area. Define using enclosure grid
	TONGUE-FLICKING	**Aerial**		**TF - A**	Movement of the tongue in the air, from its appearance outside the mouth to complete retraction
		Touching		**TF - B**	Tongue flick with tongue touching something (object, conspecific, prey item)
	OTHER	**Deep breathing**		**DB**	Visible inflating and deflating of the body, in absence of an aversive, with no audible hissing.
		Flattened head/ body		**Fl**	Flattened body with little to no muscle tone, spine visible
		Mating behaviour		**MA**	Two snakes are mating, joined at the cloaca/ vent.
0. NOT	**NOT VISIBLE**			**NA**	Snake is not visible in the enclosure

P. molurus molurus (Fig. 3) have been recorded in rocky habitats, and climbing, foraging and resting in trees in the wild (Babar *et al,* 2019). Climbing behaviour was minimal in the recorded observations. Individuals were primarily observed using regular substrate (RS), aggregated together in the back left (BL) of the enclosure. Aggregation could be to conserve heat and humidity, or provide protection. (Graves and Duvall, 1987; Costanzo, 2013; Noble and Clausen, 1936; Graves, 1991). Additionally, *P. molurus molurus* are typically found close to sustained water bodies (Babar *et al.,* 2019). The observed individuals were never observed in or around the provided water body, which likely means it likely did not suit their needs.

In the wild, *O. hannah* (Fig. 4) do not show strong preferences for any particular habitat type, though they likely prefer high leaf litter and areas close to streams with high relative humidity (Rao, 2013). Observed individuals were found to prefer the provided bamboo leaf litter substrate; this is in line with their natural ecology (Dolia, 2018; Rao, 2013). Resting behaviours superseded locomotion in recorded individuals, which is atypical of a species observed climbing and foraging, and often moving through the day (Cantor, 1836; Bhaisare *et al.,* 2010). The observed preference for the back left (BL) of the enclosure is likely due to the presence of leaf litter in the area. POTZ for *O. hannah* is unknown. Recorded average temperatures within the enclosure were between 25.4 and 25.0, so instances of aggregation

in the bamboo leaves were likely to conserve heat and humidity (Graves and Duvall, 1987; Costanzo, 2013).

Snakes housed in sufficiently large enclosures are expected to display greater levels of activity, exploration and stretching. (Mellor, 2015). However, resting behaviours superseded all other recorded behaviours in the observed species. Further, the authors believe that suboptimal temperatures and lack of hides and safe retreats could possibly explain why the *D. russelii, P. molurus molurus* and *O. hannah*, which are all solitary species (Kanagraj, 2019; Babar *et al.,* 2019) spent extended amounts of time aggregated together, in close proximity of each other within the enclosures.

The study reiterated the need to introduce high welfare and husbandry standards for ophidian species. Few essential requirements form the base of reptile keeping. A recommended safe thermal gradient range within 5°C of temperatures relevant to species natural history must be provided within the enclosure. Regulation of body temperatures is critical to ectothermic species to meet physiological and behavioural requirements. Lighting periodicity, i.e., providing a day-night cycle, and provision of UV lighting will allow for photo-stimulation, avoidance of photo-invasive conditions and improved vitamin D3 synthesis and immunity (Baines *et al.,* 2016). Observations and welfare assessments for nocturnal species should be within the times they are active. Natural substrate, water pool(s) of sufficient size and depth will allow for maintenance, drinking, bathing, or swimming for relevant species (Warwick *et al.,* 2019, Table 6). Behavioural diversity could be encouraged by introducing more enclosure complexity, naturalistic furnishings, providing choice in the form of temperature and humidity gradients and opportunities for retreat and concealment (Spain *et al.,* 2020). It would be beneficial to account for the crypto-overcrowding principle, i.e., all animals must be able to use any facility or furnishing (e.g., water bowl, branch, retreat site, basking site) at any one time (Arena *et al.,* 2013). After habitat modifications, it is key to conduct post-occupancy evaluations in order to determine how the changes impact animal behaviour and other indicators of welfare (Gaalema *et al.,* 2011).

Developments in understanding and appreciation of cognition and welfare of reptiles is indicative that that these animals are far more sensitive to their environmental circumstances than was previously thought. So, simply meeting spatial requirements is not sufficient. Providing enclosure complexity can be both enriching and beneficial to behaviour and welfare, and should be a default provision for captive snakes. Captive facilities must take into account new studies demonstrating traits of sophisticated communication, problem solving, parental care, play, and complex sociality in reptiles. Zoos and aquariums are unique in their ability to offer resources to study these elusive animals, and facilitate a number of research opportunities through a wide range of methods and technology now available. A study is proposed to further investigate our results, and enhance care and welfare of captive snakes at the zoo.

Conflict of Interest

We declare no conflict of interest.

Acknowledgements

We would like to thank the Goa Forest Department for granting us permission and providing facilitation and support through the course of the study; Shri Subhash Chandra, PCCF; Shri Santosh Kumar, APCCF, Chief Wildlife Warden; Mr Jebastin A., DCF, for their support and encouragement and the staff and keepers at Bondla Zoo, Goa, for their presence and guidance through our observation periods.

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SUCCESSFUL REARING OF CRITICALLY ENDANGERED GHARIAL (*Gavialis gangeticus*) IN CAPTIVITY AT CHENNAI SNAKE PARK, TAMIL NADU, INDIA

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Abstract

Rearing of captive-bred gharial hatchlings at the Chennai Snake Park, hatched for the first time is described. Rearing practices viz. housing, feeding and care of 23 gharial hatchlings from a single clutch from May 2020 to September 2021, are discussed. Observation on the 20 surviving hatchlings reared for about 1½ years, necessitated for consideration of factors like age/size-dependent housing, enrichment of enclosure, feeding and growth rate monitoring for successful management.

Key Words Gharial hatchlings, Rearing protocol, Feeding regimen, Captive management.

Introduction

Gharial (*Gavialis gangeticus*) is a critically endangered crocodilian, Schedule-I species, inhabiting severely fragmented Indo-Gangetic River system (Indus, Ganga, Yamuna, Bharamaputra and Mahanadi rivers) including Nepal and Bangladesh (Lang *et al.,* 2019; Saikia, 2013; Stevenson & Whitaker, 2010; Whitaker & Basu, 1982). Gharial feed exclusively on freshwater fishes (Singh, 1995, 2018; Thorbjarnarson, 1990). Excessive fishing and habitat degradation have pushed the gharial towards extinction. As the most important candidate of Project Crocodile in India, gharial are being intensively researched for conservation action (deVogs, 1984; Singh, 1999). Despite rigorous attempts to revive the population in the last decades (e.g. Acharya *et al.,* 2017; Cadi *et al.,* 2005; Hussain, 1999; Rajbhandari *et al.,* 2017), there was a sudden dip in the gharial population once again in 2007 (Ballourd *et al.,* 2010; Nawab *et al.,* 2013; Mohanty *et al.,* 2010; Stevenson, 2015; Suman, 2008; Whitaker, 2007).

One of the foremost steps is to streamline the protocol for productive captive-rearing of gharial (Bustard, 1990; Maskey *et al.,* 2003; Rao & Sharma, 1986; Singh & Bustard, 1982). Genetic bottleneck syndrome, disease and the consequent early neonatal mortality are some challenges in captive breeding (Gairhe, 2007; Kumal & Maharajan, 2014; Lal, 1982; Maskey *et al.,* 1998; Mehrotra *et al.,* 2000; Rao & Bustard, 1979; Sharma *et al.,* 2020, 2021). Captive breeding is the first step towards ensuring maximum recruitment of the progeny (Wilson & Price, 1994). This can be applied both to zoos and facilities like breeding centres that operate within the native range of the species, aiming to rear the hatchlings for a few years so as to release them in the wild (Khadka, 2013; Khadka *et al.,* 2020).

The information available on this topic stems from the scanty documentation of early rearing of captive gharials in a few facilities. A lot of research and conservation attention, including funding, are needed for in-situ and ex-situ conservation of gharial (Gill, 2010). Documentation of breeding, egg clutches, successful hatching as well as rearing are key ingredients to ensure successful progenies (Bustard, 1980; Bustard & Basu, 1982; Bustard & Singh, 1977; Singh & Bustard, 1977). Movements and post-release

monitoring studies have also been done in a few sites (Ballourd *et al.,* 2005; Maskey, 1989). Early attempts at breeding documentation of gharials in captive facilities have fetched some notable results. Khadka & Bhashyal, 2019 offer the latest compilation of current knowledge on this topic. Food and feeding habits are some of the most vital parameters that determine gharial rearing success (Choudhury & Bustard, 1983; Singh, 1983, 1995).

In the Chennai Snake Park (CSP), on 26th May 2020, a total of 23 gharial hatched out of 25 eggs with two stillborn. The eggs were laid by a female in early March 2020. Adult gharials that parented these offsprings are a captive stock from the Nandankanan Zoological Park, Odisha procured by CSP under an animal exchange scheme in 1993. The age of the gharials at the time of receiving them by CSP was 3 years.

The eggs were allowed to remain in-situ and were incubated inside the enclosure, mostly under the care of the female, in near-natural conditions. There were four more adult females apart from the adult male and female that bred. These adult gharials are kept in an enclosure measuring 63 x 32 x 9 feet (lbh) with a water body of 20 x 10 x 6 feet (lbd) size. Adult gharials were fed a range of freshwater fish, including Tilapia species.

After over two and half months, the eggs hatched. The main gharial enclosure is open to air and the gharial hatchlings would have been at risk from predatory birds. Hence the hatchlings were immediately transferred to a secluded and closed enclosure. Hatchlings were examined by a veterinary doctor. The breeding pair of gharials were from the same clutch and hence siblings. Since their arrival in 1993, there have been multiple attempts for breeding and many times only infertile eggs were laid. In 2020, the adult female laid fertile eggs. It is the first successful breeding of gharials in Chennai Snake Park.

Prelude

Since this was the first breeding in the park, we visited the gharial enclosures, especially nurseries, in the Arignar Anna Zoo and Madras Crocodile Bank in order to understand the husbandry details as the first step. We held academic discussions with their biologists and animal keepers to know their rearing protocols. Pertinent literature and publications were also perused subsequently.

Housing

A nursery enclosure measuring 14 x 8 x 8 feet (lbh) with a closed roof was chosen as the first enclosure to house the gharial hatchlings (Fig. 1). Enclosure enrichments consisting of a running water that flowed through a rockery and into a long, linear pond, circulating through a filtration unit were provided. Additionally, some suitable hides and shelter sites made of large palm leaf thatch and sand bars for movement on land were provisioned. In late May, all the 23 hatchlings were shifted to this enclosure. Sexing or individual recognition markings were kept pending as per the veterinary advice to avoid any invasive procedures. This enclosure has a recycled running water system that involved flow from an elevated rockery down to the sand bars.

After three months (June-August) of age, in September 2020, 10 gharial hatchlings that were visibly larger than the rest were shifted to another larger, open-moat enclosure (Fig. 2). However, as a precaution against avian predators and wild monitor lizards, mongoose, a mesh with small netting was cast on top to seal it well. This enclosure had sand substrate and is a rhomboidal pen measuring 17 x 9 x 6 feet (lbh) circumference by a brick wall topped with a chain-linked fence.

In October 2020, all the remaining gharial hatchlings were also transferred to this circular mesh-top enclosure, as those babies too had attained a good size. In late November, during the Nivar Cyclone, all the hatchlings were again shifted to the enclosed nursery enclosure for safety, but they were transferred back after a brief stay of one week. At present, in September 2021, having completed 1 ½ year of age, all the yearlings were shifted to a bigger open enclosure (26 x 21 x 7 feet, lbh) adjacent to the adult gharial enclosure. This enclosure is big enough to accommodate the yearlings for a further period of one year or more. The enclosure (Fig. 3) has a running water system with a waterbody of 18 x 9 x 4 feet (lbd).

Feeding

Feeding is one of the most important aspects of rearing and has a direct bearing on the survival rate of the hatchlings. Informed by technical discussions with fellow zoo biologists and curators, as well as a perusal of literature, it was understood that the hatchling absorbs the egg yolk as nutrition during the first month after hatching. Since June 2020, feeding trials were commenced, starting with chopped pieces of freshwater fish. But no feeding was observed. Subsequently, live fish fingerlings of freshwater species were added. Fish species such as Catla, Labeo and Channa were fed. Since the gharial hatchlings use the movement, scent and activities of the live fishes as the feeding cues, this method was successful in inducing foraging. In some cases, when a few hatchlings appeared to be dominated by others in the group, isolated feeding was done to ensure adequate nutrition. Hand feeding was also attempted after one month of hatching and discontinued when they started feeding on their own. Despite easy availability exotic Tilapia fish fingerlings (native to Africa) were not fed to the gharial hatchlings. It is suspected that Tilapia are capable of accumulating toxic water pollutants in their muscles which may prove to be toxic and lethal (Singh *et al.,* 2011).

Disease

In early April 2021, an eye problem with redness and swelling on the left eye in a gharial hatchling was observed. As per the advice of veterinarians, the hatchling was quarantined for diagnosis. Subsequently, antibiotic eye drops (Ciprofloxacin, Moxifloxacin and Tobramycin (0.3% w/v) + Fluorometholone (0.1% w/v)) were applied thrice a day at 9.30 am 1.30 pm, 5.30 pm for 10 days. By mid-April 2021, the hatchling recovered and returned back to its original enclosure.

Hatchling casualty

Three hatchlings died, one in July, one in September and one more in November 2020. A distinct shrinkage of the neck region was noted in the hatchlings that died. The death of three hatchlings may be due to the overcrowding of hatchlings in enclosure. This is a low percentage of mortality (1.3%) which is not uncommon. Similarly, in Arignar Anna Zoological Park where gharial are breeding for the past three years, some of the hatchlings that died early on, invariably developed similar shrinkage of the neck region (Fig. 4) (Dr. M. Manimozhi, Senior Biologist, AAZP, pers. comm.).

Fig. 1 Gharial nursery that housed the fresh hatchlings; provided with recycled running water system.

Fig 2: Intermediate gharial baby yearling enclosure, where the yearlings were housed from late 2020-mid 2021.

Fig 3: Simulated new enclosure with running water system for housing 1½ year old gharial babiesyearling, located next adjacent to the adult gharial enclosure.

Fig. 4 a & b. Profiles comparing the neck regions of baby gharial hatchlingss showing the normal condition vs. affected bywith neck shrinkage.

Growth rate

During the first year, the hatchlings were gently restrained and measured (in cm) and weighed (in g). Gharial hatchlings were put into a cloth bag, one at a time and weighted using a spring balance (L.C. 5 g) and were measured by gently restraining and placing them straight, parallel next to a graduated scale (LC. 0.5 cm). Care was taken to schedule the morphometry a few days after feeding. Soon after birth, in July 2020, the minimum-maximum values ranged from 45-54 cm length and 110-210 g weight. By December 2020, the minimum-maximum values ranged from 58-80 cm in length and 330-785 g in weight. By April 2021, the minimum-maximum values ranged from 65-89 cm in length and 530-1230 g in weight. Except for the three casualties, the remaining animals remained consistently healthy. A considerable range of variation in the morphometry of the hatchling persisted throughout the one-year period.

Table. Length (cm) and weight (g) of the gharial babies hatched and raised in the Chennai Snake Park.

Age / Month	Min. length	Max. length	Mean length	Min. weight	Max. weight	Mean weight
1-2 months (May-Jul.' 20)	45	54	49.5	110	210	160
3-4 months (Aug.-Sept. '20)	45	65	55	120	365	242.5
5-6 months (Sep.-Oct.' 20)	52	72	62	140	485	312.5
7-8 months (Nov.-Dec.'20)	58	80	69	330	785	557.5
9-10 months (Jan.-Feb.'21)	61	83	72	425	905	665
11-12 months (Mar.-Apr.'21)	65	89	77	530	1230	880

(Note: sample sizes of values were n=23 till Aug. 2020, and became n=20 from Sept. 2020 onwards)

Concluding Remarks

Species with special habitat and food preferences are difficult to maintain in captivity. Breeding of such resource-specific species is difficult. However, excellent management measures have been instrumental in maintaining them well. It is hoped that the detailed sketch of the captive rearing at CSP sheds light on this much-needed topic aiding in both ex-situ and in situ gharial conservation.

Acknowledgements

We convey our thanks to the Dept. of Environment, Govt. of Tamil Nadu, corporate sectors (M/s. HCL Foundation, Ramco Cements, CavinKare) and other donors for their grant-in-aid assistance to the Chennai Snake Park Trust, during the pandemic period 2020-21. We thank the Board of Trustees, CSPT for all their cooperation and suggestions; the team of doctors, notably Trustee Dr. K. Senthil Kumar for their veterinary advice; the Director and Biologists of Arignar Anna Zoological Park (AAZP) and Madras Crocodile Bank Trust (MCBT) for their information on gharials in their holdings. Animal keepers of CSPT are also appreciated for their active and timely care of the baby gharials.

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AN OUTBREAK OF CANINE DISTEMPER IN ASIATIC LION AND ITS SUCCESSFUL PROPHYLAXIS

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Introduction

Canine distemper virus (CDV), a morbillivirus that causes one of the most contagious and lethal viral diseases known in canids, has an expanding host range, including wild animals (Nagao, Y *et al,* 2011). Domestic dogs are the main reservoir of CDV, a multihost pathogen. This virus of the genus Morbillivirus in the family Paramyxoviridae occurs in other carnivorous species including all members of the Canidae and Mustelidae families and in some members of the Procyonidae, Hyaenidae, Ursidae, and Viverridae families. Canine distemper also has been reported in the Felidae family and marine mammals. The spread and incidences of CDV epidemics in dogs and wildlife are increasing worldwide (Kapil, S. *et al,* 2011). Recently this disease has been confirmed in tiger, lion and leopard. Outbreaks have devastated lion populations in Serengeti National Park, Tanzania in year 1994 (Guiserix, M., *et al,* 2007). The major mode of CDV transmission is through aerosolization of respiratory exudate containing virus, although other body excretions and secretions (e.g., urine) can result in infection in susceptible hosts if aerosolized. There appear to be only few reports of CD documented in large wild cats in India same is sero-prevalence to CDV has been reported 94.59% (35/37) in Asiatic lions, 76.92% (10/13) in hybrid lions and 91.96% (22/24) in leopards (Anand, R., 1999). This is an emerging disease in wildlife with a rare occurrence.

Etawah Safari Park at a glance

Etawah Safari Park is situated in the historical 'Fisher Reserve Forest area', located on the Etawah-Gwalior Road, about 5 km from Etawah town in Uttar Pradesh. The Etawah Safari Park is spread over an area of 350 ha and has been developed as per guidelines of CZA, New Delhi. All the Asiatic lions were brought from Rajkot Zoo and Sakkarbagh Zoo of Gujarat and Nehru Zoological Park, Hyderabad.

Canine distemper outbreak at breeding centre of Asiatic lions

At the Breeding Centre of Asiatic Lions, Etawah safari Park, Etawah, four adult lions succumbed to canine distemper. Onset of disease was very acute. The symptoms noticed were hind quarter paresis, high fever, convulsions, chorea, very poor control on defecation and urination, anorexia etc.
In October and November 2014 Lioness 'Laxmi' (Stud Book No. 000-6B72163/ 445) and Lion 'Vishnu' (Stud Book No 000-61146E09/670) died during the course of treatment. They were found positive for Canine distemper confirmed by IVRI, Bareilly.

In April 2016, Lion, 'Kuber' (Stud Book No 0006CC-31FA/761) fell ill and was immediately treatment on advice from IVRI Bareilly. Blood samples were sent to IVRI for analysis. IVRI, Bareilly

confirmed Canine Distemper virus. On 2nd June 2016, during treatment, Kuber succumbed to death.

In July 2016, Lioness, 'Greeshma' (Stud Book No 00-06BB-ADFB/719) was dull and unable to stand on her hind legs and treatment was initiated on advice from IVRI Bareilly and the blood sample was sent to IVRI for analysis. IVRI, Bareilly confirmed that the animal was positive for Canine Distemper virus and treatment continued as based on expert advice for 4 months till Lioness, 'Greeshma' succumbed to death on 8th November 2016.

Realizing the danger of outbreak of Canine distemper samples from healthy animals were also tested. Blood samples, ocular swabs, nasal swabs, faecal samples of all the lions (Greeshma, Pataudi, Gigo, Manan, Jessica, Kunveri, Hir) were collected in suitable RNA/DNA shield media at different intervals and sent for testing.

Blood samples of all lions were found positive for CDV by RT-PCR by using universal and F-gene primers, and also confirmed by sequencing results by IVRI, Bareilly.

Strategic Vaccination to control CD in Asiatic lions

As per guidelines provided by CZA, New Delhi, live vaccines are not safe in wild animals and are viewed with concern. Killed vaccines against canine distemper are not available worldwide. On the advice of experts live vectored (recombinant) vaccines provide hope for safe and effective vaccination of wild animals, and Canarypox vectored canine distemper vaccine by Merial Pharmaceutical Co., USA was recommended for use in lions. Recombinant Canarypox vectored canine distemper vaccine does not carry live canine distemper virus and has been used in clinical trials in large cats. No adverse reactions were reported and cats developed detectable antibody titres to canine distemper virus. This vaccine provides the best option available to provide some protection against canine distemper virus in large felids. A suitable vaccination pattern involves primary vaccination at eight weeks of age with subsequent double revaccination at 3-week intervals followed by annual revaccinations. All the 6 adult Asiatic lions were vaccinated with Distemper Ferret canarypox vectored vaccine first time in India at Etawah safari park.

Vaccination Schedule

Adult lions vaccinated with 1ml CDV ferret vaccine primary dose, 3 weeks apart 1st booster 1ml dose and 3weeks after 1st booster 1ml 2nd booster dose given. It is tabulated below:-

Table 1:

	Name of lions	Date of First CD vaccine	1st booster	2nd booster	Annual vaccination (due)
1.	Gigo	11.07.2016	01.08.2016	22.08.2016	11.07.2017
2.	Pataudi	11.07.2016	01.08.2016	22.08.2016	11.07.2017
3.	Hir	11.07.2016	01.08.2016	22.08.2016	11.07.2017
4.	Manan	18.07.2016	08.08.2016	29.08.2016	18.07.2017
5.	Jessica	18.07.2016	08.08.2016	29.08.2016	18.07.2017
6.	Kunveri	23.07.2016	13.08.2016	03.09.2016	23.07.2017

Discussion

The evaluation of vaccination efficacy in our study was based on a virus-neutralization test. The virus-neutralization test has still been regarded as a standard serological test providing a good correlate of protection against viral infection [10]. Serum samples of vaccinated animals were subjected for canine distemper virus neutralization test and results obtained were as shown in table 2.

All the lions showed increasing antibody titres for CDV with respect to day zero indicating protection level against canine distemper and effectiveness of current vaccination schedule. Antibody titres of lion named Gigo, Pataudi, Hir were comparatively less so they were revaccinated six months after 2nd booster of

Table 2:

Name of Lion	SN titre as per IVRI report			SN titre as per Vienna Institute	
	0 days	21st day post primary (dp)	42nd day post primary	30.08.16	06.09.16
Gigo	No titre	No titre	1:64	1:6 (50 dp)	1:11 (57 dp)
Manan	1:8	1:512	1:1024	1:91 (42 dp)	1:64 (50 dp)
Pataudi	No Titre	1:4	1:64	1:32 (50dp)	1:32 (57 dp)
Hir	No titre	1:4	1:64	1:23 (50 dp)	1:23 (57 dp)
Jessica	>1:128	1:1024	1:512	1:256 (42dp)	Not estimated
Kunveri	>1:128	1:1024	1:2048	1:362 (42 dp)	Not estimated

vaccination to improve their antibody titre for better protection. The whole analysis covered the period of 1year from primary vaccination. No symptoms of disease were observed in the lions throughout the study. Three lions Gigo, Pataudi, Hir had zero virus neutralization antibody titre before primary vaccination and increasing trend of serum antibody titre showed protection level against canine distemper due to vaccination. In Manan, Jessica, Kunveri presence of CDV antibody titre prior to vaccination may be due to CDV infection. In these three lions, serum antibody titre also increased after successive vaccinations without any clinical manifestation of disease. Regarding the results obtained we do not consider the recommended vaccination with a recombinant ferret CDV vaccine is a solely effective method to control CDV in population of Asiatic lions. This test will be the subject of another study on a larger population of animals.

Suggestions

There is an immediate need to test all cats for CDV exposure or virus presence. All the zoos, safari, parks should consider implementing rigorous quarantine and vaccination program. It is very clear that CDV is present in the population of Asiatic lions and it is spread amongst institutions probably because little attention is being paid to pre-ship testing, confirmatory diagnosis, and a rigorous vaccination program. There is also a need to collect and bank serum and tissues (primarily from animals that succumb to CDV) for future testing. Serum banks will allow institutions to trace the origins of a disease retrospectively. Canary pox vectored CDV vaccine is safe and provides the best option available to provide some protection against canine distemper in lions.

Acknowledgements

We offer special thanks to Central Zoo Authority, New Delhi for helping and introducing us to Smithsonian Institute (USA). We are thankful to Smithsonian Institute (USA), San Diego Zoo (USA), Institute of Virology and Veterinary Medicine, Vienna (Austria), IVRI, Bareilly, Veterinary University, Mathura for their valuable suggestions, help in diagnostics and in thorough vaccination programme.

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STANDARDISING ARTIFICIAL INSEMINATION IN LEOPARDS (*Panthera pardus*)

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Abstract

In the recent past, many wildlife species are facing serious threat to their survival due to multiple anthropogenic disturbances. Therefore, besides in situ conservation, adaption of newer conservation breeding strategies such as assisted reproductive technologies is vital for maintaining and reinforcing viable populations of threatened wild species. Artificial insemination (AI) is the most widely used reproductive technology in wildlife and has been exceptionally effective in conservation breeding of several endangered species globally. In India, similar efforts have been made in CSIR-Centre for Cellular and Molecular Biology - Laboratory for the Conservation of Endangered Species (CCMB-LaCONES) and have been successful in producing live births of small ungulates and birds using AI. In the present study, we have attempted for employing AI in leopard as a model species for wild felids. We further highlight the challenges and obstacles associated with the development of AI procedures in leopards.

Key Words Leopard, Semen, AI, Estrus induction, Ovulation

Introduction

Except domestic cat, all the 36 species of the family-Felidae are classified as threatened, vulnerable or endangered (Thongphakdee e*t al.,* 2018). This may be attributed to the various anthropogenic factors and poaching. Apart from anthropogenic activities, it has been evidenced that disease is also one of the driving factors to the decline of wild cat populations e.g. Canine distemper virus outbreaks in Serengeti (Roelke-Parker et al., 1996) and Gir (Mourya et al., 2019) lions. Recent evidence of natural SARS-CoV-2 infection in large felids (McAloose *et al.,* 2020; Mishra *et al.,* 2021; Fernández-Bellon *et al.,* 2021; Karikalan *et al.,* 2021) is an alarm of threats of emerging diseases to the endemic populations, particularly the Gir lions. Although natural breeding is the best choice to increase the population size of any species, it may not be possible in wild populations due to limited number of individual animals and incompatible mating partners. Apart from management practices, application of assisted reproductive technologies (ART) such as artificial insemination (AI), multiple ovulation and embryo transfer (MOET), in vitro maturation and fertilization (IVM-IVF), cryopreservation of gametes and embryos, somatic cell nuclear transfer (SCNT)and genetic resource banks (GRB)would be necessary for the success of

conservation breeding programs (Holt and Pickard, 1999; Shivaji *et. al.* 2003; Andrabi and Maxwell 2007; Sontakke, 2018). The success of ARTs in species conservation and management is dependent on the ability to produce a viable offspring. The basic knowledge on reproductive anatomy and physiology of a species concerned is an essential prerequisite before applying any ART. However, lack of availability of fundamental reproductive biology of a species concerned and limited number of individuals limits the application of ARTs in wild cat species (Shivaji *et al.* 2003). The development of a reliable ART for domestic cat facilitated the successful AI and IVF in nine cat species, whereas, SCNT success remained limited to only three species of wild cats (Thongphakdee *et al.,* 2018) but unfortunately, these techniques are not advanced to routine use. The overall efficiency of ART in felid species remains inconsistent, which could be attributed to several factors such as a) anatomical constraints of the female reproductive system (narrow vaginal lumen, torturous nature of cervical canal) that causes difficulty for normal passage of AI/ET catheter into uterus (Zambelli *et al.,* 2005), b) behavioural signs such as overt aggression, intractability, and occurrence of 'silent' estrus in some females, and c) issues related to seasonality, ovulatory mechanisms (spontaneous vs induced), limits sperm production, poor understanding of sperm transport in female reproductive system, embryo developmental requirements and appropriate ovarian stimulation regimes (Swanson, 2012).

Nevertheless, AI has been playing an important role in conservation and propagation of endangered wildlife species due to simplicity of its procedures compared to more complex technologies of IVM-IVF, MOET and SCNT (Andrabi and Maxwell 2007). AI is the most convenient method when natural mating is not successful and particularly in the populations that have low genetic variation. The success of AI in cat species is determined by the time of anaesthesia and insemination with respect to ovulation, efficiency of ovarian induction protocol, site of sperm deposition in female reproductive tract, and the number of viable spermatozoa in the reproductive tract and timed ovulation are critical for success of AI (Swanson, 2006)

It has been reported that restraint, high temperature or administration of adrenocorticotropic hormone (ACTH) reduces embryo survival in mice, rats, sheep and pigs (Doney *et. al.* 1976; Kittinger *et. al.* 1980; Hemsworth *et. al.* 1986). In pigs, administration of ACTH interferes with the ovulation by altering luteinizing hormone (LH) peak (Hennessy & Williamson, 1983). Generally, a combination of ketamine hydrochloride and xylazine hydrochloride, or medetomidine or inhalation anaesthetic like halothane are commonly used anaesthetic drugs for semen collection and AI in wild cats (Sontakke *et. al.* 2009). The increased circulatory levels of ACTH associated with animal restraint (Hemsworth *et. al.* 1986) and the administration of ketamine hydrochloride (Carter et al., 1984) may alter the pre-ovulatory luteinizing hormone (LH) surge and subsequent ovulation in cats (Howard *et al.,* 1992). A relatively high pregnancy rates were recorded in domestic cat (Howard *et al.,* 1992), and live cubs were produced in leopard cat and cheetah when the anaesthesia/AI was performed after ovulation (Howard *et al.,* 1991b; 1992, 1997).

Furthermore, the site of semen deposition during AI is crucial. Semen is usually deposited at three different sites in the female reproductive tract; viz. anterior part of vagina (intravaginal), uterus (intrauterine) or in the oviduct (intra-oviductal) in cat species (Zambelli *et al.,* 2005; Howard and Wildt, 2009). The success of intravaginal insemination in wild felids is limited

to Siberian tiger (Chagas *et al.,* 2000), however; the repeatability of this method is questionable (Howard and Wildt, 2009). The low conception by intravaginal insemination was attributed to altered sperm transport in female reproductive tract of anaesthetised animal, or timing of insemination or both with respect to ovulation (Howard *et. al.* 1992, 1997). The sperm of wild felids are sensitive to pH and have short viability. The trans-cervical intrauterine insemination showed better conception and birth rates compared to intravaginal approach in domestic cat (Chatdarong *et. al.* 2007). In wild felids, live births were recorded after trans-cervical intra-uterine insemination of naturally occurring estrus in Persian leopard (Dresser *et al.,* 1982) and Asiatic Golden cat (Leuders *et al.,* 2014). The constraints associated with the intravaginal approach was bypassed by depositing sperm closer to the fertilization site (ampulla-isthmus junction or utero-tubal junction) through either laparotomy or laparoscopy and livebirths were successfully achieved in domestic cat (Conforti *et al.,* 2013) as well as several wild felids including leopard cat, snow leopard, clouded leopard, cheetah, ocelot (Howard *et al.,* 1991; Barone *et al.,* 1994; Swanson *et al.,* 2012; Lambo *et al.,* 2013; Conforti *et al.,* 2013).

The estrus and ovulatory patterns in felid species are highly variable from induced to spontaneous ovulators or both, and these patterns are not only species-specific but also individual specific in few species (Brown *et al.,* 2006). In induced ovulators, a neuronal stimulus from vagina during copulation is necessary to release sufficient quantity of LH required for ovulation; whereas in spontaneous ovulators, LH released by a positive feedback mechanism is controlled by an endogenous ovarian steroid.The tiger, puma, snow leopard, cheetah, tigrina, ocelot and lynx appear to be exclusively induced ovulators, however spontaneous ovulation, at least occasionally, is prevalent in lion, leopard, Pallas's cat, clouded leopard, fishing cat, margay and some domestic cats (Brown *et al.,* 2011). A recent study has shown that jaguar (*Panthera onca*) is an induced ovulator with moderate incidence of spontaneous ovulation (Barnes *et al.,* 2016). The circulatory LH surges are directly related to the frequency of copulations during the estrus. In AI/ET, ovulation in cats is triggered either by mechanical stimulation of vagina as in natural mating to release LH, or by administering exogenous human chorionic gonadotropin (hCG) or LH. In spontaneous ovulators, designing of estrus induction protocol isa big challenge due to sporadic production of progesterone thereby compromising the effectiveness of gonadotropins (Howard and Wildt, 2009).

The ovarian response to exogenous hormones is one of the key successes in AI. The ovarian sensitivity to gonadotropins is highly variable in felids species, and successful ovarian stimulation protocol for one species may not be suitable for another species (Pelican *et al.,* 2006). Therefore, an optimal dosage of hormones used for induction of estrus and ovulation is essential. In felids, a combination of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) is commonly used exogenous hormones for induction of estrus and ovulation. According to previous studies, the dose of eCG and hCG for induction of estrus and ovulation, respectively for successful AI was 100 IU and 75 IU for leopard cat, tigrina and clouded leopard, 600 IU and 300 IU for snow leopard, 200 IU and 200IU for cheetah and puma, and 100IU and 750IU for tiger (Thongphakdee et al., 2018). However, in fishing cat, the combination of PMSG (150/200 IU) and hCG (100 IU) failed to achieve pregnancy. The endocrine changes associated with the development of postovulatory follicles and corpus lutea (CLs) following administration of hCG alter the tonicity of the oviductal/uterine conditions as in natural estrus

resulting in delayed fertilization and subsequent implantation and fetal development (Swanson *et al.,* 1996). To suppress the postovulatory follicular development, a combination of eCG and porcine luteinizing hormone (pLH) was evaluated in domestic cats as an alternative to eCG/hCG (Conforti *et al.,* 2013). Higher pregnancy rates were recorded in eCG/pLH treated domestic cats than eCG/hCG treated females and also the number of ancillary CLs on day 20 post AI was more in eCG/hCG treated group. Healthy offspring were successfully produced after AI with eCG/pLH treated females in Ocelot (*Leopardus pardalis*), Pallas cat (*Otocolobus manul*), and tiger (*Panthera tigris*) after AI with eCG/pLH treated females (Conforti *et al.,* 2013), and more recently, a combination of eCG (200 IU) and pLH (1000 IU) supported the establishment of pregnancy in clouded leopard (Tipkantha *et al.,* 2017).

India houses 15 wild cat species that includes five big cats, eight medium-sized wild cats and two small cats, as per the IUCN Red List of Threatened Species (IUCN 2021), majority of which are listed as threatened, vulnerable, near threatened or endangered. In order to protect the existing wild cat population as well as to increase the population size of these wild felids, several government and non-government agencies have been working together to implement various management practices. In India, studies on reproductive physiology and ARTs in big cats started three decades ago by collecting semen from Asiatic Lions of Gir forest in Sakkarbaug zoo, to compare the reproductive and genetic consequences of isolated populations of Asiatic lions and African lions by American Group of researchers (Wildt *et al.,* 1987). Semen characteristics, serum testosterone levels and allozyme heterogeneity studies revealed that Asiatic lions in Gir forest are highly inbred and have low genetic variation (Wildt *et al.,* 1987). However, subsequent molecular studies using randomly amplified polymorphic DNA, microsatellite analysis and multi locus finger printing indicated that low genetic variability was a characteristic feature of Indian tigers and lions and not associated with inbreeding (Sankarnarayanan *et al.,* 1997). Further, to ascertain the inbreeding depression in Indian big cats, CCMB carried out semen and hormonal studies in captive wild felids from various Indian zoos. Shivaji *et al.,* (1998) estimated serum testosterone levels, semen profiling and also fertilizing capacity of spermatozoa of captive lions and tigers from three Indian zoos and suggested that Asiatic Lions and Indian Tigers were not inbred. Jayaprakash *et al.,* (2001) demonstrated spermatology and fertilizing capacity of fresh and cryopreserved semen of leopards. Subsequently, attempts have been made to optimize the protocols for AI in big cats in which the females were induced to estrus and ovulation by administration of eCG followed by hCG, respectively (Shivaji *et al.,* 2003). The behaviour and length of estrus in Asiatic lion during natural and induced estrus was also studied by estimating fecal estrogen and progesterone (Umapathy *et al.,* 2007). The estrus behaviour and average length of estrus (5.4 d) were similar in both, natural and induced estrus lions.

In continuation of our previous studies on assessment of inbreeding depression in Indian lions, tigers and leopards, attempts were made in big cats with the objective to develop efficient estrus induction and AI protocol. The leopard was chosen as a model species for optimizing estrus induction and AI protocol for big cats. Leopard is the only Indian big cat abundantly present in wild as well as in captivity. The Leopard (Panthera pardus fusca) is listed as "Near Threatened" in IUCN Red List of Threatened Species (IUCN 2021) and also declared as an endangered species under Schedule I of Indian Wildlife (Protection) Act, 1972.

Methods

All the culture media and chemicals used in this study were purchased from Sigma Chemical Co., USA and plastic ware from Nunc, Denmark. The medium used for diluting semen ejaculates were supplemented with penicillin (100 IU/ml) and streptomycin (0.1 mg/ml) and filter (0.22 μm) sterilized. The anaesthetic drugs used were procured from Troy laboratories Pty. Ltd., Smithfield, NSW, Australia.

Care and maintenance of leopards-

All the experiments on leopards were carried out in assisted reproduction unit (Fig.1.A) in CCMB-LaCONES with prior approvals from the Central Zoo Authority of India (CZA), Forest Department, Government of Telangana and Institutional Animal Ethics Committee (IAEC) of CCMB. A total of six adult females from NZP, Hyderabad, SVZP, Tirupati and IGZP, Visakhapatnam were shifted to LaCONES-CCMB during 2009-2014 in a phase manner. Six leopards (3 males and 3 females) were replaced with new animals. One female was not used for AI due to its abnormal reproductive behaviour. The management and health of leopards were taken care as per the guidelines provided by the CZA. All the leopards were provided with enough space, feed (2 kg beef and 1 kg chicken) daily for six days in a week and potable water 24x7.

Anaesthesia

Male and female leopards were anaesthetized with a ketamine-xylazine combination as described earlier in our laboratory for semen collection (Jayaprakash *et al.,* 2001; Shivaji *et al.,* 2003; Sontakke *et al.,* 2009). Briefly, all leopards were withheld feed and water for 24 h and 12 h respectively before administering anaesthetic drugs. A combination of xylazine hydrochloride (1.1 mg/kg body weight), and ketamine hydrochloride (2.2 mg/kg) was used as anaesthesia (Sontakke et al., 2009). All the anaesthetized leopards were recovered from anaesthesia by an intravenous administration of yohimbine hydrochloride (0.15 mg/kg body weight) at the end of semen collection and artificial insemination (Sontakke *et al.* 2009).

Semen collection

Semen was collected by electroejaculation procedure (Fig.1.B) as optimised in our laboratory and described earlier (Jayaprakash *et al.,* 2001; Shivaji et al., 2003). Briefly, after the attainment of surgical plane of anaesthesia, leopard was placed in the lateral recumbence on the surgical table/stretcher. After thorough cleaning of the surrounding areas of rectum, scrotum, prepuce and penis with mild disinfectant solution and distilled water, a pre- lubricated rectal probe of 3 cm diameter with having 3 longitudinal electrodes was gently inserted into the rectum of the animal. The electrodes of the probe were positioned ventrally (against the male accessory sex glands). A total of eighty electrical (2 to 4V) stimuli divided in three series were applied to induce ejaculation. Each stimulus at the desired voltage was given for 2 seconds with a gap of 2 seconds between the stimuli. The animals were rested for 5 minutes between each series of stimuli. Ejaculates from each series of stimuli were evaluated separately for each animal. Ejaculates with 60-70 percent of motile spermatozoa (Fig.2. A) were immediately diluted with pre-equilibrated HAM'S F-10 medium supplemented with BSA and penicillin (100 U/ml) and streptomycin (100 mg/ml) and pooled for insemination.

Induction of estrus and ovulation

Adult female leopards were administered intramuscularly with 100 IU of pregnant mare serum gonadotropin (PMSG) followed by 80 IU of human chorionic gonadotropin (hCG) 80 h later, for induction of estrus and ovulation respectively.

Trans-cervical artificial insemination

Insemination was carried 36-40 h after hCG

administration. Leopard was placed in dorsal recumbency with raised hind quarters. The entire area surrounding rectum and vagina was cleaned with disinfectant and water. Processed semen containing about 50 million live sperm was loaded into a sterile insemination catheter which is commercially available for intra uterine inseminations in humans. The catheter was inserted deep into the cervix through endoscopy and semen was deposited in the female tract (Fig.1 C). After insemination, the female was allowed to keep for 10-15 min in the same position to avoid back flow of semen.

Intra-uterine insemination

Endoscope-guided laparoscopic intrauterine insemination (LUI) technique was used for deposition of semen into the uterine horn as described in cat species (Swanson et al., 2006). After aseptic preparation of the abdominal region, pneumoperitoneum was created by pumping CO2 through veres needle inserted through the abdominal wall (Fig.1.D). After obtaining sufficient pneumoperitoneum, one mid ventral laparoscopic port (5 mm) was made for inserting rigid laparoscope. After visualization of the reproductive tract and confirmation of ovulation, another port of 5 mm diameter was made lateral to the mid ventral port (either right or left) for inserting grasping forceps. The uterine horn was grasped gently by grasping forceps at utero-tubal junction. Fresh diluted semen was deposited into the lumen of the uterine horn through a 22 G IV catheter which was inserted into the uterine horn through the abdomen while observing through laparoscope. The inseminated uterine horn was thoroughly checked for any bleeding points before closing the incisions. Post-operative care including parental administration of antibiotics was taken care until the incision wound was healed.

Results and Discussion

We have tried different approaches for AI with limited number of animals however; unfortunately, we did not achieve any pregnancy. Here we are summarizing our research experiences on estrus induction and AI studies in leopards carried out in CCMB-LaCONES. Initially, AI in leopards was started with two females (Animal IDs. -Deepa and Shriya) and three males (Animal IDs. -Srinivas, Ayyappa and Venky) in the year 2009. The procedures for anaesthesia, electro ejaculation technique for semen collection and evaluation and handling of leopard semen were followed as optimised in CCMB and described earlier (Shivaji *et al.,* 1998; Patil *et al.,* 1998; Jayaprakash *et al.,* 2001; Shivaji *et al.,* 2003, Sontakke *et al.* 2009). The semen ejaculates of three male leopards were thoroughly evaluated before AI. The ejaculate volume ranged between 0.8 - 3 ml having sperm motility of 25-85% and mean sperm concentration of 31.05 ± 22.33 million per ml (20-75 x106/ml). Due to old age and presence of higher percentage of abnormal spermatozoa (>50%) in the ejaculates, two male leopards (Srinivas and Ayyappa) were replaced with two new leopards (Deva and Shyam). All the females were monitored behaviourally for 2-3 naturally occurring estrous cycles for any reproductive abnormalities before inducing estrus. Accordingly, estrous was induced in females having regular cyclicity, using injections eCG (100 IU) and hCG (75 IU) at an interval of 80h between them. Insemination was carried out 36-40 h after hCG administration. If the female was not conceived, the next estrus induction cycle was initiated 3-4 months after first AI. This transient period allowed females to neutralize antibodies produced against the exogenous hormones of the previous estrus induction (Howard *et al.,* 1992). After two AI attempts, two females have shown purulent discharge from vagina due to development of cystic endometrial hyperplasia (CEH) like signs. One of the females was recovered after clinical treatment

but second one could not be recovered. The incidence of CEH in cats is not common, but in nulliparous cats, prolonged exposure of uterus either to estrogen during anovulatory cycles or to progesterone during ovulatory cycles(pseudopregnancy)cause endometrial proliferation (Potter *et al.,* 1991; Graham et al., 2000). Whereas in these two leopards the source of progesterone may be from the ancillary CLs formed by postovulatory follicles following the administration of hCG (Swanson *et al.,* 2006). We have observed that some of the eCG/hCG treated females showed prolonged oestrous behaviour or short cycles even after the administration of hCG. We suspected that 80h following administration of eCG might not sufficient for follicular development, so in next two induction cycles, hCG was given 96 h after eCG. Unfortunately, one of the females died during the study period. On post-mortem, both the ovaries were found to have approximately 100 CLs (Fig.2B). Presence of unexpected number of CLs following the treatment of eCG/hCG indicates hyper-stimulation of ovary as reported earlier in domestic cat and cheetah (Howard *et al.,* 1992; 1997). Keeping in view of ovarian hyper-stimulation, pLH was suggested as an alternative to hCG, and live kittens were born in domestic cat, Ocelot, Pallas cat and tiger with AI after eCG/pLH treatment (Conforti *et al.,* 2013). We have also tried this combination in one of the female but did not succeed in inducing estrus.

In the present study, failures to establish pregnancy by AI in leopards may be attributed to various reasons-
1. Limited number of females (06) with unknown breeding history were used for this study. Moreover, all these animals were acquired from zoo exhibits and majority of them were aggressive in nature, obese, and no history of pregnancy. In previous study elsewhere, 18 cheetahs and 23 clouded leopards were used for optimizing the doses of gonadotropins for AI, and only 6 of 13 (46%) cheetahs became pregnant but none of the clouded leopards became pregnant with the same dose of hormones (Howard *et al.,* 1997) indicating that the hormonal doses are independent of body mass and may vary from species to species.

2. In our study, females were repeatedly used for induction of estrus that might resulted in chances of developing either ovarian refractoriness or hyper-stimulation to gonadotropins, and indeed, the later was evidenced in one of our leopards that died. More than half of the induced females (3/5) have shown short estrous cycles (10-13 days) in the subsequent cycles for more than six months. Optimal time for AI in felids was immediately after ovulation (Howard et al., 1992;1996;1997). In the present study, AI was performed after 36-40 h after hCG. We have observed one or two anovulatory follicles present on ovaries of one of the females during laparoscopy guided intra-uterine insemination (LUI) which indicates it is necessary to establish optimal time for AI in leopards with respect to anaesthesia.

3. The site of semen deposition and the approach play an important role in the success of AI in felids. From the literature, it has been observed that laparoscopic intrauterine/oviductal insemination has more advantages over transcervical insemination, specifically the availability of normal spermatozoa per insemination and the disturbances associated with ovulation, fertilization and migration of gametes and embryo with respect to anaesthesia. Laparoscopic insemination procedures require minor surgical interventions to access reproductive organs. Importantly, length and diameter of the laparoscopic instruments must be redesigned according to the size of animal (Swanson *et al.,* 1996). Live kittens were successfully produced by laparoscope-guided intrauterine or oviductal insemination in eCG /pLH treated domestic cat as well as in wild cats (Conforti *et al.,* 2013).

Figure 1 Artificial insemination Approaches in leopard: Assisted reproduction unit at CCMB-LaCONES (A), Semen collection through electroejaculation procedure (B), endoscope-guided transcervical artificial insemination (C) and laparoscopic-guided intrauterine insemination (D).

After several unsuccessful attempts of non-invasive intrauterine insemination with different approaches (endoscopic guided, elevated hind quarters as in the natural mating), we decided to inseminate using LUI and two attempts were made using LUI but pregnancy did not ensue. A combination of a reliable ovarian stimulatory regime, insemination approach and time of AI with respect to ovulation and anaesthesia is an essential prerequisite for successful AI in wild felids. At the same time, collection of uncontaminated (devoid of urine) sperm ejaculates and optimization of species-specific sperm extender and its processing is equally important in males before attempting any AI in wild felids. Therefore, basic research on reproductive physiology is always warranted to optimize responsiveness of dosages of ovarian stimulating hormones as well as sperm physiology in each species to develop reliable reproductive techniques.

Future Directions

Apart from the biological constraints, logistic issues also need to be addressed. The most important one is the access to wild animals for experimental research in assisted reproduction. A strong co-ordination between stakeholders such as the Central Zoo Authority, a statutory body of the Ministry of Environment, Forest and Climate Change (Government of India) which regulates the zoos across the country, the state forest departments and zoos is the most critical and essential to resolve this issue. The forest departments and zoos should come forward to support developing and adopting new technologies for wildlife species conservation breeding programs.

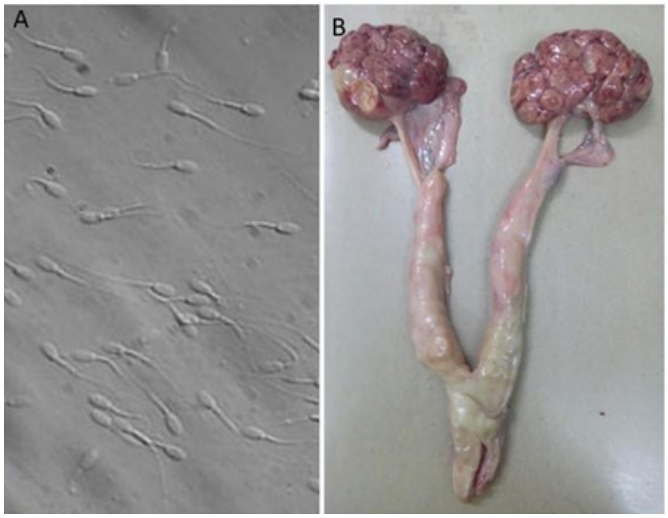

Figure2. A) Spermatozoa of leopard collected through electroejaculation. B) Hyper-stimulation of leopard ovaries, the reproductive tract was collected post-mortem from a leopard induced with human chorionic gonadotropin hormone for artificial insemination.

Acknowledgments

This research was supported by grants from the Central Zoo Authority, Government of India, and Council of Scientific and Industrial research (CSIR), Government of India. The authors thank the Chief Wildlife Warden of Telangana and the Director of Nehru Zoological Park, Hyderabad.

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HAND REARING OF HYENA CUBS AT NANDANKANAN ZOOLOGICAL PARK, ODISHA

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Abstract

Five rescued striped hyena cubs were hand reared at Nandankanan Zoological Park, Odisha between November 2015 and March, 2018. The present communication reports housing and husbandry practices, and growth rate of the hyena cubs during hand rearing.

Introduction

The striped hyena (*Hyaena hyaena*) are carnivorous mammals. They have grayish coat transverse with stripes of black or brown on their body and legs (Crandall, 1964). Nandankanan Zoological Park, Odisha (NKZP) received four rescued hyena cubs (3F:1M) of one litter from Phulnakhara area of City Forest Division, Bhubaneswar on 21.11.2015 with an estimated age of 10 days and 592.5±33g of weight. Eyes of one cub were partially open at the time of receipt which opened on the 3rd day of hand rearing and the other three cubs had opened eyes. Heptner and Sludskii (1992) reported that striped hyenas are born blind and start seeing clearly at the seventh to eighth day of age. Another female striped hyena cub was received in the Park from Malkangiri Forest Division on 17.05.2017 at an estimated age of 15 days and 815g of weight. Physical examination was carried out for the presence of any injury or deformities. The cubs were found emaciated/dehydrated. The communication is an attempt to put on record of our experience of hand rearing these hyena cubs.

Housing: The hand rearing of the hyena cubs in both the cases were carried out under similar housing conditions. In the first case all four cubs were housed together and in the later case the single cub was housed solitarily. Initially, the cubs were kept in a ply board box of 2'X2'X2' size having a wire meshed roof with cotton towels as bedding material. One 60 watt electric bulb was placed at a height of about 2' from the box to give warmth to the cubs. A thermometer was kept inside the box to monitor temperature fluctuation and accordingly the height of the bulb was adjusted. The cubs were shifted to a wooden box of 4'X4'X3' size towards the end of the first month of hand rearing. On completion of the third month of hand rearing they were shifted to kraal with 12'X6' soil substrate floor area and surrounded by 7'heigh chain link mesh.

Feeding: The cubs were bottle fed with reconstituted Royal Canin baby dog milk powder (Fig.1.). On the first day the milk was mixed with glucose and electrolyte to restore energy and electrolyte balance. The reconstituted milk was prepared by diluting 50gm of milk powder in 120ml of lukewarm water. Initially, the cubs were fed 12 times a day at 2hrs intervals with 10ml of milk per feeding, through a feeding bottle.

The feed was supplemented with one ml of Gripe water, one ml each of Proviboost and Provical pet3 syrups per day. In the 3rd week of hand rearing, the late night feeding was discontinued and 10 feedings were given in a day from 6AM to 12AM. Gradually, the milk quantity per day was increased to 450ml/ day and the number of feedings per day was reduced to 6 feedings/ day in the 7th week of hand rearing. Solid food (minced chicken meat) was introduced in the 8th week of hand rearing @ 50gm per cub. With the introduction of solid food (meat), milk consumption was reduced gradually to 150ml/day in 4 months of hand rearing and meat consumption gradually increased to 200gm per cub per day. Milk was completely withdrawn at 6 months age and replaced with chicken meat @ 500g per cub per day.

Health care: On receipt, the infants were examined for external injury, hydration status, body temperature and response to handling. Examination of faecal samples and measurement of body weight of cubs were carried out on arrival and continued at regular intervals. On 03.01.2016 (6th week of hand rearing), biting injuries on the ear tip were observed in the group housed hyena cubs. The wounds were caused as littermates were suckling each other's pinna. The wounds were dressed aseptically with Betadine lotion. To get rid of the vice, chewing bones were provided in the rearing box at rate one commercially available artificial bone per cub per week. The cubs were observed busy with chewing bone and the wounds healed up in a few days. No such injuries occurred in the second case of hand rearing of a single hyena cub. The pinna injury may be associated with social housing and eruption of teeth. The cubs were vaccinated with multivalent vaccine Megavac-6 against Canine distemper, Hepatitis, Parvo and Leptospira on completion of two months of hand rearing and repeated again on completion of four months followed by annual booster.

Fig. 1. Bottle feeding of hyena cubs under hand rearing at Nandankanan Zoological Park.

Results and discussion

The hyena cubs showed apparently steady growth rate during hand rearing (Fig.2 a,b) At the first episode of rearing of four cubs in November, 2015, growth was recorded as from 592.5±33g during first week of hand rearing, to 7195±182.3g during 14th week of rearing (Table-1). In the second episode of rearing in May 2017, the lone hyena cub had shown a growth from 850g in 2 weeks of age to 14250g in 27 weeks of age (Table-2).

Though hyenas breed well in captivity, the main problem with this species is the large failure rate in rearing young (Rieger, 1979) and may need hand rearing. Rao *et al.* (1995) reported the hand rearing of striped hyena cubs at Arignar Anna Zoological Park, Chennai and studied its growth rate from day 7 to day 84. Kholkute (2001) reported the rearing of orphaned striped hyena cubs from the age of one month. Batwe

(1988) reported a hand rearing episode of two striped hyena cubs at Sanjay Gandhi National Park, Mumbai. Mahodaya (1997) reported hand rearing of three zoo born hyena cubs at Indore Zoo that are segregated from the mother due to lack of maternal care. Senthilkumar and Thirumurugan (2006) reported hand rearing of two zoo born striped hyena cubs at Arignar Anna Zoological Park, Chennai that were weaned following aggressive behavior of the mother hyena towards them.

In the present case the five striped hyena cubs were found abandoned in the forest, rescued and brought to NKZP for hand rearing. The hyena cubs were fed with reconstituted 'Royal Canin Baby dog milk' which contains 31% crude protein, 37% crude fat, 4.5% moisture and 6% ash (manufacturer's information). Rao et al (1995) and Kholkute (2001) reported raising of hyena cubs with boiled and cooled cow's milk. Batwe (1987) recommended diluted toned milk with 3.0% fat and 8.5% SNF for hyena cubs. Mahodaya (1997) reported the start of dentition at 5th week of age. Here at Nandankanan, in the first case eruption of teeth was observed at about 6 weeks of age which accompanied development of a vice of biting each other's pinna.

Hand rearing of abandoned, orphaned or rescued young wild animals is one of the important and challenging tasks carried out in the zoological parks. Successful hand rearing experiences serve as a guide to manage such situations that will arise in future and give confidence to the concerned staff.

Table 1. Body weight of four striped hyena cubs (received on 21.09.2015) during hand rearing period

Date	Estimated age in weeks	Average weight in gram ± Standard deviation(n=4)	Range (Maximum and minimum weight in gram)
21.11.2015	10 days	592.5±33	545-615
28.11.2015	17 days	865±55	810-930
07.12.2015	3 weeks	1149±79	1056-1240
15.12.2015	4 weeks	1499±125.5	1390-1615
21.12.2015	5 weeks	1720±78.3	1640-1820
28.12.2015	6 weeks	2316±105	1935-2135
03.01.2016	7 weeks	2018±84.7	2230-2450
12.01.2016	8 weeks	3227±133	3095-3395
18.01.2016	9 weeks	3576±78	3470-3640
24.01.2016	10 weeks	4115±162	3970-4270
01.02.2016	11 weeks	4830±417.5	4300-5220
07.02.2016	12 weeks	5239±588.7	4420-5675
14.02.2016	13 weeks	5926±427	5420-6350
22.02.2016	14 weeks	7195±182.3	6995-7435

Table 2. Body weight of the lone striped hyena cub (received on 17.05.2017) during hand rearing period.

Date	Estimated age in weeks	Weight in gram (n=1)
17.05.2017	2 weeks	815
24.05.2017	3 weeks	1320
01.06.2017	4 weeks	1620
09.06.2017	5 weeks	1750
16.06.2017	6 weeks	2300
22.06.2017	7 weeks	2750
28.06.2017	8 weeks	3200
06.07.2017	9 weeks	3620
12.07.2017	10 weeks	4370
18.07.2017	11 weeks	4780
24.07.2017	12 weeks	5200
01.08.2017	13 weeks	5500
11.08.2017	14 weeks	5750
22.08.2017	16 weeks	6220
31.08.2017	17 weeks	7820
13.09.2017	19 weeks	9960
22.09.2017	20 weeks	10330
04.10.2017	22 weeks	11200
21.10.2017	24 weeks	12370
11.11.2017	27 weeks	14250

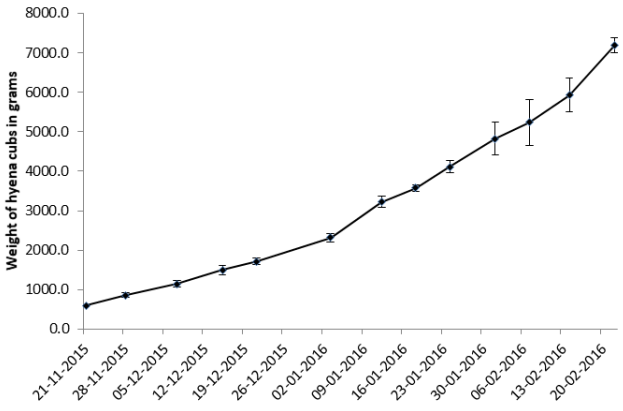

Fig.2a. Growth rate of four striped hyena cubs hand reared at Nandankanan Zoological Park received on 21.09.2015.

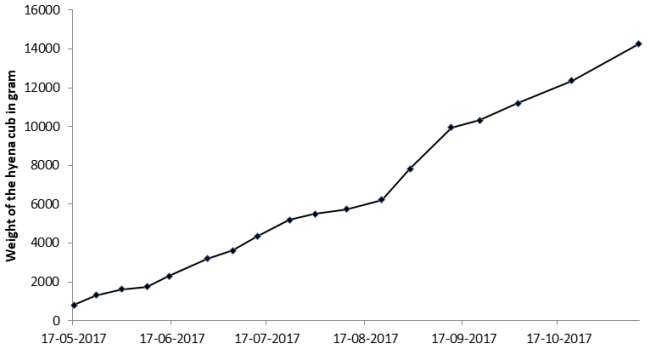

Fig.2b. Growth rate of the lone striped hyena cub hand reared at Nandankanan Zoological Park received on 17.05.2017.

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MOLECULAR IDENTIFICATION OF NUBIAN GIRAFFE (*Giraffa camelopardalis camelopardalis*) IN THE ZOOLOGICAL GARDEN ALIPORE, KOLKATA, INDIA

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Abstract

India holds a total of 29 giraffes (*Giraffa spp.*) across its zoological parks. A traditional opinion-based pelage pattern-based classification of India's captive giraffe population has been used to date. However, this classification is unreliable and highlights the need for genetic and phylogenetic analyses. This pilot study on captive giraffes in India investigated genetic composition of giraffes at the Zoological Garden Alipore, Kolkata (Alipore Zoo), to assess their taxonomic status. Two mitochondrial genes i) Cytochrome b (CYTB) of 1140 base pair region ii) Control Region (CR) of 416 base pair region, were amplified and sequenced using standard protocols. The results show that the giraffes at Alipore zoo are the critically endangered Nubian giraffe (*G. camelopardalis camelopardalis*), a subspecies of the northern giraffe (*G. camelopardalis*).

Key Words Captive Giraffe, Alipore Zoo, Mitochondrial DNA, Species Identification, Nubian giraffe

Introduction

The taxonomy of giraffe has been questioned for more than a century (Thomas, 1901; Lydekker, 1904; Dagg & Foster, 1976; Brown et al. 2007; Groves & Grubb, 2011). Whilst the International Union for Conservation of Nature (IUCN) Red List assessment for giraffe currently recognizes only one species of giraffe (*G. camelopardalis*) and nine subspecies, a taxonomic review of the taxon has been recommended (Muller *et al.,* 2018). The first detailed genetic analyses of all major populations across their current range in Africa established that there are four distinct giraffe species (Fennessy *et al.* 2016; Winter *et al.* 2018; Coimbra *et al.* 2021). The validated four species are: Masai giraffe (*G. tippelskirchi*) (including the proposed Luangwa giraffe (*G. t. thornicrofti*)), Northern giraffe (*G. camelopardalis*) (including three subspecies: Kordofan (*G. c. antiquorum*), Nubian (*G. c. camelopardalis*) synonym: Rothschild's (*G. c. rothschildi*), and West African giraffe (*G. c. peralta*)), Reticulated giraffe (*G. reticulata*), and Southern giraffe (*G. giraffa*) (including two subspecies Angolan (*G. g. angolensis*) and South African giraffe (*G. g. giraffa*). Although Brown *et al.* (2007) considered Rothschild's giraffe a separate species, they did not analyzed Nubian giraffe or make a clear distinction between them and Rothschild's giraffe. In last two decades increased taxonomic studies assessing morphology, ecology and genetics has clearly indicated that the Nubian and Rothschild's giraffes are undistinguishable (Kingdon, 1997; East 1999; Brown *et al.* 2007; Hassanin *et al.* 2007; Groves & Grub, 2011; Thomassen et al. 2013; Ciofolo, & Le Pendu 2013; Fennessy *et al.* 2016; Winter et al. 2018; Coimbra *et al.* 2021). As such, Rothschild's giraffes were synonymized with the Nubian giraffe as per taxonomic protocol (Fennessy *et al.,* 2016, Winter *et*

al. 2018; Coimbra *et al.* 2021).

The updated taxonomy of giraffes questions the genealogy status of captive giraffes in Indian zoos, as their provenance in the wild or initial acquisitions are largely unknown. The current captive population of 29 giraffes in India is made up of individuals from various age classes. The Zoological Garden Alipore, Kolkata (Alipore zoo) and the Sri Chamarajendra Zoological Gardens, Mysore (Mysore zoo) house the largest herds, ten and seven giraffes, respectively. In the present genetic study on captive giraffes in India, two mitochondrial genes of giraffes at the Alipore zoo were investigated for better understanding of their taxonomic status.

Materials and Methods

Alipore zoo is located in West Bengal, India, hosts the largest captive herd of 10 giraffes (4M: 6F) among Indian zoos (Table 1). Their provenance from the wild was largely unknown at the time of sample collection.

Using a non-invasive sampling method, i.e., fresh dung samples of the above ten giraffes were collected following standard protocol and sent for laboratory analysis. Genomic DNA was isolated from the peripheral mucus of the samples using the QIAamp DNA Mini Kit (Qiagen). The genomic DNA concentration and purity was estimated by the NanoDrop 2000 nanophotometer (Thermo scientific™). A 1140 bp long Cytochrome B (CYTB) gene and 416 bp section of the Control Region (CR) were subsequently amplified using the Real Time PCR (Applied Biosystems™ StepOne™) following standard protocol. The details of primer sequences used for these two loci are given (Table 2). The CYTB gene was amplified in two sections considering the length of the target sequence.

Amplification conditions followed initial denaturation at 94°C for 30 seconds. Each cycle included a 30 second extension at 72°C and was followed by a final extension at 72°C for ten minutes. Sequencing was done by the Sanger di-deoxy method using the ABI automated sequencer (ABI 377, Applied Biosystems) employing the same primers (Sanger et al. 1977). The sequences were deposited in NCBI GenBank, and accession numbers were generated (Table 3).

The chromatograms of all ten giraffes were aligned in the R package for Multiple Sequence Alignment (msa R) (Bodenhofer et al. 2015) and found to be identical for all. Therefore, only one sequence was used from each mitochondrial region for further analysis. FASTA sequence formats were created using MEGA 7.0 software (Kumar et al. 2015). Sequences of each gene were aligned using MUSCLE Software (Edgar, 2004). FASTA Sequences were BLASTed in NCBI BLAST (Johnson et al. 2008). The 1140 bp of CTYB and 416 bp of CR regions of all the downloaded sequences (Table 3) and Alipore Zoo sequences were aligned and concatenated using MEGA 7.0 software.

Results and Discussion

The 1140 bp sequence of the CYTB gene Alipore zoo giraffe showed 99.91% similarity to Rothschild's giraffe sequences deposited in NCBI GenBank (NCBI accession No - HG975130.1 deposited by Bock et al. 2014) and 99.82% to the Nubian giraffe sequences deposited in NCBI GenBank (NCBI accession No - MG262281.1 deposited by Winter *et al.* 2018).

The 416 bp Control region (CR) sequence of Alipore zoo giraffe showed 100% similarity to Rothschild's giraffe sequences deposited in NCBI GenBank (NCBI accession No - HG975232.1 deposited by Bock et al. 2014) and 99.28% to the Nubian giraffe sequences deposited in NCBI GenBank (NCBI accession No - LT628369.1 deposited by Fennessy *et al.* 2016).

Table 1. Name, Sex, Age Class and Date of birth of the ten giraffes in Alipore zoo, Kolkata

(*Age Class - Calf: 0-1 year; Subadult: 1-5 years; Adult: 5+ years)

Sr. No.	Giraffe Name	Sex	Age Class*	Date of Birth
1	Roshan	Male	Adult	05-05-2000
2	Sabuj	Male	Adult	28-01-2002
3	Trina	Female	Adult	24-01-2005
4	Mangal	Male	Adult	02-02-2010
5	Laxmi	Female	Adult	21-12-2011
6	Bithi	Female	Subadult	21-05-2013
7	Chitra	Female	Subadult	06-12-2014
8	Calf -1(Not named)	Male	Subadult	27-06-2015
9	Calf - 2 (Not named)	Female	Subadult	25-05-2016
10	Calf -3 (Not named)	Female	Calf	13-02-2017

Table 2. Details of primer sequences and annealing durations of the captive giraffe genomic DNA of Alipore zoo

Targeted Region	Primer Id	Oligo Sequence	Annealing Duration / No. of Cycles
CTYB – I Fragment	CBIF	5'ATAATCGCCCCCAAAACAGT3'	52^0C for 30 Sec (3 Cycles) 50^0C for 30 Sec (3 Cycles) 48^0C for 30 Sec (24 Cycles)
	CBIR	5'GTGCCGATTTATGGGATTGC3'	
CTYB – II Fragment	CBIIF	5'TGGGGCATCCACATTCTTCA3'	57^0C for 1 Min (35 Cycles)
	CBIIR	5'GGCAATGGCTCCTTCCTT3'	
CR	CRF	5'CCACTCTCCCTAAGACTCAA3'	54^0C for 30 Sec (3 Cycles) 52^0C for 30 Sec (3 Cycles) 50^0C for 30 Sec (24 Cycles)
	CRR	5'AAAATACCAAATGCCTTACA3'	

Table 3. Details of Mitochondrial gene deposited, and the corresponding accession number generated by NCBI upon deposition

Sr. No.	Mitochondrial gene	NCBI GenBank Accession Number
1	Cytochrome B (CYTB)	MW991403.1
2	Control Region (CR)	MW991402.1

Table 4: Accession numbers of downloaded sequences related to giraffes from NCBI.

Accession No	Deposited by	Sub(species)	Mitochondrial Gene
HG975130.1	Bock *et al*., 2014	*rothschildi*	CYTB
MG262281.1	Winter *et al.,* 2018	*camelopardalis*	CYTB
EF442272.1	Hassanin *et al*., 2007	*reticulata*	CTYB
EF442263.1	Hassanin *et al*., 2007	*angolensis*	CTYB
EF442268.1	Hassanin *et al*., 2007	*antiquorum*	CTYB
EU088345.1	Brown *et al*., 2007	*giraffa*	CTYB
EF442274.1	Hassanin *et al*., 2007	*peralta*	CTYB
HF571167.1	Fennessy *et al*., 2016	*thornicrofti*	CTYB
EF442269.1	Hassanin *et al*., 2007	*tippelskirchi*	CTYB
EU088352.1	Brown *et al*., 2007	*Okapia johnstoni*	CTYB
HG975232.1	Bock *et al*., 2014	*rothschildi*	CR
LT628369.1	Fennessy *et al*., 2016	*camelopardalis*	CR
LT628385.1	Fennessy *et al*., 2016	*reticulata*	CR
LT628388.1	Fennessy *et al*., 2016	*angolensis*	CR
HG975281.1	Bock *et al.,* 2014	*antiquorum*	CR
LT628392.1	Fennessy *et al*., 2016	*giraffa*	CR
HG975290.1	Bock *et al.,* 2014	*peralta*	CR
HF571176.1	Fennessy *et al*., 2016	*thornicrofti*	CR
HG975264.1	Bock et al., 2014	*tippelskirchi*	CR
AY135359.1	Baysdorfer *et al.,* 2002	*Okapia johnstoni*	CR

The similarity of the Alipore zoo giraffe sequences (CYTB, CR) with those deposited in NCBI GenBank clearly shows that the Alipore Zoo giraffe sequences match most with Nubian and Rothschild's cluster than any other sub (species) of giraffe.

The concatenated tree output placed the query sequences of the Alipore Zoo giraffe in the Nubian and Rothschild's giraffe cluster with 96% bootstrap support (Figure 1). As only two genes (CYTB and CR) of mitochondrial DNA of Alipore zoo giraffe were sequenced during this study, the concatenated phylogenetic tree recognised reticulated giraffe in the northern giraffe cluster resulting in three separate species; Northern giraffe, Masai giraffe and Southern giraffe (Figure 1). However, Coimbra *et al.* (2021), Winter *et al.* (2018) and Fennessy *et al.* (2016) undertook mitochondrial, nuclear and genomic level analysis of giraffe from across their range in the wild which clearly showed distinct separation between these two species (reticulated giraffe and northern giraffe), thus delineating the giraffe into four different species.

Fennessy *et al.* (2016) for the first time genetically analysed samples from all major giraffe populations across their range in Africa, including Nubian giraffes from the wild for the first time. An extended study by Winter *et al.* (2018) and (Coimbra *et al.* 2021) of detailed nuclear and genomic analyses corroborated the findings of Fennessy *et al.* (2016). In 2020 Petzold and Hassanin, and Petzold *et al.* proposed three species taxonomies based on a varied analysis of Fennessy *et al.* (2016) and Winter *et al.* (2018) data, as well as inclusion of some additional museum samples. However, based on the latest updated genomic level analysis of Coimbra *et al.,* (2021), building on from the findings of Fennessy *et al.* (2016) and Winter *et al.* (2018), four distinct species and five subspecies of giraffe (subsuming Rothschild's giraffe within

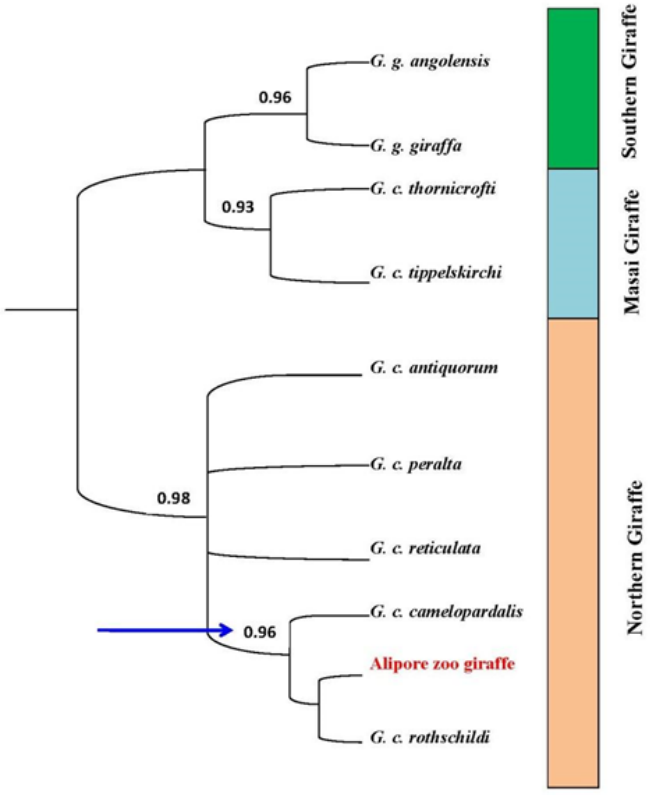

Figure 1: Phylogenetic analysis to identify the affinities of the ten giraffes in Alipore Zoo, India

Nubian giraffe) are identified based on the primary mitochondrial, nuclear and genomic data.

Inferring the results of this study with those of Coimbra *et al.* (2021), Winter *et al.* (2018) and Fennessy *et al.* (2016), we conclude that the giraffes at Alipore Zoo are Nubian giraffes. The outputs from this study will hopefully aid in future breeding and management programs of giraffes at Alipore Zoo and the Indian zoos that acquired giraffes from the said zoo. Undertaking similar genetic studies on giraffes at other Indian zoos is a matter of future study.

Conclusion

We conclude that the giraffes at Alipore Zoo are the Nubian subspecies belonging to Northern giraffe species and native to areas of Ethiopia, Kenya, South Sudan and Uganda. Future genetic sampling of giraffes in all Indian zoological facilities is recommended to understand their taxonomic status in support of their long-term management and

conservation of genetic diversity.

Acknowledgements

I wish to thank the West Bengal Zoo Authority, Kolkata, West Bengal, for their permission to undertake the study (order no: 515/WBZA/T-2/17-18 dated 16 October 2017). I would like to thank Mr. Vinod Kumar Yadav (West Bengal Zoo authority), Mr. Asis Kumar Samanta, Dr. Kanailal Ghosh, Mr. Ramchandranath, Mr. Sitaram, Mr. Mohammed Jamaluddin (Zoological Garden Alipore), Head of the Department and staff at the Molecular Biodiversity Lab, Department of Zoology & Wildlife Biology, Government Arts College, Ooty, Dr. Prayag Hodigere Siddalingappa (Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru), Dr. Sven Winter (Senckenberg Biodiversity and Climate Research Centre (SBiK-F), Germany) and Dr. Julian Fennessy (Giraffe Conservation Foundation) for their valuable support during the course of this study. I am thankful to Molecular Biodiversity Lab, Department of Zoology & Wildlife Biology, Government Arts College, Ooty, India, for lab work and funding the above project.

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BEHAVIOURAL STUDY OF ZOO ANIMALS

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Abstract

The behaviour of zoo animals differs from their conspecifics in the wild. Understanding behaviour is a key for better husbandry and management. Behaviour defined, and behavioural sampling methods like Ad libitum sampling, Focal sampling, All occurrence sampling, one zero sampling and instantaneous sampling methods for qualitative and quantitative measurement of behaviours were described in this communication. Besides, various methods for development of ethogram, behavioural categories were explained. The role of this behavioural study was to describe the activity pattern, behavioural need, stress, welfare effect of environmental enrichment on stereotypic behaviour, and captive breeding of zoo animals. The significance of behavioural studies in zoo conditions in understanding of species biology, promoting husbandry, breeding and welfare were discussed. Limitations in behavioural research and insurmountable difficulties in zoos discussed. Suggestions were made for collaborative research and scientific management of zoos.

Key Words Behaviour, Captivity, Zoo, Ethogram, Sampling method, Activity pattern

1. Introduction

Zoo animals are wild animals in captivity. They are maintained in zoos for education, conservation, research, and recreation (Mench & Kreger, 1996). Behaviour is the exhibition of a phenotypic trait within the environmental context for which primary selective forces have shaped it, the outcome of which being maximal, inclusive fitness (Eisenberg, 1981). Behaviour includes all the processes by which an animal responds to stimuli in its environment. Behavioural studies in captivity provide important information on the physical and psychological wellbeing and reproductive status of the animal necessary for their propagation. These studies include the monitoring of baseline behaviour (e.g. maintenance behaviours) or a change in instances (e.g. in response to environmental enrichments) using appropriate behavioural sampling techniques. A complete understanding of phenotypic traits of a species requires a combination of long-term field studies with observations and experiments on their captive counterparts. Field studies allow the formulation of realistic hypotheses but have the disadvantage that they do not allow the complete control of many potential confounding variables (Lambrechts *et al.,* 1999). So, studies with captive animals are important because they allow tests and observations that cannot be conducted in the field. Experiments with captive animals have the disadvantage, as the artificial environment may provoke abnormal behaviour (Dawkins, 1998). In zoo contexts, behavioural research usually focused towards collecting baseline information and addressing

management problems. Both are important and contribute in their way to understand the biology of animals and their conservation. This communication gives a generalized account on the significance of behavioural study in management and breeding of zoo animals.

2. Measuring behaviour

Behaviour of an animal is studied to understand the animal and the species. Behavioural study not only describes the behaviours displayed by the animals, but also asks questions regarding what, who, why, where and when the patterns in question occur (Lehner, 1996). Such study may include a description of behaviours in form of ethogram, estimation of activity time, behavioural bout and average time spent in different behaviours, seasonal variation of behaviours and effect of environmental parameters on it. Preliminary data collection is of importance as this provides baseline information to plan and project the detailed research work. Methodology of recording behavioural data depends on the question in hand and selection of appropriate sampling technique having accuracy, repeatability and relevance to the study. Depending on the research question, observers may record environmental parameters (e.g., temperature, humidity, intensity of light, rainfall), space utilization and seasonal variation in behaviour. There are two research methods used in the study of behaviour. Both methods are useful and have unique characteristics that help determine when each one is more suitable.

The first method, qualitative analysis, seeks to understand the behaviour of an animal in descriptive terms. Usually without the use of any numerical analysis, qualitative ethology presents a holistic view of animal behaviour. It is a common approach taken by scientists who study ecological aspects of animal behaviour in the wild. This qualitative ethology is especially useful when 1) the animal being examined is a new species whose behaviour is unknown, 2) when the animal is exhibiting previously unobserved behaviours, and 3) when the observer wishes to develop a narrative explanation of behaviour, an ethogram (Mohapatra & Panda, 2014a). Better explanations and descriptions of behaviour of animals help better understanding and prediction.

The second method, quantitative analysis, is an experimental approach to ethology. It requires collection of numerically analysable data about the behaviour of animals. These data can then be used to make concrete statements about how animals behave. Quantitative analysis is considered the most useful method to learn about animal behaviour today, and it is different in many ways from qualitative analysis. One crucial difference is the amount of work required of the researcher prior to the observation of behaviour (Mohapatra & Panda, 2013). Quantitative analysis requires much forethought and planning of any successful behavioural study. Details of behavioural data collection methodology including ethogram, sampling methods are described.

2.1 Ethogram

An ethogram is "a complete inventory of the behaviour patterns of a species" (Tinbergen, 1951). An animal's behaviour is naturally a continuous flow from one event or state to another. Martin and Bateson (1993) defines "event" as a short behaviour pattern like movement of the body or sound production at a particular point of time. The important feature of an event is in its frequency of repetition. A "state" is a long behaviour pattern or prolonged activity, e.g. resting, sleeping or near to any object. Often, the most important characteristic of a state is its time duration (Martin & Bateson, 1993). Breaking it down into categories allows a researcher to make measurements and comparisons. This is also useful for understanding the behavioural repertoire

of a species and for recording behaviour easier. When developing a catalogue of behaviours, names are applied to behaviours with an implicit description. Familiarity with the animal's behaviour and insights into its function help to understand the complexity of what an animal does.

2.2. Describing Behaviour

It can be based on physical form and structure of the behaviour in terms of posture, movement and sound or on the presumed effects of the subject's behaviour on itself, on its environment, or on other organisms or basing on spatial relation i.e., where or with whom the subject is interacting? Behavioural description must be clear, precise and comparable with those collected by other researchers, without any anthropomorphic interpretation. Therefore, a preliminary study and the drawing of an ethogram can be a great help.

There are two types of behavioural descriptions: empirical and functional descriptions (Lehner, 1996; Martin & Bateson, 1993). The empirical description deals with the structure, posture and body movement e.g. "tiger licking its body hairs with its tongue." Empirical description is useful in preliminary studies and constructing an ethogram. The functional description is an account of the behavioural movements and function e.g. "tiger is grooming."

2.3. Behavioural categories

Each behaviour is represented by a range of several movements and postures, making it difficult to obtain a definitive measurement. It is advisable to split behaviours into categories for easy collection and with precision. For example, to describe and measure the resting behaviour, it is better to divide this activity into its various components: resting awake, sleeping, roll over and laying on back etc. Martin and Bateson (1993) suggested features such as number, definition, independence and homogeneity for behavioural categories.

Number: It should have a complete description to indicate the assigned behavioural patterns in accordance to the research objectives.

Definition: It should be objective, clear, concise and complete.

Independence: It should be independent so that a behaviour pattern can be assigned to it without any ambiguity.

Homogeneity: All behaviour patterns included in a category should have similar features.

2.4. Sampling Methods

There are many methods of sampling to obtain a true picture of behaviour. It is not possible, also not necessary to record all types of behaviours. Most behaviours are classified as events or states lasting an appreciable amount of time, which can summarize the occurrence of both with counts or frequencies. There are following sampling techniques for behavioural data collection.

2.4.1. Ad Libitum (ad lib) Sampling: It is an informal method of sampling while making field notes (Altmann, 1974).As the observer can't record all behaviours and there may be observational bias in recording the behavioural pattern, it is difficult to get reliable quantitative information based on Ad libitum. However, this method helps in the development of ethograms and studying rare behaviours. Das (1980) described reproductive behaviours of captive tigers following ad lib sampling. Besides, observations on rare but significant behaviours like egg laying and territoriality in reptiles were collected using ad lib sampling (Bustard and Maharana, 1982; Maharana and Pati, 1983). Mohapatra and Panda (2014a) developed the ethogram of Indian pangolins following this sampling method.

2.4.2. Focal Animal Sampling: Focal behavioural sampling records all occurrences of specified actions

of one individual during a predetermined period (e.g., one hour) (Altmann, 1974).The observer also records the length of the sample period and the amount of time the focal animal is in view. It provides unbiased data relevant to a wide array of questions if the study animal remains in the field of view. The method has been used for studying mating behaviours (Mohapatra et al. 2015a, 2015b), stereotypic behaviour of zoo animals (Vaz. & Narayan, 2017). Mohapatra and Panda (2013) used the method for standardization of instantaneous sampling intervals.

2.4.3. All Occurrence Sampling: It focuses on a particular behaviour instead of a particular individual e.g. number of alarm calls in monkey troop. It is also useful for providing the frequency of behaviour in the group as a whole. This method is suitable for studying the behavioural synchrony within a group (Altmann, 1974). Behaviours like sexual mounting and grooming can be successfully scored by using this method but behaviours like 'avert gaze' or similar brief or subtle responses cannot be scored following this method. The result will degrade in case of large group size and space occupied (Bernstein, 1991).

2.4.4. One-Zero Sampling: The sampling method records an occurrence or non-occurrence (rather than frequency) of the behaviour in each sampling period. An observer makes single entry during a predetermined time unit if any instance of behaviour of interest is seen during that time unit regardless of its duration or number of times it occurs (Bernstein, 1991).It can provide good indication about the rate of occurrence of behaviour per unit time (e.g. hourly rate). But if the observer is interested in recording frequency and duration of behaviours and percent of time spent in various states as variables of interest, alternative sampling methods like focal and instantaneous sampling methods should be considered (Altmann, 1974).

2.4.5. Instantaneous or Scan Sampling: It is a method of recording behaviour as it occurs in a predetermined time or as the sample is in the act of doing it. It is used to study the percent of time spent in a certain activity. If the behaviours of all members of a group are surveyed within a short period of time, it is called a scan sampling. This provides data on the distribution of behavioural states in a group. Instantaneous or scan sampling is for sampling of easily identifiable behaviours. The sample intervals should be ideally shorter than the behaviour of interest.

After collection of behavioural data following a suitable sampling method, appropriate statistical methods are used for analysis. The sampling method was used to collect activity pattern, seasonal variation in behaviour, evaluation of environmental enrichment programmes for Indian pangolins and tiger (Mohapatra *et al.* 2010, 2014; Mohapatra & Panda, 2013; Mohapatra *et al.,* 2016), behavioural correlates for breeding in Indian black buck (Rajagopal, *et. al.,* 2011; Archunana & Rajagopal, 2013), effect of enrichment, personality and life history on the welfare of Asiatic lions (Goswami *et. al.,* 2020, 2021), stereotypic behaviour of giraffes (Kulkarni, 2020) and influence of zoo visitors on Indian gaur (Sekar & Rajagopal, 2008).

3. Significance of behavioural study of zoo animals

The missions of the zoos are complex but generally include conservation, education, research and recreation. Zoos are making a difference for wildlife conservation by bringing people face-to-face with living animals and inspiring them to care about and care for the natural world. The management of captive animals requires basic knowledge of the needs and information about its reproduction. However, this information is often lacking in endangered species (Wildt *et al.,* 2003). Zoos offer

unique opportunities to study animal behaviour, physiology, growth, development of a wide variety of taxa under semi-controlled conditions. Many of these studies would be difficult, if not impossible, to conduct in nature because of practical and ethical limitations. Behavioural studies in zoos provide valuable information about the inhabitants ultimately contributing towards a better understanding of species biology, promoting husbandry and welfare of the animals. The particular method of behavioural study preferred in zoo condition, as it is easily observed, non-invasive, and non-intrusive measure of welfare and can provide good cues about the internal, subjective state of animals, with their preferences and needs (Mench & Mason, 1997; Dawkins, 2004). Behavioural studies in zoos are usually carried out on the following aspects.

3.1. Ethogram, time budget and activity pattern

Ethogram represents behavioural repertoire of animals. This is essential for starting a behavioural study focusing on a species or specific behavioural category (Mohapatra & Panda, 2013; Mohapatra & Panda 2014a). The time budget in the behavioural repertoire is a reflection of resources (Young, 2003), thus the pattern of activity and time budget is an important basis for understanding behaviour. Hence, behavioural studies can be an important element for successful captive management of a species (Kleiman, 1994). These can be even more crucial for developing effective conservation strategies in many species. Studies of free ranging animals are often hampered by the difficulty of direct observation of the subjects (Emmons, 1988; Law *et al.,* 1997). For example, scientific study of captive felids is equally applicable to the conservation of wild felids as well (Law et al., 1997). Study of activity pattern and time budget are essential to know the baseline activity and change of the same in positive and negative state of welfare by exhibiting stereotypic behaviour or increased exploration in response to enrichment (Mohapatra *et al.,* 2010; Mohapatra et al., 2014).

3.2. Behavioural needs

Behavioural needs are activities that individual animals of a species in captivity show a strong motivation to perform, and which may lead to frustration, stress, health problems and/or abnormal behaviours when they are hindered. A comparison of the natural behaviour in captivity helps assessment of positive and negative welfare states of the animal. Besides, the display of abnormal behaviour or natural behaviour in irregular frequency indicates disturbance (Mench & Mason, 1997). When control systems are overtaxed and there is an actual or potential reduction in fitness, the animal is stressed (Broom & Johnson, 1993). Therefore, behavioural study must include not only the observation of what animals do, but also examination of general biological activities that help to form and develop an animal's behaviour. Regular observations help understanding what animal wants to fulfill its biological needs. It also facilitates chalk out management strategies to address the issues.

3.3. Animal welfare, environmental enrichment and space utilization

Animals continuously explore their environment to stay aware of food and water sources, shelters, trails, predators, hazards, territory intruders and potential mates. Zoo animals are typically maintained in static environments and have limited opportunities to explore (Mench, 1998). The ability of a species to respond to captive conditions with behaviour from its normal repertoire depends on the degree to which the particular captive condition resembles its natural environment (Carlstead & Shepherdson, 1994). The use of behaviour in stress and welfare assessment is based on knowledge of normal species-specific behaviour as well as on the nature of deviations in response to different stimuli and emotions (Keeling &

Jensen, 2002; Squires, 2003). The zoo community was among the first to raise concerns over abnormal and stereotypic behaviors in captive animals and began developing environmental enrichment strategies to deal with the perceived problem (Swaisgood & Shepherdson, 2005). Stereotypies are relatively invariant, repetitive behaviors that seem to have no immediate function (Mason, 1991). Useful behaviours for welfare assessment include activity levels, posture and movement patterns, vocalization, aggression, sleep patterns and ingestion (Squires, 2003). They may not have the motivation, opportunity or need to display the range of behaviours necessary to succeed in its natural habitat (McPhee, 2002).

In the zoo community, environmental enrichment has become almost an accepted method for husbandry with specific aim of improving wellbeing and as such is the method of choice for reducing stereotypic behaviour (Swaisgood & Shepherdson, 2005). Any object or stimulus that attracts the animals' interest can be considered as an environmental enrichment (Shepherdson et al., 1998). It is generally designed to permit the animals to display their natural behavioural repertoire (Mellen & MacPhee, 2001). Increasing physical and temporal complexity may add biologically relevant information to an animal's enclosure, resulting in increased opportunities for exploration. It can also offer an animal to learn and achieve a desired goal through the performance of appropriate behaviour (Sambrook & Buchanan-Smith, 1997). Animal enclosures can be enriched by simply adding appropriate substrates such as sand, dirt or vegetation. The substrates can also induce foraging and exploratory behaviour by hiding food, adding scents or naturally occurring life forms e.g. insects. Proper hideouts and landscaping can offer privacy, encourage territoriality, give escape gateways, and therefore can make scope for better social interactions. Climbing structures allow more efficient use of space and provide shade and temperature gradients for choice of microclimate. They can also provide hiding places from conspecifics, the visitors, and zoo keepers. Novel objects like toys and cardboard box, scents, and exhibit changes are techniques used to stimulate exploratory behaviour. Zoo biologists now accept the responsibility to design programmes and techniques carefully that will contribute to the 'psychological well-being' and enhance the lifestyles of captive animals. Behavioural observation before and during environmental enrichment sessions are useful to develop the baseline behavioural budget and to evaluate the behavioural response of the animals to the enrichment object, respectively.

It is important to assess how the animal is using the enclosure in relation to the available space and/or the ecological resources of the enclosure. There are two methods usually used for this purpose. Firstly, by dividing enclosures into several equal area zones and calculating Spread Partition Index of those areas to find any difference in observed occupancy time or frequency compared to the expected time or frequency (Mallapur *et al.* 2005). Secondly, dividing the enclosure based on the resource value (may be of unequal area) and calculating the utilization time/ frequency of specific resources in relation to its overall availability in that resource (Lechowicz, 1982).

3.4. Captive breeding programme

Many endangered species are currently involved in conservation breeding programs worldwide. Conservation breeding deals with propagation of captive populations, often with the ultimate aim of releasing animals into the wild. Zoos provide an opportunity to determine the behavioural patterns associated with reproduction of many elusive endangered animals that are difficult to study in the wild. Research estimating parameters such as age, onset of oestrus, duration of oestrus, inter oestrus

duration, breeding season, mating behaviour, courtship, copulation, post copulatory aggression, gestation period, litter size, interbirth interval, weaning age and weight, behaviour of primiparous mother with infants and needs of lactating mother have inherent benefit in management, breeding and propagation of endangered species in captivity (Das, 1980; Bustard & Maharana, 1980; Maharana & Bustard, 1981; Mohapatra & Panda, 2014a,b; Mohapatra *et al.,* 2016; Mohapatra, 2016). Study of behavioural aspects for conservation breeding is useful in maintenance of the available small population to make self-sustaining viable populations in captivity without compromising the success of reintroductions. The need for behavioural studies to improve husbandry and management for breeding endangered species has been highlighted (Håkansson, 2007; Mohapatra & Panda, 2014b; Sutherland, 1998). Behavioural study gives crucial insights on animals' biology needed for conservation breeding (Bustard & Maharana, 1980; Maharana & Bustard, 1981; Mohapatra & Panda, 2014b). With the technological advancement, installation of infra-red enabled CCTV cameras provide important biological information of nocturnal and elusive species like Indian pangolins greatly help in their conservation breeding programme in zoo (Mohapatra, 2016; Mohapatra & Panda, 2013).

4. Limitations in behavioural research in zoos

The zoo, as a living laboratory, presents a unique and valuable avenue for research. It often lacks special facilities and financial support to carry out serious research on behaviour. Zoo animals are kept in an artificial environment with less opportunity to display their natural behaviours. Though the conditions have improved in many zoos where animals are displayed in large naturalist enclosures simulating their natural habitat for fulfilment of their biological needs, still some zoos exhibit in small artificial environments like cages. Besides, in zoo conditions many events like feed, feeding time, visitor's presence and interaction with zookeeper are predictable events for animals and can impact their behaviour (Mohapatra et al., 2010). In zoos, sample size is usually small and when several groups of multiple zoological institutions are considered for study to get a larger sample size, other variables like housing conditions, husbandry practices, group composition create inter-group differences. In addition, observer does not have control over variables like time of feeding, movement of animal from exhibit to back kraal and vice versa, presence of zoo visitors and other animals (e.g. free living monkeys) that impact the exhibition of behaviour in unpredictable and undesirable ways (Hosey, 1997). At times, there is a possibility of addition (acquisition, birth and introduction) or reduction (separation, death and disposal) of animals during the study period. Besides, empirical research needs manipulation of variables. The most important difficulty is the reluctance of zoos to allow experimental manipulation of their animals or cages (Hosey, 1997). Such difficulties in zoo-based research could be overcome by considerable negotiation, cooperation, good will of both parties and collaborative research. Choosing a research subject which helps to solve management problems that encourage carrying out research in zoos. Above all, adopting non-invasive methodology and following legal and ethical standards rationalize the study.

5. Conclusion

Zoo has the potential for development of a database for efficient management of wild animals in captivity. The observations can be safely extrapolated to nature if enclosure enrichment simulates near to their habitat. Ultimately, zoos can contribute immensely to standard research on a wide range of topics, both on pure and applied fields, in collaboration with reputed research institutes with minimum financing for zoo management on scientific lines.

Conflict of interest

Authors declare no conflict of interest.

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PERSPECTIVES FOR POPULATION MANAGEMENT OF FELIDS IN INDIAN ZOOS

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Abstract

The family: Felidae (hereafter referred to as felids) is among the commonly represented species in animal collections in Indian zoos. Of the globally recognised 45 species, 15 species (>30%) are housed in Indian zoos. Since 2007, the Central Zoo Authority has laid emphasis on ex situ conservation for seven threatened species by initiating planned breeding programmes. We investigated the demographics of felids housed in Indian zoos using data from CZA annual inventory records. Between 1995-96 and 2019-20, the population of large felids have remained stable with a mean growth rate (λ) of 1.01; whereas the population of small felids have a marginally higher mean growth rate (λ) of 1.03. We further use Sustainability-index analysis to investigate whether the observed growth patterns arise from intrinsic (i.e. births/deaths) or extrinsic (acquisition/disposal) factors. The management of felids in Indian zoos requires careful consideration of many factors including space, hybridisation, lack of pedigree knowledge, addition of wild-rescued specimens and color-morphs. We provide the first insights on how felids populations have fared at the family-level and species-level based on analysis of longitudinal data. The said analysis intends to inform plans to manage felid collections in Indian zoos. It should further present an outlook and also guide ongoing planned breeding programs of felids. Given the relatively large collection size and the corresponding conservation attention accorded to felids, our analysis will aid in setting priorities for collection planning, conservation education messaging, integration of in situ and ex situ efforts in the context of IUCN One Plan Approach.

Key Words Felidae, Sustainability, Captive Breeding, Population Management

Introduction

The contribution of zoos to wildlife conservation has been emphasized in the recent years (IUCN SSC 2014). This includes providing animals for reintroductions (Gilbert *et al.* 2017), field- based monitoring and research (Che-Castaldo *et al.* 2018), and management of ex situ animal populations (Balmford *et al.* 1996; Conway 2011). An integrated approach is emphasised, that promotes an active contribution of lessons learnt from ex situ conservation to in situ conservation (Redford *et al.* 2012; IUCN SSC 2014). To be able to successfully realize the conservation goals, ex situ populations should be demographically and genetically stable (Lees and Wilcken 2009; Ballou *et al.* 2010). To this end, zoos have coordinated the breeding and exchange of animals among facilities through coordinated breeding programs since the 1980s (Ballou *et al.* 2010; Ballou and Traylor- Holzer 2011).

Central Zoo Authority (CZA) is an apex statutory body that recognizes and regulates operation of zoos in the country. Among other functions, the CZA coordinates breeding programs for threatened species and acquisition/transfer of animals between zoos, thereby, regulating animal collections in zoos. The overall goal is to ensure that collections are demographically robust and genetically representative of wild counterparts for the foreseeable future. Standardised inventory information maintained by zoos offers insights on dynamics of zoo populations. Recognised zoos submit inventory of collections detailing acquisitions, births, disposals and deaths annually to the Central Zoo Authority.

In this paper, we analyse longitudinal inventory data of species belonging to the family *Felidae* housed in Indian zoos between 1995 and 2020. The analysis is an attempt to get an overview of population trends, growth rates and holding patterns of felids. The results of this retrospective analysis would provide perspectives for managing felid populations in Indian zoos sustainably.

Methods

In this study, we selected all the species belonging to the family Felidae housed in Indian zoos (Table 1). The data pertaining to these species was obtained from inventory records submitted by recognised zoos to the Central Zoo Authority, subsequently digitised and publicly available (www.cza.nic.in). The inventory was available for each fiscal year i.e., from 1st April of a given year to the 31st March of the following year. It comprised of species-wise opening and closing stock including details of births, acquisitions, disposals and deaths for each fiscal year. In total, data from 143 zoos housing one or more target species for at least one fiscal year was used for the study.

The nature of the data available i.e. basic inventory with records of births, deaths, acquisitions and disposals was apt for conducting S-index analysis (Lynch 2018). We computed the following S-index metrics for species mentioned in Table 1, where N = number of individuals in the collection, t = time step, B = births, D = deaths, I = imports (acquisitions) to the collection, and E = exports (dispositions) from the collection.

1. Total lambda (λ_T) = The proportional change in population size from one year to the next, calculated as:

$$\lambda_T = \frac{N_{t+1}}{N_t}$$

2. Intrinsic lambda (λ_I) = The proportional change in population size due to Births/Hatches and Deaths, calculated as:

$$\lambda_I = \frac{N_t + B - D}{N_t}$$

3. Extrinsic lambda (λ_E) = The proportional change in population size due to Acquisitions and Disposals, calculated as:

$$\lambda_E = \frac{N_t + I - E}{N_t}$$

Since Sumatran Tiger Panthera tigris ssp. sondaica, Cougar Puma concolor, Siberian Tiger Panthera tigris ssp. altaica, Serval Leptailurus serval, Caracal Caracal caracal and Asian Golden Cat Catopuma temminckii, Leopard (melanistic) Panthera pardus are either not housed in Indian zoos anymore or data includes fewer than 10 individuals, only descriptive account of the population trends is presented. For the remaining

species, the S-index metrics were computed for each fiscal year and summarised for investigating patterns of population growth.

All the analyses were performed using R Statistical Program (R Core Team 2020) using the packages 'tidyverse' (Wickham *et al.,* 2019), 'ggpubr' (Kassambara, 2020) and 'viridis' (Garnier *et al.,* 2021).

Since Sumatran Tiger *Panthera tigris ssp. sondaica*, Cougar *Puma concolor*, Siberian Tiger *Panthera tigris ssp. altaica*, Serval *Leptailurus serval*, Caracal *Caracal caracal* and Asian Golden Cat *Catopuma temminckii*, Leopard (melanistic) *Panthera pardus* are either not housed in Indian zoos anymore or data includes fewer than 10 individuals, only descriptive account of the population trends is presented. For the remaining species, the S-index metrics were computed for each fiscal year and summarised for investigating patterns of population growth.

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Results

(a) Population trends and housing patterns

In total data from 143 Indian zoos housing felids between 1st April 1995 to 31st March 2020 including 14852 observations of births, acquisitions, disposals and deaths were analysed. The species-wise census trends of felids species in captivity and corresponding number of holding institutions is indicated in Figure 1.

The captive population of viz. Leopard *P.pardus*, Asiatic Lion *P.leo ssp. persica* and Jungle Cat *F. chaus* has steadily increased and has more than doubled during the said time period. The captive population of Bengal Tiger *P.tigris ssp. tigris*, Leopard Cat *P.bengalensis* has remained relatively stable. Non-native species viz. *Puma P. concolor*, Sumatran Tiger *P. tigris ssp. sumatrae* and Siberian Tiger *P. tigris altaica* were housed briefly and are no longer housed in any zoos. All the remaining felid species have continued to remain as small collections without significant growth. The captive population of Bengal Tiger *P.tigris* (leucistic) has steadily increased, whereas Hybrid lion *P. leo ssp.* has progressively decreased. Leopard *P. pardus* (melanistic) has continued to remain as a small collection without any significant growth.

The holding patterns by the zoos have remained consistent over the years across species (Figure 1). As on 31st March, 2021, 85 zoos are housing at least one of the species listed in Table 1. Large felids viz. Asiatic Lion *P. leo ssp. persic*a, Bengal Tiger *P.tigris ssp. tigris*, Leopard *P. pardus* and small felids Jungle Cat *F. chaus* and Leopard Cat *P.bengalensis* are the most commonly housed species in Indian zoos. All the remaining felid species are housed as small collections in few zoos in comparison to hybrid and color morph specimens that have a relatively higher representation. The distribution of felids across zoos at the end of fiscal year 2019-2020 is represented in Figure 2.

For each fiscal year, the proportion of acquisitions, births, disposals and deaths were computed and plotted across time to investigate their patterns (Figure 3). Acquisitions have been predominant in *P.pardus* and *F.chaus* and *P.bengalensis*; further, the birth:death ratio was less than zero in these species and also in the case of *N.nebulosa*, *P.uncia* and *P.t ssp. tigris*.

We fitted a localised polynomial regression (loess) to investigate trends in collection sizes across years for select species of felids (Figure 4). The populations of *P. leo ssp. persica, P. viverrinus, P.pardus, F.chaus,*

P.tigris (leucistic) show increasing trends, while the remaining species show variable, yet, declining trends.

(b) Total growth rate, intrinsic and extrinsic lambda

The mean growth rate across species is 1.05 (range = 0.97 – 1.36). To investigate changes in population growth across species, we calculated annual growth rates for each fiscal year and summarised it across species. Realised annual growth rates were highly variable across years (Figure 5). However, in general, growth rates remained close to replacement rate (growth rate [λ] ≥1). To understand drivers of the realised growth rates across species, we calculated intrinsic lambda (λ_I) and extrinsic lambda (λ_E) for each species. The values of λ_E was significantly higher than λ_I in all the species except Snow Leopard P.uncia and Bengal Tiger *P.tigris* (leucistic) (Figure 6). In the case of *P.unica* both the parameters had nearly equal values, whereas, in the case of *P.tigris* (leucistic), λ_I was significantly higher than λ_E, indicating an intrinsically driven increase in the collection size.

Discussion

Our analysis provides the first insights on long-term trends of felid populations in Indian zoos. While more than 50% of the zoos in the country continue to house one or more felids from this study, very few species demonstrate signs of long-term sustainability. Non-native species including *P.t ssp. sondaica, P. concolor, P.t ssp. altaica* are not housed in any zoos, whereas others such as *P.onca, L. serval, P. leo* and *A.jubatus* continue to remain as small collections without significant growth. Comparatively, native species have fared better. However, majorly, they too are housed as small collections in a few zoos. The trends of species that are housed in sizeable numbers in zoos are variable indicating fluctuating breeding successes correlated with a birth:death ratio of less than zero.

Generally, for species housed in large numbers the growth rate is indicative of an increasing population. However, given the significantly higher rate of extrinsic lambda λ_E observed in most species, growth of the collection is therefore driven by acquisitions/ disposals within the collection.

It is essential that demographic and genetic characteristics of collections are frequently evaluated to ensure that the population characteristics are aligned with the overall goal (e.g. insurance, display and education, conservation breeding etc.).

Conversely, it is also important that goals are met, and outputs assessed, and results of the evaluation are used to adaptively manage populations.

The results presented in this paper offer long-term trends that can be adaptively used to manage collections and drive policy-level decisions addressing the following aspects:

1) Several species of felids are still housed as small collections with fewer than 20 individuals. Since, small populations are more susceptible to demographic stochasticity, genetic drift, and the effects of inbreeding depression (Lacy 1997; Ballou *et al.* 2010). The future management should achieve breeding success and promote population growth to shield them from these effects.

2) Several species (e.g. *P.tigris spp. tigris, P.rubiginosus*) are species prioritised for coordinated breeding by the Central Zoo Authority. The results of this analysis can be used to formulate conservation breeding plans for the identified species. Further, the results should also inform identification of felid species for initiation of conservation-oriented captive breeding programs.

3) This analysis provides a broad indication of species that can be successfully housed and bred (especially native species). It also presents how collections in zoos have varied over the years, thus allowing informed decisions pertaining to species acquisitions, movement of animals between zoos, management of rescued animals and in determining surplus collections.

4) The results presented here should serve as a baseline for further analysis and precise understanding of the factors that drive changes in collection size that will allow management of animal collections for sustainability.

Figure 1: Census of felids housed in Indian zoos: 1995-96 to 2019-20. Census details are collated for fiscal years i.e. from 1st April of a given year to the 31st March of the following year. The line indicates the total number of males, females and unsexed individuals housed in all the zoos at the end of each fiscal year. Bars represent the number of zoos housing the species in the respective fiscal year.

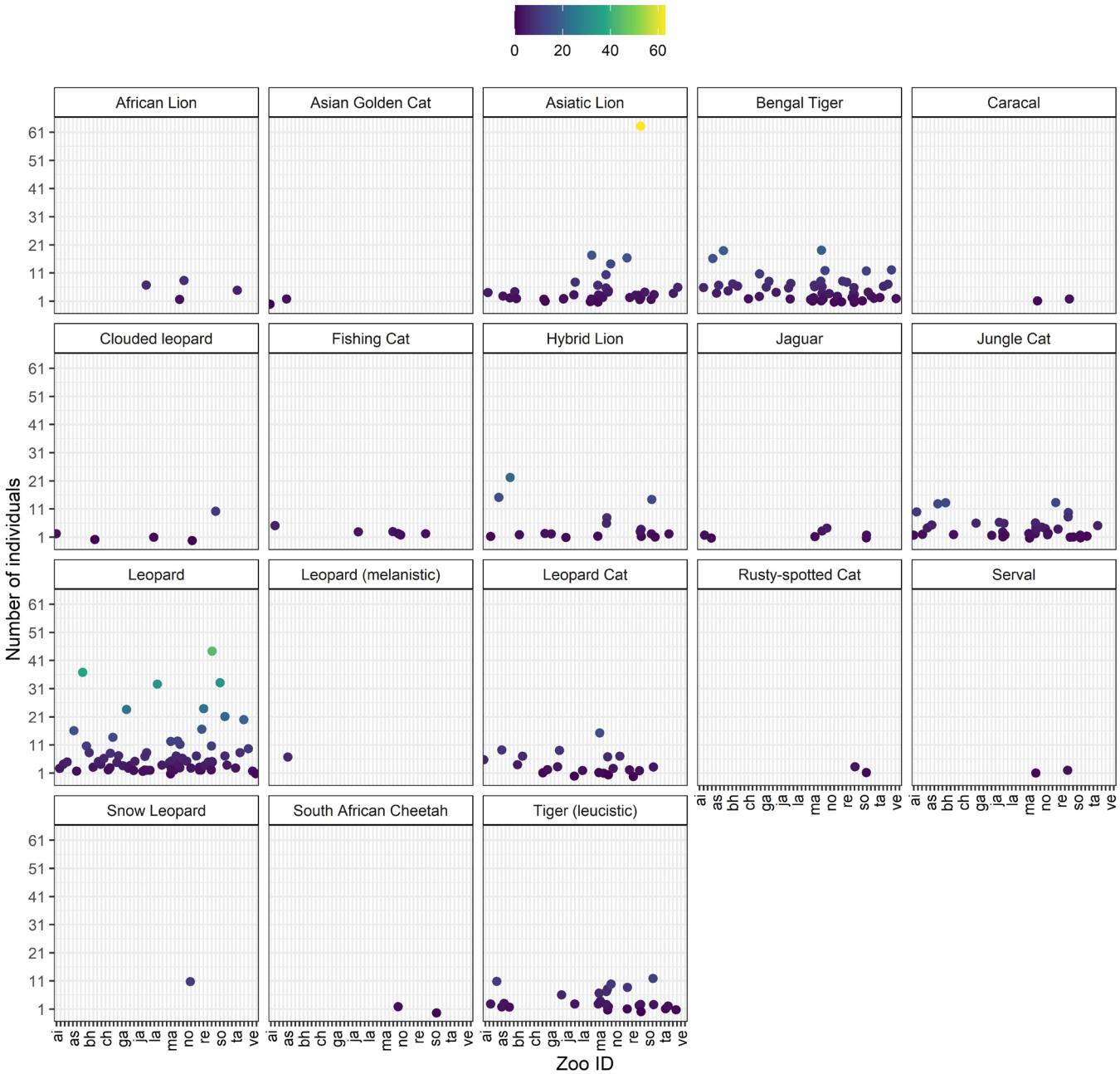

Figure 2: Housing patterns of felids in Indian zoos at the end of 2019-20 fiscal year. Zoo ID's are abbreviations derived from zoo names. Colour gradient represents the number of individuals in respective zoo.

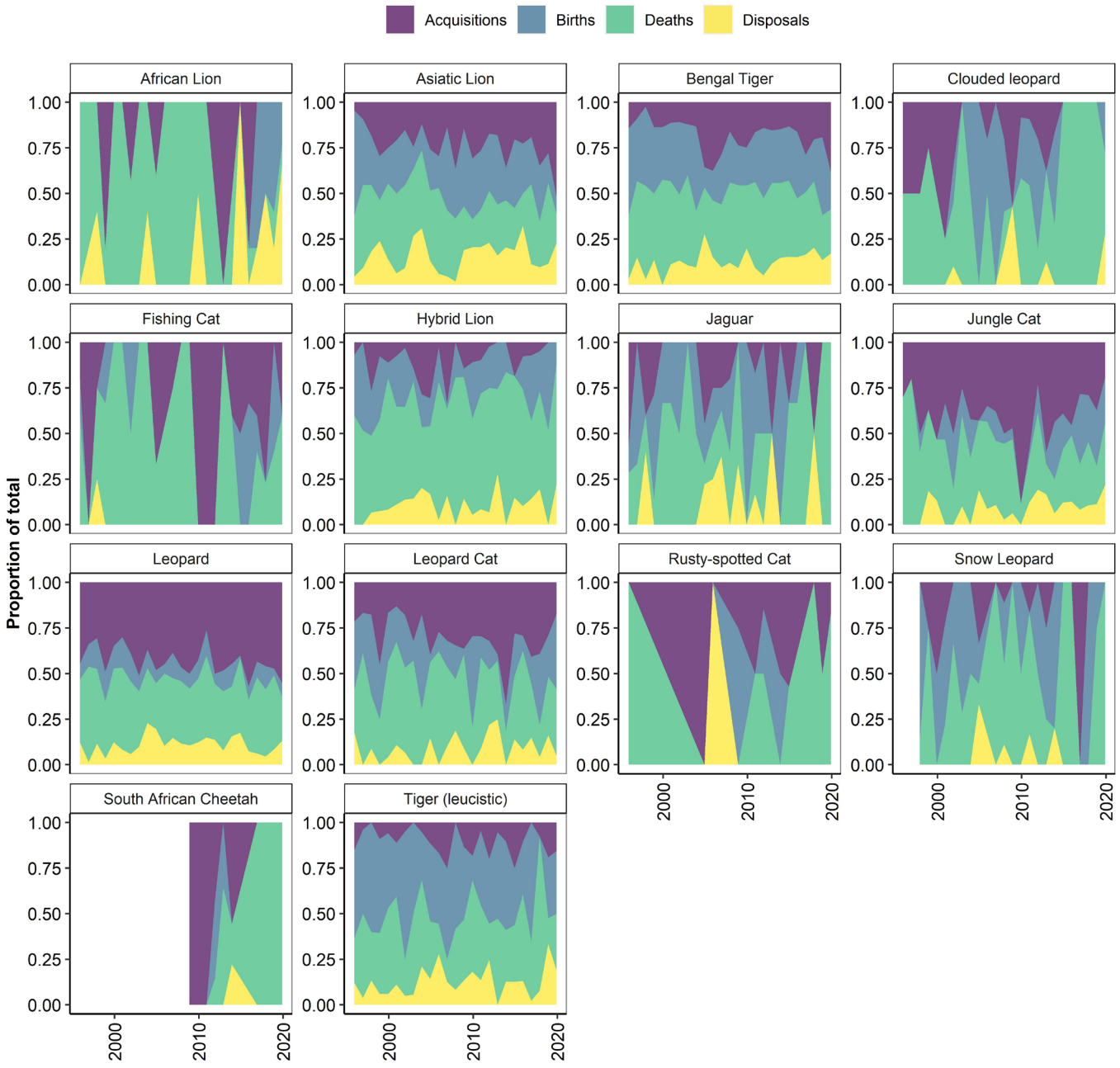

Figure 3: Proportion of acquisitions, births, deaths and disposals in select felids from 1995-96 to 2019-20.

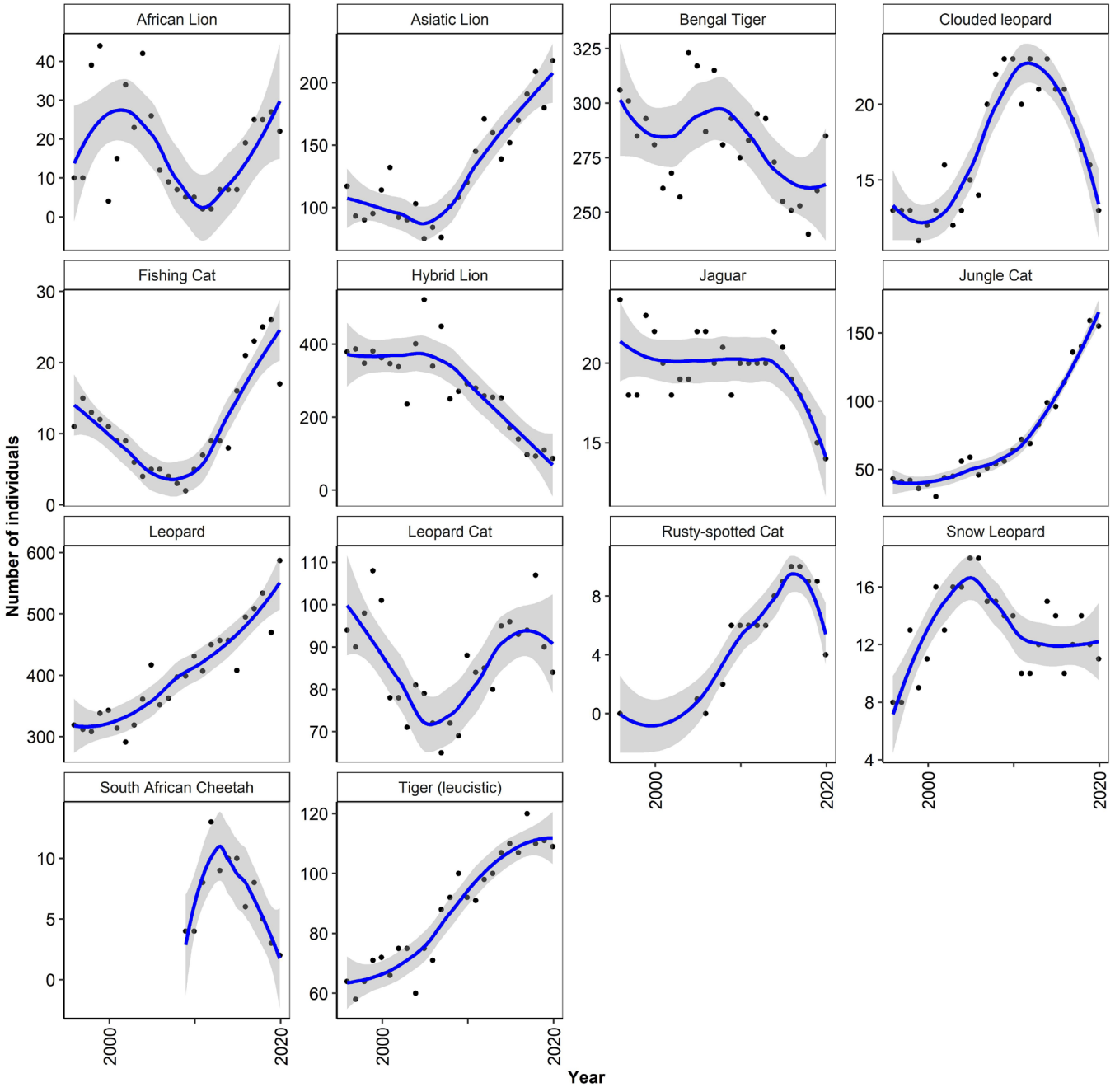

Figure 4: Trends (with loess smoothing and pointwise confidence intervals) of population sizes of select species of felids housed in Indian zoos from 1995-96 to 2019-20.

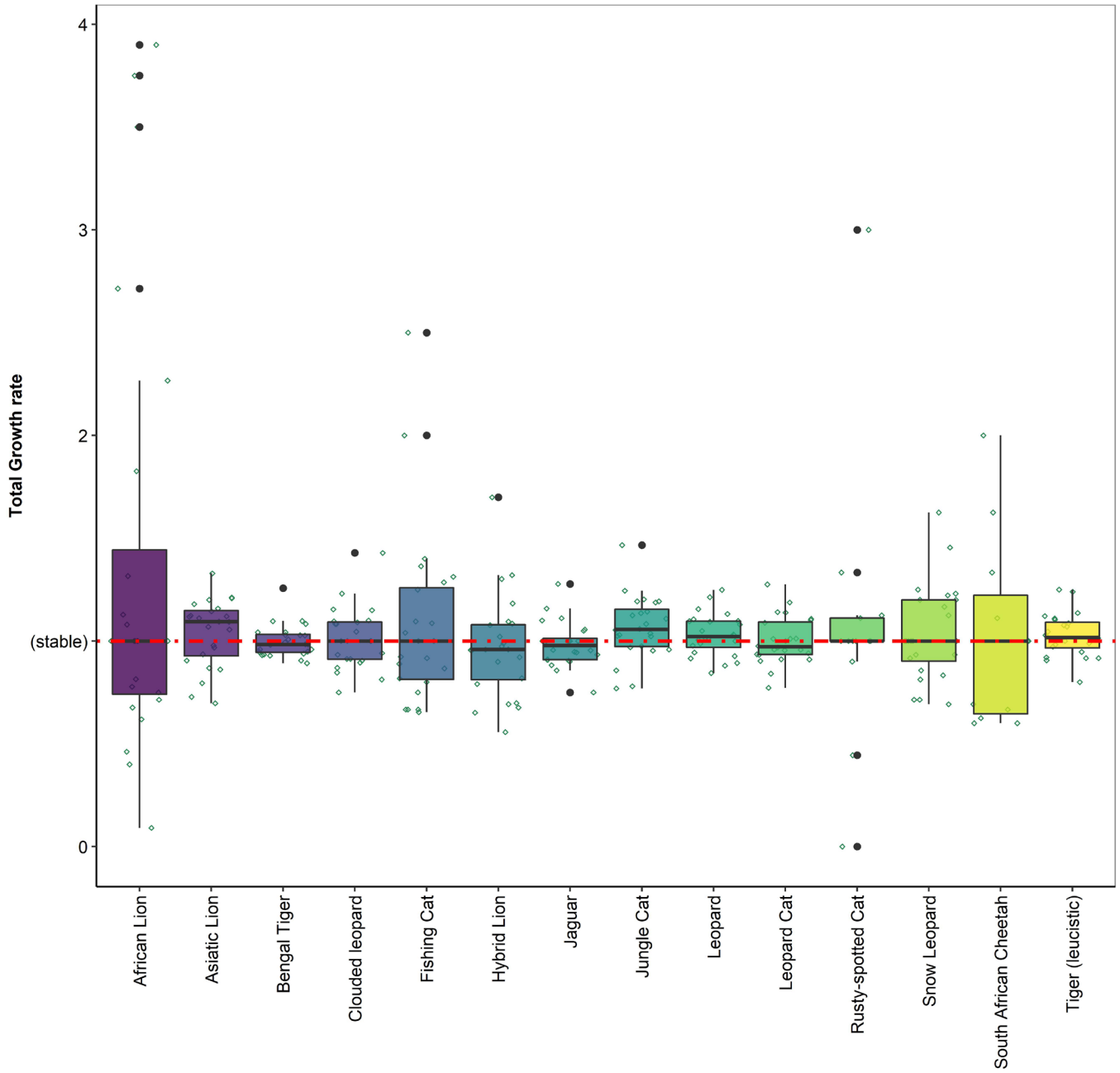

Figure 5: Species-wise growth rate (lambda [λ]) calculated from annual census data of felids housed in Indian zoos from 1995-96 to 2019-20. Red line indicates the stable growth rate. Data points in green indicate distribution of annual growth rates.

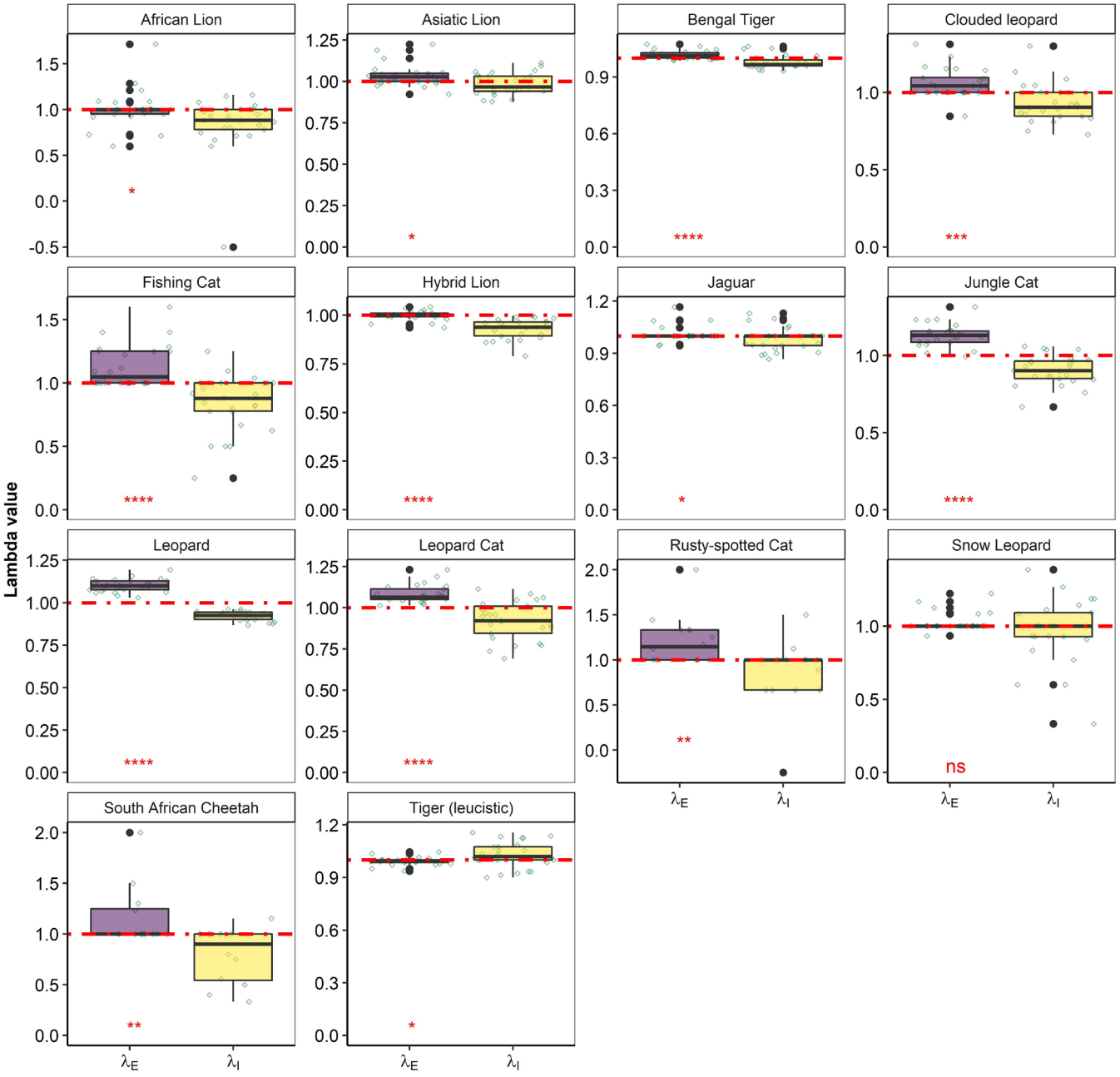

Figure 6: Species-wise intrinsic growth rate (λI) and extrinsic growth rate (λE) calculated from annual inventory records of felids housed in Indian zoos from 1995-96 to 2019-20. Red line indicates the stable growth rate. Data points in green indicate distribution of annual growth rates. Asterisk in red indicates significance levels, ns is non-significant.

Table 1: Species and data availability used in the current study.

No.	Species	Data availability on housing	Category
1	African Lion *Panthera leo*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	Large felids
2	Asiatic Lion *Panthera leo persica*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
3	Bengal Tiger *Panthera tigris*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
4	Jaguar *Panthera onca*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
5	Leopard *Panthera pardus*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
6	Puma *Puma concolor*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2009 – Mar 31, 2010	
7	Siberian Tiger Panthera tigris altaica	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2013 – Mar 31, 2014	
8	Snow Leopard *Panthera uncia*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
9	South African Cheetah *Acinonyx jubatus*	Apr 1, 2008 – Mar 31, 2009 to Apr 1, 2019 – Mar 31, 2020	
10	Sumatran Tiger *Panthera tigris sumatrae*	Apr 1, 2016 – Mar 31, 2017 to Apr 1, 2018 – Mar 31, 2019	
11	Clouded Leopard *Neofelis nebulosa*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	Small felids
12	Asian Golden Cat *Catopuma temminckii*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
13	Serval *Leptailurus serval*	Apr 1, 2017 – Mar 31, 2018 to Apr 1, 2019 – Mar 31, 2020	
14	Caracal *Caracal caracal*	Apr 1, 2016 – Mar 31, 2017 to Apr 1, 2019 – Mar 31, 2020	
15	Leopard Cat *Prionailurus bengalensis*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
16	Rusty-spotted Cat *Prionailurus rubiginosus*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
17	Fishing Cat *Prionailurus viverrinus*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
18	Jungle Cat *Felis chaus*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
19	Hybrid Lion *Panthera leo*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	Hybrid and colour morph specimens
20	Leucistic Tiger *Panthera tigris*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	
21	Melanistic Leopard *Panthera pardus*	Apr 1, 1995 – Mar 31, 1996 to Apr 1, 2019 – Mar 31, 2020	

Acknowledgements

We like to thank zoo operators/directors for submission of Annual Inventory information of respective zoos. We would like to thank officials and staff of the CZA Secretariat, especially Vivek Goel, for curation and maintenance of inventory datasets, and Sruthy Boopathy for assistance is digitisation of inventory records.

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