Amputation of Gangrenous Tails in Four Elephants - Case Report

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Four elephants of 20-30 years age group, weighing 3-4 tons were suffering from tail gangrene. Three of them were dry gangrene and the remaining was moist. All the tails amputated by putting U shaped incision and disarticulation of joint under medetomidine-ketamine anaesthesia. The amputated tail end was sutured and bandaged. The tail was tied with jute rope and fastened to the farra in the neck of the elephant for 14 days. Skin suture was removed in 10th day of operation. All the tails of elephants healed without any complication.

Introduction

Gangrene is the death and putrefaction of extensive volumes of tissue in a living body. Traumatic gangrene occurs as results of compression, interfering blood supply to the part. As a result the involved tissues are deprived of nutrition and subsequently lose their vitality. Gangrene of tails in elephants is observed frequently following injury during logging, biting by fellow mates of the herd, other animals like tigers and rhinos or due to insect bites. Once gangrene started, infected with organisms and gradually spread in ascending way towards the base of the tail. If whole of the tail got damaged, it will affect the epidural nerves as well as posterior part of the elephant including anus. In unattended cases the gangrene can become severe and requires surgical intervention. Otherwise it might lead to septicemia and death.

Case report: Four elephants belonging to Kaziranga National Park, Pobitora Wildlife Sanctuary and private owners were suffering from tail gangrene. Two of them were injured during logging, the third one was bitten by another elephant and the fourth one developed gangrene as a result of insect bites. The elephants were of 20-30 years age group, weighing 3000-4000kg. Two of them are of forest camp elephants used for patrolling inside forest and the remaining two were of private owner's used for logging. All the elephants were in good working condition. Examination of the injured tail revealed that the distal parts of the tails were missing and in three case the ends were dry and necrotic whereas, in the forth it was moist. The process did not respond to prolonged treatment by local veterinarians. These cases were diagnosed as dry gangrene of the tails in three and moist in fourth cases.

In one of the cases in Pobitora the gangrene had progressed involving most of the tail and leaving only 1.5 feet of healthy tissue. This case needed immediate surgical intervention. Without which the gangrene will progress to the base of the tail and this will lead to fatality of the elephant. However till the time of operation, no sign of septicaemia was developed.

All four elephants were anaesthetized standing with Medetomidine and Ketamine (5µg/kg + 50µg / kg). The tail above the affected parts was prepared for aseptic surgery. A rubber tourniquet was applied 30 cm above the gangrene. Two "U" shaped skin incisions were placed 15 cm above the gangrene, one on the dorsal and one on the ventral side of the tail. An intervertebral space was palpated proximal to the skin incisions and skin and the muscles were reflected to this point, and the scalpel was inserted under the flap to the joint in order to disarticulate the tail. The vertebrae were disarticulated and the affected part dissected. The tourniquet was relaxed momentarily and bleeding vessels were identified and ligated with Catgut No. 1 , followed by suturing of the muscles with simple continuous sutures. The skin was sutured in a simple interrupted pattern by using Black Silk No. 2, "Tincture Benzene" compound seal was applied to cover the suture end and the wound was bandaged with gauze cloth. The bandage was covered with adhesive tape. Then a jute rope was tied at the base of the tail and pulled toward the neck, where it fastened to the farra and kept for 14 days. Farra is the belt made of jute and used to be fastened in the neck of elephant where the mahout sit. After completion of the operation the elephants were reversed from anaesthesia with intravenous injection of atipamezole (5µg /kg). The tourniquet was removed 24 hours after surgery. A course of antibiotic (Cefavet, 4mg/kg body weight/ day) and antihistaminic (Zeet, 20ml/day) were injected intramuscularly for seven days. The bandage was removed after first 48 hours of operation, the wound was dressed with povidone iodine solution and the wound was rebandaged and kept for another 7 days. On day 10 after the operation the skin sutures were removed. The tails were amputated in all four elephants and the same protocol was followed in all four cases. The tails healed without any complications in all elephants.

Discussion

Combination of Medetomidine and Ketamine at a dosage of 5µg/kg + 50 µg /kg produced standing immobilization and analgesia of the elephants, which allowed to perform the surgery aseptically.

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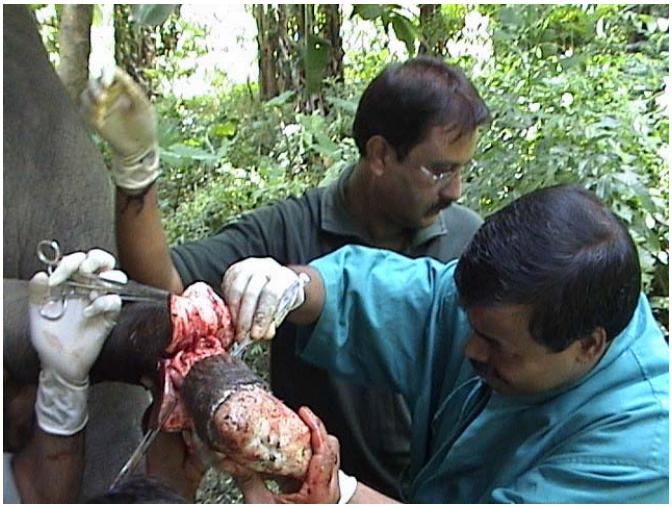
Elephant with gangrenous tail



Docking of gangrenous tail

Sarma and Pathak (2004) also reported excellent standing anaesthesia following Medetomidine and Ketamine in Asian elephants with the same dose as mentioned in this case reports. The possibility to revert the effects of Medetomidine with atipamezole (5µg/kg body weight) reduced.

Conclusion

Amputation of the infected parts has proved to be an efficient way to control tail gangrene in elephants. The surgery should be conducted under aseptic conditions and care should be taken to prevent infection and retraumatization after the operation to allow for fast uncomplicated primary wound healing. Gangrenous tails should be amputated as early as possible, otherwise the process will progress towards the base of the tail, possibly affecting the spinal nerves, in which treatment is not possible, and might lead to the death of the elephant. No sign of septicaemia was observed in the elephant affected with tail gangrene.

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Management of Hydrocyanic Acid Poisoning In Asian Elephants

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Hydrocyanic acid poisoning in domestic herbivores animal is common in most countries due to easily available jowar (Sorghum) plants. Poisoning of animals usually occurs after ingestion of large quantity of plants containing cyanogenetic glycosides. The content of cyanogenetic glycosides in the plants varies between season and between different parts of the plants. Sorghum species are used extensively in some countries for forage and may cause heavy mortalities in particular circumstances (Radostits et al, 1995). Cyanogenetic glycosides as such is not poisonous, they become toxic only after release of HCN by enzyme or acid hydrolysis and intestinal micro flora was found to cause decomposition of glycosides and release of HCN. The glycoside content is highest in the leaves and stalks, when plants grow rapidly after a period of retardation. This is common after autumn rains, which increases the growth of stunted plants during summer or crop is eaten back by livestock.

Five adult captive elephants were fed a large quantity of fresh jowar, during the stay of a circus at Jabalpur. After sometime all the elephants started showing the symptoms of restlessness and diarrhea. The local Vets gave the routine anti-diarrheal treatment to these elephants, but the diarrhea could not be controlled, and condition of these elephants was gradually become serious.

The manager of the circus approached the Principal Investigator of the Wildlife Health Monitoring Disease Diagnosis and Research Cell for the needful. All the five Asian elephants were examined thoroughly and the history of feeding and watering was carefully recorded. During examination it was observed that plants of jowar were fed to these elephants. The feeding of jowar was immediately stopped. The condition of elephants was gradually going down. Clinical examination of elephants was done, and fecal examination did not reveal any significant result. The body temperature was in between 97° – 98° F.

Out of five Asian elephants the condition of one aged elephant was critical. Soon after the tentative diagnosis all the elephants were given about 8-10 litres of dextrose saline with 30 ml MVI through ear vein. Sodium thiosulphate 50gm was dissolved in water and given orally through drinking water. The same treatment was repeated after 12 hrs. Four elephants started showing signs of recovery after the 2nd dose of the above treatment. The condition of the aged elephant was still constant. This elephant was given 3rd dose. The animal was still showing little diarrhea. So the animal was again given 10 litres of cold dextrose saline along with small pieces of ice and Tab. Diadin through rectum with the help of tube. Next morning the 5th elephant had shown signs of recovery and started drinking water and eating fresh green grass.

The HCN poisoning in elephants may be an accident, when circus authorities fed large quantity of Sorghum plants due to non availability of regular food. Also these animals were not accustomed to Sorghum.

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Screening of Captive Elephants for Leptospirosis

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In the present study serum samples were collected from 51 captive elephants kept in the three different forest ranges (19-Bandipur, 12-Shimoga, and 20-Mudumalai). The samples were subjected to screening for leptospirosis by Dark Field Microscopy (DFM), Polymerase chain reaction (PCR) and isolation studies in EMJH (Difco) semisolid and liquid media. It was found that none of the serum samples was positive by all the three tests indicating the absence of active infection.

Leptospirosis is also known as Weil's disease, canicola fever, canefield fever, nanukayami fever, 7-day fever and many more is a bacterial zoonotic disease caused by spirochaetes of the genus *Leptospira*. It was first described by Adolf Weil in 1886 when he reported an "acute infectious disease with enlargement of spleen, jaundice and nephritis. Number of animal species including man is susceptible to leptospira infection. Rodents, wildlife, sometimes large animals including dog, swine and horse has been incriminated as reservoirs of leptospires (Upadhyaya, *et al.,* 1979). The complexity of epidemiology of leptospires has not been fully studied in India and the information on the incidence of this disease in elephants is not sufficient (Narayana Bhatt, *et al.,* 1998). The importance of the disease in the public health aspects acquires more significance especially in countries like India because of large livestock, rodent and wildlife populations and close association between man and animals providing a congenial environment for the spread of the disease.

Advances in the zoonosis research have greatly contributed mans to continues effort to wipe endemic, epidemic and panzootic communicable disease throughout the world, which took considerable loss of human and animal life in the past. Many diseases are becoming increasingly recognized as a threat to wildlife conservation especially as zoonotic diseases in the free range and capture wild animals. Transmission of zoonotically important diseases like leptospirosis is commonly encountered either by direct with human being (tourists, researcher, veterinarians and domestic field staff) or from domestic animals grazing in the surrounding areas. As captive elephants are being close proximity with human, thus increasing the chances of cross infection. Hence a detailed prevalence, molecular detection and isolation study was carried out.

Materials and Methods

Serum sample were collected from 51 captive elephants kept in different forest ranges. (19-Bandipur (Karnataka) 12- Shimoga (Karnataka), 20-Madumalai (Karnataka- Tamil Nadu border)). All the samples were initially subjected to dark field microscopic examination using Dark field microscope. Dark field microscopy was done as per Chandrasekhar and Pankajalakshmi, 1997 with minor modification. It was conducted on a minute drop, approximately 10 µl of the processed sample and covering it with cover slip to meticulously look for the typical spiral leptospirea organisms with spinning hooked ends and showing high corkscrew motility.

Then the samples were subjected to the polymerase chain reaction as per the method of Grave kemp, *et al.* 1993 using G1 (5' CTG AAT CGC TGT ATA AAA GT 3') and G2 (5' GGA AAA CAA ATG CTC GGA AG 3') primers. The DNA from the suspected blood/serum sample was extracted by hot-cold lysis method. The reaction was set up in 50 µl reaction volume, containing 5 µl of 10X buffer, 1 µl of primers each, 0.5 µl of dNTPs, 0.5 µl of Taq DNA polymerase, 5 µl of template and final volume was made up using double distilled water. The PCR was performed in a MJ research thermal cycler, for 32 cycles, each consisting of denaturation at 94°C for 90 sec, annealing at 55°C for 60 sec and polymerization at 72°C for 2 min. PCR products were finally electrophoresed on 1.5% agarose gels after staining with ethidium bromide and then visualized with ultraviolet light using Gel documentation system (Biorad). If the template is amplified then it will yield a product of 285bp.

Apart from this the samples were filtered and inoculated into EMJH (Difco) semisolid and liquid medium for isolation as per the procedure of Venkatesh *et al.* 1997. A loopful of processed sample was inoculated aseptically into screw cap tubes containing EMJH semisolid medium. The tubes were incubated at the room temperature for 4-6 weeks and were examined at weekly interval for the presence of Dingers ring to check the growth of any leptospires. The cultures were also observed under DFM for viable organisms and to ensure purity and cultural stability of the isolation.

Results and Discussion

All the 51 samples didn't reveal any leptospira like organisms on DFM examination and none of the samples produced an amplicon size of 285 bp indicating that all the serum samples of captive elephants screened in the present study were

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Sarus Crane in Chimnabai Sarovar, North Gujarat

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Sarus Crane *Grus antigone* is said to be a resident, nomadic and locally common (in Central and NW India, Uttar Pradesh, Gujarat, Rajasthan, Assam, North Andhra Pradesh; Pakistan; Nepal; Bangladesh; Myanmar; breeds in Pakistan, N India, Nepal and Myanmar) of Indian Subcontinent (Grimmett *et al*. 1998 and Arun Kumar *et al*. 2005). Of three cranes found in Gujarat (Common Crane, Demoiselle Crane and Sarus Crane), Sarus is the most popular among local people. In fact, this bird has a place in the local traditions, culture and folklore (Pandey 2006). The love and compassion of the local community towards this graceful bird have played a decisive role in its survival. It is a resident bird for Gujarat which moves locally with variations and drought conditions and water availability. Chimnabai Sarovar is situated in North Gujarat ($23^0$55'N & $72^0$38'E) at Mehsana district. It is located 6 km far from Kheralu. On 3rd January, 2009 at 11:00 AM, when we visited Chimnabai Sarovar, sighted a pair of Sarus Crane in Black gram farm field, which is situated on the bank of Sarovar in feeding posture. After a week on 10th January 2009, a pair was also sighted at Kharodo, is located between at Miyasana and Nandali village. During our observations of three year duration, it was first sighting of Sarus Crane at above area. This area is virgin for Ornithology point of view. So, no literatures are found on sighting of the Sarus Crane.

The species is listed on Appendix II of CITES. It receives full legal protection in all range countries. It was further suggested that the species be upgraded from CITES Appendix II to Appendix I. In India, the species should be moved from Schedule IV to Schedule I of Wildlife Act (BirdLife Int. 2001) (Arun Kumar *et al*. 2005).

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negative for leptospires. The same was further confirmed by isolation studies as none of the EMJH tubes showed the formation dingers ring even after six weeks. Indicating that all the 3 tests used in the present study for the screening of Leptospirosis were correlating with each other as none of the elephant serum samples was found positive for leptospires in the present study by all the tests. It can be concluded that the elephants screened for the study were free from leptospira active infection.

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Prevalence of Parasitic infestations in Captive wild Carnivores at Dhaka Zoo

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Fecal examination of different captive wild carnivores at Dhaka Zoo showed numerous parasitic infestations even after treatment with Ivermectin (1% soln.), Albendazole (600 mg tablet), Mebendazole (100 mg tablet) and Levamisole (600 mg tablet), recurrent within 4 months. Single animal exhibit showed less chance of re-infection than multiple animal exhibits. *Toxocara* sp., *Strongyloides* sp. and *Ancylostoma* sp (hook worm) were found in higher prevalence in almost all group of carnivores while *Diphylobothrium latum*, *Dipylidium* sp. and *Dirofilaria immitis* rarely found as infestation in unnatural host to Asiatic lion during the study (From July, 2005 to October, 2005) which could be considered as one of exciting findings.

Introduction

Wild animal harbor numerous parasites in their natural environs, but seldom lead to harmful infection unless stressed (Gaur *et al.*, 1979). Sporadic reports of parasitic infestation in different zoo animals (Chauhan *et. al.*, 1973 and Gaur *et. al.*, 1979) and systemic study in different species of animals in Assam state zoo (Chakraborty *et al.*, 1994) and Nashiruddullah and Chakraborty 2001) in India have been reported but a systemic study in zoo animals in Bangladesh was lacking. The present study, reports the prevalence of various parasitic infestation in captive carnivores through fecal examinations at Dhaka Zoo research section. The Dhaka zoo has a collection of 1829 number of animals, birds and reptiles, of 140 species in both semi natural settings and confinement. A total of 61 animals of 11 species were observed for this study out of 73 carnivores.

Materials and Methods

Fecal samples of different species of carnivores of Dhaka Zoo were collected between 2nd July to 16th July, 2005 and treatment given subsequently on the following day of feces examination for this study period. The fecal samples of all carnivores were re-checked for infection between 2nd October to 16th October, 2005.

Collection of Feces: Feces were collected in the morning for examination. They were processed for concentration by direct smear and sedimentation - flotation techniques (centrifugation). Presence of helminthic ova and/or segment (proglottid) of Cestode/tape worm (*Dipylidium* sp., and *Diphylobothrium* sp.) and ova of *Dirofilaria* sp. for one or more examinations were taken as positive infestation.

Treatment: Ivermectin (1%) solution, Albendazole (600 mg tablet), Mebendazole (100 mg tablet) and Levamisole (600 mg tablet) were used for treatment at higher dosage than recommended dosage by the manufacturer to overcome the early experienced resistance during the phases of treatment following fecal examination. This procedure was attempted based on past personal experiences.

Results and Discussion

The prevalence of endo-parasitic infections of captive wild carnivores is presented to Table-1.

The dosage of drugs details are given in Table-2.

Ivermectin was used for lion, leopard, cheetah, Bengal tiger, Asiatic black bear, ratel, striped hyena and Spotted hyena with the dosage of 40 mg (1.43 times), 30 mg (2.14 times), 25 mg (2.08 times), 50 mg (1.39 times), 35 mg (1.25 times), 7.5 mg (3.13 times), 10 mg (1.10 times) and 12.5 mg (1.25 times higher than recommended dosage for cats and dogs) respectively.

Albendazole was administered to fishing cat-900 mg (1.5 times higher than recommendation); Mebendazole to palm civet- 500 mg (1.25 times higher than recommendation) and Levamisole to terrier dog- 300 mg (1.5 times higher than recommendation) to cover the risk of drug resistance due to repeated use of same anthelmintics.

The most frequently observed parasites were *Toxocara* sp*., Strongyloides* sp*.* and *Ancylostoma* sp. and *Toxocara* sp was found to be most prevalent among carnivores. Samples from males and females showed no distinction in infections. These findings are supported by the earlier report of Ravindran, *et al.* (2006), Chauhan *et al.*, (1973), Tripathy *et al*. (1971) and Chakraborty *et al.* (1994). Tigers and lions were more prone to parasitic infection and even after treatment, in 2nd phase of fecal examination, these animals showed high percentage of *Toxocara* and *Strongyloides* sp. Out of 12 samples of tigers, 4 samples were negative for worm infestation in 2nd phase of examination and in case of lion, 3 samples were negative in 2nd phase of examination.

It was seen that re-infection did not occur incase of the singly inhabited animal enclosure where contamination of food with ova were less. In case of ratel no ova were found in 2nd phase of

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examination. Among tiger, lion, leopard, cheetah, fishing cat, hyeana, and terrier dog, *Toxocara* infections were common even after treatment.

In Asiatic black bear, tape worm infection prevailed even after treatment which indicated that these animals need more specific and intensive treatment.

Some exciting exceptions of infecting unnatural host have emerged out of this study, i.e. *Diphylobothrium, Dipylidium* and *Dirofilaria* in Asiatic lion**.**

As there have been no previous reports on the prevalence of endo-parasites of captive wild carnivores in Bangladesh, the result of the present study can only be compared with those reports elsewhere. Helminthes occupy an important place among the parasites in the pathology of wild animals (Hediger, 1964). His study suggested periodic treatment to reduce the percentage of infection to the minimum since it is difficult to eradicate completely. Chakraborty and Maity (1995) reported the death of five 1½ month old Himalayan wolf pups at Darjeeling Zoo caused by *Toxocara cani*s. Successful treatment of clinical cases of *Toxocara* sp. infection in snow leopards caused by *Toxocara cati* has been reported from Darjeeling Zoo (Maity *et al.,* 1994). An Asiatic lion of Bikaner Zoo which suffered from parasitic gastritis caused by *Toxocara leonina* was successfully treated by Tanwar *et al.,* 1984. A survey of fecal samples of wild mammals of Kanpur Zoo revealed the presence of *Toxocara leonina* in both African and Asiatic lions, tiger and fishing cat. *Ascaris felis* was noticed in Asiatic lion in Nandankanan zoo. *T. leonina* from Asiatic lion and tiger; *T. cati* from leopard and jungle cat and *T. canis* from tiger jackal at Assam State zoo have been demonstrated (Nashiruddullah & Chakraborty, 2001). *Toxocara mystax* from tiger, leopard, jungle cat and leopard cat and *T. transfuga* from Himalayan black bear, sloth bear and red panda have also been reported (Chowdhury, 2001). Chowdhury (2001) described, hookworm infection has also been recorded from different species of wild carnivores from India.

Summary: A study of Intestinal helminthic infestation in captive carnivores of Dhaka Zoo was conducted through examination of feces i.e. during July'05 to Oct'05. The result obtained from this survey indicated that periodic examination of feces and suitable treatment on fecal examination is an essential part for the management of carnivore animals.

Concluding remarks:

1) It is important to check the presence of immature parasites even after treatment.
2) Use of combination of anthelmintics may help in avoiding resistance that has been observed to occur on use of a single drug over long periods of time.

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Table-1: The prevalence of endo-parasitic infections of captive wild Carnivores

No. of Animals	No. of sample examined (in 2 phases)	No. of sample showed positive/negative		Ova detected / represented parasites with level of significance with extent and prevalence	
		1st Phase	2nd Phase	1st Phase	2nd Phase
Bengal tiger (*Panthera tigris*), 12 nos.	24 nos.	12 positive	8 positive, 4 negative	*Toxocara*+++, *Paragonimus*+, *Strongyloides*++	*Toxocara (Rare), Strongyloides*+
Asiatic lion (*Panthera leo persica*), 21 nos.	42 nos.	20 positive 1 negative	18 positive 3 negative	*Diphylobothrium latum*+, *Toxocara*+++, *Dipylidium*+, *Strongyloides*++, *Dirofilaria immitis on PM*	*Toxocara*++, *Strongyloides*+, *Dipylidium* +
Leopard (*Panthera pardus*), 3 nos.	6 nos.	3 positive	2 positive 1 negative	*Toxocara*++, *Strongyloides*+++	*Toxocara*+, *Strongyloides*++
Cheetah (*Acinonyx jabatus*), 2 nos.	4 nos.	2 positive	1 positive 1 negative	*Toxocara*++, Larva of *Strongyloides* present+	*Toxocara*+
Fishing cat (*Felis viverrina*), 4 nos.	8 nos.	4 positive	4 positive	*Toxocara*+++, *Hookworm*++ *(Ancylostoma sp.)*	*Toxocara*++
Striped hyena (*Hyaena hyaena*), 4 nos.	8 nos.	4 positive	3 positive 1 negative	*Toxocara*++, *Strongyloides*+	*Toxocara*+, *Strongyloides*+
Spotted hyena (*Crocuta crocura*), 3 nos.	6 nos.	3 positive	2 positive 1 negative	*Toxocara*+++, *Strongyloides*++	*Toxocara*++, *Strongyloides*+
Terrier dog, *Canis lupus familiaris* sp. 7 nos.	14 nos.	7 positive	5 positive 2 negative	*Toxocara*+, *Strongyloides*++	*Toxocara*+, *Strongyloides*+
Asiatic black bear (*Selenarctos thibentanus*), 3 nos.	6 nos.	3 positive	2 positive 1 negative	*Segment of Tapeworm*+, *Strongyloides*+++	*Segment of Tapeworm*+, *Strongyloides*+
Ratel (*Mellivora capensis*), 1 no.	2 nos.	1positive	1 negative	*Toxocara*++, *Strongyloides*+++	No Ova found
Palm Civet (*Viverra zibetha*) (Bagdas), 2 nos.	4 nos	2 positive	2 positive	Larva of *Strongyloides* Present+	*Strongyloides*+

Note: + indicates level of significance and extent of infestation (1-2 ova has got single +, 3-5 double +, 6-more triple + in a single slide field though higher number of ovum didn't always considered highly significant).

Table-2 : Treatment details of the study.

Drugs and unit composition	Animals treated and average wt. considered	Recommended dosage for cats and dogs and total requirement (Fraser C. M. *et al.*,1986))	Dosage applied and Route of administration
Ivermectin 1% (10 mg/ml)	Asiatic Lion (140 kg), Leopard (70 kg), Cheetah (60 kg), Bengal tiger (180 kg), Asiatic black bear (140 kg), Ratel (12 kg), Stripped hyena (45 kg), and Spotted hyena (50 kg)	200-300 µg/kg bw parentral, .003-.006 mg/kg oral (Canids) (Lion- 28 mg/head, Leopard- 14 mg/head, Cheetah- 12 mg/head, Bengal tiger- 36 mg/head, Asiatic black bear- 28 mg /head, Ratel- 2.4 mg/head, Stripped hyena- 9 mg/ head and Spotted hyena- 10 mg/ head)	Asiatic Lion - 4 ml/head, *Leopard - 3 ml/head, *Cheetah – 2.5 ml/head, Bengal tiger- 5 ml/ head, Asiatic black bear- 3.5 ml /head, Ratel- ¾ ml/head, Stripped hyena- 1 ml/ head and Spotted hyena- 1.25 ml/head. I/M instead of s/c or oral
Albendazole (600 mg/tab)	Fishing cat (30 kg)	15-20 mg/kg bw @ 20 mg (600 mg)	1.5 tab/head with meat/feed
Mebendazole (100 mg/tab)	Palm civet (8 kg)	15-50 mg/kg bw @ 50 mg (400 mg)	5 tab/head with meat/feed
Levamisole (600 mg/tab)	Terrier dog (10 kg)	15-20 mg/kg bw @ 20 mg (200 mg)	½ tab/head with meat/feed

***Large swelling was found to the injection site in Leopard and Cheetah but disappeared within 96 hrs.**