Prevention of Trypanosomiasis: Lessons Learned from Nandankanan Zoo, Orissa

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Introduction

Nandankanan ("Garden of Gods"), famed for the breeding of white Tiger, Gharial, Crocodile, Asiatic Lion, Leopard, Lion-tailed Macaque, Nilgiri Langur, Indian Pangolin, Mouse Deer and many land and water birds etc., received a set back to its creditability in a rare catastrophe that took 12 of its valued tigers (8 white and 4 normal-coloured) during June-July, 2000. Nandankanan was affected by the dreaded blood-borne disease 'Trypanosomiasis'. Efforts made to treat and save the affected tigers were in vain. This was earlier preceded by the unprecedented super cyclone of October 1999, which had devastated the zoo's landscape.

Trypanosomiasis

Trypanosomiasis is a protozoan infection of the blood in humans and other animals caused by trypanosome parasites (Caddis/PAAT). The parasite is a flagellate protozoan. Members of this genus occur in vertebrates principally in the blood, although, a few may invade tissue cells. They are transmitted by blood-sucking arthropods in which their developmental stages occur. A few species are transmitted mechanically (i.e. there is no cyclical development in arthropod hosts). The species of trypanosomes consist of *Trypanasoma brucei*, *T. vivax*, *T. congolense*, *T. semiae* and *T. evansi* of which only two are known in India, namely, *T. equipedrum* and *T. evansi*, most of other species are confined to African countries and a few to South America. Geographically, *T. evansi* occurs in the Indian subcontinent typically, but is now widespread in far east, near east, North America, Central and South America (Soulsby, 1986). In Africa, the chief vector of the disease is Tse-tse Fly (*Glossina* spp.). In India, the main vectors are Tabanids *viz.*, *Tabanus*, *Stomoxys*, *Haematoptoa* and *Lyperosia* spp. Animal trypanosomiasis represents one of the most serious veterinary problems in the world. While most other animal diseases have been successfully controlled during the 20th century, trypanosomiasis continues to represent a major threat to animal production and species conservation at large.

Generally, parasites do not pose any problem as far as the mortality of tigers are concerned (Tilson & Seal, 1987). As far as we have known, trypanosomiasis does not occur in tigers or even in lions. It occurs in man and herbivores in Africa. It has not been recorded from lions. The symptoms of the tigers that died in Dhaka Zoo do not match with those of trypanomiasis either. Besides, 'Tse-tse' fly does not exist in Bangladesh (Masih-ul Alam, Director, CESTI, Alliance Francáise, Dhaka, *pers. comm.*). During 1947, a tiger weighing 200lb owned by a circus troupe at Nellore District, Andhra Pradesh died of *T. evansi* despite treatment with Antrypol. But a younger tiger of 16 months could be saved with the same drug (Raju & Swaminathan, 1947-1948). In 1973, six tigers of a circus were affected with *T. evansi*, out of which four were treated with Inj. Berenil (0.8g/100lb b.wt. I/M) and two were treated with Antrycide successfully (Reddy *et al.*, 1975). In 1964, a male tiger at Trichur Zoo, Kerala, administered with Antrycide s/c recovered (Nair, 1967). In 1965, a 10-year old tiger was treated with Antrycide and supported with i/v fluid therapy (Krishnan, 1982). A tiger cub was cured of trypanosomiasis with injection of Triquin at National Park, Madhya Pradesh (Saxena, 1993). Nine tigers, five Pumas and one Indian Lion affected with trypanosomiasis were successfully treated with Antrycide at 2mg/lb S/C for five nos. and Berenil at 0.8g/100kg b.wt. (Khan *et al.*, 1982). In 1978, Berenil 2.4g total dose with supportive therapy for trypanosomiasis in a white tiger was successful in Nehru Zoological Park, Hyderabad, India. A case of sudden death in a male tiger cub with trypanosomiasis was reported by Rao (1986). An adult male tiger 'Chadu' of nine years in Maharajabag Zoo, Nagpur, was successfully treated against trypanosomiasis with Berenil (total dose of 3g) and dextrose 10%.

An essential factor in mechanical transmission is, interrupted feeding in flies, which go from one host to another quickly. *Trypanosoma* do not survive more than 10-15 minutes in the proboscis of a fly. This is crucial related to the quick and successful transmission of parasites between animals which are crowded together in small cages. Since, tigers are solitary animals in wild, they are naturally protected against such vector transmitted diseases.

Trypanosoma evansi affects a wide range of hosts including horses, cattle, camels, buffaloes, elephants, pigs, cats, Tapirs, Capybara and deer. Laboratory rodents such as mice, guinea pigs, rats and rabbits can be readily infected. In the Indian subcontinent, this disease caused by the trypanosomes, particularly in horses is known as 'surra'. Surra is nearly fatal if treatment is not carried out on time. The parasite *T. evansi* is believed to cause chronic infection in big cats. The important clinical signs of the disease is high rise in temperature, staggering gait, anaemia, nervous signs, discharge from eyes, oedematous swellings under the skin, bloody urine, etc. The syndrome is similar to chronic renal failure. In dogs and felids, the disease tends to be acute and fatal (e.g. Wild Dogs in Chennai Zoo, tigers in Dhaka Zoo, reported recently). Oedema is marked. Bi-lateral opacity of cornea is common. Due to oedema of larynx, volce changes similar to rables may be noticed.

Early treatment of trypanocidal drugs can be essentially used. The parasites successfully manage to escape the host immune system, which happens to be a very complex mechanism of antigen switching (Hunt, 2000). This particular parasite is well known for its antigenic variation in that it can change its surface coat antigens a number of times. It is said that once the parasites enter, they remain in

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Table 1: Particulars of report on tiger deaths

Name	Encl. No.	Phenotype	Sex	Age	Date of death	Time of death	Post-mortem findings
Debasish	31A	NCT	M	12	23.6.2000	16.30hr	Enlargement of both the kidneys with fibrotic changes. Blood samples and urine examination revealed *Trypanosoma* positive *Microfilaria* and *Leptospira* positive
Sagar	33A	NCT	M	8	4.7.2000	18.30hr	Preliminary PM report indicated to be trypanosomiasis. Sample tissues sent to Orissa Veterinary College and later confirmed to be *Trypanosoma* positive.
Arun	White Tiger Safari	WCT	M	8	5.7.2000	22.00hr	-do-
Chandan	-do-	WCT	M	8	5.7.2000	6.00hr	-do-
Durga	32D	WCT	F	14	5.7.2000	6.00hr	-do-
Petu	32C	NCT	M	13	5.7.2000	8.00hr	Enlarged lymph node. Congested spleen.
Surya	White Tiger Safari	WCT	M	3	5.7.2000	8.00hr	-do-
Aneesh	-do-	WCT	M	3	5.7.2000	22.45hr	-do-
Janak	33C	WCT	M	6	5.7.2000	11.00hr	-do-
Bandhabi	32C	NCT	F	10	5.7.2000	12.00hr	-do-
Shyama	32C	WCT	M	13	6.7.2000	8.00hr	Congested liver, lymph gland and enlarged spleen.
Abinash	White Tiger Safari	WCT	M	3	7.7.2000	10.30hr	Enlarged lymph node, congested and enlarged spleen. Congested liver, both kidneys enlarged and edematous.

the body for a very long time, intermittently causing problems. Although the host immune system kills most of the parasites, a few survive by rapidly changing the antigen surface coat. The trypanosomes have a very odd nuclear genome. The chromosomes do not condense in nuclear division hence, their number is not known. The nuclear genome includes 1000-2000 genes that encode the variable surface antigens that allow the host of the organism to be changed regularly so that it can avoid the host's immune response (Hunt, 2000). These few parasites then multiply rapidly to cause another cycle of parasitaemia. The killed parasites are equally dangerous as their break down results in the release of several endogenous enzymes that destroy RBC and cause lesions in other organs. Hence, it is important to diagnose the disease before treatment is ensured.

A vaccine to control or prevent the disease has not been developed yet. For treatment, several trypanocidal chemotherapeutic drugs such as Antrypol (Bayers), Antrycide (Quinapyramine; ICI), Berenil (Hoechst), Quinapyramine Sulphate and Chloride are available in different brand names (Triquin, Tribexin, Trypanil).

Trypanosomiasis in Tigers at Nandankanan

The post-mortem examination of one tiger Debasish on 23 June 2000 confirmed the cause for death to be due to trypanosomiasis. It was the first signal for a disaster in the offing. On 3 July, 16 tigers were given the drug Berenil (Hoechst) injection as prophylaxis, *i.e.*, 11 tigers (adults) were administered 1.6g in 10ml distilled water i/m rt. and five tigers (cubs and juveniles) 0.8g in 5ml distilled water i/m rt. Out of them, all the adults died while all the young ones survived. Prior to the deaths, the zoo supported 56 tigers. The symptoms prior to death were high temperature, bloody urine, staggering gait or hind leg paralysis, etc. On 4 July between 0630-1200hr, Sagar (NCT) was seen staggering and sinking in the wet moat. Seven animals (5 WCT & 2 NCT) died in succession and finally on 7 July morning at 1030hr, one more tiger 'Abinash' (WCT) died.

Study and observation

Report on prevalence of trypanosomiasis in the peripheral vicinity:

With the assistance of the Department of Animal Husbandry and Veterinary Services, reports were sought from veterinary dispensaries (Chandaka, Saheednagar, Nayapalli & Baranga) around Nandankanan. As per the reports, these zones were free from trypanosomiasis and no previous case had been observed in livestock.

Comparative analysis of inbreeding co-efficient and pedigree of the dead tigers to those that were healthy (WII report):

As part of the Central Zoo Authority's studbook compilation program of five endangered animals in Indian zoos, the Wildlife Institute of India had collected primary data on pedigrees of the tigers of Nandankanan zoo. These data were used to trace the genealogy of the recently dead tigers and calculate the inbreeding coefficient for each dead tiger.

Primary data collected from the zoo were compiled and analyzed with the help of a computer program – Single Population Analysis and Record Keeping System (SPARKS). This program is exclusively used for studbook maintenance, demographic and genetic analysis.

Output obtained after analysis of data (Table 2) is as inbreeding co-efficient report and pedigree chart report (only dead tigers). Ten out of 12 dead tigers exhibit certain level of inbreeding. The inbreeding coefficient in the dead tigers varied from 0.1171 to 0.2812 (mean ± SE = 0.1598 ± 0.025676). The inbreeding coefficient of the remaining two tigers was assumed to be zero as they came from a different population and their percentage is not known. These two tigers were acquired from Shivpuri, Madhya Pradesh on 13 August 1998 to add new blood line to the existing tiger population of Nandankanan.

Like the dead ones, the surviving tiger population of Nandankanan also exhibits some level of inbreeding. Their

Table 2: Inbreeding coefficient of dead tigers at Nandankanan during June-July 2000

Local stud #	Sex	Age	Sire	Dam	Location	Death date	Inbreeding	Name of the Tiger
201	Female	7y, 10m,29d	64	57	Nandankanan		0.1875	
202	**Male**	**7y, 10m,10d**	**102**	**90**	**Nandankanan**	**5 Jul. 2000**	**0.1914**	**Arun**
214	**Male**	**6y, 10m, 10d**	**113**	**55**	**Nandankanan**	**5 Jul. 2000**	**0.2812**	**Chandan**
223	Female	6y, 7m, 17d	92	59	Nandankanan		0.2812	
224	Female	6y, 7m, 17d	92	59	Nandankanan		0.2812	
226	Male	6y, 1m, 14d	64	57	Nandankanan		0.1875	
236	**Male**	**5y, 8m, 7d**	**156**	**78**	**Nandankanan**	**5 Jul. 2000**	**0.1562**	**Janak**
241	Male	5y, 1m, 11d	165	101	Nandankanan		0.2031	
243	Male	5y, 1m, 11d	165	101	Nandankanan		0.2031	
248	Female	4y, 11m, 12d	50	54	Nandankanan		0.2500	
253	Male	4y, 7m, 0d	64	57	Nandankanan		0.1875	
252	Female	4y, 7m, 0d	113	55	Nandankanan		0.2812	
254	Male	4y, 4m, 22d	64	57	Nandankanan		0.1875	
260	Female	2d	181	187	Nandankanan	18 Apr. 1996	0.3281	
265	Female	3y, 0m, 4d	181	187	Nandankanan		0.3281	
266	Female	3y, 0m, 4d	181	187	Nandankanan		0.3281	
267	**Male**	**2y, 9m, 14d**	**157**	**78**	**Nandankanan**	**7 Jul. 2000**	**0.1562**	**Abinash**
268	**Male**	**2y, 9m, 3d**	**157**	**78**	**Nandankanan**	**5 Jul. 2000**	**0.1562**	**Aneesh**
270	**Male**	**2y, 8m, 19d**	**165**	**101**	**Nandankanan**	**5 Jul. 2000**	**0.2031**	**Surya**
280	Male	2y, 3m, 5d	92	201	Nandankanan		0.2421	
281	Male	2y, 3m, 5d	92	201	Nandankanan		0.2421	
283	Female	2y, 2m, 28d	226	224	Nandankanan		0.2617	
284	Female	2y, 2m, 28d	226	224	Nandankanan		0.2617	
288	Male	1y, 10m, 3d	112	88	Nandankanan		0.2500	
289	Male	1y, 10m, 3d	112	88	Nandankanan		0.2500	
290	Male	1y, 10m, 3d	112	88	Nandankanan		0.2500	
292	Female	1y, 8m, 1d	181	187	Nandankanan		0.3281	
295	Female	1y, 0m, 1d	199	223	Nandankanan		0.2617	
297	Female	6m, 21d	165	101	Nandankanan		0.2031	
298	Female	6m, 21d	165	101	Nandankanan		0.2031	
299	Female	6m, 21d	165	101	Nandankanan		0.2031	
300	Male	6m, 21d	165	101	Nandankanan		0.2031	
301	Unknown	3m, 24d	226	224	Nandankanan		0.2617	
302	Unknown	3m, 24d	226	224	Nandankanan		0.2617	
303	Unknown	3m, 24d	226	224	Nandankanan		0.2617	
304	Unknown	3m, 24d	226	224	Nandankanan		0.2617	
286	**Male**	**Unk Birth**	**Unk**	**Unk**	**Nandankanan**	**5 Jul. 2000**	**0**	**Petu**
287	**Female**	**Unk Birth**	**Unk**	**Unk**	**Nandankanan**	**5 Jul. 2000**	**0**	**Bandhabi**
237	Male	Unk Birth	Wild	Wild	Nandankanan		0	
1*	Male	19y	Wild	Wild	Rewa	19 Dec. 1969	0	
2*	Female	18y, 2m, 9d	Wild	Wild	Ahmedabad	3 Sep. 1969	0	
3*	Female	18y, 11m, 27d	1*	2*	Delhi	2 Apr .1974	0	
1	Male	16y, 10m, 26d	Wild	Wild	Nandankanan	23 Nov. 1973	0	
2	Female	17y, 10m, 21d	Wild	Wild	Nandankanan	18 Aug. 1974	0	
11*	Male	18y, 9m, 24d	1*	3*	Delhi	19 Aug. 1977	0.2500	
13*	Female	19y, 0m, 1d	1*	3*	Delhi	26 Oct. 1977	0.2500	
84*	Male	19y	Wild	Wild	Delhi	18 Jun. 1979	0	
7	Female	16y	Wild	Wild	Nandankanan	23 Sep. 1983	0	
6	Male	18y, 6m, 17d	1	2	Nandankanan	10 Feb. 1986	0	
97*	Female	30y, 3m, 15d	11*	13*	Delhi		0.3750	
19	Male	10y, 11m, 17d	6	7	Nandankanan	6 Jun. 1984	0	
17	Female	19y, 6m, 6d	1	2	Nandankanan	15 Jun. 1992	0	
21	Female	25y, 4m, 16d	6	7	Nandankanan		0	
22	Female	13y, 9m, 24d	19	17	Nandankanan	11 Feb. 1991	0.1250	
26	Male	15y, 4m, 0d	6	7	Nandankanan	17 Sep. 1992	0	
27	Female	21y, 8m, 29d	84*	97*	Nandankanan	28 Feb. 1999	0	
33	Female	15y, 1m, 25d	6	21	Nandankanan	28 Feb. 1995	0.2500	
35	Male	10y, 6m, 8d	6	21	Nandankanan	14 Jul. 1990	0.2500	
34	Female	10y, 5m, 10d	6	21	Nandankanan	8 Jan. 1991	0.2500	
50	Male	13, 6m, 23d	26	22	Nandankanan	3 May 1995	0.1875	

Local stud #	Sex	Age	Sire	Dam	Location	Death date	Inbreeding	Name of the Tiger
51	Female	18y, 9m, 2d	6	27	Nandankanan		0	
52	Male	7y, 9m, 24d	6	27	Nandankanan	12 Aug. 1989	0	
54	Female	16y, 5m, 18d	19	17	Nandankanan	11 Apr. 1998	0.1250	
55	Female	14y, 10m, 29d	6	21	Nandankanan	2 Nov. 1996	0.2500	
56	Male	17y, 9m, 23d	6	21	Nandankanan	27 Sep. 1999	0.2500	
57	Female	16y, 7m, 11d	6	21	Nandankanan		0.2500	
59	Female	11y, 8m, 26d	6	21	Nandankanan	3 Sep. 1997	0.2500	
64	Male	13y, 1m, 13d	35	27	Nandankanan	6 Feb. 1997	0	
67	Female	16y, 4m, 4d	26	22	Madras		0.1875	
78	Female	14y, 8m, 26d	35	27	Nandankanan		0	
88	**Female**	**13y, 10m, 21d**	**52**	**33**	**Nandankanan**	**5 Jul. 2000**	**0.1875**	**Durga**
90	Female	7y, 9m, 4d	52	33	Nandankanan	3 Jun. 1994	0.1875	
92	Male	12y, 0m, 11d	52	55	Nandankanan	7 Dec. 1998	0.1875	
101	Female	12y, 10m, 29d	35	27	Nandankanan		0	
102	**Male**	**12y, 10m, 18d**	**64**	**67**	**Nandankanan**	**7 Jul. 2000**	**0.1171**	**Shyama**
111	Male	12y, 2m, 17d	56	51	Nandankanan		0.1875	
112	Male	12y, 2m, 17d	56	51	Nandankanan		0.1875	
113	**Male**	**12y, 2m, 6d**	**56**	**51**	**Nandankanan**	**23 Jun. 2000**	**0.1875**	**Debashish**
135	Female	11y, 1m, 20d	64	57	Nandankanan		0.1875	
147	Female	10y, 3m, 13d	50	54	Nandankanan		0.2500	
149	Male	10y, 2m, 27d	35	27	Nandankanan		0	
153	Male	10y, 1m, 10d	26	78	Nandankanan		0.1250	
154	Male	10y, 1m, 10d	26	78	Nandankanan		0.1250	
156	Male	10y, 1m, 6d	26	34	Nandankanan		0.2500	
157	Male	10y, 1m, 6d	26	34	Nandankanan		0.2500	
165	Male	9y, 10m, 24d	56	51	Nandankanan		0.1875	
175	Male	8y, 11m 6d	26	78	Nandankanan		0.1250	
181	**Male**	**8y, 7m, 19d**	**113**	**55**	**Nandankanan**	**4 Jul. 2000**	**0.2812**	**Sagar**
187	Female	7y, 2m, 29d	92	59	Nandankanan	6 Jul. 2000	0.2812	
189	Male	8y, 3m, 11d	56	51	Nandankanan		0.1875	
199	Male	7y, 10m 29d	64	57	Nandankanan		0.1875	

Animals that died at Nandankanan Zoological Park are in bold; * National stud-book numbers of individuals; Numbers without * are local identification numbers given to them in the zoo.

Inbreeding coefficient varied between 0 to 0.3281 (mean ± SE = 0.212222 ± 0.010526; n = 45). Mean inbreeding coefficient when compared between the dead and the live tigers did not show any significant difference (Z = /-1.888, P = 0.06, n = 12, Mann-Whitney U test).

With the present information, it is not evident that tiger mortalities were a consequence of inbreeding. Inbreeding might have played a role in the death of the tigers through suppressed immunity to disease, but it cannot be held as the sole reason behind the deaths. In fact, some of the surviving individuals at Nandankanan show a higher level of inbreeding than the dead ones.

Defence Research and Development Establishment (DRDE) Study and Recommendations:

A team of three scientists, Dr. B.D. Parashar, Dr. Vijay Veer and Dr. Shri Prakash deputed by the Defence Research and Development Establishment, Gwalior undertook pioneering studies in and around the zoo environment on prevalence and population of incriminating vectors from 21–25 August 2000. Their studies and observations brought an insight to the preventive and control measures against diseases especially vector borne, at Nandankanan thereafter. The study and observation by DRDE team was repeated in 9-15 June 2001.

Tables 3 to 5 give details on prevalence of insects, population density and trappings by the specially-designed canopy traps for these insects. The suggested control measures were commenced before the onset of summer season in 2001 for a full year.

As a result of these control measures on tiger and in tiger dens, not a single fly belonging to *S. cancitrans* and *H. irritans exigua* species has been recorded since June 2001 (Table 5), while, before implementation of control measures, their density was 16.2 and 7.5/tiger respectively. This indicates the impact of control measures recommended by DRDE, Gwalior and implemented by the zoo authorities successfully.

Recommendations and suggestions

The guidelines for improving animal housing, healthcare, zoo management, prophylactic treatments and preventive measures by various inspecting committees were taken up expeditiously. Besides there were suggestions/ recommendations from large number of individuals, NGOs and other institutions, many of which were beyond the ambit of zoo management and present organizational structure. They are described here in two parts - by Central Zoo Authority and by others.

Table 3: Species composition of flies and ticks collected from Nandankanan Zoo, Bhubaneswar during 21-25 August 2000

Family	Species name	Found on or near	Trypanosomiasis vector status
Tabanida	*Tabanus rubidus*	Tiger, deer enclosure, Raghunathpur village, Mithun enclosure	Potential Vector
	Tabanus tricepus	Tiger	
	Tabanus sp.	Tiger, Raghunathpur village	
	Tabanus optatus	Tiger area	Vector
	Tabanus striatus	Deer enclosure, Mithun enclosure	Potential Vector
	Atylotus cryptotaxis	Raghunathpur village school camp	
Muscidae	*Stomoxys calcitrans*	Tiger, Deer, Mithun enclosure	Vector
	Stomaxys dubitalis	Tiger enclosure	
	Haematobia irritans exigua	Tiger, Deer, enclosure	Vector
	Musca (Philaematomyia) crassirostris	Tiger, Deer, enclosure	Vector
	Musca sp. (under determination)		
Hard ticks	*Haemaphysalis* sp. (under determination)	Tiger	

A. Guidelines by Central Zoo Authority

1. Upgradation of animal housing, healthcare facilities:
(a) Individual feeding cell
(b) Determining parasitic load and regular interval de-worming
(c) Broken boundary walls may be reconstructed soon.
(d) In-built squeeze cage for individual animal.
(e) Isolation ward at zoo hospital be upgraded and functional.
(f) Feeding cubicles at ground level be raised and drainage be improved.
(g) Moats of enclosures be kept dry and supply of potable water to each enclosure.

2. Prevention of disease:
(a) Immunization of all live stock within 5km of zoo.
(b)Ante-mortem and post-mortem examination of animals slaughtered for beef.
(c) Supply of meat in hygienic and closed containers.

3. Administrative and Financial matters:
(a) Direct flow of funds from CZA to Zoo administration.
(b) Whole time Zoo Director, single line command and proper accountability.
(c) Veterinarians having experience in zoo, preference of trained persons for treatment of zoo animals.
(d) Zoo shall maintain required stocks of life saving drugs and replenish it regularly.
(e) The veterinarians shall be authorized to procure drugs directly in emergency.

4. General:
(a) The State Government shall form a broad-based Managing Committee to supervise functioning of the zoo.
(b) CZA would constitute a team of vets who shall visit the zoo every 2 months for one year and every 6 months there after. Committee should examine PM report of all large cats.
(c) CZA would create a regional health facilities at veterinary colleges and help them to acquire latest diagnostic facilities in respect to wild animals.
(d) Suggestion box at appropriate place to give weightage to different suggestions coming from visitors.

Expert Committee Report

For monitoring and guiding the follow-up actions on guidelines of July, 2000, the Central Zoo Authority constituted an Expert Committee of three members constituting Dr. A.T. Rao, Dr. L.N. Acharjyo, Dr. A.B. Srivastav. The following as per their recommendations were taken up for zoo management.

1) Blood samples, screening for bacteriological, bio-chemical and hematological examination. De-worming and de-ticking includes white washing of wall, sprinkling of 1:3 salt and turmeric powder, sanitization of drain, bush cleaning.
2) Similar test for lions suggested, individual squeeze cage for carnivore recommended, incompatible groups of lions should be separated.
3) Screening of suspected herbivores like deer, zebra.
4) Animal Husbandry Department be contacted to obtain report on occurrence of trypanosomiasis in adjoining areas.
5) Transfer of other cages be made from isolation ward at the hospital.
6) Enclosure design including moat, kraal be reviewed and improved for some of the animals.
7) Conditions of animal house should be improved.
8) Veterinarians having zoo and wild animal health care and management training and exposure be appointed.
9) White tiger safari fence damaged during super cyclone should be raised without delay to check cattle entry.
10) Surrounding sterile belt be created by continued immunization.
11) All enclosures, kraals should have bigger pools and daily changing of water is desired.
12) Animals should be fed in feeding cubicles.
13) ¼ inch G.I. mesh surrounding animal house.
14) Ante-mortem and post-mortem examination of beef be done by qualified veterinarians.
15) Feed should be despatched to each animal house in closed containers.
16) Large population of tigers and lions be reduced.
17) The inbreeding among white tigers leading to mass causalities may be doubtful since out of 12 died, 2 were wild caught. CCMB, Hyderabad, India, be involved in determining inbreeding co-efficient of each felid.
18) For the functioning of such a size of zoo, an officer at least of the cadre of Dy. Conservator of Forests is desired to stay inside the Park.
19) Delegation of authority at various levels.

Table 4: Number of blood sucking flies on different animals and traps in Nandankanan Zoo

Location	SC (per animal)	HIE (per animal)	T (in traps)(*)
Tiger Den	16.2± 5.1	7.5±0.7	6
Lion Den	13.± 1.4	7± 1.4	-
Sambar Enclosure	16.3± 6.3	-	-
Mithun Enclosure	8.5± 0.7	16±1.4	1
Raghunathpur	-	-	-

SC - *Stomoxys calcitrans*; HIE - *Haematobia irritans exigua*; T - Tabanids
(*) trap: specially designed canopy by DRDE, India with black and white colour combination that specifically attract flies

20) Technical Advisory Committee may be formed and keeping liaison with local veterinary institutions.
21) Water from nearby lake should not be given to animals for drinking purpose. Instead bore-wells may be made for the said purpose.
22) Disposal of garbage and leftover bones are to be incinerated/burnt.

B. Recommendation by DRDE

Insects control measures to be implemented at Nandankanan Zoological Park for prevention of vectors incriminating zoo animals.

The studies of the scientists of Defence Research and Development Establishment, (DRDE), Gwalior on the pestiferous species of flies belonging to family Tabanidae, Muscidae and ticks have recommended measures to be adopted for control/management of flies, vectors of trypanosomiasis. Presently, following measures are being executed with effect from January, 2001 with all arrangements made for comprehensive action.

1. For control of insects like Haemotobia (which mostly hovers and come into body contact), Butox formulation of 0.05% or 50 ppm solution in 1 or 2L is applied on the animal body completely once a month. The zoo veterinarians supervise the application on all the animals for observing any hyper-sensitivity action on skin of any tiger or lion or other carnivores. As the supplied eco-formulation of Butox contains 1.25% deltamethrin, 4ml. of this should be diluted to 1L of water and applied on the animal.
2. Walls, fences, inside feeding chambers and other surfaces of all carnivore and omnivore enclosures are treated with K-Othrine WP 2.5% at the concentration of 30mg m-2 once in two months as residual spray.
3. Excreta collected in a pit is covered with soil to prevent contact of insects or is to be burnt at one place either on a daily- or weekly-basis in presence of one forest guard.
4. Canopy traps for the tabanids are to be placed near each enclosure of carnivore, omnivore, herbivore, two of lion safari kraals and 10 each for safaris. The record of traps are to be maintained location-wise and trapped insects are to be burnt daily.
5. The stopping of trespass across boundary and movement of any cattle inside park is ensured.
6. Measures taken in the face of tiger death:

i. Studying hematological and bio-chemical picture in felids

As recommended, physiological, hematological and biochemical profiles have been assessed wherever possible. Fresh blood smear examinations were done every month randomly (see Table 6). In the event of blood smears showing no parasites, immunological and biological tests were supposed to be carried out but facilities were not existing for serological diagnosis of disease in wild animals, especially, carnivores.

Table 5: Population density of blood sucking flies in enclosures of different animals and in traps, in Nandankanan Zoo during 9-15 June 2001.

Location	SC (per animal)	HIE (per animal)	T (in traps)(*)
Tiger Den	0	0	6
Lion Den	14.8	11.6	-
Sambar Enclosure	11.4	4.0	8
Zebra	-	-	2
Raghunathpur village (cattle)		12.6	35.4
6			
Daruthenga village (cattle)	7.5	26.8	2

SC - *Stomoxys calcitrans*; HIE - *Haematobia irritans exigua*; T - Tabanids
No tick was recorded on the tiger's body
(*) trap: specially designed canopy by DRDE, India with black and white colour combination that specifically attract flies

ii. Rat/mice inoculation test for detecting trypanosomiasis

For carrying out the biological tests like mice/rat inoculation, vector isolation test etc. mice inoculation test was performed on normal-coloured tiger Ram, aged about 10 years at Encl. No. 31(A), located at the central place among tiger enclosures. For the purpose white rats were reared at the zoo. During mid September, 2000 blood was collected aseptically from Ram and was inoculated intra-peritoneal to two white mice. Observation made for the fortnight could conclude that the mice were safe having no such symptoms.

iii. Introduction of prophylactic treatment:

In the face of the established cases of trypanosomiasis and the aftermath losses of big cats, a lot of suggestions have come from many quarters regarding the drugs of choice for prophylactic and curative use i.e. Quinapyramine sulphate chloride (trade name TRIQUIN). After consultation with the members of health committee from OUAT, Bhubaneswar and the health committee deputed by Central Zoo Authority, decision was taken to go for triquin injection at four months interval. Though, such prophylaxis is practiced in many zoos over a long period, the Nandankanan management had not earlier needed such intervention. Both animal keepers and zoo vets were motivated to carry out the recommended regime besides vaccination schedules to the fullest of their ability which they did it with finesse. At first, Triquin was administered to group – four of the lions at Safari as a trial. In the long history of captive carnivore management at Nandankanan, Triquin was first administered to lions on 6 September 2000 with supportive treatment to avoid hypersensitiveness. Since introductory action went safe, triquin was administered to normal coloured tigers from 7 September 2000 and to white tigers from 15 September 2000 onwards. However, by end of August 2001, third protocol on Triquin administration was accomplished. Even then short- comings like sloughing of skin at site of

injection, stress, sprain, broken teeth, aggressive behaviors etc. was also experienced. On the subsequent occasions, care were taken to minimize it.

iv. Vector control measures – Butox as body spray:
As decided earlier the vector load of the zoo was well studied by Entomology Division of DRDE (Defence Research Development Establishment). On prevalence and taxonomic identification of Tabanids and associated flies, this organization from Government of India did extensive studies and as per their recommendation on the kind of drugs and dosage, Butox 0.005% or 50ppm (Deltamethrine) now in higher concentration was sprayed on the body of all carnivores in continuous phases as per the recommended dilutions. No case of hypersensitivity has been observed and instead the tigers having more number of ticks and lice felt relieved after the treatment and almost of all tigers were seen free of flies sitting on the body within a month of application. Here it is worth mentioning that *Haematobia irritants exigua* remains attached to the body of animals for major period of their life while *Stomoxys calcitrans* hovers around the animals. Comparative observations shows reduction of number of blood suckling flies on different animals after usage of Butox (Table 4 & 5).

v. Vector control measures – K. Othrine spray at feeding chamber, enclosure and its vicinity:
Spraying of K. Othrine W.P. 2.5% at the above sites with recommended dosages, dilution and precautions were also carried out as per the guidelines of DRDE, Gwalior, which has shown good result. The entry of flies into the enclosures and resting on walls, fences and surfaces were checked. In this case, care was taken to spray the chemical in the morning hours. The feeding cubicle floor was washed by flushing Dettol solution. However, in cases of old animals and cubs, spraying of feeding chambers floor was avoided. The man in operation used spectacles, apron, cap and face mask to avoid the ill effects of the chemical. The walls, wall fencing, inside walls of feeding chambers are sprayed because *Stomoxys* and *Haematobia* flies rest on these places. The ground soil inside the enclosure and the trees (up to 6' height) were also treated with the same concentration. Hence, observations have shown that ground soil was free of ticks and load of Tabanids. Besides, other associated flies have also substantially been reduced as shown in the comparative tables 4 and 5.

vi. Vector control measures – installation of canopy and insect flashers:
The control of tabanids is a most difficult task as they come in contact of animal body only for short duration of blood meals. Many of the species are capable of dispersal up to a distance ranging from 500m to 7km. To trap these flies, DRDE has designed and developed a canopy or umbrella like white and black coloured device which was used in tiger line and a few in rest of the zoo. 10 traps were installed in the vulnerable pockets of tiger line and daily the trapped flies were collected and recorded. These traps are simple, eco-friendly, easy to operate and cost-effective. Hence, thousands of flies which were trapped by these canopies is supposedly an approach to understand the intensity and type of vector load as well as to reduce the overall vector load from the area.

vii. Routine deworming measures:
To reduce the overall parasitic loads in all the felids, faecal sample of each individual is examined regularly at the zoo hospital. The positive cases were treated with pyrantel palmoate and Albendazole with requisite dosage. After a fortnight the faecal samples from the treated cases were again examined. It was observed that more than 90% cases responded to the above drugs and few cases which continued to carry the parasites were treated with Ivermectin with requisite dose. In this way 100% F/S cased were routinely examined in tigers in particular and the animals were kept parasite free as far as practicable in a way not to facilitate to attack of trypanosomiasis.

viii. Ante-mortem and post-mortem examination of the cattle at site of slaughter:
Since cattle and buffaloes are considered to be main reservoirs and the meat is the principal feed of the tigers, it was necessary to have both ante- and post-mortem examination. Immediately after slaughter, the blood smears were collected at random and examined at zoo hospital and at times, at Orissa Veterinary College. Immediately after the beef reached the feed-receiving centre of the zoo, random beef smears were also examined and all samples have been found till date to be negative against trypanosomiasis. It is worthwhile to mention that the slaughter place (Village-Jadupur) is 30km away. The hygiene and functioning at the place of slaughter was enforced to be modified from September, 2000 as follows:
(a) The cattle were slaughtered on a pucca floor (402 / 202) with wire-net covering.
(b) Drainage of blood was facilitated by a newly built passage.
(c) The offals were disposed at a distant place.
(d) After slaughter formalities, the place was washed by Dettol/bleaching powder wherever necessary.
(e) A water tank was provided by the zoo to ensure proper washing of the spot.
The weak and emaciated animals were discarded at site of inspection. Also after slaughter, strict measures were taken to discard the diseased animals. The place was kept free from flies and to avoid hovering of enroute flies, the beef was transported at the quickest possible time with closed and well-ventilated containers.

ix. Transfer of carnivores to other zoos:
Transfer of tigers (6:3) and lions (3:5) to reduce the captive population at Nandankanan was carried out with all cares and formalities to different zoos of the country.
(1) To Andhra Pradesh (first transferred to Vishakhapatnam and then distributed to Zoos of Hyderabad, Tirupathi and Vishakhapatnam) - on 20.09.2000 NCT (2:3) and 22.09.2000 WCT (2:0) lions (2:4)
(2) To Bihar (Sanjay Gandhi Biological Park, Patna)- on 06.03.2001 WCT (1:0)
(3) To Karnataka - on 07.09.2001 WCT (1:0); lions (1:1)

Transfer of white tiger (1:0) to Tamil Nadu, and lion (0:1) to Karnataka is yet to take place. Tigers (1:1) and lions (1:2) were transferred to Jharkhand State in April, 2002.

Results and discussion
Although, the vaccine against Trypanosomiasis is not available, the drug Triquin (Quinopyramine Sulphate and

Table 6: Brief of tigers blood sample examinations

Month	A	B	C	D
September, 2000	38	15	41	41
October, 2000	3	2	33	90
November, 2000	18	1	28	70
December, 2000	4	2	24	15
January, 2001	42	4	30	91
February, 2001	20	5	36	60
March, 2001	-	-	38	60
April, 2001	7	1	42	86
May, 2001	25	1	36	64
June, 2001	-	-	32	32
July, 2001	40	-	34	99
August, 2001	4	1	22	61
September, 2001	5	2	24	49

A - Number of blood smears examined for intra and extra cellular parasites; B - number of blood smears examined for bio-chemical and hematological studies; C - number of beef smears examined for intra and extra cellular parasites; D - number of faecal samples examined.

Chloride) administered to the tigers on a routine basis (3-4 months gap) have been quite successful. Even then some parasites might have remained in the body to cause interim parasitaemia but possibly the drug quinopyramine could have blocked the antigen switching, a complex mechanism as described earlier. Further, no symptoms of Trypanosomiasis have been noticed. Also the interim blood film examination and bio-chemical profiles did not give any clues of the disease occurrence. Simultaneous administration of pheneramine malate/dexamethasone sodium also checked the drug sensitiveness to drug Triquin was observed except during the first protocol on 17.09.2000 i.e. a lion aged about 15 years from group-12 showed prominent drug reaction viz: severe rigor, salivation followed by abdominal respiration (whistling sound with peritoneal dribbling). But after 20 minutes of water bath, repeated enhanced dosage of pheneramine malate brought them back to normalcy.

The other way vector control measure to check transmission of disease have been effectively carried out through installation of canopy, insect flasher, slaughter area management, proper disposal of faecal matters (burial/ burning), burning of carcass, spraying of Deltamethrine to the body of animals and enclosure in different proportions, keeping water moat dry, vaccination of peripheral village cattle, cleaning weeds around the enclosure and overall maintaining of good hygienic measures etc.

As a result from September 2000 to September 2001, only two tiger deaths were experienced and which is very low in comparison to the previous five years' tiger deaths. That too these two cases i.e. one normal coloured tiger 'Joy' about 17 years of age died on 04.06.2001 due to shock as a sequel to epileptic convulsion and trauma and other one 'Visakha' about 20 years age died on 05.07.2001 due to pneumonia as a result of inclement weather.

Overall parasitic load have been checked through routine de-worming measures and hence the flare up of any infectious disease is controlled. Transmission of disease through beef if any have been effectively controlled through proper slaughter practises.

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Abbreviations

CZA - Central Zoo Authority
DRDE - Defence Research and Development Establishment
GoI - Government of India
MoEF - Ministry of Environment and Forests
NCT - Normal-coloured tiger
OUAT - Orissa University of Agriculture and Technology
OVC - Orissa Veterinary College
VSA - variable surface antigen
WCT - white-coloured tiger