

## TRYPANOSOMA INFESTATIONS IN ROYAL BENGAL TIGER (*PANTHERA TIGRIS TIGRIS*) AT LAHORE ZOOLOGICAL GARDENS AND ITS THERAPY

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### ABSTRACT

The aim of this study was to describe the signs and symptoms of Trypanosomiasis and its effective therapy. A large population of Royal Bengal Tigers (*Panthera tigris tigris*) in many zoos of the world including those in India, Bangladesh and Pakistan, have suffered a lot from this disease. The present study was conducted on three white and three brown tigers infested by *Trypanosoma* at Lahore Zoo. Two doses of Fa.try.banil injection (Fatro, Emilia, Italy) containing *Diminazene aceturate* as active ingredient, was injected at a recommended dose rate for treatment of sick animals and as prophylactic therapy to others to make possible the survival of endangered species of Royal Bengal Tigers (*Panthera tigris tigris*) kept in captivity.

**Keywords:** Royal Bengal Tigers, Trypanosoma, Diminazeneaceturate, Lahore Zoo.

### INTRODUCTION

*Trypanosoma* is a protozoan blood parasite of mammals which is transmitted mechanically by biting of flies of the genera *Tabanus*, *Lyperosia*, *Stomoxys* and *Atylotus* (Brun *et al.* 1998). People of every age and race could be infected by Trypanosomiasis same the way. *Trypanosoma* is pathogenic in most domesticated animals and some wild animals, including deer, elephants, capybara, jaguars (Choudury *et al.* 1972) and tigers (Manoharet *al.* 2003) The generally known infections in cattle, horses, dogs, buffalo and camels causes characteristic signs like edema, anemia, lymphadenomegaly, sudden death and weight loss (Aquino *et al.* 1999). If these infections are not treated and analyzed initially, the parasitic infections affect brain, consequential lead to confusion and sleep disorders. These all ultimately caused serious losses, death and coma (Maya *et al.* 2007). Carnivores may also get affected after feeding on infected meat (Raina *et al.* 1985). A large number of Tigers has died in different zoos of the world due to *Trypanosomiasis*. The number of this magnificent creature is also decreasing due to human activities the most remarkable among them are deforestation, illegal hunting and poaching. Other problems which have got attention are the health problems of tigers in captivity. The dramatic decrease in the number of tigers has brought into notice that there is

an urgent need to recognize the infectious diseases affecting the big cats. The proper handling of the problems relevant to the tigers' health with their diagnosis, treatment and prevention will decide the destiny of this magnificent creature. In 2000 summer a deer and twelve tigers died at Nandankanan Zoological Gardens, Orissa (Chattopadhyay, 2000). This national loss hoists some indispensable queries relevant to epidemiological protocols of infectious diseases in captivity (Parija *et al.* 2001). This disease also affected the National Zoo in the Bangladesh and killed four tigers. This parasite attacked the tigers in Lahore Zoo, twice in the past and the zoo lost its five tigers (two brown and three white tigers). The tigers are listed as an Endangered species, at IUCN Red Data Book and it is an Appendix I species under CITES (The IUCN Red List of Threatened Species 2008). This study was conducted when this parasite attacked the tiger population at Lahore Zoo, Pakistan for third time. To describe the signs and symptoms of Trypanosomiasis in big cats in captivity and to determine efficacy of *Diminazene aceturate* (Fa.try.banil) therapy for survival of tigers.

### MATERIALS AND METHODS

**Study Site:** The study site selected was the tiger house at Lahore Zoo.



**Fig.1: Tiger house at Lahore Zoo.**

**Animals:** A total of six tigers (*Panther tigris tigris*), three brown (Tiger 1, Tiger 2, Tiger 3) and three white tigers (Tiger 4, Tiger 5, Tiger 6), at Lahore zoo, were included

in this study. The details about ages, feeding scale and origin is given in Table-1.

**Table 1: Details of Experimental Animals**

Study Animals	Gender	Ages	Feeding Scale	Origin
Tiger1	Male	11 years	5-8 Kg Beef + 1 litre Milk/day(6) One Whole Chicken occasionally	Tuesday Off Zoo Breed
Tiger2	Male	8 years	5-8 Kg Beef + 1 litre Milk/day(6) One Whole Chicken occasionally	Tuesday Off Zoo Breed
Tiger3	Female	9 years	5-8 Kg Beef + 1 litre Milk/day(6) One Whole Chicken occasionally	Tuesday Off Shifted from Bahawalpur Zoo, Pakistan
Tiger4	Female	11 years	5-8 Kg Beef + 1 litre Milk/day(6) One Whole Chicken occasionally	Tuesday Off Shifted from Lahore Zoo Safari, Lahore Pakistan
Tiger5	Female	12 years	5-8 Kg Beef + 1 litre Milk/day(6) One Whole Chicken occasionally	Tuesday Off Zoo Breed
Tiger6	Female	10 years	5-8 Kg Beef + 1 litre Milk/day(6) One Whole Chicken occasionally	Tuesday Off Zoo Breed

**Symptoms:** Infection was suspected in all tiger’s population at Lahore Zoo. The clinical signs observed, were anorexia (off feed) with intermittent temperature i.e. 102-105<sup>0</sup>F, watery eyes and pale mucous membrane. There was bilateral enlargement of prescapular lymph nodes in three tigers (Tiger 1, 2 and 4). All tigers were showing clinical signs other than two (tiger # 2 and 4)

who were showing the seizures means rapid bursts of disorganized electrical activity which interrupt the normal working of the brain, leading to uncontrolled movements in the body and sometimes a transitory change in consciousness. Tigers were reluctant to walk as well. The incubation period of the disease is 1-4 weeks.

**Diagnosis:** The complete blood count of all animals was performed to have idea about nature of problem and the confirmatory diagnosis of infection was based on two things, first the thick and thin blood smears (Zeenat Medical Laboratories, Lahore) of three Tigers (Zablotskij *et al.* 2003), and secondly the therapeutic diagnosis was confirmed by the response of specific therapy by using Diminazene aceturate (Fa.try.banil) which given as intramuscular injection.

**Treatment:** Injection Diminazene aceturate as 7% aqueous solution (Fa.try.banil) was administered at a dose rate of 3.5mg/kg body weight by deep intramuscular injection to all tigers. Second dose of same drug was given 48 hours later to first injection. This all was accompanied by supportive therapy (Ringer lactate®, Methycobal®, dextrose 25%® and Hepamerz®) at the same time. Ringer lactate was also administrated to overcome the hypoglycemia which a common finding of Trypanosomiasis as suggested by Laha *et al.* 1991 in buffalo calf. The possible reason for hypoglycemia is utilization of glucose by Trypanosoma to maintain its life cycle. Other reason could be the disturbance of host physiology and improper functioning of carbohydrate metabolism.

## RESULTS

In addition to the specific therapy with Fa.try.banil injection, supportive therapy (as explained

above in Treatment) was also given at the same time which resulted in the observation that 35 minutes later the tigers took their meals spontaneously for the first time in last 7 days. The treatment was repeated after 48 hours. At the end of the 4<sup>th</sup> day, the appetite had been improved to almost normal level, the tigers were now much livelier and could walk properly. Unfortunately one brown Tiger (tiger 2) expired on third day. The most remarkable thing was that the white tiger showing nervous signs recovered from disease and did not show nervous signs again. The laboratory tests for different biological samples of tigers (tiger # 2 and 4) with nervous signs were also conducted shown in Table-4.

The confirmatory diagnosis has been shown in table 2. The table 3 shows the complete blood count of all the tigers out of which three were suffering from Trypanosomiasis in blood (Tiger 1, Tiger 2 and Tiger 4), and three tigers were negative for hemoparasites (Tiger 3, 5 and 6). The normal values have been adopted from (Duncan and Prasse, 1986; Feldman *et al.* 2000).

All the animals were under close observation with supportive therapy being continued. Blood analysis was done repeatedly to check the presence of parasite but result was negative for Trypanosoma infection in tigers. Precautionary measures were adopted to control the spread of disease including placement of nets over cages, repeated white wash of cages, burning of crevices of cage walls and the Fipronil Spray (Frontline® Spray- Merial) on all tigers for external parasites.

**Table 2: Complete Blood Count (CBC) of Six Tigers during the Disease**

Tests	Units	Normal	Tiger 1	Tiger 2	Tiger 3	Tiger 4	Tiger 5	Tiger 6
Hemoglobin	g/dL	8 - 15.0	15.5	13.5	14.5	13.7	14.1	13.7
RBC	× 10 <sup>6</sup> /μL	5 - 10.0	8.00	8.1	7.6	7.2	7.2	6.9
Reticulocytes	%	0-1	0.00	0.00	0.00	0.00	0.00	0.00
MCV	fL	39-55	39	38.00	38.00	39	42.54	41.05
Platelets	× 10 <sup>5</sup> /μL	3 - 7.0	1.1	1.3	2.23	1.1	4.31	3.9
TLC	× 10 <sup>3</sup> /μL	5.5-19.5	8.20	5.00	13.90	8.30	14.70	11.30
Neutrophils (segmented)	%	35-75	36.00	24.00	58.00	37.00	62.00	64.00
Lymphocytes	%	20-55	56.00	71.00	34.00	60.00	33.00	29.00
Monocytes	%	1-4	3.00	4.00	6.00	2.00	3.00	2.00
Eosinophils	%	2-12	5.00	1.00	2.00	1.00	2.00	5.00
Basophils	%	Rare	0.00	0.00	0.00	0.00	0.00	0.00

**Table 3: Confirmatory Diagnosis for the presence of Haemoparasites (Trypanosomes) of the Tigers after Lab Tests and Therapeutic Diagnosis**

Tests for Heamoparasite	Infected Tiger 1	Infected Tiger 2	Infected Tiger 3	Infected Tiger 4	Infected Tiger 5	Infected Tiger 6
<b>Blood Smear (Thin) *</b>	+ tive	+ tive	- tive	+ tive	-tive	-tive
<b>Blood Smear (Thick) *</b>	+tive	+tive	+tive	+tive	-tive	-tive

\* Giemsa Stain was used to stained blood smears G9641-Sigma-Aldrich Corp. USA)

**Table 4: Laboratory Findings of Biological Samples of Tiger with Nervous Signs**

Tests	Tiger #2 Findings	Tiger#4 Findings	References Value
<b>Biochemical Parameters</b>			
Serum Creatinine mg %	0.59	0.47	2.7±0.9 <sup>^</sup>
Serum Calcium mg %	6.8	7.9	10.6±0.6**
Serum Phosphorus mg %	4.2	3.9	5.5±1.3**
Serum Bilirubin (Total ) mg %	1.1	0.8	0.2±0.4 <sup>^</sup>
<b>Serum Protein and Enzymes</b>			
Serum Protein g %	7.9	6.4	7.1±0.6 <sup>^</sup>
Serum Albumin g %	3.9	3.7	3.7±0.5 <sup>^</sup>
Serum Globulin g %	1.9	2.9	3.3±0.6
Aspartate amino transferase (AST) IU/L	33.1	33.0	11±2**
Alanine amino transferase (ALT) IU/L	21.7	19.7	NA
Alkaline phosphatase (ALP) IU/L	108	115	90±45**
<b>Fecal Examination</b>			
Parasitic ova/protozoan oocysts	Not found	Not found	-

(Adopted from Singh *et al.*, 1999; Fowler & Miller, 2003)

## DISCUSSION

Trypanosomiasis in non-human creatures is very common disease. The species of protozoans linked with non-human infection due to trypanosoma are *T. vivax* (infects ruminants), *Trypanosoma evansi* (pigs, cattle, sheep and goats in Asia and camels& buffaloes Africa), *T. equiperdum* (could infects horses when they transmitted sexually), *T. theileri* (infects mammals in India). The vectors for trypanosomiasis in South American and Africa are tsetse flies (*Glossina spp.*) and reduviid bug (*Triatoma spp.*). The zoonotic species in India includes non-tsetse trypanosomiasis the *T. theileri*. The mechanical vectors in these cases are suspected as ticks and flies (Touratier *et al.* 1999; Rogers *et al.* 1993).

A naturally occurring Trypanosoma infection was detected in tigers and treated with Diminazene aceturate (Fa.try.banil® Fatro) a drug registered for the treatment of Trypanosomiasis. Diagnosis in this case was based on clinical signs, microscopic detection of Trypanosoma (Lab Diagnosis) and by reaction to treatment (Therapeutic Diagnosis). The speedy clinical improvement escorted with the removal of Trypanosoma from blood film, carried out after treatment inveterate the findings (therapeutic diagnosis).

One of the very important physical finding in these affected animals was the swollen prescapular lymph nodes. This enlargement of prescapular lymph nodes was seen in three animals only; the one was suffering from Trypanosomiasis. The similar findings about prescapular lymph nodes in bovines were reported by Gupta *et al.* 2003.

The complete blood count shows many important supportive changes for the presence of haemoparasites, but this is to be noted that the changes in

CBC are never considered confirmatory alone for the presence or absence of any infection or infestation but they always give a sort of indication about the nature of problem in blood e.g., bacterial, viral or fungal or some parasitic problem (Thrall *et al.* 2004)

The TLC was less than normal in Tiger 2 and in lower limits in Tiger 1 and 4 (these three were suffering from Trypanosomiasis) while the platelet count was markedly low in all three affected tigers, some similar results were reported by Manohar *et al.* 2003 and Feldman *et al.* 2000. The hemoglobin and the TEC were in normal upper ranges in all affected animals as well in animals negative for blood parasites. The mean corpuscular volume (MCV) was on extremely lower limits in all affected animals as shown in table 2. The trend in blood picture of affected animals shows Neutropenia and Lymphophilia, the two major supportive signs of the blood parasite presence. As the neutrophils are increased only in bacterial infections and the lymphocytes increases indicate the presence of some parasitic problems, as is evident in this case. This was also presented by Maya *et al.* (2007).

Transmission of infection to big cats (Tigers) is easily possible. On comparison with other mammals, tiger may get infection either by the flies' bites or having infected beef (Raina *et al.*, 1985). Poor appetite, anorexia, and difficulty in standing were noticed in all big cats but fever was observed in one tiger only. According to Brun *et al.*, (1998) reluctant to walk or even standing is very often in many receptive species. But these all were very obvious in the case of these feline. This is inconsistent with the findings of Finol *et al.*, (2001) that incongruity of muscle fibers along with weakening of endothelial cells of capillaries are common due to infections caused by *Trypanosoma*. These clinical signs and symptoms indicate that trypanosomiasis in tigers may display

indefinable non-specific symptoms in their endemic places. The Diminazene aceturate (Fa.try.banil® Fatro) may also be useful in the treatment of *Trypanosomes* in felines. The tiger comprises a vital role in the food chain to control the population of herbivores. The extinction of big cats would not only jeopardize the ecology but also disturb the bio-diversity of planet earth.

Due to non-availability of an efficient vaccine and practical chemo-prophylactic procedures, alternative measures should be designed to cope with trypanosomiasis in big cats. Trypano tolerant individuals could be produce in captivity through selective breeding plan (d' Ieteren *et al.*1998).

**Conclusion:**The present study has shown that there is history of trypanosomiasis infection in Lahore Zoo. The parasitic infection kill five tigers previously, the same episode have been repeated this time. Five infected tiger has been cured successfully with treatment of Diminazene aceturate (Fa.try.banil® Fatro).

**Recommendations:**The death of wild animals in captivity or in forest reserve is not an uncommon incident. The noticeable reasons could be poor maintenance, substandard sanitary conditions and imbalanced diet. Some policy or guideline to handle such crisis should be framed out for:-

- Diagnosis of an unusual disease in wild animal species
- Appropriate treatment of captive animals and control zoonosis to other animals of the similar species or others wild animals
- Selection of a suitable drug to treat heamoparasites.
- To avoid the drug resistance.
- To provide hygienically safe dwelling.

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