VI SUMMARY

The present study was conducted for serological diagnosis and molecular characterization of trypanosomosis in captive wild animals. Three important zoos of Karnataka formed the base for the study viz., Bannerghatta Biological Park, Bangalore, Shri Jayachamrajendra Zoological Gardens, Mysore and Tiger-Lion safari at Thyavarekoppa, and elephant camp at Sakarebyalu.

In the investigation, conventional methods such as blood smear examination, serodiagnosis by PHA and molecular diagnosis by PCR were employed for the detection of trypanosomosis in captive wild animals. The genetic profile of canine, lion and leopard isolates of trypanosomes was studied.

A total of 113 captive wild animals including 11 camels were screened for trypanosomosis in the present investigation.

In the present study three isolates of trypanosomes were obtained from canine, leopard and lion which were maintained in mice and also cryopreserved. The virulence of these isolates was also observed. It was observed that the canine isolates were most virulent and the mice died on the 5th day of post inoculation. In the leopard isolates, the organisms appeared on the 5th day post inoculation and parasitaemia increased on the 6th-7th day and on 8th day post inoculation, mortality of mice was noticed. The lion isolates were least virulent and the mice survived upto the 26th day with peak parasitaemia.

During the present study, conventional methods such as blood smear examination, did not reveal organisms. The PHA was used to screen the serum samples for trypanosome specific antibodies in the captive wild animals. The soluble antigen of *T.evansi* of canine origin was harvested from rats. The DEAE cellulose Chromatography method was used to separate trypanosomes and the pure extract of *T.evansi* was collected sonicated, centrifuged
and the supernatant used as soluble antigen. The protein concentration of the soluble antigen was 760 µg/ml.

In the PHA test the titre of 1:32 was taken as cut off to declare positive or negative. Out of 113 samples of different wild animals species screened, 64 serum samples were positive by PHA.

Out of 15 leopard serum samples screened for *T.evansi* antibodies by PHA test from different zoos, 9 of them were positive. Three leopards belonging to the Bannerghatta Biological Park Bangalore, 5 leopard serum samples of Thyavarekoppa Safari, Shimoga and one leopard of Mysore zoo were positive. Two Jaguars of Mysore Zoo were also found positive. Out of the total 29 tiger serum samples screened, 17 were found positive by PHA test. Of them nine were from Bannerghatta Biological Park, one tiger from Mysore zoo and seven tigers of Thyavarekoppa safari showed positive titre. A total of 45 lion serum samples were screened and thirty out of 43 lions of Bannerghatta Biological Park and two lions of Thyavarekoppa safari were positive. Out of 11 elephant serum samples 3 were positive and out of 11 camel serum samples only one sample was found positive.

The DNA was extracted from frozen blood of all 113 animals and was subjected to polymerase chain reaction. The primer pair (Urakawa *et al.*, 2001) for amplification of 488 bp fragment was used for detection of camelian trypanosomosis was used for detection of trypanosomes in the captive wild animals. In the present study, DNA which was extracted from blood of mice showing parasitaemia which were inoculated with canine, leopard and lion isolates and showed amplicon at 488 bp. Out of 11 elephants, five samples showed amplicon at 488 bp and nine out of 11 DNA samples of camels proved to be positive showing the clear amplicon at 488 bp. None of direct blood samples of tigers, lions, leopard and jaguar showed amplicon of 488bp probably to the variation RoTat 1.2 VSG gene in *T.evansi* or due to presence of PCR inhibitory blood samples. Thus may be biometrical alteration of *T.evansi* isolates where change from one host to the other leading to change in the target gene sequence would have resulted in non-amplification of 488 bp.

The incidence of trypanosomosis at Shri Jayachamarajendra zoological park at Mysore was low and reflecting the effective prophylactic regimen followed. The highest incidence was recorded from lion-tiger safari of Thyavarekoppa, Shimoga. The leopards and lions were in the carrier state which was confirmed by obtaining trypanosome isolates from these animals. Since this safari is located in a highly endemic area, the role of vectors is indicative in persistence of the infection.
The homology studies with the nucleotide sequences among canine, leopard and lion isolates was studied. The similarity and diversity ranged from 92 to 99.6 and 0.4 to 3.5 percent respectively. The phylogenetic tree indicated that the canine and lion isolates belonged to different clusters. The leopard isolate and reference strain (NO.AF 317914) were different and belonged to the cluster of canine isolate.

This epidemiological study helped to correlate the virulence of the different isolates with the help of phylogenetic tree. The results of PCR of lion, tigers and leopards blood samples and their respective isolates suggest that host factors and genetic level adaptability of *T.evansi* organisms in lab animals should be considered for analysis of epidemiology in trypanasomosis.