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Prevalence of mammalian schistosomes in captive elephants of central India

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Abstract
Captive Asian elephants are pivotal in wildlife conservation pertaining to patrolling of distance places and chemical immobilization of flagship species of protected forest areas. They are excellent swimmer and prefer water wallowing for keeping body cool during resting time while pickup water borne infections. Blood flukes are only unisexual and dimorphic trematodes transmit through water snails cercariae invade soft skin of the susceptible host and finally reach in the blood vessels where they become adult migrate in the haepato-trachial route. The female blood flukes shed spiny eggs forms granuloma followed by necrosis in the liver and small intestine. During health monitoring and disease diagnosis of captive elephants of tiger reserves of central India screened 51 captive elephants that revealed 35.2% occurrence of blood flukes. The prevalence of mammalian schistosomes (*Bivitellobilharzia nairi*) eggs were recorded highest in the elephants of Satpura tiger reserve (67.0 %) followed by Kanha tiger reserve (46.6%), Bandhavgarh tiger reserve (33.3%) and Pench tiger reserve (25.0%) whereas lowest infection was encountered in Panna tiger reserve (14.2%). Remarkable decrease in haemoglobin (7.5-11.8, 10.06±1.47 g/dl), elevated aspertate aminotransferase (AST=65-102 85±21.6 U/L), alanine aminotransferase (ALT=85-105 91±17.4 U/L) and blood urea nitrogen (BUN=46.2-65.5 58.2±13.7 mg/dl) were observed as compare to elephants found negative for eggs of blood flukes. Infected animals showed clinical manifestations characterized as dullness and depression with emaciated body condition specifically in elephants those harbor higher EPG (> 1200-2300 egg/g) counts of B. *nairi* eggs.

Keywords: *Bivitellobilharzia nairi*, Blood flukes, captive elephant, Haematobiochemistry, Granuloma

1. Introduction
Mammalian Schistosomiasis enlisted in the NTD (Neglected tropical diseases) as the most devastating water borne infection of wild and domestic herbivores including humans (WHO 2022) [5]. Schistosomes are blood flukes reporting consistently from African and Asian Elephants (Brant *et al*. 2013) [4]. These blood flukes are unisexual and dimorphic flukes develop in the blood vessels and their spiny eggs are responsible for erosion of parenchymatous tissue causes granuloma and necrosis in the liver followed by small intestine of the host. The transmission of infection in the definitive host occurs through active skin penetration of furcocercal cercariae, which develops further as an adult parasite in the portal veins. The worm load may lead to morbidity and mortality depending upon intake of furcocercal cercariae of blood flukes and its sustenance in the host (Agrawal & Shah 1998) [1]. In Asian Elephants, *Bivitellobilharzia nairi* has been encountering albeit without information about the disease manifestations (Bhoyar *et al*. 2014) [3]. However, the study of pathogenic effect of schistosomosis in elephants seems still obscured while observing granuloma in the hepatic parenchyma followed by necrosis or sometimes gastrointestinal bleeding with obstructive uropathy and severe anaemia (Singh & Agrawal 2000) [11]. Subsequently, infected elephants become clinically unfit for forest oriented works owing to reduction in their agility and potentials (Anchal, 2017) [7]. Seeking to biodiversity conservation mission and their need base programme, the trained elephants of different tiger reserves and national parks has been using as front line worker where vehicle mobility is utmost negligible.

Thus, agility and alertness of trained elephants would be monitored for such important tasks of wildlife management in the protected and non-protected forest areas of India. The present research article deals with the disease manifestation, impact of schistosomiasis on haematology and serum biochemistry in captive elephants of different Tiger Reserves.

Material and Methods

Prior to begin with the collection of biological samples of each elephant from different tiger reserves of Madhya Pradesh, the information of their age, sex and area from where they captured or purchased was gathered. The body evaluation of captive elelphants of different tiger reserves were conducted following the guidelines of Ramnathan and Mallapur (2008) [6]. For coprodiagnostics, 20 g fecal samples from freshly lead defecated dong bolus were collected from each elephants of either in the sterilized container and divided in two parts; one part was collected without preservatives and another was kept in 10% buffered formalin and brought to the laboratory of School of wildlife Forensic and health for qualitative and quantitative analysis of fecal samples. Sedimentation method of Singh and Agrawal (2000) [11] was used to screen the *Bivitellobilharzia nairi* eggs in the processed samples of elephants. Furthermore, 4-5 ml of blood of each elephant was also collected by ear vein puncture using 18 gauge needles aseptically in the vaccutainer both in EDTA (Ethylene Diamine Tetra Acetate) coated and non EDTA serum tubes following the guidelines of Jain (1986) [10]. The blood smears were prepared soon after the blood collection and stained with Romanwsky stain for differential leukocytes counts (DLC) as well as screening of haemoprotozoans. Haematological parameters were conducted using semi auto haematology analyzer (PG-6800 VET) within 12 h of collection of blood while harvested serum samples were stored at 4-8 ºC until further analysis. The serum biochemical's were mainly attributed with liver and kidney function tests and estimated using ERBA diagnostic kits with semi auto analyzer (ARK diagnostics, Mumbai) and interpretations of the findings were commenced as per Benjamin (1978) [2].

Results and Discussion

Impact of schistosomiasis on health status with reference to hematological and biochemical parameters was envisaged and considerable outcomes were obtained in pursuance of diagnosis of blood flukes and their effect on haematobiochemical parameters. Overall 35.2% prevalence of elephant schistosomosis was recorded in different Tiger Reserves of Madhya Pradesh. The occurrence of *B. nairi* infection in elephants is an indicative of natural nidus of intermediate hosts for animal schistosomiasis that might be owing to presence of water snails i.e. *Indoplanorbis exustus* and *Lymnaea luteola* in these national parks and adjoing endemic areas. Agrawal & Shah (1998) [1] also observed water snails (*Indoplanorbis exustus* and *Lymnaea luteola*) in central India those responsible for mammalian schistosomiasis in livestock. Nonetheless, stagnant water resources are used for grooming and bathing of captive elephants in different tiger reserves of central India where snail's population breed (Plate 1a). The highest occurrence of infection was recorded in Satpura (67.0%) followed by Kanha (46.6%), Bandhavgarh (33.3%), Pench (25%) and lowest (14.2%) in Panna, tiger reserve of M.P. (Table 1). Singh and Agrawal (2000) [11] have also recorded higher prevalence (32.8%) of *B. nairi* infection in captive elephants of Kanha Tiger Reserve while Islam (1994) recorded only 16% infection of schistosomosis in captive elephants of Kajiranga National might be owing to difference in geographical distribution and timing of wallowing and bathing of elephants in the stagnant water resources as release if furcocercal cercariae takes place during morning hours (Agrawal and Shah 1998) [1]. Hence the prevalence rate of Schistosomes differs with seasonal variations and might be owing to availability of stagnant waterholes.

The complete blood count (CBC) analysis showed decreases in hemoglobin percentage (7.5-11.8, 10.06±1.47 g/dl) in schistosomes infected elephants. Changes in differential leukocyte count including eosinophilia were recorded, particularly in elephants in which higher EPG of *B. nairi* eggs was reported. Biochemical changes included elevation of aspartate aminotransferase (AST=65-102 85±21.6 U/L), alanine aminotransferase (ALT=85-105 91±17.4 U/L) and blood urea nitrogen (BUN=46.2-65.5 58.2±13.7 mg/dl), indicated in the obstruction in the livers and kidney function showed chronic phase of the disease (Table 2). Singh & Agrawal (2000) [11] have also encountered increased level of SGPT and SGOT in infected elephants of Kanha National Park. The considerable alteration in the blood profiles are indicative of effects of parasitimia on liver, small intestine, mesenteric veins probably causing granuloma followed by necrosis. Similar findings have been reported by Bhoyar *et al*. (2014) [3] during haematobiochemical studies of elephants infected though B. *nairi*. Therefore, schistosomiasis may be controlled specifically in captive elephants through change in routine bathing in the stagnant river pockets as the water snails mostly release mammalian cercariae after sunrise early in the morning hours (Dorsey *et al*. 2002) [8]. The present study may be useful in determination of haematobiochemical parameters of infected and non-infected elephants with diagnosis of schistosomosis in elephants to control the disease burden in different tiger reserves in addition to health monitoring and diseases diagnosis of captive elephants in different tiger reserves for smooth functioning and conducting the wildlife health management aspects.

Table 1: Occurrence of *Bivitello bilharzia nairi* eggs in captive elephants

Tiger Reserves	No examined	Found positive	EPG Range	Mean EPG±SD
Kanha	15	7(46.6 %)	1800-2300	2000±64.41
Bandhavgarh	12	4(33.3%)	800-1200	1000±34.62
Panna	14	2(14.2 %)	600-1400	1000±50.85
Satpura	6	4(67.0%)	1200-1800	1500±74.8
Pench Tiger	4	1(25.0%)	800-1200	1000±0.51
Total	51	18 (35.2 %)	600-2300	1500±42.8

Table 2: Haematological profile of *B. nairi* infected and non-infected captive elephants

S. No	Parameters	Unit	Non-infected Elephants		Infected Elephants	
			Range	Mean ±SD	Range	Mean ±SD
1.	TEC	10^6/µl	2.61-5.34	3.57±0.15	2.66-4.77	3.43±0.13
2.	Hb	g/dl	12-18.4	14.25±0.69	7.5-11.8	10.06±1.47
3.	PCV	%	33.1-64.3	44.59±1.67	33.5-50.2	40.70±1.22
4.	MCV	fl	107.6-153.3	124.54±1.99	107.6-128.2	120.88±1.47
5.	MCH	pg	33.3-45.8	40.40±0.63	33.3-40.6	38.122±0.45
6.	MCHC	g/dl	26.6-37.4	32.54±0.55	26.6-34.6	31.57±0.40
7.	PLT	10^3/µl	226-744	458.65±29.04	263-665	433±28.90
8.	TLC	10^3/µl	7.5-41.1	23.01±1.63	11.1-31.6	20.57±1.34
9	DLC					
I.	Polymorph	%	43-71	55.21±1.66	69-85	75.27±1.38
II.	Lymphocytes	%	15-38	26.52±1.22	15-34	24.5±1.21
III.	Monocytes	%	4.0-24	11.65±1.09	6-17	10.88±0.83
IV.	Eosinophils	%	2.0-12	6.60±0.63	8.0-16	10.88±0.72
V.	Basophiles	%	00	00	00	00

Table 3: Serum biochemistry of *B. nairi* infected and non-infected captive elephants.

S. No.	Parameters	Unit	Non-infected elephants		*B. nairi* infected elephants	
			Range	Mean ± SD	Range	Mean ± SD
1.	AST	IU/L	10.56-81	40.30±5.08	65-102	85±21.60
2.	ALT	IU/L	12.17-45.4	6.31±0.54	85-105	91±17.40
3.	ALP	IU/L	34-118.8	65.30±4.63	34.2-101.4	66.06±4.74
4.	TBIL	mg/dl	0.1-1.4	0.68±0.08	0.1-2.9	0.96±0.17
5.	TP	g/dl	4.68-10.2	7.72±0.28	4.52-9.8	7.89±0.27
6.	CRE	mg/dl	1.2-2.44	1.89±0.08	1.2-3.2	1.89±0.12
7.	BUN	mg/dl	9.5-40.1	23.13±1.96	46.2-65.5	58.2±13.70
8.	UA	mg/dl	10.4-12.8	11.08±0.13	12.4-16.4	13.41±0.26

1A 1B 1C

Fig 1: Showing (1A) Blood extraction from ear vein of captive elephant (1B) Lymnaea luteola snail's intermediate host of schistosomes (1C) *Bivitellobilharzia* nairi egg in elephant faeces

Conflict of Interest
Not available

Financial Support
Not available

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