Original Research

Incidence of Endoparasitic Infestation in Free-Ranging and Captive Asian Elephants of Odisha

Biswal Chichilichi[1], Chitta Ranjan Pradhan[1], Lakshman Kumar Babu[1], Niranjan Sahoo[2], Mitra Ranjan Panda[3], Sumanta Kumar Mishra[4], Kumaresh Behera[1*], Abinash Das[5] and Ananta Hembram[3]

College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha-751003, INDIA

[1]Department of Livestock Production and Management
[2]Department of Epidemiology and Veterinary Preventive Medicine
[3]Department of Veterinary Parasitology
[4]Department of Animal Nutrition
[5]Fisheries and Animal Resources Development Department, Govt. of Odisha

***Corresponding author:** kumaresh.ouat@gmail.com

Rec. Date:	Sep 18, 2018 10:17
Accept Date:	Nov 05, 2018 16:14
DOI	10.5455/ijlr.20180918101740

Abstract

The study was conducted to evaluate the incidence of endoparasitic infestation in free ranging and captive Asian elephants in Odisha covering three seasons namely, summer (April-June), rainy (July-September) and winter (November-January). The locations were Chandaka Wildlife Sanctuary, Khordha; Satkosia Wildlife Sanctuary, Angul; Similipal Biosphere Reserve, Mayurbhanj for free-ranging, and Nandankanan Zoological Park, Bhubaneswar for captive elephants. Faecal samples were analysed by direct smear method, sedimentation technique and floatation method. The captive location showed a markedly lower endoparasitic infestation rate than any of the free-range locations, in every season; the differences being significant ($P \leq 0.01$) in summer and rainy only. Thus, as regards to the seasonal influence, for each of the free-range and captive locations, the highest rate of infestation was observed in summer followed by rainy and winter.

Key words: Endoparasitic Infestation, Asian Elephant, Faecal, Free Ranging, Captive, Seasonal Influence

How to cite: Chichilichi, B., Pradhan, C., Babu, L., Sahoo, N., Panda, M., Mishra, S., Behera, K., Das, A. & Hembram, A. (2019). Incidence of Endoparasitic Infestation in Free-Ranging and Captive Asian Elephants of Odisha. International Journal of Livestock Research, 9(2), 302-310. doi: 10.5455/ijlr.20180918101740

Introduction

Asian elephants, the majestic animals, are now-a-days imperiled to several adversities affecting their very survivability and longevity, which include increased parasitic infestation as well. Parasitic infestations in wild animals act as a potential stressor afflicting their health status. Parasitic study can serve as an effective, practical, non-invasive early warning system for the health of individual hosts, host populations and the ecosystems of which they are a part, because environmental changes impact upon both hosts and their parasites (Lafferty, 1997; Marcogliese, 2005). Parasitic study in wild animals is often a neglected area, though it serves as an important tool of conservation science from the perspective that, ecosystem and animal health are linked and hence, monitoring parasites can be an indicator of both (Lafferty, 1997; Marcogliese, 2005; Howells *et al*., 2011). This study was conducted to ascertain the incidence of endoparasites from the faecal samples and the influence of seasonality in both free-ranging and captive Asian elephants.

Materials and Methods

Study Locations and Duration

The field study was carried out in both geographically distinct free-range areas and captive locations in Odisha, India involving Chandaka Wildlife Sanctuary (WLS), Bhubaneswar, Khordha; Satkosia Wildlife Sanctuary, Angul and Similipal Biosphere Reserve (BR), Mayurbhanj for free-ranging Asian elephants, and in the Nandankanan Zoological Park (ZP), Bhubaneswar, Khordha for captive ones. The three naturally classified seasons namely, summer (April-June), rainy (July-September) and winter (November-January) were covered during the trial.

Collection of Sample

Core samples were collected within few hours of defecation from faecal boli of elephants to reduce contamination by soil helminthes, and stored in 10% formalin. Since parasite eggs may not be evenly distributed among the boli of a dung pile or even within a bolus, each sample comprised of separate portions from the surface and the interior of different boli of a dung pile. Due care was taken to prevent cross-contamination among the samples by putting on gloves during sample collection and changing them after each collection. The age and sex of the elephants were unknown for samples collected from free range. The samples were collected labeling with place, date and time of collection.

Direct Smear Method

Samples of 10-15 g were mixed thoroughly in a clean glass mortar and pestle by adding little amount of water and poured through a sieve of mess size 500-800 micrometer to remove debris and large lumps. The strained fluid was collected in a beaker. The sieve was rinsed with water and the debris left on the screen was discarded. A few drop of strained fluid was placed on a slide with removal of any fibers or particles present followed by placing a cover slip on it while avoiding any air bubble. The slide was investigated using a magnification of 10X and 40X.

Hosted@www.ijlr.org DOI 10.5455/ijlr.20180918101740

Sedimentation Technique

The strained suspension was transferred to centrifuge tube and centrifuged at 1500 rpm for 2 minutes. The sediment was mixed well after discarding the supernatant. A small quantity of it was taken and mixed with a drop of water on the slide. A cover slip was applied and observed under low power objective of the microscope to identify the parasitic ova present, through their morphological characteristics (Bowman, 1999).

Flotation Method

About 5 g of faeces was taken in a glass mortar and pestle and small quantity of saturated flotation solution (magnesium sulphate/ zinc sulphate/ sugar solution) was added and mixed well. Suspension was strained to remove the debris and was centrifuged at 1500 revolutions per minute (rpm) for 2-3 minutes. The surface layer was examined under low power microscope by covering with a cover slip. The presence of eggs was identified through their morphological characteristics (Bowman, 1999).

Statistical Analysis

The data generated in the experiment was statistically analyzed using the Statistical Package for the Social Sciences (SPSS) Version 20.0. For comparison of groups, ANOVA procedure and Duncan's multiple range tests were used (Steel and Torrie, 1980). Pearson's Chi- square test was conducted to analyze the seasonal and locational differences in parasitic incidence.

Results and Discussion

Infestation Rate (%)

A comparison of endoparasitic infestation rates, denoted by percent of samples positive for parasitic ova, among different locations and seasons are presented in Table 1.

Table 1: Endoparasitic infestation rate in different locations and seasons

Season	Free Range (%)			Captive (%)	P value
	Chandaka	Satkosia	Similipal	Nandankanan	
Summer	76.67^{Aa}	82.86^{Aa}	72.50^{Aa}	22.86^{Ab}	< 0.01
Rainy	62.86^{Aa}	54.29^{Ba}	60.00^{Aa}	20.00^{ABb}	< 0.01
Winter	6.67^{B}	10.71^{C}	6.25^{B}	2.86^{B}	> 0.05
P value	< 0.01	< 0.01	< 0.01	< 0.05	

^{a,b,c} Means along rows differ significantly (P≤0.05 or 0.01); $^{A, B,C}$ Means along columns differ significantly (P≤0.05 or 0.01)

It was revealed (Table 1) that there was no difference (P≥0.05) among the free-range locations in any season, suggesting that the free-range locations, though geographically different, did not influence infestation rate. It indicated that the elephants, irrespective of geographical locations, were exposed to

 DOI 10.5455/ijlr.20180918101740

parasitic infestation in the wilderness. Exposure to parasitic infestation might have arisen from contact with other animals and use of common waterholes. The range of infestation rates (6.25 to 82.86%) in free-range locations as found in the present study, across locations and seasons, falls within the reported figures from different parts of the country. Kashid *et al*. (2002) reported 25% helminthic infestation incidence in elephants at Kanha National park while, Nishanth *et al*. (2012) reported endoparasitic infestation of 80%, 88% and 80%, respectively, in free ranging elephants of Tamil Nadu state at Mudumalai sanctuary, Anamalai sanctuary and Sathyamangalam forest. As regards to the comparison between free range and captive locations, it was found that the captive location, as compared to any of the free-range locations, showed a much lower infestation rate in every season, the differences being significant ($P \leq 0.01$) in summer and rainy. The lower infestation rates in captive elephants could be ascribed to the stringent bio-security measures followed resulting in the elimination of intermediate hosts affecting transmission of parasites.

In the present study, the range of infestation rates (2.86 to 22.86%) in the captive location across seasons, were found similar to or different from the reported figures. Saseendran *et al*. (2004) observed an incidence 17.17 percent of helminthic infestation in the captive elephants of Thrissur district in Kerala. Pandit *et al*. (2015) reported a lower incidence (*Fascioloides magna*- 9%, *Strongyloides westeri*- 23%) of parasitic infestation in captive elephants of Nepal. In both the cases, the animals were regularly dewormed with Albendazole @ 2.5 mg/ kg body weight. In contrast, higher infestation rates than in the present study have also been reported. The reported figures were; 41-43 percent for the elephants of Gemini circus (Kashid *et al*., 2002), 62.5 percent for the temple elephants of Gujarat (Jani, 2008) and 100 percent parasitic infestation in the captive elephants of Nepal (Karki, 2008). The differences in the reported findings might be due to variation in geographical locations, environmental conditions, bio-security measures adopted and deworming practices followed.

Effect of Seasons

As regards to the seasonal influence, it was found that, for each of the free-range locations, the highest rate was in summer followed by rainy and winter. For each of the free-range locations, summer or rainy showed significantly ($P \leq 0.01$) higher rates than winter, there being fluctuating trends between summer and rainy. It was found that, while for Chandaka or Similipal, there was no significant ($P \geq 0.05$) difference between summer and rainy; for Satkosia, summer showed a significantly ($P \leq 0.01$) higher rate than rainy. For the captive location, a similar pattern was observed, in that, the highest rate was recorded in summer followed by rainy and winter. However, while there was no significant ($P \geq 0.05$) difference between summer and rainy, the difference between summer and winter was significant ($P \leq 0.05$), there being no difference ($P \geq 0.05$) between rainy and winter. Thus, it was found that for free-range or captive locations, the highest rate was found in summer followed by rainy and winter.

 DOI 10.5455/ijlr.20180918101740

As regards to the seasonal influence, the highest rate of infestation was recorded in summer followed by rainy and winter, for both free-range and captive locations. The parasite incidence was comparable between summer and rainy seasons for all the study locations. Very low incidence in winter months might be due to the unfavourable environmental temperature and humidity affecting shedding of eggs in faeces. The periodical larval hypobiosis phenomenon is a characteristic of the parasite species belonging to Trichostrongylidae family; therefore, the results of fecal samples examination from autumn-winter season were negative (Gundlach and Sadzikowski, 2004). Increased faecal shedding of the nematode ova during spring time or early summer might be attributed to the 'spring-rise phenomenon'. The higher prevalence of parasitic infestation during summer could be attributed to the prevalence of conducive climatic conditions (temperature and humidity) for faster rates of hatching of eggs and rapid development to the infective stage as suggested by English (1979). Higher stress in elephants during summer as detected from the faecal cortisol level might be another reason of higher infestation percentage owing to the fact that stress affects host immunity thereby predisposing animals to parasite infestation (Dhabhar and McEwen, 1997; Agarwal and Marshall, 2001). Further, increased susceptibility of individuals to parasites and pathogens due to nutritional stress such as limited food availability and deficiencies in dietary components was reported by Chapman *et al*. (2006b).

The highest rate of infestation in summer followed by rainy and the lowest in winter, as observed in this study, was similar to the findings of Vanitha *et al*. (2011), with reports of highest infestation rate during summer (49.0%), followed by monsoon (38.0%), and the lowest infestation rate during winter (7.0%). The findings were further corroborated by those of Pechimuthu (2014), where captive elephants showed a mean higher prevalence during summer (55.0%), followed by monsoon (37.5%), pre-monsoon (25.0%) and post-monsoon (22.5%). Similarly, higher parasitic load during dry season was reported for captive and wild Indian elephants by Watve (1993) and Vidya and Sukumar (2002). In contrast, Mbaya *et al*. (2013) quoted typically peak helminthic prevalence during the rainy season in African elephants in Nigeria. Unlike the above studies, Baines *et al*. (2015) observed no strong associations between season (rainy, flood and dry) and the prevalence or density of parasite stages in wild African elephant faeces. The differences in the reports could be due to variation in the climatic conditions of the study locations.

Effect of the Locations

The infestation rates for the free-range locations ranged from 72.50 to 82.86 percent in summer, 60.00 to 62.86 percent in rainy, and 6.25 to 10.71 percent in winter. No significant ($P \geq 0.05$) difference was observed among the free-range locations across the seasons. For the captive location of Nandankanan Zoological Park (NZP), the infestation rates were 22.86%, 20.00% and 2.86% in summer, rainy and winter, respectively. The captive location showed a markedly lower infestation rate than any of the free-range

 DOI 10.5455/ijlr.20180918101740

locations, in every season. The differences between the captive and any free-range location were found significant (P≤0.01) in summer and rainy, while in winter, no significant (P≥0.05) difference was observed between any of the free range and captive locations.

Spectrum of Endoparasitic Infestation

The spectrum of infestation in different locations and seasons are presented in Table 2. Single as well as mixed endoparasitic infestations were encountered. Single infestations comprised *Strongyle spp.*, *Strongyloides spp.*, *Paramphistomum spp.*, *Fasciola spp.* and ciliates; while mixed infestations comprised *Strongyle spp. + Strongyloides spp.*, *Strongyle spp./Strongyloides spp. + Fasciola spp./ Paramphistomum spp.* and *Strongyle spp. + Strongyloides spp. + Fasciola spp./ Paramphistomum spp.*

Table 2: Spectrum of endoparasitic infestation in different locations and seasons

Season	Location	Total no. of samples	Single Infestation (%)						Mixed Infestation (%)		
			Positive for endoparasites (%)	*Strongyle spp.*	*Strongyloides spp.*	*Paramphistomum spp.*	*Fasciola spp.*	Ciliates	*Strongyle spp. + Strongyloides spp.*	*Strongyle spp./ Strongyloides spp. + Fasciola spp./ Paramphistomum spp.*	*Strongyle spp. + Strongyloides spp. + Fasciola spp./ Paramphistomum spp.*
Summer	Chandaka	30	76.67	23.33	30	13.33	3.33	-	6.67	-	-
	Satkosia	35	82.86	28.57	22.86	14.29	2.86	5.71	5.71	2.86	-
	Similipal	40	72.5	30	12.5	15	7.5	-	7.5	-	-
	Overall Free-range	105	77.14	27.62	20.95	14.29	4.76	1.9	6.67	0.95	-
	Nandankanan	35	22.86	5.71	5.71	2.86	2.86	-	2.86	2.86	-
Rainy	Chandaka	35	62.86	17.14	22.86	8.57	5.71	-	-	5.71	2.86
	Satkosia	35	54.29	20	17.14	8.57	2.86	-	2.86	-	2.86
	Similipal	25	60	20	16	8	8	-	4	4	-
	Overall Free-range	95	58.95	18.95	18.95	8.42	5.26	-	2.11	3.16	2.11
	Nandankanan	35	20	8.57	5.71	-	2.86	-	-	2.86	-
Winter	Chandaka	30	6.67	6.67	-	-	-	-	-	-	-
	Satkosia	28	10.71	7.14	-	3.57	-	-	-	-	-
	Similipal	32	6.25	3.13	3.13	-	-	-	-	-	-
	Overall Free-range	90	7.78	5.56	1.11	1.11	-	-	-	-	-
	Nandankanan	35	2.86	2.86	-	-	-	-	-	-	-

It was found that location did not influence spectrum of parasites in any season. Season influenced spectrum, winter showing the lowest and rainy the highest. The *Strongyle* spp. was the only parasite to be observed during winter season, across locations, though *Strongyloides* spp. and *Paramphistomum* spp. were

  DOI 10.5455/ijlr.20180918101740

found only sporadically. The spectrum of infestation in different locations and seasons are presented in Table 2. It was found that location did not influence spectrum of parasites in any season. In contrast to the present finding, Karki (2008) reported different spectrum and intensity of infestation in location-wise samples of elephants from four different locations namely, Chitwan National Park, Suklaphanta, Bardiya and Koshi Tappu wildlife reserves in Nepal owing to the difference in bio-geographical conditions. Season influenced spectrum of parasites, winter showing the narrowest and rainy the widest. The presence of wider spectrum of endoparasites during summer and rainy might be due to the favorable warm and humid environmental conditions required to complete the life cycle, while the same not being available during winter might be the reason of lower endoparasitic spectrum. Very low incidence in winter months might be due to the unfavourable environmental temperature and humidity affecting shedding of eggs in faeces. The periodical larval hypobiosis is a phenomenon, characteristic of the parasite species belonging to Trichostrongylidae family, therefore the results of fecal samples examination from autumn-winter season were negative (Gundlach and Sadzikowski, 2004). The higher prevalence of parasitic infestation during summer can be attributed to the prevalence of conducive climatic conditions (temperature and humidity) for faster rates of hatching of eggs and rapid development to the infestive stage (English, 1979). The *Strongyle spp*. was ubiquitous across seasons and locations studied. A similar finding was observed by Vanitha *et al*. (2011) who reported presence of *Strongyle spp*. in summer (49%), rainy (41%) and winter (15%) seasons in temple, private and forest department elephants in Tamil Nadu.

The spectrum of parasites observed in the present study (*Strongyle spp.*, *Strongyloides spp.*, *Paramphistomum spp.*, *Fasciola spp.* and ciliates) was previously reported in the free range and captive elephants. In free range locations, they consisted of *Strongyloides spp., Strongyle spp., Bivitellobilharzia spp.* and *Anoplocephala spp*. (Vimalraj *et al*., 2012; Nishanth*et al*., 2012; Hing*et al*., 2013; Vimalraj and Jayathangaraj, 2015; Heinrich, 2016). The same for captive locations comprised *Strongyloides spp.*, *Strongyle spp.*, *Fasciola spp.*, *Paramphistomum spp.*, *Fascioloides magna, Balantidium coli* and *Oesophagostomum spp.* (Suresh *et al*., 2001; Saseendran *et al*., 2004; Stremme *et al*., 2007; Jani, 2008; Chandrasekharan *et al*., 2009; Vanitha *et al*., 2011; Rahman *et al*., 2014; Pechimuthu, 2014; Pandit *et al*., 2015; Heinrich, 2016), while *Strongyle spp.*, *Strongyloides spp.* and *Paramphistomum spp.* were reported in semi-captive elephants (Lynsdale *et al*., 2015). The order of predominance by single or mixed infestations was more or less similar between locations as well as seasons. In free-range or captive locations, and in all seasons, the most predominant species were nematodes (*Strongyle spp.* and *Strongyloides spp.*) followed by trematodes (*Paramphistomum spp.* and *Fasciola spp.*), which were followed by mixed infestations of different combinations.

 DOI 10.5455/ijlr.20180918101740

Conclusion

The captive location showed a markedly lower endoparasitic infestation rate than any of the free-range locations, in every season. The differences between the captive and any free-range location were found significant ($P \leq 0.01$) in summer and rainy, while in winter, no significant ($P \geq 0.05$) difference was observed between any of the free range and captive locations. As regards to the seasonal influence, it was found that, for each of the free-range locations, the highest rate was in summer followed by rainy and winter. For each of the free-range locations, summer or rainy showed significantly ($P \leq 0.01$) higher rates than winter, there being fluctuating trends between summer and rainy.

Acknowledgements

Authors are thankful to PCCF (Wildlife) and Chief Wildlife Warden, Odisha for providing scholarship and necessary facilities in the field to conduct the research work. The authors also thank Director, Nandankanan Zoological Park, Bhubaneswar, Odisha for permission to carry out research.

References

1. Agarwal SK and Marshall GD Jnr. 2001. Stress effects on immunity and its application to clinical immunology. Clinical & Experimental Allergy. 31: 25−31.
2. Baines L, Morgan ER, Ofthile M and Evans K. 2015. Occurrence and seasonality of internal parasite infestation in elephants, *Loxodonta africana*, in the Okavango Delta, Botswana. International Journal for Parasitology: Parasites and Wildlife. 4: 43–48.
3. Bowman DD. 1999. Georgis' Parasitology for Veterinarians, 7th Edn. W.B. Saunders. Philadelphia.
4. Chandrasekharan K, Radhakrishnan K, Cheeran JV, Muraleedharan KN and Prabhakaran T. 2009. Review of the incidence, aetiology and control of common diseases of Asian elephants with special reference to Kerala. In: Ajitkumar G, Anil KS, Alex PC (eds) Healthcare management of captive Asian elephants. Kerala Agricultural University Press, Pookode, pp. 92-95.
5. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes MJ, Saj TL and Ziegler TE. 2006b. Do food availability parasitism and stress have synergistic effects on red colobus populations living in forest fragments? American Journal of Physical Anthropology. 131: 525−534.
6. Dhabhar FS and McEwen B. 1997. Acute stress up regulates while chronic stress down regulates cell mediated immunity in vivo. Brain, Behavior, and Immunity. 11: 286−306.
7. English AW. 1979. The epidemiology of equine Strongylosis in southern Queensland 1. The bionomics of the free-living stages in faeces and on pasture. Australian Veterinary Journal. 55(7): 299-305.
8. Gundlach JL and Sadzikowski AB. 2004.Parazytologia i parazytozyzwierząt.PWRiL, Warszawa.
9. Heinrich L. 2016. Prevalence and molecular identification of helminths in wild and captive Sri Lankan Elephants, *Elephas maximus maximus*, Bachelor of Veterinary Medicine- Research Project 2, Royal Veterinary College, University of London.
10. Hing S, Othman N, Nathan SKSS, Fox M, Fisher M and Goossens B. 2013. First parasitological survey of Endangered Bornean elephants *Elephas maximus borneensis*. Endangered Species Research. 21: 223-230.
11. Howells ME, Pruetz J and Gillespie TR. 2011 Patterns of gastrointestinal parasites and commensals as an index of population and ecosystem health: The case of sympatric Western Chimpanzees *Pan troglodytesverus* and Guinea baboons *Papio hamadryas papio* at Fongoli, Senegal. American Journal of Primatology. 73: 173- 179.
12. Jani RG. 2008. Prevalence and Haemato-Biochemical Studies of Gastro-intestinal Parasites of Indian Elephants (*Elephas maximus*). Veterinary World. 1(10): 296-298.

 DOI 10.5455/ijlr.20180918101740

13. Karki K. 2008. Incidence of gastrointestinal helminths in captive Elephants in Wildlife Reserves of Nepal.Article base. Wildlife Disease Association. Available at: http:// www.articlesbase.com/health-articles/incidence-of-gastrointestinal-helminthes-in-captive-elephants-in- wildlife-reserves-of-nepal-335689.html
14. Kashid KP, Shrikhande GB and Bhojne GR. 2002. Incidence of gastro intestinal helminths in captive wild animals at different locations. Zoos print. 18(3): 1053-1054.
15. Lafferty KD. 1997. Environmental parasitology: What can parasites tell us about human impacts on the environment? Parasitology Today. 13: 251−255.
16. Lynsdale CL, Santos DJF, Hayward AD, Mar KU, Htut W, Aung HH, Soe AT and Lummaa V. 2015. A standardized faecal collection protocol for intestinal helminth egg counts in Asian elephants, *Elephas maximus*. International Journal for Parasitology: Parasites and Wildlife. 4: 307-315.
17. Marcogliese DJ. 2005. Parasites of the super organism: Are they indicators of ecosystem health? International Journal for Parasitology. 35: 705−716.
18. Mbaya AW, Ogwiji M and Kumshe HA. 2013. Effects of host demography, season and rainfall on the prevalence and parasitic load of gastrointestinal parasites of free-living elephants (*Loxodonta africana*) of the Chad Basin National Park, Nigeria. Pakistan Journal of Biological Sciences. 16(20): 1152-1158.
19. Nishanth B, Srinivasan SR, Jayathangaraj MG and Sridhar R. 2012. Incidence of endoparasitism in free-ranging elephants of Tamilnadu state. Tamilnadu Journal of Veterinary & Animal Sciences. 8 (3): 171-173.
20. Pandit A, Dhakal IP and Gairhe KP. 2015. Prevalence of endoparasitic diseases in private elephants of buffer zone of Chitwan National Park, Nepal. International Journal of Recent Scientific Research. 6(8): 5768-5771.
21. Pechimuthu D. 2014. Seasonal variation in prevalence of helminthic infestation in captive Asian elephant, *Elephas maximus*. Applied Biology and Biotechnology. 2(2): 8-14.
22. Rahman SM, Dey AR, Kundu UK and Begum N. 2014.Investigation of gastrointestinal parasites of herbivores at Dhaka National Zoological Garden of Bangladesh. Journal of Bangladesh Agricultural University. 12 (1): 79–85.
23. Saseendran PC, Rajendran S, Subramanian H, Sasikumar M, Vivek G and Anil KS. 2004. Incidence of helminthic infestation among annually dewormed captive elephants. Zoo's Print Journal. 19(3): 1422.
24. Steel RGD and Torrie JH. 1980. Principles and Procedures of Statistics, Second Edition, New York: McGraw-Hill.
25. Stremme C, Lubis A and Wahyu M. 2007. Implementation of regular veterinary care for captive Sumatran elephants. Gajah. 27: 6-14.
26. Suresh KP, Choudhuri PC, Nalini Kumari K, Hafeez Md and Hamza PA. 2001. Epidemiological and clinico-therapeutic studies of Strongylosis in elephants. Zoo's Print Journal. 16(7): 539-540.
27. Vanitha V, Thiyagesan K and Baskaran N. 2011. Prevalence of intestinal parasites among captive Asian Elephants *Elephas maximus*: effect of season, host demography, and management systems in Tamil Nadu, India. Journal of Threatened Taxa. 3(2): 1527-1534.
28. Vidya TNC and Sukumar R. 2002. The effect of some ecological factors on the intestinal parasite intensities of the Asian elephant (*Elephas maximus*) in southern India. Journal of Bioscience. 27: 521–528.
29. Vimalraj PG and Jayathangaraj MG. 2015.Endoparasiticinfestations in free-ranging Asiatic elephants of Mudumalai and Anamalai Wildlife Sanctuary. Journal of parasitic diseases. 39(3): 474–476.
30. Vimalraj PG, Jayathangaraj MG, Sridhar R, Senthilkumar TMA and Latchumikanthan A. 2012. Elephant schistosome (*Bivitellobilharzia nairi*) in free-ranging Asian elephants (*Elephas maximus*) of Sathyamangalam forest division of Tamil Nadu State. Journal of Veterinary Parasitology. 26(1): 80-81.
31. Watve MG. 1993. Helminth parasites of elephants: Ecological aspects. In Daniel, J.C (ed) A Week with Elephants; Proceedings of the International Seminar on Asian Elephants, Bombay, India, 13-15 June. Oxford University Press, New Delhi, pp.289-295.