Archives of Agriculture and Environmental Science 8(3): 290-294 (2023)
https://doi.org/10.26832/24566632.2023.080303

e-ISSN: 2456-6632

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: journals.aesacademy.org/index.php/aaes

ORIGINAL RESEARCH ARTICLE

Prevalence of gastrointestinal parasites in endangered captive Asian Elephants (*Elephas maximus*) of Chitwan National Park in Nepal

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ARTICLE HISTORY

Received: 26 June 2023
Revised received: 20 August 2023
Accepted: 05 September 2023

Keywords

Asian elephants
Chitwan National Park
Gastrointestinal parasites
prevalence

ABSTRACT

In order to ascertain the comprehensive prevalence of gastrointestinal parasites among captive Asian elephants in Chitwan National Park, a cross-sectional investigation was conducted. A total of 103 samples was purposefully collected. Demographic details encompassing age and gender, along with epidemiological information concerning deworming status, timing intervals, and nutritional condition for both government-owned and privately-owned elephants, were procured through a structured questionnaire survey. The process involved microscopic identification and quantification of gastrointestinal parasites through sedimentation, centrifugal floatation, and MacMaster Egg Per Gram (EPG) count methods. The resultant data indicated an overall prevalence of gastrointestinal parasites at 47.57% (49 out of 103 samples). The dominant class of parasites observed was Nematodes (n=30, 61.22%), followed by Trematodes (n=14, 28.57%) and Cestodes (n=5, 10.20%). Six distinct parasite genera were identified with positive results: Strongylus (26.53%), Trichostrongylus (24.48%), Fasciola (16.35%), Paramphistomum (12.24%), Anoplocephala (10.20%), and Ascaris (10.20%). Notably, the prevalence was markedly higher in females (39.80%) in comparison to males (7.76%), with the disparity being statistically significant ($p>0.05$). Additionally, a noteworthy correlation was observed between parasite prevalence, age groups, and deworming history, with statistical significance ($p<0.05$). The Egg Per Gram (EPG) count analysis demonstrated that the majority (87.75%) of the positively identified samples exhibited mild infection (100-500 eggs), while a relatively low percentage (6.12%) displayed heavy infection (1000-1500 eggs). The mean EPG was calculated as (248.39 ± 54.25). Consequently, the heightened prevalence of gastrointestinal parasites in captive elephants within Chitwan National Park underscores the necessity for targeted interventions to mitigate the risk of parasitic infestations.

Citation of this article: Dahal, G., Sadaula, A., Gautam, M., Magar, A. R., & Adhikari, S. (2023). Prevalence of gastrointestinal parasites in endangered captive Asian Elephants (*Elephas maximus*) of Chitwan National Park in Nepal. *Archives of Agriculture and Environmental Science*, *8*(3), 290-294, https://dx.doi.org/10.26832/24566632.2023.080303

INTRODUCTION

The Elephantidae lineage, situated within the realm of the animal kingdom, encompasses elephants, which stand as the most sizable terrestrial mammals. The emergence of elephants is posited to have transpired roughly 50 million years in the past. At present, approximately a third of the worldwide elephant populace, totaling roughly 15,000 individuals, pertains to captive Asian elephants (Fernando, 2013). Only three species of elephants remain today: the Asian elephant, the African bush elephant, and the African forest elephant (Barnes *et al*., 1995; Dudley, 1999; Ishwaran, 1981). These organisms belong to the

lineage encompassing approximately 350 species spread across 44 Genera. Their distribution spans 13 nations within Asia, encompassing Nepal, India, Bhutan, Bangladesh, China, Myanmar, Thailand, Indonesia, Laos, Vietnam, Cambodia, Malaysia, and Sri Lanka (Olivier, 1978). In the area surrounding Chitwan National Park, privately-owned elephants are mainly used for tourism, while government-owned elephants are utilized for patrolling and research. Moreover, private elephants are commonly used for forest excursions and entertaining tourists in buffer zones of parks and reserves (Dangol *et al.*, 2020). However, Frequent issues that captive elephants in Chitwan National Park encounter are parasites, tuberculosis, and inadequate foot health. Thereafter, to detect endoparasites such as trematodes, cestodes, and nematodes - which are widespread in Asian elephants at risk of extinction - the Mc Master approach would be the best (Abhijith *et al.*, 2018). Since 1978, the Department of National Parks and Wildlife Conservation (DNPWC) has been the government agency in charge of tamed elephant management (Pradhan *et al.*, 2011).

Currently, Chitwan National Park has 208 captive elephants, owned by different entities (Pradhan *et al.*, 2011). The government's Protected Areas owns 94 elephants, the National Trust for Nature Conservation owns 8, and 106 elephants are privately owned (Pradhan *et al.*, 2011). These elephants serve diverse functions including park patrols, forest expeditions, tourism, and research. It's crucial to emphasize that even seemingly robust wild creatures can harbor protozoa and helminthic parasites without displaying evident symptoms of illness. (Fowler *et al.*, 2006). Helminthic parasites are frequently found in different types of vertebrates and can have a significant effect on the health, management, and conservation of both captive and wild populations (Du *et al.*, 2008). This is particularly true for endangered or endemic species, where infection can interact with additional factors, such as poaching or habitat fragmentation, leading to population decline. Coprological techniques, like faecal floatation technique, are used to detect gastrointestinal helminth eggs and estimate parasite abundance within individual hosts (Du *et al.*, 2008). Faecal floatation allows for microscopic identification and quantification of helminth eggs in a faecal sample, expressed as faecal egg count (FEC) in eggs per gram of feces (EPG) (Lynsdale *et al.*, 2015). FEC is an estimate of parasite burden that is subject to between-host variation in parasite development and population structure, as well as adult parasite sex ratio number and fecundity (Morgan *et al.*, 2005). Host sex, age and location can also influence infection and cause variation in FEC.

However, FEC is a useful tool for quantifying gastro-intestinal helminth burden where invasive methods (such as post-mortem sampling or endoscopy) are impractical and has been proven to provide a reliable estimate of individual parasite burdens in a range of host species (Lynsdale *et al.*, 2015). Assessing the levels of helminth infections is crucial for creating suitable strategies for treating or controlling these issues in confined groups of hosts and for gaining improved insights into the trends of contagious illnesses and the present well-being of untamed animal groups. In this study, we investigate the presence and severity of intestinal parasites in captive elephants residing in Chitwan National Park (CNP).

MATERIALS AND METHODS

Origin of samples

Chitwan National Park has area of 932 Square kilometer with total of about 208 captive elephants according to the data of Department of National Park and Wildlife conservation (DNPWC) (Pradhan *et al.*, 2011). A total of 103 samples were collected from captive elephant of Chitwan National Park. Captive elephant samples were gathered from private and government elephants in Chitwan National Park's buffer zone. This area is distinguished by great habitat variability, with crop fields, irrigation reservoirs, villages, and woodland areas intermingled. Elephants inhabit most of the terrain, resulting in frequent human-elephant conflict. We obtained 103 samples from captive elephant poo heaps near water sources and per rectum. Purposive sampling was carried out and the Research was carried out from 1st Jan 2019 to 30 march 2019 (Figure 1).

Sample collection

An elephant dung pile is made up of separate boli. The samples were taken from newly deposited dung heaps of captive elephants. Elephant samples were collected from dung piles believed to be less than 3 hours post-defection. Scrapings from a dung pile were combined to create a 50 g composite sample, which was placed in a tiny plastic container, sealed and taken to NTNC lab and transported to Veterinary Teaching Hospital, Paklihawa for parasitological examination.

Lab techniques

The sample was incubated in the dark at room temperature for 7 days, checked periodically and moistened if dry. Floatation and sedimentation method were used and samples were processed and analyzed using faecal culture and harvesting through Baermann technique (Abeysinghe *et al.*, 2012). Nematode, Trematode and Cestode eggs were identified and quantified based on morphology and morphometry (Condy, 1974).

Statistical analysis

Data was entered in MS Excel 2007 and data analysis was done using software Open Epi Open-Source Epidemiologic Statistics for Public Health (Version 3.01) and SPSS- V25 and MS Excel. Value of $p< 0.05$ were considered significant at 95% of confidence interval.

Figure 1. *Area of Chitwan National Park and Its Buffer Zone (Source: DNPWC).*

RESULTS AND DISCUSSION

Overall prevalence of Gastrointestinal parasites in captive Asian Elephants was 47.57% (49 out of 103 samples) and 52.48% were found to be negative (54 out of 103 samples) (Table 1 and Figure 2). Similarly, of the total 49 positive samples class wise prevalence was found higher in Nematode n=30 (61.22%) and this was followed by class Trematode n=14 (28.57%) and class Cestode n=5 (10.20%). This is due to success of survivability of 3rd stage larva of nematode and flushing out of the variable intermediate host of other different parasitic stages (Table 1 and Figure 3). Higher prevalence was found in strongylus (n=13,) followed by trichostrongylus (n=12), Fasciola(n=8), then paramphistomum (n=6) and ascaris and anoplocephala each with (n=5) were observed (Table 1 and Figure 4). The prevalence of gastrointestinal helminths in elephants showed highly significant with higher prevalence rate in private elephants which were dewormed in every six months (75.56%) ($p< 0.05$) and low in government elephants which were dewormed in every 4 months back (24.44%). i.e.; $\chi2=4.499$ $p=0.033$ (Table 1). Age wise prevalence of Gastro intestinal parasites showed higher prevalence in calf of age up to 15 years and retired adult (above 45 years) than elephants of age above 15 years. Result is highly significant ($p < 0.05$) i.e., $\chi2=12.67$, $p=0.005$ (Table 1). Result of Sex wise prevalence is statistically non-significant (with $\chi2=0.2336$, $p=0.628$ ($p> 0.05$). out of 88 total samples 41 were found positive whereas out of 15 samples from male 8 of them were found positive (Table 1). Level of mild infection was 87.75% (n=43), then that of moderate infection and severe infection as 6.1% (n=3) were observed. Mean EPG= 248.39, total EPG= 248.39± 54.25 Minimum =100 and Maximum=1300. Reference value of EPG is different as per the severity as for Mild: 50-500, for a Moderate:500-1000 and Severe: above 1000 (Soulsby and animals, 1968) Infection level of nematode is seen more in private elephants than governmental elephants.

Table 1. Prevalence of gastrointestinal parasite in elephant.

Parameters		Positive result	P- Value
Overall prevalence	Helminth	49 (47.57%)	
Class	Nematode	30 (61.22%)	ND
	Trematode	14 (28.57 %)	
	Cestode	5 (10.20%)	
Species	Strongylus	13(26.53%)	P<0.05*
	Trichostrongylus	12 (24.48%)	
	Fasciola	8 (16.32 %)	
	Paramphistomum	6 (12.24%)	
	Anoplocephala	5(10.20%)	
	Ascaris	5(10.20%)	
Organization	Private Elephant	37 (75.56%)	P=0.005**
	Government Elephant	12 (24.44%)	
Age	Calf	15 (30.61%)	P=0.005**
	Sub adult	5 (10.2%)	
	Adult	12 (24.48 %)	
	Retired adult	17 (34.69 %)	
Sex	Male	41 (83.6%)	P>0.05
	Female	8 (16.3%)	
Severity	Mild	43(87.75%	p>0.05
	Moderate	3(6.12 %)	
	Severe	3(6.12 %)	

P-value < 0.05, P-value<0.01, p-value <0.001 indicates significance.

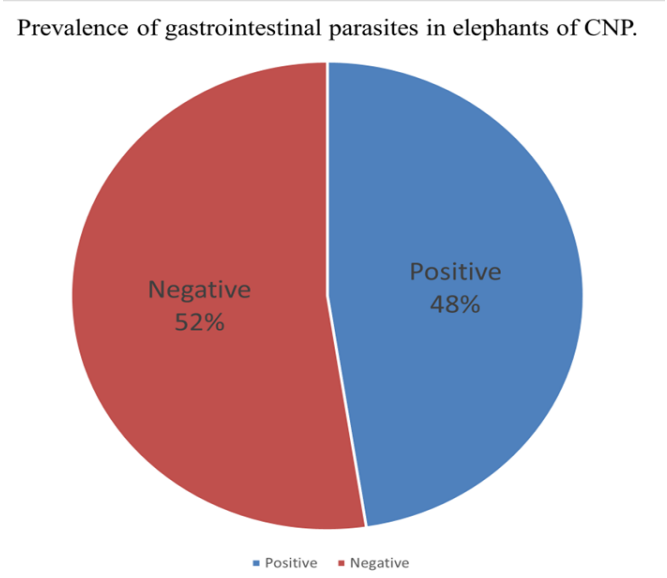

Figure 2. *Overall prevalence of gastrointestinal parasites in elephants of CNP.*

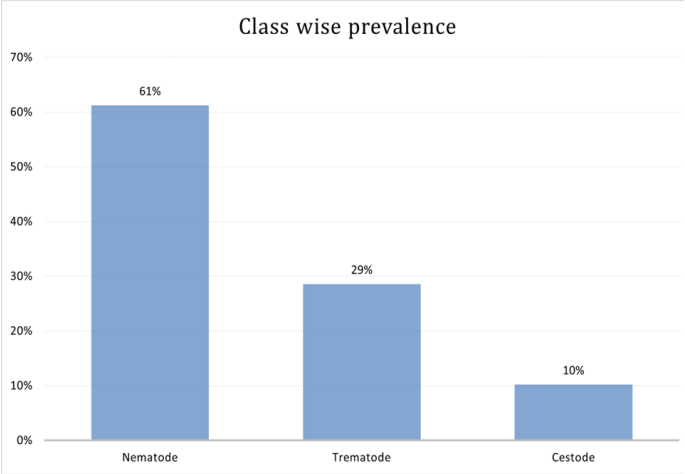

Figure 3. *Class wise prevalence of GI helminths.*

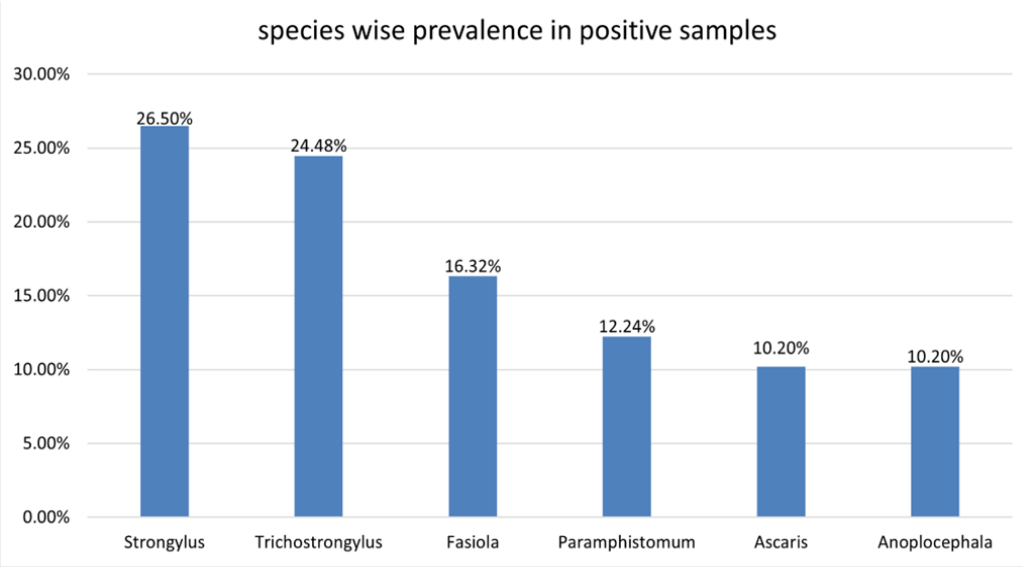

Figure 4. *Species wise prevalence in positive samples.*

Result was statistically non-significant i.e., $\chi 2=1.255$, p=0.534, (p> 0.05). Out of 103 samples, overall prevalence of gastrointestinal parasites in captive Asian elephants was 47.57% which is lower than finding by (Hing, 2012; Jani, 2008; Karki & Manandhar, 2008) who found the prevalence of 100%,62.5% and 60% respectively. This difference might be due to some of the intrinsic and extrinsic factor such as sampling technique, deworming status, climate, season and diagnostic employed. Trend of reducing helminths infection was attributed to an increasing routine use of anti-helminthic (Vidya and Sukumar, 2002). Moreover, the overall prevalence was found to be lower but the incidence seems to vary widely between sites. Most of the research states that histocompatibility complex is one of the most significant factors determining the susceptibility of the parasitic infection in the animal. Not only this immune status, physiological condition and general health of an individual also play great contribution in the disease prevalence (Lilly *et al.*, 2002). Large scale studies would be needed for the identification and determination of the factor responsible for the observed variance in the incidence and parasite load in captive elephant. Nematodes are frequently found with hookworms as particularly reported to cause pathological lesions and hemorrhages in the bile ducts and liver, as well as the intestines (Obanda *et al.*, 2011). This is due to success of survivability of 3rd stage larva and flushing out of the variable intermediate host of different parasitic stages as supported by (Raghavan & Arunachalam, 2022).

Age was significantly associated with the prevalence of gastro intestinal parasites with higher prevalence with age group 1-15 years i.e., Calf, and retired adult. Therefore, parasite prevalence increased with increasing elephant age (Kosaruk *et al.*, 2023). In this study, elephants do not appear to develop parasite immunity with age, or if they do, it is not robust enough to induce a discernible decline in infection levels in previously exposed animals. Similarly, a study on wild elephants in Namibia found that within family groups, parasitic burden increased with age and this was attributed to older elephants eating more, and therefore being exposed to a greater number of parasites (Baines *et al.*, 2015; Le Boeuf *et al.*, 2019). However, sex was non-significantly associated with prevalence of gastro intestinal parasites this may be due to unhygienic stable with poor urine drainage, dung storage closely to the stable confined to the small areas and friendly nature with are responsible for the parasitic infestation. Similarly, prevalence is higher in private elephants than Governmental elephants because there is regular deworming of government elephants generally practiced in four months of interval but some are only dewormed after shown of clinical signs and symptoms in private elephants. Besides, there is no practice of grazing for private elephant and are mainly under rice straw stall feeding, only government elephant offers for grazing It is in accordance to that dried straw fed to buffaloes might play important role to carry the dormant stages of Fasciola parasites (Mahato *et al.*, 2000; Mahato *et al.*, 2005; Nishanth *et al.*, 2012). Gastrointestinal parasite control in animals has mostly been designed to target highly intensive livestock production and is based on frequent anthelmintic medication therapy. Although productivity is rarely a consideration, livestock parasite control strategies are frequently adapted straight to captive management of wild species. In captive elephants, for example, totally removing internal parasites by rigorous anthelmintic treatment is the norm, and several studies have shown that albendazole is beneficial against strongyles in captive elephants (Abeysinghe *et al.*, 2017).

Life span of most of the livestock species is less than 10 years but in contrast the life span of the captive Asian elephant is usually exceed 70 years (Lahdenperä *et al.*, 2014). Long-term use of intrinsic antihelminth medication-built drug resistance in the farm animals which is also an interest of global concern (Stringer and Linklater, 2014). However, enhancing the animal to develop natural immunity against helminth infection is gaining more topic of interest for the researcher worldwide and the research also explain that the exposure with helminth parasite lowers the allergic disease (Sutherland *et al.*, 1999).

Conclusion

In summary, the investigation of Asian elephants in Chitwan National Park (CNP) revealed their infection with various gastrointestinal parasites. The prevalence of these parasites among captive/domestic elephants was determined at 47.57%. These parasites, identified based on egg morphology, encompassed three nematode genera, two trematode genera, and one cestode genus. Notably, private elephants exhibited a higher parasite burden compared to government-owned elephants near CNP. Significant associations were established between parasites and age groups as well as deworming history, while no significant correlation was found with gender. The study concludes that six different helminth parasites were identified, with Strongylus spp. being the most predominant. The average Eggs Per Gram (EPG) count was 248.39 ± 54.25, and although not significantly correlated with organizational prevalence, this finding underscores the susceptibility of CNP's Asian elephants to various gastrointestinal parasites. Such susceptibility could lead to illness under heavy infestations, a concern also applicable to captive/domestic elephants with elevated parasite counts.

ACKNOWLEDGEMENT

We express sincere gratitude to all the members of the advisory committee and all the research team.

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