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Prevalence and chemotherapeutic management of gastrointestinal nematodes in captive wild carnivores of Kanan Pendari Zoo, Bilaspur (Chhattisgarh)

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Abstract
The present study was carried out to find prevalence and chemotherapeutic management of gastrointestinal nematodes in captive wild carnivores of Kanan Pendari Zoo, Bilaspur (Chhattisgarh). Faecal samples (n=148) were collected aseptically from zoo enclosures of captive wild carnivores housed in zoo and subjected to examination by direct, sedimentation and floatation method followed by calculating eggs per grams by using McMaster slide. The findings of our study revealed prevalence of gastro-intestinal nematodes in captive wild carnivores was reported to be 29.05% (43/148). Amongst all species of nematodes observed in captive wild carnivores, *Strongyle* spp. was the most prevalent nematode (52.00%). Therapeutic efficacy of combination of fenbendazole along with praziquantel against concurrent infection of *Ancylostoma* spp. and *Spirometra* spp. in captive Royal Bengal Tiger was recorded to be 85.63% effective in eliminating gastrointestinal nematodes.

Keywords: Gastrointestinal nematodes, captive wild carnivores, prevalence, therapeutic efficacy, fenbendazole, praziquantel

Introduction
Zoological parks have been established for the purpose of public amusement and education with their respect to the conservation of biological diversity (Cuaron, 2005) [3]. Zoological parks are considered as the nodal center for conservation of various wild animal species for various activities related to imparting education to community for wildlife conservation, carrying out research activities as well as recreational activities for various age group of humans (Varadharajan and Pythal, 1999) [14]. Government of India has also framed and implemented the National Zoo Policy (1998) with objectives to supplement and reinforce the efforts in the conservation of varied wild fauna biodiversity across country (Moudgil, 2015) [8]. Like domestic animals, zoo animals have also been observed to incur various infectious and non-infectious diseases. Moreover, presence of diseases in wild animals is always considered as a risk for domestic livestock as well as human population living in vicinity of wild fauna and vice-versa. In free ranging wild animals, natural resistance against endoparasites is commonly noticed as well as a balanced living in harmony with helminths in ecosystem is observed (Thawait *et al.,* 2014) [13]. Regular surveillance of gastrointestinal parasites along with adoption of various control measures, effective therapeutic management and proper prophylactic measures can play a vital role in maintaining health of captive animals which in turn will also be helpful to restore ecological balance. However, adoption of such practices is not being followed in our country even in its initial phases (Nayak *et al*., 2018) [9]. Incorporation of existing knowledge of endoparasitic control as well as preventive measures can prove to be a major milestone to reduce morbidity and mortality arising due to helminth infection in wild animals (Barmon *et al*., 2014) [1]. Even though availability of variety of anthelminthic drugs in Veterinary medicine has been observed during last few decades but complete elimination of all infective stages of helminthes from zoo environment as well as host is not practically possible due to various reasons which are beyond control of mankind (Sahoo *et al*., 2009) [10].

Materials and methods
Place of study: Kanan Pendari Zoo, Bilaspur is located 9 km from Bilaspur city on Jabalpur – Bilaspur highway.

In 1975, this area was being used as nursery for forest department and later on it was converted into wild animal rescue centre. This centre started attracting people and was developed as a breeding centre for cheetal and this centre was promoted to status of small zoo in the year 1997. The present study duration was conducted from March, 2018 to August, 2018 for a span of 6 months.

Sample collection

Freshly passed faecal samples (n=148, Table no. 1) were collected randomly from the enclosures of various captive wild carnivores in clean polythene bags and placed inside ice box until transported to laboratory. All faecal samples were given a label depicting species of wild animal, age, sex and enclosure details for appropriate data keeping of samples.

Table 1: Captive wild carnivore population of Kanan Pendari Zoo, Bilaspur

Sl. No.	Species	Total population	Male	Female	Young
1.	Asiatic Lion (*Panthera leo persica*)	13	07	06	00
2.	Royal Bengal Tiger (*Panthera tigris tigris*)	14	07	07	00
3.	Leopard (*Panthera pardus*)	08	06	02	00
4.	Jackal (*Canis aureus*)	15	05	06	04
5.	Hyaena (*Hyaena hyaena*)	04	02	02	00
6.	Fox (*Vulpes bengalensis*)	15	05	05	05
7.	Jungle cat (*Felis chaus*)	05	01	02	02

Direct method of faecal examination

A small amount of faecal sample was evenly spead over glass slide after adding a drop of water. A cover slip was placed over slide followed by direct examination at low power (10X) under microscope. A total of 3 slides prepared from different parts of the individual faecal sample were screened for presence of helminth ova (Soulsby, 1982) [12].

Floatation method

Faecal sample (3 grams) was added to pestle and mortar and water was added followed by complete mixing to make suspension. The sample suspension was strained and strained material was transferred into a plastic centrifuge tube followed by centrifugation at 2000 rpm for 5 minutes. Supernatant was discarded and refilling of tube with water was done followed by centrifugation for 2 to 3 times until clear supernatant was observed. Finally, sediment was mixed with saturated sugar solution and centrifuged again. A drop of fluid from top of supernatant was taken and examined under low power microscope (10X) after placing coverslip over slide.

Sedimentation method

Approximately 3-gram faecal sample was added in pestle and mortar and triturated well after mixing 15 ml water. Faecal emulsion was then filtered using strainer into a centrifuge tube for centrifugation at 2000 rpm for 5 minutes. The supernatant was discarded and tube was refilled with water again and centrifuged again for 2 to 3 times so as to obtain clear supernatant. The left-over sediment was examined under the low power (10X) microscope after putting a cover slip to rule out presence of helminth ova.

McMaster method for Eggs per gram count

Small carnivores: Faecal sample (2 gram) was added into a beaker with 20 ml of water and left to soak for half an hour. Then sample was transferred into the pestle and mortar followed by thorough mixing. The sample was again poured back into the same beaker followed by addition of 40 ml of saturated salt solution into beaker (Dilution factor is 1 in 30). One ruled chamber of the Modified McMaster slide was charged and all eggs were counted under microscope.

Area of each chamber = 16 mm (length) x 12.5 mm (breadth) x 1.5 mm (height)= 300 cmm = 0.3 cc = 0.3 ml

$$\text{Eggs of each chamber} = \frac{\text{Number of eggs counted}}{\text{Area of one chamber}} \times \text{Dilution Factor}$$

$$= \frac{\text{Number of eggs counted}}{0.3} \times 30$$

$$= \text{Number of eggs counted} \times 100$$

Large carnivores

Approximately 3gm faecal sample was added into beaker with 20 ml of water and left for about 30 minutes for soaking. The sample was transferred to pestle and mortar and mixed thoroughly followed by transferring sample back into same beaker. About 40 ml saturated salt solution was poured into beaker taking care not to form air bubbles (Dilution factor is 1 in 20). One ruled chamber of the Modified McMaster slide was charged and all eggs were counted under microscope.

Area of each chamber = 40mm (length) x 12.5 mm (breadth) x 2mm (height)

= 1000 cmm = 1 cc = 1 ml

$$\text{Eggs of each chamber} = \frac{\text{Number of eggs counted}}{\text{Area of one chamber}} \times \text{Dilution Factor}$$

$$= \frac{\text{Number of eggs counted}}{0.2} \times 20$$

$$= \text{Number of eggs counted} \times 10$$

Therapeutic management

Royal Bengal Tigers (n=6) found positive for moderate to severe (EPG 920-1360) with nematode infection (02 *Stronglye* sp. and 02 *Ancylostoma* sp.) and nematode and cestode mixed infection (02 *Ancylostoma* sp. and *Spirometra* sp.) were treated with fenbendazole in combination with praziquantel for evaluation of therapeutic efficacy. Fenbendazole @ 10 mg/kg body weight and praziquantel @ 5mg/kg body weight given as single dose orally (SID) mixed with meat.

Assessment of therapeutic efficacy

Therapeutic efficacy of drug combination was evaluated on the basis of decrease in EPG recorded on day 0 (pre-treatment) as compared to 7th and 14th day post treatment.

Statistical analysis

The data regarding prevalence and efficacy of anthelmentic drug between two groups were subjected to statistical interpretations as per standard protocol (Snedecor and Cochran, 1994) [11].

Result and discussion

Prevalence of gastrointestinal nematodes

A total of 43 samples were found to be positive out of 148 faceal samples screened for the presence of endoparasitic ova thereby reporting 29.05% prevalence of nematodes in Kanan Pendari Zoo, Bilaspur. The findings of current study are in corroboration with the findings of Thawait *et al.* (2014) [13] who have reported 28.33% prevalence of helminthic infection in captive wild carnivores.

Species wise prevalence of gastro-intestinal nematodes

Species wise prevalence of gastrointestinal nematodes observed in our study is being presented in table no. 2.

The findings of species wise prevalence revealed that 9 samples (9/26) obtained from lion, 04 samples (4/16) of leopard, 28 samples (28/28) of Royal Bengal tiger, nil faecal sample (0/8) of hyaena, 01 sample each out of 30 samples of jackal and hyaena and no sample (0/10) of jungle cat revealed presence of nematode infection. Prevalence of nematode infection in different species under study was observed to be 34.61%, 25.00%, 100%, 0%, 3.33%, 3.33% and 0% in lion, leopard, Royal Bengal tiger, hyaena, jackal, fox and jungle cat respectively. The findings of cent percent prevalence of nematode infection in Royal Bengal tiger is contrastingly higher than those stated by Deshmukh *et al.* (2009) [4] wherein prevalence of 50% of helminthic infection in tigers at Van Vihar National Park, Bhopal (M.P.) have been reported. Similarly, much lower prevalence of 32.85% for nematode infection in captive tigers has been reported by Chandrakar *et al.* (2020) [2]. In another study, Khutey *et al.* (2021) [5] have also reported much lower prevalence of 51.56% of gastrointestinal nematodes in captive wild herbivorous species housed at Kanan Pendari Zoo, Bilaspur, Chhattisgarh.

Table 2: Species wise prevalence (%) of gastro-intestinal nematodes

Sl. No.	Species	Total no. of samples examined	No. of positive samples	Prevalence (%)
1.	Lion (*Panthera leo persica*)	26	09	34.61
2.	Leopard (*Panthera pardus*)	16	04	25.00
3.	Royal Bengal tiger (*Panthera tigris*)	28	28	100
4.	Hyaena (*Hyaena hyaena*)	08	Nil	0
5.	Jackal *(Canis aureus)*	30	01	3.33
6.	Fox (*Vulpes bengalensis*)	30	01	3.33
7.	Jungle Cat (*Felis chaus*)	10	Nil	0

Sex wise prevalence of gastro-intestinal nematodes

Sex wise prevalence of gastrointestinal nematodes in captive wild carnivores of Kanan Pendari Zoo, Bilaspur is being presented in table no. 3.

Out of total of 148 fecal samples examined in our study, 66 samples were obtained from male animals while 60 samples were obtained from captive female carnivores. The findings of fecal sample examination revealed that 22 samples belonging to male animals were found to be positive while out 14 samples obtained from female animals were found to be positive for gastrointestinal nematodes. Hence, prevalence rate was 33.33% and 21.21% in male and female animals respectively. A similar findings of sex wise prevalence of gastrointestinal parasites in 03 different zoos of Chhattisgarh has been reported by Chandrakar *et al.* (2020) [2] where in sex wise prevalence of to be 31.75% in male and 25.00% in female animals was documented.

Table 3: Sex wise prevalence (%) of gastro-intestinal nematodes

Sex	Number of faecal samples examined	Number of positive samples	Prevalence (%)
Male	66	22	33.33
Female	60	14	21.21

Season wise prevalence of gastro-intestinal nematodes

Season wise prevalence of gastrointestinal nematodes observed in our study has been presented in table no. 4.

A total of 148 faecal samples were screened out of 74 samples each were examined during summer (March-May) as well as monsoon (June-August) season. The findings of our study revealed that 15 samples were positive for the presence of gastrointestinal nematodes in summer season while 25 samples were observed to harbour nematode infection in monsoon season, indicating season wise prevalence of 20.27% (15/74) in summer and 33.78% (25/74) in monsoon season respectively.

Season wise prevalence of gastrointestinal nematodes during summer season was observed to be 20.27% in our study which is contrastingly lower than those stated by Modi *et al.* (1997) [7] who have reported 33.33% prevalence in captive wild carnivores of Bihar Zoo, Patna during summer season. The prevalence of gastrointestinal nematodes during monsoon season was 33.78% which is much lower than those documented by Sahoo *et al.* (2009) [10] who have recorded 47.62% prevalence of endoparasitic infection in captive animals of Nandankanan Zoological Park, Orissa during pre-monsoon season.

Table 4: Season wise prevalence (%) of gastro-intestinal nematodes

Season	Number of faecal samples examined	Number of positive samples	Prevalence (%)
Summer (March-May)	74	15	20.27
Monsoon (June-August)	74	25	33.78

Age wise prevalence of gastro-intestinal nematodes

Age wise prevalence of gastrointestinal nematodes in captive wild carnivores of Kanan Pendari Zoo, Bilaspur have been presented in table no. 5.

A total of 148 faecal samples were screened out of which 22 samples were obtained from young wild carnivores while 126 faecal samples were collected from adult counterpart. Faecal sample examination revealed that 36 samples collected from adult animals were positive for gastro-intestinal nematodiasis while only 8 samples obtained from young animals harboured gastrointestinal nematode eggs, thereby indicating prevalence of 28.57% (36/126) in adults and 36.36% (08/22) in young animals respectively.

The results of our study reported 36.36% prevalence of gastro-intestinal nematodes in young animals less than 12 months of age, which is in close proximity with the findings of Kumar and Rao (2003) [6] have also documented prevalence to be 41.00% in animals aged less than 1 2months at Animal Rescue Centre at Vizag Zoo, Visakhapatnam, Andhra Pradesh.

Table 5: Age wise prevalence (%) of gastro-intestinal nematodes

Age group	Number of faecal samples examined	Number of positive samples	Prevalence (%)
Adult	126	36	28.57
Young	22	08	36.36

Therapeutic management

The findings of therapeutic efficacy of drug combination used in our study in relation to reduction to eggs per gram count have been presented in table no. 6.

Egg per gram values in the treatment group revealed highly significant ($p<0.01$) reduction in values on day 7th and 14th post-treatment as compared to baseline values (day 0). Hence, fenbendazole in combination with praziquantel was found to be 85.63% effective in eliminating mixed *Ancylostoma* spp. and *Spirometra* spp. infection in captive Royal Bengal Tigers.

Table 6: Therapeutic efficacy of the drug combination used in our study

Group	EPG (Before treatment)	EPG (After treatment)	
	0 day	7th day	14th day
I (Positive Control)	911.67 ± 56.83	1003.33 ± 51.62	995 ± 58.12
II (Treatment)	1086.67 ± 80.69	191.67** ± 41.99	156.67** ± 15.42

** Values differ highly significantly ($p<0.01$)

Conclusion

The present study reported prevalence of gastro-intestinal nematodes in captive wild carnivores housed at Kanan Pendari Zoo, Bilaspur to be 29.05% (43/148). *Strongyle* spp. was the most prevalent nematode (52.00%) in our study. Evaluation of therapeutic potential of fenbendazole along with praziquantel against mixed infection of *Ancylostoma* spp. and *Spirometra* spp. in captive Royal Bengal Tigers was observed to be 85.63%. Hence, it can be concluded that captive wild carnivores housed in zoos are infected with various species of gastrointestinal helminths which needs to be eliminated by adopting planned therapeutic and managemental interventions.

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Conflict of interest

The authors declare that there is no conflict of interest in regards to research, authorship, and publication of this research manuscript.

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