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SHORT COMMUNICATION

Efficacy of oxyclozanide and levamisole treatment on the gastrointestinal parasites in captive Lions *Panthera leo*

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Abstract: A study was carried out to determine the efficacy of anthelmintics on gastrointestinal parasites in lions under captivity at Bannerghatta Biological Park (Bengaluru), Sri Chamarajendra Zoological Garden (Mysuru) and Tiger-Lion Safari Tyavarekoppa (Shivamogga) during the period from January to June, 2018. Out of 20 faecal samples subjected to qualitative and quantitative methods, 66.6% were found positive for eggs of *Ancylostoma* spp., 60.0% for *Toxascaris leonina*, 20.0% for *Spirometra* spp. and 13.3% for *Balantidium coli* cysts with an overall infection rate of 75.0%. A combination of oxyclozonide 6% w/v and levamisole 3% w/v (Neozide plus) at the rate of 1ml per 4kg body weight revealed egg per gram counts for *Ancylostoma* spp., *T. leonina* and *Spirometra* spp. to be reduced from 100, 11,450 ± 11,250 and 100 to zero respectively on subsequent 3, 7, 10 and 21 days post treatment and proved to be cent per cent effective.

Keywords: Anthelmintics, efficacy, gastrointestinal parasites, Lions.

In captive wild carnivores, the change in the environment and living conditions influences the ecology of the animal and might increase the susceptibility to many of the diseases, viz., bacterial, viral, parasitic and rickketsial diseases (Goossens et al. 2005). Especially, carnivores kept in captivity in zoos usually suffer from several parasitic infections, such as from nematodes, cestodes, trematodes, and protozoans. In particular, among nematodes ascarids constitute the major parasitic infection in wild carnivores and are established as a problem in most of the zoos throughout the world (Sayid & Mohammed 1997–1998).

Among parasitic diseases, particularly helminthic infections have a greater ramification and significant impact on host survival, growth and reproduction through direct and indirect pathological effects. The subclinical infections may not cause any immediate alarming signs of disease but in the long course, they would render the animals susceptible to other concurrent infections (Muraleedharan et al. 1990). In addition, gastrointestinal parasites of wild carnivores include zoonotic species to humans and may raise public health concern (Acharjyo 2004).

Though helminthic diseases are a major constraint to zoo animals, the occurrence of parasitic infections may vary depending on the type of husbandry practices, viz., nutritional status, physiological condition, implementation of disease control programmes, and treatment administered (Singh et al. 2006). In most of the zoological gardens, the prevention and control of

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gastrointestinal parasitic infections is mainly dependent on the short term deworming programmes. A range of antiparasitic drugs has been used to reduce parasitism in wild animal populations. In particular, most drug treatment experiments using anthelmintic has been carried out by targeting nematode infections (Pedersen & Fenton 2015). The commonly used anthelmintic includes thiabendazole, piperazine citrate and adipate, pyrantel pamoate, albendazole, fenbendazole, levamisole, and ivermectin. Many studies have provided information on the effects of treatment on the target parasite, assessed either in terms of the prevalence of infection (proportion of hosts infected), mean parasitic abundance (mean number of parasites or parasitic eggs shed per host), or mean parasite intensity (mean number of parasite eggs shed per infected host).

The regular examination of faeces, assessment of parasitic load, and the assessment of drug efficacy, however, are not frequently carried out in many zoological parks or gardens. Therefore, the present study was undertaken to determine the efficacy of anthelmintic on the gastrointestinal parasites in captive lions.

Material and Methods

Study area

The study area included Bannerghatta Biological Park (BBP) which is located 22km south of Bengaluru in the hills of the Anekal range with 26356.16 hectares area with zoo, a pet corner, an animal rescue centre, a butterfly enclosure, an aquarium, a snake house, and a safari park. Sri Chamarajendra Zoological Garden (SCZG) is around 63.53 hectares located near the palace in Mysuru, is one of the oldest zoos in India and is home to a wide range of species (168). Tiger-Lion Safari Tyavarekoppa (TLST), Shivamogga is Karnataka's second safari park, after BBP with an area of 250ha.

Collection of samples

During this study, a representative faecal sample of about 10g was collected from each enclosure and the methodology was followed as per Soulsby (1982) and Taylor et al. (2015). However, in Dehuri et al. (2013), 2g of faeces was collected and also for the methods the reference Soulsby (1982) is cited. A total of 20 faecal samples were collected from lions under captivity at BBP (12), SCZG (4), and TLST (4) during the period January–June 2018. The faecal samples were examined macroscopically and were subjected to microscopic examination by using qualitative (direct and concentration) and quantitative (Mc Master's) methods to assess the severity of different parasitic infections (Soulsby 1982; Taylor et al. 2015). The parasitic eggs/larvae/cysts/oocysts were identified based on the standard morphological characters (Soulsby 1982; Zajac & Conboy 2012; Bowman 2014).

Determination of anthelmintic efficacy

An anthelminthic efficacy against gastrointestinal parasitic infections was determined based on the eggs per gram (EPG) of faeces using Mc Master's method. During this study, a combination of oxyclozonide 6% w/v and levamisole 3% w/v (Neozide plus) @ 1ml per 4kg body weight in meat was administered during May 2018. The EPG was carried out during pretreatment and on 3rd, 7th, 10th, and 21st days post treatment. The percentage of efficacy of drug was determined by the following formula:

Efficacy % = (Pre treatment EPG – Post treatment EPG / Pre treatment EPG) x 100

Statistical analysis

The statistical analysis of data was carried out by Fisher's exact and one way ANOVA tests using graph pad prism software, version 5.01.

Results

During this study, out of 20 faecal samples examined by direct and concentration methods, 15 samples were found to be positive for helminthic infections of *Ancylostoma* spp. (66.6%), *T. leonina* (60.0%), *Spirometra* spp. (20%), and *Balantidium coli* (13.3%) cysts, with an overall infection rate of 75%. In BBP, SCZG, and TLST, 10 (83.3%), four (100%), and one (25.0%) samples were found to be positive for gastrointestinal parasitic eggs/cysts, respectively (Table 1). The statistical differences between the infections in the different locations were found to be nonsignificant at $P< 0.05$. The mixed infections of *Ancylostoma* spp. and *T. leonina* (5), *Ancylostoma* spp. and *Spirometra* sp. (2), and *Ancylostoma* spp. and *B. coli* (2) were commonly observed with an overall infection rate of 70%.

Anthelmintic efficacy

The average mean pretreatment EPG counts was found to be 100 ± 0 for *Ancylostoma* spp., 11,450 ± 11,250 for *T. leonina* and 100 ± 0 for *Spirometra* spp. Subsequently, after 3, 7, 10 and 21 days post treatment, the EPG counts were reduced to zero. An examination of faecal samples was negative for gastrointestinal parasitic infections with cent per cent efficacy on 21 days post treatment.

Table 1. Number of faecal samples positive for gastrointestinal parasites in lions at different locations.

	Locations	No. of animals examined	No. of animals infected	No. of positive samples			
				Ancylostoma spp.	*T. leonina*	*Spirometra* spp.	*Balantidium coli*
1	Bannerghatta Biological Park, Bengaluru	12	10 (83.3%)	8 (80.0%)	6 (60.0%)	3 (10.0%)	2 (20.0%)
2	Sri Chamarajendra Zoological Garden, Mysuru	4	4 (100.0%)	2 (50.0%)	2 (50.0%)	0	0
3	Tiger-Lion Safari Tyavarekoppa, Shivamogga	4	1 (25.0%)	0	1 (25.0%)	0	0
	Total	20	15 (75.0%)	10 (66.6%)	9 (60.0%)	3 (20.0%)	2 (20.0%)

Discussion

During this study, one faecal sample from TLST and two from SCZG were found to be positive for eggs of *Ancylostoma* spp. and *T. leonina* with very low parasitemia (EPG = 0). Therefore, anthelmintic efficacy was carried out at BBP. All the animals irrespective of the infection and whether the animals were positive or negative were administered a combination of oxyclozonide (6% w/v), a broad spectrum antitrematodal drug and levamisole (3% w/v) which has activity against nematodes and an immunostimulant at the rate of 1ml per 4kg body weight. In the present study, subsequently after 3rd, 7th, 10th and 21st day post treatment, the EPG counts were reduced to zero per cent and the faecal samples were negative for infections indicating 100% efficacy. During this study, though the *Spirometra* infection was recorded, the animals were not administered with anticestodal drugs. Therefore, based on the present findings zoo veterinarians received the suggestion that wild carnivores under captivity should be regularly dewormed with anthelmintic only after an examination of faecal samples for the presence or absence of gastrointestinal parasitic infections to avoid unnecessary dosing with anthelmintic thereby reducing the cost of treatment and development of resistance in future. Many authors, however, have reported varied efficacy with different modes of action of anthelmintic—Sayid & Mohammad (1997–1998) at Khartoum Zoo, Sudan reported complete clearance of infection with piperazine but incomplete clearance of parasites on 28th day post treatment with thiabendazole in lions, leopards, jackal, and dwarf mongoose infected with *T. leonina*; Sur et al. (2000) treated lions infected with *Toxocara* and *Ancylostoma* with ivermectin injection (Ivomec) @ 1ml per 50kg body weight and recorded an absence of eggs on the 7th day post treatment; Kumar et al. (2005) treated 22 lions which showed the occurrence of *Toxocara* eggs in the faeces with piperazine @ 220mg per kg orally at M.C. Zoological Park, Chhatbir, Patiala District, Punjab and later, with ivermectin at the dose rate of 1ml per 50kg body weight subcutaneously twice at one week intervals for piperazine resistant *T. cati* and observed the egg count to be reduced by 98.71%; Moudgil et al. (2017) reported that fenbendazole @ 10mg per kg body weight once daily for three consecutive days was ineffective to eliminate the infection in Asiatic Lions infected with *T. leonina* and observed eggs reduction by 69.35% at day 3 post treatment with three consecutive treatment schedule, however, extended period of time with fenbendazole for five days and ivermectin @ 100µg per kg body weight once daily for three alternative days resulted in eggs reduction by 95.34% and 95.74%, respectively, and proved to be effective.

The differences in the efficacy of each anthelmintic drug may be attributed to the fact that the vehicle of drug to captive wild animals (especially wild felines) play an important role in administering exact dosage the reason could be that the total dosage of anthelmintic drug cannot be calculated according to their body weight (Moudgil et al. 2017).

In conclusion, though helminthic and protozoan infections are known to occur in captive wild carnivores, the control measures undoubtedly would depend upon several factors. Further, the rationale behind the control of parasitic infections in wild carnivores of Indian zoos presumes that in as much as each carnivore is infected and that the zoo environment cannot be changed frequently because of space confinement, overcrowding, and the movement of keepers from one enclosure to another and the presence of stray dogs and cats which may act as a source of infection (Acharjyo 2004). The present findings indicated that quarterly deworming (once in three months) of all the animals and examination of faecal samples before and after deworming should be followed regularly to confirm the efficacy of treatment. In addition, a change of anthelmintic should be instituted from time to time to avoid drug resistance in captive wild carnivores.

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