Study on Gastrointestinal Parasitism of Wild Animals in Captivity at the Zoological Garden of Haramaya University, Ethiopia

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Abstract
Captivity of wild animals and restriction of their movement can lead to stress, resulting in suppressed immune response and reduced disease resistance ability. Wild animals in zoological gardens can predominately affected by various gastro-intestinal parasites. This study was therefore, conducted to determine occurrence, identify types and assess seasonal variation of gastrointestinal parasites in captive animals at the Zoological garden of Haramaya University. The study involved a retrospective investigation of zoo records and fecal examinations. Twenty two fecal samples were collected in December, 2013 from all the animals in the garden and examined using concentration and fecal culture techniques. Sampling was repeated after four months in April, 2014 to assess seasonal variations of the parasites. The result from the retrospective study showed death of 21 animals in the last five years. Fecal examination confirmed presence of gastrointestinal parasites with an overall mean prevalence rate of 73.8%. A total of 9 parasite taxa were identified of which 6 (66.67%) and 4 (33.33%) were helminths and protozoa respectively. No Cestode and Trematode species were recovered. Trichuris spp., Toxocara spp., Strongyloides spp., Toxascaris leonina, Passalurus ambiguous and Ascaridia spp. were the helminth parasites observed in the garden. Entamoeba spp., Isospora spp. and Eimeria spp. were the protozoan parasites encountered. No remarkable seasonal variation in parasitic infection was noticed in the garden. The study revealed occurrence of gastrointestinal parasites in the zoological garden, most of which are zoonotic and hence future studies are needed to determine risks of cross-transmission.

Keywords
Gastrointestinal Parasite, Captive Wild Animals, Zoological Garden
1. Introduction

Large populations of wild animals in captivity are affected by various diseases. Parasite infections are one of the major problems in the wild animals in captivity. Gastrointestinal parasites are frequently reported to affect the health and the wellbeing of the animals in zoological gardens [1]. The species of the gastrointestinal parasites infecting wild animals in captivity might differ with the type of animals and the management practices [2] [3].

In captivity, animals appear to be less resistant to parasitic infections than under their natural habitats [4]. The changes in environment and living conditions from the natural habitat to captivity alter the life styles of animals, reduce disease resistance ability and increase their susceptibility to parasitic infections [5] [6] [7]. Vulnerability of captive wild animals to parasitic infections depends on factors, like feeding, keeping conditions, animal management and environmental conditions, such as temperature and humidity. Confining captive animals in cages accompanied with improper feeding systems and poor sanitation may lead to contamination of environment, which makes the animals more prone to different parasitic infections [4] [6] [8] [9]. Proximity and close contact of humans with captive animals provide opportunity for the transmission of new parasite species which would not normally come in contact with them in the natural habitat [10]. Most of the deaths of wild animals in controlled areas are amounted to parasitic diseases. Parasitism, especially endoparasitic infection produces ill effects like weakness, emaciation, inappetance in zoo animals that can cause immune suppression and potentially predispose the animals to other diseases [11].

Although many parasitological investigations are conducted in domestic animals for long period of time, it has been recently recognized that parasitic infections are also common and important in wild animals [12] [13]. Incidence of gastrointestinal parasites in wild and zoo animals has been reported from different parts of the world [9] [14]. Several studies have been carried out on gastro-intestinal parasites in non-human primates [15] [16] [17] [18] [19], wild carnivores [20] [21] [22], wild herbivore animals [6] [7] [23], birds in captivity [11] [24] [25] [26], reptiles [27] and many other zoo animals across the globe.

Haramaya University zoological garden was established in 1966 for conservation of wild animals, recreational purpose, wild life education and research (Beadles and Ingersol, 1968, Haile Sellassie I University (HSIU) Experiment station Publication). Up on foundation, the garden started conservation with a total of 129 animals representing 51 different species. However, most of the species of animals that the garden started conservation with do not exist today. The number of species in the garden has been gradually reduced to 9, consisting of a total of 25 animals. Lions (Panthera leo), Hamadryas baboon (Papio hamadryas), Vervet monkey (Cercopithecus aethiops), Lesser kudu (Tragelaphus imberbis), Spotted hyena (Crocuta crocuta), Kit foxes (Vulpes macrotis), Japanese rabbit (Oryctolagus cuniculus), Helmeted guinea fowl (Numida meleagris) and East
African Giant Tortoise (*Geochelone sulcata*) are the species of wild animals currently found at the zoological garden.

There are incidences that the health problems and death of the zoo animals is usually connected with infection of gastrointestinal parasites. However, the attention given to investigate and document the type of gastrointestinal parasites infecting zoo animals in the country in general and at Haramaya University in particular is minimal. This study was therefore conducted to determine the occurrence, identify species and assess seasonal variation of gastrointestinal parasites in captive animals at the Zoological garden of Haramaya University.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted at the Zoological garden of Haramaya University, 9°26’N latitude and 42°03’E longitude at an altitude of 1980 m.a.s.l., 520 km east of the capital, Addis Ababa. The area has three common seasons; the two rain periods and one dry period. The rain seasons are from June to September (*Kiremt* rains) and February to May (*Belg* rains) while the dry period extends from October to January (*Bega* season). The mean annual rainfall is 780 mm with average minimum and maximum temperatures of 8.25°C and 23.4°C, respectively. In the garden, animals are housed in different cages individually and/or in small groups based on species and age of the animals.

2.2. Study Methodology

The study involved observation of the general management practices and retrospective investigation of zoo records, detection and identification of the parasites infecting the animals at the zoological garden. Fecal samples were analyzed for the presence and identification of nematodes, cestodes and protozoan oocysts. Samples from the East African Giant Tortoise (*Geochelone sulcata*) were excluded due to the failure to obtain fresh samples.

2.3. Fecal Sample Collection

Twenty two fresh fecal samples were collected from eight species, consisting of 22 zoo animals two times in December 2013 by using a sterile spatula. The samples were grossly examined immediately for the presence of adult worms and their larva and then placed into labeled sterile screw cap plastic containers for other parasitological techniques. Sampling was repeated in the same way after four months in April 2014 to assess seasonal variations of the parasites and samples were analyzed at parasitology laboratory of School of Animal and Range Sciences, Haramaya University. Only twenty animals were examined in April due death of two animals during the project work.

2.4. Identification of Parasite Oocyst

Zinc sulphate centrifugal floatation technique was used for the identification of
About 3 gm of fecal sample was mixed with 15ml of ZnSO₄ solution and the resulting solution was strained through a nylon tea strainer to remove coarse fecal material. The solution was poured into a centrifuge tube and centrifuged at 2000 rpm for 5 minutes. The tubes were removed from the centrifuge and filled with the flotation solution to the top; and then cover slips were placed over the top of the tube and left for 10 minutes. Finally, a drop of iodine solution was put on each slide and covered with cover slide and examined under microscope at 40X objective. Identification of helminths and protozoan oocysts were done by standard parasitological criteria [28] [29].

On the other hand fecal sedimentation technique was used for the examination of eggs of trematodes and cestodes. A small quantity of faeces (3 gm) was mixed well with water (15 ml) and the resulting emulsion was strained through a nylon tea strainer to remove coarse faecal material. The filtrate was poured into a centrifuge tube and centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded and the tube was refilled with water and centrifuged 2 - 3 occasions until clear supernatant was observed. Then a drop of the sediment was taken on a clean, dry glass slide and examined under low power (10X) of the microscope. Identification of the helminth eggs were carried out by standard parasitological procedures [28] [29] [30].

2.5. Parasite Larval Identification

About 10 gm of fecal samples were collected for coproculture examination. The samples were broken up in a Petri dish and mixed with vermiculite and distilled water to form a homogenous wet mixture. The dishes were closed and placed in an incubator for 14 days. The larvae were then recovered by a Baermann apparatus where the samples were placed in double layer of gauze and suspended in a spring clipped funnel containing tape water. The fecal cultures were left to stand on the bench at ambient temperature for 24 hours. Approximately 15 ml of the fluids were taken into tubes by releasing the spring clip; the tubes were put in refrigerator for 3 hours. The supernatants were removed leaving about 1 ml of the sediment in the tube. The larvae in the sediment were transferred to microscope slides and examined. The identification keys described in Soulsby [28] and van Wyk and Mayhew [31] were employed to identify the larvae.

3. Results and Discussion

The data from retrospective study indicated that most of the disease cases of wild animals at Zoological garden of Haramaya University were related to parasite infections. Records revealed the death of 21 animals over the last five years (Table 1). The death for the animals might be due to gastrointestinal parasites accompanied with poor management practices and poor nutrition. Previous studies on feeding and management of zoo animals reported that the poor nutritional status and poor management practices can diminish resistance to parasitic disease [21] [32] [33].
### Table 1. Number of wild animals died from 2008-2012.

| Species of Animals died                | Number of animals died | Total |
|----------------------------------------|------------------------|-------|
|                                        | 2008  | 2009  | 2010  | 2011  | 2012  |       |
| Vervet monkey (Cercopithecus aethiops) | 1     | 1     |       |       |       | 2     |
| Grey Duiker (Sylvicapra grimmia Abyssinia) | 1     | 3     | 1     | 4     |       | 4     |
| Ethiopian Hare (Lepus aethiops)        | 1     | 1     | 2     | 4     |       | 4     |
| Civet (Civettictis Civetta)            | 1     |       |       |       |       | 1     |
| Helmeted Guinea fowl (Numida meleagris) | 1     |       |       |       |       | 1     |
| Greater Kudu (Tragelaphus strepsiceros) | 1     |       |       |       |       | 1     |
| African Black Duck (Anas sparsa)       | 2     |       |       | 2     |       | 4     |
| Barn Owl (Toyto alba)                  | 1     |       |       | 1     |       | 2     |
| Leopard (Panthera leo)                 | 1     | 2     |       | 3     |       | 6     |
| Cheetah (Acinonyx jubatus)             | 1     |       |       | 1     |       | 2     |
| White-tailed Mongoose (Ichneumia albicauda) | 1     |       |       | 1     |       | 2     |
| Caracal Lynx (Felis caracal)           | 1     |       |       | 1     |       | 2     |
| Spotted Hyena (Crocuta crocuta)        | 1     |       |       | 1     |       | 2     |
| **Total animals died**                 | **21** |       |       |       |       |       |

**Source:** Haramaya University Zoological Garden animal status recording book.

### 3.1. Occurrence of Gastrointestinal Parasites

Out of the 22 animals (15 males and 7 females) examined in December, 16 (72.72%) were infected with gastrointestinal parasites. In the same way, out of the 20 animals (13 males and 7 females) examined in April, 15 (75%) were found infected. All the animals examined in December of the species kit fox, spotted hyena and Japanese rabbit were found infected. Similarly kit fox and helmeted guinea fowl had also shown 100% parasitic infection in April. No gastrointestinal parasites were found in Lesser Kudu both in December and April (Table 2).

The high rates of parasitic infection in the zoological garden in the present study might be contributed by management problems, improper feeding and sanitary conditions as well as environmental factors. The feeding habits of the animals were considered to be the potential source of infection as animals are usually fed on leftovers from the University student’s cafeteria. In the natural and free ranging environment, wild animals have wide choice of feeds which is actually free from human contacts. However, in enclosures animals are restricted of movement and prohibited access to natural feeds in the natural environment and forced to hand-feed. This condition may suppress the immune system of the animals to fight against the parasites and other disease causing agents. The possibility of cross-transmission of parasites from human beings to the zoo animals were considered to be higher.
Table 2. Overall prevalence of gastrointestinal parasites of animals at the zoological garden of Haramaya University in December and April.

| Animal species               | Incidence of parasite infection in December | Incidence of parasite infection in April |
|------------------------------|--------------------------------------------|-----------------------------------------|
|                              | No. examined | No. infected (%) | No. examined | No. infected (%) |
| Hamadryas baboon (*Papio hamadryas*) | 9            | 7 (77.78%)       | 9            | 8 (88.89%)       |
| Vervet monkey (*Cercopithecus aethiops*) | 2            | 1 (50%)          | 2            | 1 (50%)          |
| Lions (*Panthera leo*)        | 3            | 2 (66.67%)       | 3            | 1 (33.33%)       |
| Kit fox (*Vulpes macrotis*)   | 2            | 2 (100%)         | 2            | 2 (100%)         |
| Spotted hyena (*Crocuta crocuta*) | 1            | 1 (100%)         | -(Died)      | -                |
| Lesser kudu (*Tragelaphus imberbis*) | 1            | 0 (0%)           | 1            | 0 (0%)           |
| Japanese white rabbit (*Oryctolagus cuniculus*) | 1            | 1 (100%)         | -(Died)      | -                |
| Helmeted guinea fowl (*Numida meleagris*) | 3            | 2 (66.67%)       | 3            | 3 (100%)         |
| **Total**                    | **22**       | **16 (72.72%)**  | **20**       | **15 (75%)**     |

3.2. Identification of Species of Gastrointestinal Parasites

The species of gastrointestinal parasites detected in the zoological garden is shown in Table 3. A total of 9 parasite taxa were identified both in December and in April, of which 6 (66.67%) and 3 (33.33%) were helminthes and protozoa respectively (Table 3). Among the parasites *Trichuris spp.*, *Strongyloides spp.*, *Entamoeba spp.* were recovered from non-human primates. Other infection including *Toxocara spp.*, *Toxascaris leonine*, *Trichuris spp.* and *Isospora spp.* were observed in carnivores. *Passalurus ambiguous* and *Eimeria spp.* from white Japanese rabbit and *Ascaridia spp.* and *Eimeria oocyts* from helmeted guinea fowl were the other significant gastrointestinal parasites present in the garden.

Among the helminths in the zoological garden, *Trichuris spp.* had the highest prevalence (46.67%) followed by *Toxocara spp.* (20%) in December. *Trichuris spp.* had similar position in April (46.15%) followed by *Strongyloides spp.* (23.08%). On the other hand, *Eimeria spp.* (60%) were found predominant protozoan parasite in December while *Entamoeba spp.* (50%) took the lead in April (Table 3). Mixed infections were noticed in some of the animals both in December (25%) and April (20%). Mixed cases of helminthes such as *Trichuris spp.* and *Strongyloides spp.* in Hamadryas baboons and *Trichuris spp.* and *Toxocara spp.* in kit foxes were observed in the garden. Similarly, combinations of helminths and protozoan infections including *Trichuris spp.* with *Entamoeba spp.* in Hamadryas baboons; *Toxascaris leonine* with *Isospora spp.* in lions; *Passalurus ambiguous* with *Eimeria spp.* in rabbit and *Ascaridia spp.* with *Eimeria spp.* in helmeted guinea fowl were also observed in some animals (Table 3). In agreement with this study, many previous works reported mixed infections in wild animals in captivity [7]. Otegbade and Morenikeji [26] reported that that mixed infections may consists of two or more parasites.

The present study revealed that nematodes take the entire share of infection...
Table 3. Gastrointestinal parasites (Helminths and Protozoa) detected in fecal samples of animals in captivity at Haramaya University zoological garden.

| Animal Species | Gastrointestinal parasites observed in December and their proportions | Gastrointestinal parasites observed in April and their proportions |
|----------------|---------------------------------------------------------------------|------------------------------------------------------------------|
|                | Helminths               | Protozoa            | Helminths               | Protozoa            |
| Hamadryas baboon (Papio hamadryas) | Trichuris spp. 5 (71.43%)* | Entamoeba spp. 1 (14.29%) | Trichuris spp. 5 (62.5%)* | Entamoeba spp. 3 (37.5%)* |
|                | Strongyloides spp. 2 (28.57%)* |                     | Strongyloides spp. 3 (37.5%)* |                     |
| Vervet monkey (Cercopithecus aethiops) | -                       | Entamoeba spp. 1 (100%) | Trichuris spp. 1 (100%) | - |
| Lions (Panthera leo) | Toxascaris leonine 1 (50%) | Toxascaris leonine 1 (100%)* | Isospora spp. 1 (100%)* |                     |
|                | Toxocara spp. 1 (50%)* |                     |                     |                     |
| Kit fox (Vulpes macrotis) | Trichuris spp. 2 (100%)* | -                       | -                       | - |
| Spotted hyena (Crocuta crocuta) | Toxocara spp. 1 (100%) | -                       | -                       | - |
| Japanese white rabbit (Oryctolagus cuniculus) | Passalurus ambiguous 1 (100%)* | Eimeria spp. 1 (100%)* | -                       | - |
| Helmeted guinea fowl (Numida meleagris) | Ascaridia spp. 1 (50%)* | Eimeria spp. 2 (100%)* | Ascaridia spp. 1 (33.33%) | Eimeria spp. 2 (66.67%) |

*Mixed infections of helminths; **Mixed infections of helminths and protozoan parasites.

(100%) among the helminth parasites encountered. There were no cestodes and trematode species recovered from the fecal samples which agrees with the result of Bezjian et al. [34], who reported that no parasites eggs found by sedimentation from forest baboons at Kibale National Park, Uganda. Similarly, Singh et al. [22] reported that nematodes had the highest prevalence among the helminthes recovered in captive wild animals. The high percentages of helminths compared to protozoan parasites can also be supported by the report of Varadharajan and Kandasamy [2], who found about 58% helminth parasites and 6% protozoan infections in wild animals at V.O.C. Park and Mini Zoo, Coimbatore, India.

Most of the gastrointestinal parasites observed in this study had also been reported by several authors. The occurrence of Trichuris and Strongyloides spp. in captive wild animals were reported by Opara et al. [35]. Trichuris trichuria and Entamoeba spp. in non-human primates were reported to be more prevalent [10] [15] [19] [36]. The incidence of Toxocara cati and Toxascaris leonine in Lions in the current study was supported by Okulewicz et al. [37], Pawar et al. [38], Mukarati et al., [39]. In line with the present study Engh et al. [40] reported that Toxocara canis was infecting hyena in the Masai Mara national reserve of Kenya while Luty [41] recovered it in the feces of foxes in the University zoological garden of south western Nigeria. The presence of Passalurus ambiguous and Eimeria perforans from white rabbit was also reported by Okumu et al. [42]. Several reports revealed Ascaridia spp. to be more prevalent in birds, particularly in helmeted guinea fowl [26] [43] [44].
Overall, the high incidences of the same species of parasites among the animals in the garden indicated that there was a share the parasites between the animals in the garden. The introduction of large numbers of visitors to the animal habitats and the closeness of humans to the animals, particularly non-human primates in the zoological gardens might have caused the exchange of parasites between humans and animals. The presence of *Trichuris*, *Strongyloides* and *Toxocara spp.* in high numbers in the animals in the current study may be due to the contact with human beings as these species of helminthes are more likely to be transmitted from humans to animals. The spread of gastrointestinal parasites among zoo animals by zoo keepers were reported by several authors [16] [26] [35]. Arafa *et al.* [45] reported that unhygienic zoo keepers can potentially transmit parasites between the animals especially when the life cycle is direct. Calvignac-Spencer *et al.* [46] stated that people who share the same habitat with nonhuman primates are considered as potential sources of transmission. Prevalence of zoonotic gastrointestinal parasites was reported in *Papio anubis* (baboon) and *Cercopithecus aethiops* (vervet) in Ethiopia [47]. Maske *et al.* [14] and Kashid *et al.* [9] reported that parasites can be transmitted from wild animals to domestic animals and man and vice-versa.

### 3.3. Seasonal Patterns of Gastrointestinal Parasites in the Garden

The pattern of parasitic infection in the garden was found nearly the same throughout a year. Examinations conducted on fecal samples during dry and wet seasons indicated that there were no marked differences in the infection rates of parasites. The number of animals infected in December (72.72%) were slightly lower than the number infected in April (75%). On the other hand, the species and number of the parasites found was almost similar both in December (dry season) and April (a wet season). This showed that the seasonal variation in parasitic infection among the zoo animals in the garden is not remarkable. The finding was in agreement with the report of Gillespie *et al.* [48], who reported that seasonal variation in parasite infections was minimum. This minimum variation in parasite loads between these seasons in the zoo may indicate that the animals are kept constantly in a contaminated environment with the parasites which was also stated by Atanaskova [4]. It can also be explained that in natural environments, wild animals live on large areas and have a low genetic resistance against parasitic infections because of low exposure. However, when the wild animals are kept in captivity in zoological gardens, the problem of parasitic infections can aggravate which agrees with the observation of Muoria *et al.* [49].

### 4. Conclusion

This paper showed that there are gastrointestinal infestations with different parasite species in the Zoological garden of Haramaya University at an overall mean rate of 73.8%. Fecal analysis revealed that nematode helminthes are the most prevalent parasites in the zoo animals. There were no Cestode and Trematocas, A. Berhanu

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tode species recovered from the fecal samples. Among the nematodes, *Trichuris* and *Strongyloides* and *Toxocara* spp. were found to be the most prevalent parasite in the zoological garden. Seasonal patterns of parasitic infection in the zoo animals were not readily apparent among the animals and the seasons of a year. The study showed that most of the gastrointestinal parasites of wild animals examined may be zoonotic and hence future studies are needed to determine risks of cross-transmission.

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**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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