An eight-year survey of the intestinal parasites of carnivores, hoofed mammals, primates, ratites and reptiles in the Ljubljana zoo in Slovenia

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Abstract: Problems with parasitic infections and their interspecies transmissions are common in zoological gardens and could pose serious health damage to captive animals. This study presents results of eight-year monitoring of intestinal parasites in animals from Zoo Ljubljana, Slovenia. A total of 741 faecal samples from 40 animal species were collected two to four times per year and examined microscopically. Intestinal parasites were detected in 45% of samples, with detection of helminths (Cestoda, Nematoda – Ascaridida, Enoplida, Strongylida, Oxyurida, Rhabditida and Trichurida) and protists (Apicomplexa and Ciliophora) in 25% and 13% of samples, respectively; mixed infection was found in 7% of samples. The mostly infected were ungulates (61%), followed by reptiles (44%), ratites (29%), primates (22%) and carnivores (7%). During the observation period, the number of infected animal species increased from 8 to 25. This is the first long-term monitoring study of intestinal parasites in zoo animals from Slovenia. Routine monitoring of parasitic infection and regular deworming and hygienic measures are necessary to prevent gastrointestinal infections in captive animals.

Keywords: helminths, protists, long-term monitoring, zoo animals, coprology

Both domestic and wild animals are hosts of a wide variety of parasite species. Although wild animals are usually infected with several species of parasites at once, they seldom suffer massive deaths, or epizootics, because of the normal dispersal and territorialism of most animal species. In the wild, natural resistance against parasitic diseases and a state of equilibrium between host and parasite generally prevent the development of clinical disease, unless in stress conditions (Mir et al. 2016). In contrast, domesticated animals are usually restricted to pastures or pens for long time, often with great stock density, so that parasite eggs, oocysts, cysts and larvae become extremely abundant in the soil. This mechanism of rising parasite density is common for all animals bred for extended periods in one place, allowing for the parasites' life cycle to be renewed without barriers. Kumar et al. (2013) mentioned that parasite infections increase with the animal load per square surface unit and therefore, for a given piece of land, parasitic infections will quadruple if the stocking density doubles.

Zoological collections are represented with exotic animal species which would never or rarely meet certain parasites amongst natural circumstances. They often have naive immune systems without possibility of coevolution with parasites and natural selection is usually absent. Keepers may play the role of mechanical vector of parasites and improper feeding systems can encourage the parasite infection. Browsing animals forced to graze or pick up food from the ground are at a greater risk of infection with geohelminths. Intensive or imperfect usage of antiparasitic agents can cause resistance to antiparasitic drugs. The risk of parasite infection also rises if animals are changed frequently, allowing the introduction of new parasites. Serious cases of parasite infection may then arise if a parasite is introduced in a new environment where fully susceptible hosts are available (Borgsteede 2011).

The same situation applies to wild animals in captivity, which are normally kept in the same enclosure for prolonged periods of time, with space limitations and under...
constant stress, leading to immunosuppression and consequent higher susceptibility to parasitic infection (Mir et al. 2016). Therefore, problems with parasitic infections and their interspecies transmission are often common in zoos and can pose a great number of negative effects.

Infections with pathogenic species can lead to the death of the infected animals, whereas other parasitic infections may become a predisposing factor for the development of secondary diseases. Impaired health condition resulting from these infections may also have a negative impact on reproduction, compromising breeding programs which are frequently of major importance in zoos. Lastly, as zoos are institutions which are opened to the public, close contact with humans which would not happen in the natural environment of the captive animals, rises the risk of development of anthropozoonosis (Panayotova-Pencheva 2013) and, as such, raises public health concern.

The present study was carried out to establish the gastrointestinal parasite profile of animals kept in the zoo in Ljubljana, Slovenia, during an eight-year time frame. Zoo Ljubljana is the only zoo in Slovenia with a large number of exotic animal species and preventive health care program.

**MATERIALS AND METHODS**

The study took place in Zoo Ljubljana, which is the only institution with a large collection of exotic mammals, birds and reptiles in Slovenia. The zoo is located on the outskirts of Ljubljana (46°03'09''N; 14°28'20''E) and covers an area of 6 hectares and has houses about 544 animals of 125 different animal species by the time of the present study. Five groups of animals were included in the study: carnivores, ungulates (autochthonous, allochthonous and domestic animals), primates, ratites and reptiles.

Carnivores had inside enclosures with concrete floor covered with straw and outside enclosure with soil and grass, and none of the carnivores’ exterior enclosures were covered. Faeces from their exhibits were removed daily. Ungulates had an indoor enclosure with concrete for giraffes and camels or wooden floor for other animals, covered with soil, grass and sand for giraffes or just with sand for domestic animals. Each group of ungulate species were housed, with an outside enclosure and wooden shelter. Similarly to the carnivores, none of the ungulates’ exterior enclosures had any kind of ceiling or cover. Faeces were removed daily from the enclosures, with the exception of autochthonous ungulates, due to the steep terrain and large size of outdoor enclosures in which these species were housed in. In their outside enclosure, there were more shade than sun in comparison to the enclosures of other ungulates.

Domestic ruminants were represented with two different groups, one enclosure with autochthonous Slovenian breeds of goats and sheep, and a children’s petting zoo where pygmy goats and sheep can be found. In the petting zoo, visitors have direct contact with the animals. Chimpanzees had an indoor enclosure with concrete floor covered with wooden shavings. The outdoor enclosure had a concrete floor without bedding and was covered with mesh. Their exhibit was cleaned daily with a water hose. The other primates had indoor enclosures with concrete floor covered with different bedding materials (wooden shavings, wooden chips or sliced straw). Faeces were removed daily and bedding was replaced entirely once to twice a week. Their outside enclosures were covered with soil and grass, with no fixed cleaning routine due to the low number of animals housed in these enclosures. These latter species also did not have any kind of ceiling or cover in the outside enclosures.

Ratites shared outside enclosures with other animal species. One pair of ostriches were housed with giraffes and another with red lechwe antelopes (Kobus leche Gray); emus (Dromaius novaehollandiae [Latham]) were housed with wallabies (Macropus rufogriseus [Desmarest]) and rheas (Rhea americana [Linnaeus]) shared their enclosure with maras (Dolichotis patagonum [Zimmermann]) and guanacos (Lama guanicoe [Müller]). These enclosures were covered with soil and grass. None of the outside enclosures had a ceiling cover. There were wooden houses with concrete floor inside the ratites’ enclosures. Faeces were removed daily. Reptiles had only inside enclosures covered with peat and some plants; faeces were removed daily. With the exception of reptiles which only had inside enclosures, all other animal species had outside enclosures which may allow the access of unwanted free ranging animals.

Animals from the zoo were repeatedly examined from 2008 to 2015 for parasitic infections. A total number of 741 faecal samples were collected from 40 animal species. All animals were examined twice a year (March and June) in first two years of survey. In years 2010 and 2011, ungulates were examined four times a year (March, June, September and November), while the other animals were examined only twice a year (March, November). In the last four years of the study (2012–2015), all animal species were examined four times a year (March, June, September and November) with the exception of reptiles which were examined only twice a year (March and October) due to hibernation.

Fresh samples of faeces from each animal species were randomly collected from the floor of the enclosure during daylight, to the amount of 5 g and were stored at 5 °C for no longer than two days before analysis. Modified Sheather’s sugar solution (— 1.27) was used for flotation based on the study of Blagburn and Butler (2006). Formol-ether sedimentation method with normal saline was used as described in a study of Suwansaksri et al. (2002).

In case of suspected coccidiosis, the samples were collected into vials containing 2.5% potassium dichromate and stored at room temperature (23 °C) for five days to sporulate. Eggs and oocysts of parasites (helminths and protists, respectively) were identified microscopically (using 100–400× magnification, with Karl Kaps Microscope, Typ KCTE14) based on their size and morphologic characteristics and parasites were classified according to proper taxonomy (Foreyt 2001). In case of parasitic infection, animals were treated and examined repeatedly to see efficacy of treatment. Treatment was done one week after coprological examination and result was evaluated 14 days after the drug application. In case of helminthoses, doramectin, praziquan tel, levamisole and flubendazol in proper dosages individually or combination of ivermectin and fenbendazol were used. Protops were treated with toltrazuril or metronidazole with dosages and treatment regime prescribed by manufacturer. All new animals received by the zoo were kept separately at least for 30 days and their faecal samples were examined for parasites by flotation, sedimentation, sporation and larvscopy to exclude possible introduction of parasites into the zoo. Flotation, sedimentation
and sporulation methods were done as previously mentioned; larvalscopy was performed using the Baermann method described by Kaminsky (1993).

RESULTS

Intestinal parasites were detected in 45% of 741 faecal samples collected during 2008 to 2015 (Table 1). Ungulates had the highest infection rate (61%), followed by reptiles (44%), ratites (29%), primates (22%) and carnivores (7%). In the studied group of ungulates, autochthonous species were demonstrated to have the highest infection rate (76%), followed by domestic animals and allochthonous species. Helminths and protists were detected in 25% and 13% of samples, respectively and mixed infection was found in 7% of samples. Table 2 demonstrates the number of animal species positive for intestinal parasitic infection during the observed period. Infected animals were all asymptomatic with low parasite load.

Helminths were represented by platyhelminths (tapheworms and trematodes similar to Dicrocoelium dendriticum Rudolphi, 1819) and Nematodiridae of six orders: Ascaridida (Parasarcis equorum [Goeze, 1782]), Enoploida (Trichuris sp.), Oxyurida (Aspiculuris sp., Enterobius sp.), Rhabditida (Nematodiridae gen. sp., Strongylida sp. and Trichostrongylidae sp.), Strongyloida (Prostrobobyldidae gen. sp., Ostertagia sp.) and Trichurida (Capillaria sp.).

Protists were represented by Apicomplexa (Eimeria sp. and Isospora sp.) and Ciliophora (Balantidium coli, Buxtonella sp., Nyctotherus sp.). Table 3 shows the list of detected intestinal parasites according to the animal species in study. All parasite genera were identified according to egg morphometrics. No regular seasonal differences regarding results were observed.

DISCUSSION

Carnivores

Intestinal parasites were not detected in faeces of carnivores in years 2008–2012, which could be explained by the fact that there was no transport of new carnivores to the zoo during that period. However, from 2013 and forward, helminths (Ascaridida, Capillaria sp. and Trichuris sp.) were found in their faeces after arrival of new pairs of cheetahs (Acinonyx jubatus [Schreber]) from Botswana in South Africa and tigers (Panthera tigris altaica Temminck). Fagioli et al. (2010) detected 43% helminths (hookworms, Strongyloides stercoralis [Bavay, 1876], Toxascaris leonina [Linstow, 1902] and Toxocara canis [Werner, 1782]) and 10% protists (Cryptosporidium sp.) in samples of carnivores from a zoo in Italy.

High prevalence of helminths (100%) and protists (80%) were found in lions from Recreation Park in Zimbabwe (Mukarati et al. 2013). In contrast, lions included in our study were negative, which could be due to the separation of the enclosure from other carnivores and regular deworming. Husbandry differences between

Table 1. Detection of intestinal parasitic infection (helminths and protists) in samples of animals from Zoo Ljubljana during years 2008–2015.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No. of samples</th>
<th>Positive (%)</th>
<th>H</th>
<th>P</th>
<th>H + P</th>
<th>Positive samples/total (%) in individual years (2008–2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnivores</td>
<td>54</td>
<td>4 (7%)</td>
<td>4 (7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0/0</td>
</tr>
<tr>
<td>Ungulates</td>
<td>378</td>
<td>231 (61%)</td>
<td>101 (27%)</td>
<td>83 (22%)</td>
<td>47 (12%)</td>
<td>3/17</td>
</tr>
<tr>
<td>autochthonous</td>
<td>139</td>
<td>106 (76%)</td>
<td>39 (28%)</td>
<td>29 (21%)</td>
<td>38 (27%)</td>
<td>1/5</td>
</tr>
<tr>
<td>allochthonous</td>
<td>142</td>
<td>70 (49%)</td>
<td>44 (31%)</td>
<td>22 (15%)</td>
<td>4 (3%)</td>
<td>1/10</td>
</tr>
<tr>
<td>domestic animals*</td>
<td>97</td>
<td>55 (57%)</td>
<td>18 (19%)</td>
<td>32 (33%)</td>
<td>5 (5%)</td>
<td>1/2</td>
</tr>
<tr>
<td>Primates</td>
<td>114</td>
<td>25 (22%)</td>
<td>21 (18%)</td>
<td>4 (4%)</td>
<td>0 (0%)</td>
<td>0/0</td>
</tr>
<tr>
<td>Ratites</td>
<td>62</td>
<td>18 (29%)</td>
<td>17 (27%)</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td>0/2</td>
</tr>
<tr>
<td>Reptiles</td>
<td>133</td>
<td>59 (44%)</td>
<td>45 (34%)</td>
<td>7 (5%)</td>
<td>7 (5%)</td>
<td>5/26</td>
</tr>
<tr>
<td>Total</td>
<td>741</td>
<td>337 (45%)</td>
<td>188 (25%)</td>
<td>94 (13%)</td>
<td>55 (7%)</td>
<td>8/45</td>
</tr>
</tbody>
</table>

H – only helminths; P – only protists; H+P – mixed infection; * domestic ruminants were represented with two different groups, one enclosure with autochthonous Slovenian breeds of goats and sheep; and a children’s petting zoo where pygmy goats and sheep can be found.

Table 2. Detection of intestinal parasitic infection in species of animals from Zoo Ljubljana during years 2008–2015.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No. of animals</th>
<th>Positive samples in individual years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnivores</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Ungulates</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>autochthonous</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>allochthonous</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>domestic animals</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Primates</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Ratites</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Reptiles</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

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these two zoos, with special regard to the food provided to the animals, may also contribute to the different results obtained. In comparison with the latter two studies, the present study demonstrates lower infection rates. Lim et al. (2008) detected Toxocara cati and also other parasites such as Cryptosporidium sp., hookworms and Spirometra sp. in felines from zoo Negara in Malaysia; felines were the most infected (89%) compared to the other animal groups.

In the present study, Trichuris sp. was found in Persian leopard male (Panthera pardus saxicolor Pocock), which was transported from zoo Chemnitz in Germany. Opportunistic infection in this case was not excluded as all later examinations of the faeces were negative. Several eggs of Trichuris sp. were also found in wolves in March 2014. At that time, the wolves were fed with whole sheep from controller breeding. Because the wolves like to eat the entire gastrointestinal tract of animals, we suppose, that it was a passage from sheep to wolves.
The later coprological examination did not prove *Trichuris* sp. in wolves anymore. Other parasites like *T. leonina* and *T. cati* were also found in faeces of other animal species (cheetahs, tigers and lynx) that were not included in the eight-year survey. The infection with the two latter parasites was first introduced into the zoo in 2012 through recently acquired cheetahs from Boras zoo with consequent spread and infection of Siberian tiger and Eurasian lynx (*Lynx lynx Linnaeus*). The infection was successfully treated in tigers and lynx after two years, but the deworming protocol had no effect on elimination of these parasites in cheetahs.

**Ungulates**

Parasites were detected in 61% of ungulate samples with differences in autochthonous (Slovenian species), allochthonous and domestic animals. Helminths and protists were detected in 27% and 22% of samples, respectively with mixed infection in 12% of samples. Fagiolini et al. (2010) examined samples of ungulates from a zoo in Italy. In artiodactyls, a higher percentage of helminths was found (57%, including *Paramphistomidae* gen. sp., *Strongylus* sp., *Toxocara vitulorum* [Goeze, 1782] and *Trichuris* sp.) when compared to protists (25%, *Cryptosporidium* sp. and *Elmeria* sp.). In perissodactyls, gastrointestinal strongyles were found in 50% of samples. Lim et al. (2008) found 46% prevalence of intestinal parasites in ungulates from zoo Negara in Malaysia, having isolated *Cryptosporidium* sp., hookworms and *Trichuris* sp. In our study, *Trichuris* sp. was detected in 12 out of the 16 animal species and several other parasites.

**Ratites**

In ratites, helminths (*Ascaridida*, *Capillaria* sp., Strongylidae and *Strongyloides* sp.) were detected in 27% of samples. In a Nigerian zoo, the most prevalent parasite in ratites was *Capillaria* sp. (14%); mixed infection of helminths and protists was found in 4% of samples (Otegbade and Morenikeji 2014). As previously mentioned, ratites shared outside enclosures with other animal species. Aside from the wallabies which were not contemplated in this study, giraffes, red lychee antelopes and guanacos were not infected by the same parasites that were found in ratites.

**Reptiles**

In reptiles, helminths (unidentified cestodes and nematodes of the genera *Aspiculuris* Schulz, *Capillaria* Zeder and the families Oxyuridae, Strongylidae and Strongyloididae) and protists (*Isospora* sp. and *Nyctotherus* sp.) were isolated in 34% and 5% of samples, respectively. Mixed infection was found in 5% of samples. Fernando and Udagama-Randeniya (2009) found 86% positive samples for one or more parasitic species from a total of 103 reptile faecal samples collected in a zoo in Sri Lanka, in which nematodes were the most abundant parasites found.

**Primates**

The faecal analysis of primates revealed the presence of helminths (*Enterobius* sp., unidentified nematodes of the families Oxyuridae, Strongylidae, Strongyloididae and *Trichuris* sp.) and protists (*Balantidium* sp.) in 18% and 4% of samples; respectively. No mixed infection was seen. The most infected were chimpanzees (*Pan troglodytes* [Blumenbach]), whereas black and white ruffed lemurs (*Varecia variegata* [Kerr]) were negative during the whole observed period. In a zoo in Italy, helminths (*Strongyloides fuelleborni* Linstow, 1905 and *Trichuris* sp.) and protists (*Cryptosporidium* sp.) were found in 100% and 67% of samples from primates, respectively (Fagiolini et al. 2010). A higher prevalence of protists (33%) compared to helminths (19%) was found in animals from a zoo in Malaysia (Lim et al. 2008), where *Ascaris* sp., *Balantium coli*, *Blastocystis* sp., *Cryptosporidium* sp., hookworm and *Trichuris* sp. were isolated. A high prevalence of intestinal parasites (61%) was found also in samples of primates from a zoo in Nigeria where *Entamoeba* sp., *Strongyloides* sp., *Strongylus* sp. and *Trichuris trichiura* (Linnaeus, 1771) were found (Adetunji 2014).

**Concluding remarks**

The results of the present study demonstrate that gastrointestinal parasites are common in zoo animals and in some cases in high prevalence. In the observed period, the number of infected animal species rose from 8 to 25. This tendency could be due to higher frequency of parasitological examinations and thus higher chance to detect more parasites. Nevertheless, regular monitoring of parasitic infection allowed Ljubljana zoo’s veterinarians to use selective treatment that resulted in reduction of death caused by parasitic infection, which was very common prior to the preventive coprology monitoring. Other explanations can be found for this situation, such as the introduction of new animals, such as the previously mentioned introduction of *T. cati* and *T. leonina* by recently acquired cheetahs; or higher stocking density.

Quarantine period in length of 30 days and including coprological examination, deworming treatment as well as determination of treatment efficacy control has proved insufficient and did not prevent spread of the parasitic infection. This suggests duration of the quarantine, disinfection protocols and deworming programs must be re-evaluated. The possibility of mechanical transmission of eggs must be prevented, as usual disinfection barriers in quarantine are aimed to prevent bacterial spread of infection but are unable sufficiently control more resistant parasite eggs. The timing of coprological exam, treatment and control exam is also important since periods when particular parasites are shedding eggs varies greatly.

The present study was done to merely present typical parasite occurrence in zoo environment and describe difficulties accompanying parasitic infections in a middle-sized zoo. Regular coprological examinations four times per year seems to be sufficient to control parasite burden in most of the animals but must be individually adjusted to sensitive animal species. Parasite control, due to the specific nature of zoological collection is one of the pillars of preventive health care of zoo animals.

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REFERENCES


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