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UNIVERSITÄT BERN

Institute of Parasitology

Endoparasites of zoo carnivores in Switzerland

Master thesis
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1 Zusammenfassung

In dieser Masterarbeit wurde die Endoparasitenfauna von wilden Carnivoren in Gefangenschaft in der Schweiz anhand von Kotproben untersucht. Einzel- und Sammelproben von den Carnivorenfamilien Felidae, Canidae, Ursidae, Procyonidae, Mustelidae, Herpestidae, Ailuridae und Otariidae wurden auf Magen-Darm Parasiten und auf Lungenwürmer hin untersucht. Hierfür wurde ein kombiniertes Sedimentation-Flotations-Verfahren, eine Ziehl-Neelsen Färbung eines Kotaustriches, das Baermann-Trichter-Verfahren und ein *Giardia*-Antigen ELISA angewendet. Es wurden diverse Nematoden (*Toxascaris leonina, Toxocara canis, Toxocara mystax*, nicht genauer identifizierte Ascarididae Spezies, *Capillaria* sp., *Trichuris* sp., Hakenwürmer, *Aspiculuris tetraptera*, *Heterakis* sp., *Crenosoma* sp. und *Angiostrongylus vasorum*) und Protozoen (*Cryptosporidium* sp., *Isospora* sp., *Giardia duodenalis* und *Hammondia* sp. oder *Neospora caninum*) nachgewiesen. Zusätzlich wurden potentielle Risikofaktoren für Infektionen mit diversen Parasitenspezies untersucht. Jungtiere oder Tiere mit Kontakt zu Jungtieren, sowie Tiere welche aus freier Wildbahn stammten, waren signifikant häufiger mit *Cryptosporidium* sp. oder *Toxocara* sp./Ascarididae Spezies infiziert. Carnivoren, welche regelmässig entwurmt wurden, jedoch ohne zuvor auf Parasiten getestet worden zu sein, waren häufiger mit *T. leonina* infiziert. Grosse Gehege waren ein Risikofaktor für *G. duodenalis*-Nachweis und Carnivoren, welche durch klinische Symptome aufgefallen sind, waren häufiger mit *Cryptosporidium* sp., Hakenwürmer oder *A. vasorum* infiziert. Einige unserer Resultate waren vergleichbar mit ähnlichen Studien, jedoch konnten Lungenwürmer in einem Europäischen Iltis *(Mustela putorius)* und *Cryptosporidium* sp. in amerikanischen Schwarzbären *(Ursus americanus)* das erste Mal in einem Zoo beschrieben werden.

Schlagwörter: intestinale Parasiten, Lungenwürmer, Carnivoren, Zoo, Risikofaktoren für parasitäre Infektion, Schweiz.

2 Summary

In this master thesis, the endoparasite fauna affecting wild carnivore kept in captivity in Switzerland was examined by analysing fecal samples. Individual and bulked samples from the carnivore families Felidae, Canidae, Ursidae, Procyonidae, Mustelidae, Herpestidae, Ailuridae and Otariidae were examined for intestinal parasites, such as lungworm infections. Therefore, combined sedimentation-flotation technique, Ziehl-Neelsen staining of fecal smears, Baermann-Wetzel technique and *Giardia* antigen ELISA were conducted. Different nematodes (*Toxascaris leonina*, *Toxocara canis*, *Toxocara mystax* and unidentified Ascarididae species as well as *Capillaria* sp., *Trichuris* sp., hookworms, *Aspiculuris tetraptera*, *Heterakis* sp., *Crenosoma* sp. and *Angiostrongylus vasorum*) and protozoan (*Cryptosporidium* sp., *Isospora* sp., *Giardia duodenalis* and *Hammondia* sp. or *Neospora caninum*) were identified. Furthermore, potential risk factors for parasitic infections were analysed. Juvenile animals or animals with contact to juvenile as well as wild born animals were statistically significant more often infected with *Cryptosporidium* sp. or *Toxocara* sp./Ascarididae species. Carnivores getting dewormed regularly without fecal examination were more likely being infected with *T. leonina.* Large enclosures were a risk factor for *G. duodenalis* detection and carnivore showing clinical signs were more often infected with *Cryptosporidium* sp., hookworms or *A. vasorum.* Most results are comparable to similar investigations, though first findings of lungworms in a captive European polecat *(Mustela putorius)* and *Cryptosporidium* sp. infections in captive American black bears *(Ursus americanus)* are reported in this master thesis.

Keywords: intestinal parasites, lungworms, carnivores, zoo, risk factors for parasitic infection, Switzerland.

3 Introduction

The order of the Carnivora is highly diverse and includes numerous animal species with different lifestyles. An entire range of Carnivora is kept in zoos worldwide, from the North American raccoon *(Procyon lotor)*, a very invasive species, to the Amur leopard (*Panthera pardus orientalis),* a critically endangered species (IUCN, 2018). Therefore, zoos play an important role not only for educational but also for conservation purposes, since many different species (295 carnivore species in total), are listed on the IUCN Red List of Threatened Species these days (2018). International breeding programs are implemented in order to support the maintenance of these endangered animal species. Also parasitic diseases have to be kept under control because they may have a negative impact on health and reproductivity of zoo animals (Dubey et al., 2010; Wang et al., 2018). In addition, as some of the parasites occurring in carnivores are zoonoses, infections not only compromise the animals' health, but can be important for employees, too (Deplazes, 2013; Juncker-Voss et al., 2004).

Many investigations about parasites in various animals housed in zoos all over the world have been performed. Most of them include carnivores. Some examined intestinal parasites in general (Ahasan et al., 2010; Deshmukh et al., 2009; Fagiolini et al., 2010; Javaregowda, 2016; Ramos, 2014; Roman et al., 2017), while others were restricted to one parasite species (Bertelsen et al., 2010; Wang et al., 2015) or one host species only (Bertelsen et al., 2010; Bindke et al., 2017; Peng et al., 2016; Wang et al., 2015). Studies from zoos abroad are only limited transferable, because of the diverse parasite spectra in different areas. Also investigations comparing wild and captive animals are reporting differences in parasite spectra, since wild carnivores normally live on large areas and have different feeding habits than zoo animals (Schaul, 2006; Szafranska et al., 2010).

This master thesis was conducted with the aim to obtain a better understanding of the endoparasite fauna affecting zoo carnivores in Switzerland and was put into context with former investigations including a master thesis performed in 2009 (Weber). Additionally, risk factors for parasitic infections in these animals were examined, since this would be of a great advantage for their management in zoos.

4 Material and Methods

4.1 Material

All zoos in Switzerland known to keep canines or felines in spring/summer 2017 were contacted via email or phone. Out of 27 zoos, 24 participated in this study. Additionally, private keepers of wild canines or felines were searched. The only circus in Switzerland that kept tigers during this period of time and all veterinary inspection offices of Switzerland were contacted. However, only three private keepings of wild Felidae were willing to participate in this investigation.
Zoos and private keepings were visited between June 2017 and March 2018 and a total of 149 fecal samples was collected from the ground, either by the stockman or directly by the author. Overall, 41 different genera, species or subspecies of 8 different carnivore families were sampled (table 1). Volume of samples varied between 5 and 50 grams. In 61.1% (91/149) of all cases enough (over 20 grams) material for ideal test conditions were obtained. While 89 were bulk samples, 55 out of 149 samples were assigned to an individual animal. For 5 samples, the author had no information about the number of animals living in sampled enclosures (table 1). Samples were collected over one to four days. During the transport to the Institute of Parasitology in Zürich the samples were kept in a cooling bag.

Table 1: Number of analysed fecal samples from Swiss zoo carnivores.

Felidae	Number of samples (single sample/ bulk sample)
Lion (*Panthera leo)*	10 (5/5)
Tiger *(Panthera tigris)*	11 (9/2)
Leopard *(Panthera pardus)*	6 (1/3; 2 unknown)
Snow leopard *(Panthera uncia)*	4 (1/3)
Cheetah *(Acinonyx jubatus)*	6 (4/1; 1 unknown)
Wildcat *(Felis silvestri)*	5 (1/4)
Lynx *(Lynx* sp.)	14 (4/10)
Cougar *(Puma concolor)*	7 (6/1)
Serval *(Leptailurus serval)*	1 (0/1)
Ozelot *(Leopardus pardalis)*	1 (1/0)
Caracal *(Caracal caracal)*	1 (1/0)
Leopard cat *(Prionailurus bengalensis)*	1 (1/0)
Jungle cat *(Felis chaus)*	1 (1/0)

Table 1: Number of analysed fecal samples from Swiss zoo carnivores.

Canidae	
Wolve *(Canis lupus)*	9 (1/8)
Red fox *(Vulpes vulpes)*	8 (1/7)
Arctic fox *(Vulpes lagopus)*	2 (0/2)
Fennec fox *(Vulpes zerda)*	1 (1/0)
African wild dog *(Lycaon pictus)*	1 (0/1)
Raccoon dog *(Nyctereutes procyonoides)*	2 (0/2)*
Ursidae	
Brown bear *(Ursus arctos)*	7 (4/3)
American black bear *(Ursus americanus)*	2 (1/1)
Asian black bear *(Ursus thibetanus)*	1 (1/0)
Spectacled bear *(Tremarctos ornatus)*	2 (1/1)
Malayan sun bear *(Helarctos malayanus)*	1 (1/0)
Procyonidae	
North American raccoon *(Procyon lotor)*	14 (0/14)*
Crab-eating raccoon *(Procyon cancrivorus)*	1 (0/1)
Coati (*Nasua* sp.)	4 (0/3; 1 unknown)
Kinkajou *(Potos flavus)*	1 (1/0)
Mustelidae	
European pine marten *(Martes martes)*	1 (0/1)
Stone marten *(Martes foina)*	2 (2/0)
Tayra *(Eira barbara)*	1 (0/1)
European polecat *(Mustela putorius)*	1 (1/0)
Ferret (*Mustela putorius furo)*	2 (0/1; 1 unknown)
European badger *(Meles meles)*	2 (1/1)
Eurasian otter *(Lutra lutra)*	4 (3/1)
Asian small-clawed otter *(Aonyx cinerea)*	5 (1/4)

Table 1: Number of analysed fecal samples from Swiss zoo carnivores.

Ailuridae	
Red panda *(Ailurus fulgens)*	1 (0/1)
Herpestidae	
Meerkat *(Suricata suricatta)*	4 (0/4)*
Helogale (*Helogale* sp.)	1 (0/1)
Yellow mongoose *(Cynictis penicillata)*	2 (0/2)*
Otariidae	
California sea lion *(Zalophus californianus)*	1 (0/1)
Total	151* (55/91; 5 unknown)

* Two times bulk samples were analysed, from two different species that shared one compound. Once from raccoon dogs and North American raccoons, and once from meerkats and yellow mongoose. Effectively, 149 samples were analysed only.

Information about the animals and their deworming management was collected, in order to identify potential risk factors for parasitic infections.
Depending on whether animals had contact to juveniles, they were divided in two groups. The first group included samples from juveniles (<1 year) or from enclosures with juveniles present, while in the second group, all samples from animals without contact to juveniles were included. Additionally, a difference was made between wild born and captive born animals. As two bulk samples included captive and wild born animals, they were not considered for the statistical analyses with this risk factor.
For 107 out of 149 samples anamnestic information about the presence of clinical signs from the examined animals were obtained. Initially, none of the animals were defined as ill due to parasitic infection, but upon more detailed request, some of the animals were reported to show symptoms like emaciation (n=3) or slight diarrhea (n=2) at the day of sampling. In three further felids, slight diarrhea was sometimes observed, but it was always associated with new or incompatible food.
Since different zoos have different deworming strategies, they were classified into three categories. Most samples (n=85) originated from animals living in zoos with a regular deworming strategy based on fecal examinations. Furthermore, in 31 cases animals were dewormed regularly (most commonly twice a year), without previous fecal examination, while 26 samples originated from animals that were never dewormed, or only sporadically.
The size of the enclosures of the animals were registered and classified as small (n=47), medium (n=50) or large (n=32) by subjective rating.

4.2 Methods

All samples were examined with four different techniques: combined sedimentation-flotation, Baermann-Wetzel technique, Ziehl-Neelsen staining of fecal smears (Deplazes, 2013), and *Giardia lamblia* (syn. *G. duodenalis, G. intestinalis*) coproantigen detection by ELISA using *Giardia lamblia* ELISA from Novitec® (Hiss diagnostics, Freiburg im Breisgau). While the Baermann-Wetzel technique was carried out on the day of feces collection or latest the day after, about half of the sampled material was kept at 4° Celsius and analysed by the combined sedimentation-flotation technique, and fecal smear latest after 14 days. Findings obtained by the combined sedimentation-flotation technique and with Ziehl-Neelsen staining were graded in a semi-quantitative scale (table 2), while larval counts were quantified with the number of larvae per gram feces. The *Giardia* ELISA from Novitec®, was conducted with frozen samples after all zoos were sampled. Results obtained with the *Giardia* ELISA from Novitec® were either classified as positive or negative.
Helminth eggs, protozoan oocysts and lungworm larvae were identified as described by Deplazes et al. (2013), Beugnet et al. (2008) and Bertelsen et al. (2010).

Table 2: Classification of the excretion intensity for the combined sedimentation-flotation technique and the Ziehl-Neelsen staining.

Excretion intensity	Combined sedimentation-flotation technique	Ziehl-Neelsen staining
-	no parasites found	no oocysts found
(+) very low	< 4 per sample	< 4 per sample
+ low	≥ 4 per sample	≥ 4 per sample
++ moderate	approx. 1 in every 4th vision field (10x)	approx. 1 in every 10th vision field (50x)
+++ high	between 1 and 10 in every vision field (10x)	1 in every vision field (50x)
++++ very high	more than 10 in every vision field (10x)	n.a.

4.2.1 Combined sedimentation-flotation technique

This technique is described to detect eggs of different nematodes, cestodes and oocysts of protozoan in feces and was performed as described by Deplazes et al. (2013) with modifications.
Ten grams of feces were used, if available. The material was suspended in tap water and mixed until it became homogenous. The suspension was filtered through a normal kitchen sieve (mesh size: 0.33 mm) into a 250 ml beaker and sedimented for at least 30 minutes. Afterwards, the supernatant was decanted and about 2 ml of the sediment were filled into a centrifugation tube. The tube was filled with about 11 ml zinc chloride (with density of 1.45 g/cm^3) and centrifuged for five minutes at 500 g instead of two to three minutes at 300 g as described by Deplazes et al. (2013). Two times 3-4 drops of the surface suspension were transferred with a metal loop onto a slide, covered with a cover slip and analysed under the microscope (20x).

4.2.2 Baermann-Wetzel technique

If possible, 10 grams of feces were used and mixed with autoclaved sawdust and wrapped into a gaze. The gaze was placed into a funnel with a rubber closed by a clip at the lower end, filled with tap water as much as almost 3/4 of the feces-sawdust-pellet was dipped, and left for 12 to 29 hours at room temperature (figure 1). Instead of analysing some drops (Deplazes, 2013), 14 ml were harvested at the lower end of the funnel by opening the clip and centrifuged for five minutes at 500 g. Then the upper 13.5 ml were discarded and the residual 0.5 ml were analysed under the microscope (4x, 10x) and larvae were counted, allowing a quantitative statement about the number of lungworm larvae detected per gram of feces.

Figure 1: Baermann-Wetzel technique with 19 fecal samples.

4.2.3 Ziehl-Neelsen staining

The Ziehl-Neelsen staining (Deplazes, 2013) was used for the detection of *Cryptosporidium* sp. oocysts. Some fecal material was transferred on microscope slides with a cotton bud. After air-drying, the slides were put into methanol for five minutes for fixation. Afterwards they were dried again, before putting them into the staining solution Carbol-fuchsin for four minutes, then into acidic ethanol until no more red staining solution was flowing off, followed by four minutes in brilliant green staining solution. Between these three steps the slides were always washed under cold running water. After the slides were dry again, they were examined under the microscope (50x) with immersion oil.

4.2.4 *Giardia duodenalis* Copro-Antigen ELISA

Frozen samples of all animals were analysed for *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis*) antigen by ELISA (Novitec®, Hiss diagnostics, Freiburg im Breisgau), according to manufactures protocol. Based on literature research, the different animal species were classified according to their probability to be infected with *G. duodenalis*, and the samples were pooled into groups of two, three or four samples. These pooled samples were diluted with specimen dilution buffer (1 ml). A subsample

of 0.2 ml of the material was transferred to the wells and the microplate was incubated at room temperature (20-25 °C) for 60 minutes. The wells were poured off and washed five times with diluted washing buffer. Four drops of enzyme conjugate were added to each well and incubated at room temperature for 30 minutes.
After decantation and washing five times for a second time, four drops of color substrate solution was added to each well. The microplate was incubated again (at room temperature) for 10 minutes before one drop of stop solution was added to each well. Within 10 minutes, color reactions were monitored visually. Positive pooled samples were retested as single samples.

4.3 Statistical analyses of risk factors

Statistical analyses were carried out using IBM SPSS Statistics (version 25). For detection of statistically significant correlations between the occurrence of a parasite and potential risk factors, cross tables and the Fisher's exact test or the Pearson's chi-squared test for statistical independence were used. The null hypothesis was defined as "being exposed to one of the risk factors does not have any influence on the probability of animals beeing infected with a specific parasite species". Tests were conducted with every single risk factor put in context to every parasite species found in this study. The significance level was set at $p < 0.05$. The risk factors which were analysed for correlation with parasite occurrence are listed in the following table:

Table 3: Evaluation of potential risk factors for parasitic infection.

Potential risk factor	**% of samples with information about the potential risk factors**	**Classification**	**Statistical test used**
number of animals in the enclosure	88.6%	≤2 animals (n=84) >2 animals (n=48)	Fishers exact test or Chi-square test
juveniles present (<1 year)	89.9%	yes (n=19) no (n=115)	Fishers exact test
origin of the animals	56.4%	born in the wild (n=9) born in captivity (n=75)	Fishers exact test
clinical signs observed	71.8%	yes (n=5) no (n=102)	Fishers exact test
time since last deworming	70.5%	≤3 month (n=28) >3 month (n=77)	Fishers exact test or Chi-square test
deworming strategy	91.9%	regular deworming based on fecal analyses (n=80) regular deworming without fecal analyses (=31) sporadic or absent deworming (n=26)	Fishers exact test
size of the enclosure	86.6%	small (n=47) medium (n=50) large (n=32)	Fishers exact test or Chi-square test

5 Results

In 79 (53.0%) of all 149 samples parasites were identified with at least one of the methods. Overall, 62 were positive by the combined sedimentation-flotation technique, 24 by Ziehl-Neelsen staining, 8 for *Giardia* specific copro-antigen detection and by Baermann-Wetzel technique each. No sample was positive by all four methods.

5.1 Parasitic findings

Nematodes were identified in 42.3% (n=63) of all samples. The most common nematode was *Capillaria* sp. (n=26), followed by *Toxocara mystax* (n=13), *Toxascaris leonina* (n=10), hookworms *(Ancylostoma or Uncinaria* sp.) (n=9), unidentified Ascarididae species (n=7), *Toxocara canis* (n=6), *Crenosoma* sp. (n=6), *Heterakis* sp. (n=4), *Trichuris* sp. (n=5), *Angiostrongylus vasorum* (n=2) and *Aspiculuris tetraptera* (n=2) (table 4).
Protozoan were identified in 26.8% (n=40) of all samples. *Cryptosporidium* sp. (n=24) accounted for more than 50% of the infections, followed by *Isospora* sp. (n=13), *Giardia duodenalis* (n=8) and *Neospora caninum* or *Hammondia* sp. (n=1).

Table 4: Parasites identified in overall 149 individual or bulked fecal samples of zoo carnivores in Switzerland.

	Nematodes											Protozoan			
	Toxascaris leonina	*Toxocara* sp.	unident. Ascarididae	*Capillaria sp.*	*Trichuris* sp.	hookworms	*Heterakis* sp.	*Aspiculuris tetraptera*	*Angio-strongylus vasorum*	*Crenosoma* sp.	unident. meta-strong. L1	*Isospora* sp.	*Neospora/ Hammondia* sp.	*Crypto-sporidium* sp.	*Giardia duodenalis*
Felidae	**10**	**13**	**-**	**7**	**2**	**2**	**3**	**-**	**-**	**-**	**-**	**6**	**-**	**8**	**5**
Lion (*Panthera leo)*	6	1	-	1	-	-	2	-	-	-	-	1	-	1	-
Tiger *(Panthera tigris)*	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-
Leopard *(Panthera pardus)*	-	-	-	1	-	-	1	-	-	-	-	1	-	-	-
Snow leopard *(Panthera uncia)*	1	1	-	1	-	-	-	-	-	-	-	1	-	1	-
Cheetah *(Acinonyx jubatus)*	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Wildcat *(Felis silvestri)*	-	1	-	-	-	-	-	-	-	-	-	-	-	2	2
Lynx (*Lynx* sp.)	1	7	-	4	2	2	-	-	-	-	-	2	-	3	1
Cougar *(Puma concolor)*	1	1	-	-	-	-	-	-	-	-	-	-	-	1	-
Ozelot *(Leopardus pardalis)*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Canidae	**-**	**6**	**-**	**11**	**-**	**6**	**1**	**1**	**2**	**1**	**-**	**6**	**1**	**9**	**3**
Wolve *(Canis lupus)*	-	1	-	3	-	3	-	-	1	1	-	1	-	2	3
Red fox *(Vulpes vulpes)*	-	5	-	5	-	3	1	1	1	-	-	3	1	5	-
Arctic fox *(Vulpes lagopus)*	-	-	-	2	-	-	-	-	-	-	-	2	-	-	-
African wild dog *(Lycaon pictus)*	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Raccoon dog *(Nyctereutes procyonoides)*	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-
Ursidae	**-**	**-**	**3**	**1**	**-**	**-**	**-**	**-**	**-**	**3**	**-**	**-**	**-**	**1**	**-**
Brown bear *(Ursus arctos)*	-	-	3	1	-	-	-	-	-	2	-	-	-	-	-
American black bear *(Ursus americanus)*	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-

Table 4: Parasites identified in overall 149 individual or bulked fecal samples of zoo carnivores in Switzerland.

	Nematodes											Protozoan			
	Toxascaris leonina	*Toxocara* sp.	unident. Ascarididae	*Capillaria sp.*	*Trichuris* sp.	hookworms	*Heterakis* sp.	*Aspiculuris tetraptera*	*Angio-strongylus vasorum*	*Crenosoma* sp.	unident. meta-strong. L1	*Isospora* sp.	*Neospora/ Hammondia* sp.	*Crypto-sporidium* sp.	*Giardia duodenalis*
Procyonidae	**-**	**-**	**3**	**2**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**-**
North American raccoon *(Procyon lotor)*	-	-	3	2	1	-	-	-	-	-	-	-	-	4	-
Nasua (*Nasua* sp.)	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-
Mustelidae	**-**	**-**	**1**	**4**	**-**	**1**	**-**	**1**	**-**	**1**	**-**	**-**	**-**	**2**	**-**
European pine marten *(Martes martes)*	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Stone marten *(Martes foina)*	-	-	1	1	-	1	-	-	-	-	-	-	-	1	-
European polecat *(Mustela putorius)*	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
European badger *(Meles meles)*	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-
Eurasian otter *(Lutra lutra)*	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Asian small-clawed otter *(Aonyx cinerea)*	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Ailuridae	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**1**	**-**	**-**	**-**	**-**
Red panda *(Ailurus fulgens)*	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-
Herpestidae	**-**	**-**	**-**	**1**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**
Meerkat *(Suricata suricatta)*	-	-	-	1	1	-	-	-	-	-	-	1	-	-	-

No parasites were found in samples from Serval *(Leptailurus serval)*, Leopard cat *(Prionailurus bengalensis)*, Caracal *(Caracal caracal)*, Jungle cat *(Felis chaus)*, Fennec fox *(Vulpes zerda)*, Asian black bear *(Ursus thibetanus)*, Spectacled bear *(Tremarctos ornatus)*, Malayan sun bear *(Helarctos malayanus)*, Crab-eating raccoon *(Procyon cancrivorus)*, Kinkajou *(Potos flavus)*, Tayra *(Eira barbara)*, Ferret *(Mustela putorius furo)*, Helogale *(Helogale* sp.*)*, Yellow mongoose *(Cynictis penicillata)*, California sea lion *(Zalophus californianus)*.

Table 5: Number of mixed infections identified in 149 individual or bulked fecal samples of zoo carnivores in Switzerland. Negative samples are excluded in this table. Bulked samples from raccoon dogs and North American raccoons, as well as from meerkats and yellow mongoose are listed as if they were separate samples.

	No. analysed (n)	No. positive for at least one parasite (n)	Percentage positive for at least one parasite (%)	Positive for one parasite species (n)	Positive for two parasites species (n)	Positive for three parasites species (n)	Positive for four parasites species (n)	Positive for five parasites species (n)	Positive for six parasites species (n)
Felidae	**68**	**36**	**53**	**21**	**11**	**3**	**1**	**-**	**-**
Lion (*Panthera leo)*	10	7	70	4	1	2	-	-	-
Tiger *(Panthera tigris)*	11	2	18	2	-	-	-	-	-
Leopard *(Panthera pardus)*	6	2	33	1	1	-	-	-	-
Snow leopard *(Panthera uncia)*	4	4	100	3	1	-	-	-	-
Cheetah *(Acinonyx jubatus)*	6	2	33	1	1	-	-	-	-
Wildcat *(Felis silvestri)*	5	4	80	3	1	-	-	-	-
Lynx (*Lynx* sp.)	14	11	79	3	6	1	1	-	-
Cougar *(Puma concolor)*	7	3	43	3	-	-	-	-	-
Ozelot *(Leopardus pardalis)*	1	1	100	1	-	-	-	-	-
Canidae	**23**	**18**	**78**	**4**	**6**	**4**	**2**	**1**	**1**
Wolve *(Canis lupus)*	9	6	67	1	2	2	1	-	-
Red fox *(Vulpes vulpes)*	8	7	88	-	2	2	1	1	1
Arctic fox *(Vulpes lagopus)*	2	2	100	-	2	-	-	-	-
African wild dog *(Lycaon pictus)*	1	1	100	1	-	-	-	-	-
Raccoon dog *(Nyctereutes procyonoides)*	2	2	100	2	-	-	-	-	-
Ursidae	**13**	**7**	**54**	**6**	**1**	**-**	**-**	**-**	**-**
Brown bear *(Ursus arctos)*	7	5	71	4	1	-	-	-	-
American black bear *(Ursus americanus)*	2	2	100	2	-	-	-	-	-
Procyonidae	**20**	**10**	**50**	**8**	**2**	**-**	**-**	**-**	**-**
North American raccoon *(Procyon lotor)*	14	8	57	6	2	-	-	-	-
Coati (*Nasua* sp.)	4	2	50	2	-	-	-	-	-
Mustelidae	**18**	**6**	**33**	**4**	**1**	**-**	**1**	**-**	**-**
European pine marten *(Martes martes)*	1	1	100	1	-	-	-	-	-
Stone marten *(Martes foina)*	2	1	50	-	-	-	1	-	-
European polecat *(Mustela putorius)*	1	1	100	1	-	-	-	-	-
European badger *(Meles meles)*	2	1	50	-	1	-	-	-	-
Eurasian otter *(Lutra lutra)*	4	1	25	1	-	-	-	-	-
Asian small-clawed otter *(Aonyx cinerea)*	5	1	20	1	-	-	-	-	-

Table 5: Number of mixed infections identified in 149 individual or bulked fecal samples of zoo carnivores in Switzerland. Negative samples are excluded in this table. Bulked samples from raccoon dogs and North American raccoons, as well as from meerkats and yellow mongoose are listed as if they were separate samples.

	No. analysed (n)	No. positive for at least one parasite (n)	Percentage positive for at least one parasite (%)	Positive for one parasite species (n)	Positive for two parasites species (n)	Positive for three parasites species (n)	Positive for four parasites species (n)	Positive for five parasites species (n)	Positive for six parasites species (n)
Ailuridae	**1**	**1**	**100**	**-**	**1**	**-**	**-**	**-**	**-**
Red panda *(Ailurus fulgens)*	1	1	100	-	1	-	-	-	-
Herpestidae	**7**	**2**	**29**	**1**	**1**	**-**	**-**	**-**	**-**
Meerkat *(Suricata suricatta)*	4	2	50	1	1	-	-	-	-

No parasites were found in samples from Serval (*Leptailurus serval;* n=1), Leopard cat (*Prionailurus bengalensis;* n=1), Caracal (*Caracal caracal;* n=1), Jungle cat (*Felis chaus;* n=1), Fennec fox (*Vulpes zerda;* n=1), Asian black bear (*Ursus thibetanus;* n=1), Spectacled bear (*Tremarctos ornatus;* n=2), Malayan sun bear (*Helarctos malayanus;* n=1), Crab-eating raccoon (*Procyon cancrivorus;* n=1), Kinkajou (*Potos flavus;* n=1), Tayra (*Eira barbara;* n=1), Ferret (*Mustela putorius furo;* n=2), Helogale (*Helogale* sp.; n=1), Yellow mongoose (*Cynictis penicillata;* n=2), California sea lion (*Zalophus californianus;* n=1).

5.1.1 Felidae

Out of 149 samples, 68 (45.6%) originated from Felidae. Thereof, 52.9% (n=36) were positive in at least one of all four methods. In Felidae, the most common parasite family was Ascarididae (n=23) and it was distinguished between *T. mystax* (n=13) or *T. leonina* (n=10) based on the morphology and size of the eggs (figure 2), as well as on the known occurrence of different *Toxocara* species in Felidae (Deplazes, 2013). Other parasites were *Cryptosporidium* sp. (n=8), *Capillaria* sp. (n=7), *Isospora* sp. (n=6), *G. duodenalis* (n=5), *Heterakis* sp. (n=3), *Trichuris* sp. (n=2) and hookworms (n=2), which were not differentiated between *Ancylostoma tubaeforme* or *Uncinaria stenocephala*.
High prevalences were observed in lynxes *(Lynx* sp.), lions *(Panthera leo),* snow leopards *(Panthera uncia)* and wild cats *(Felis silvestri)* (table 4). At the same time, lions and lynxes also showed high number of mixed infections (table 5), with up to four different parasite species in a single sample from a lynx.
While infections with hookworms never occurred as single infections but always together with *T. mystax*, half (n=4) of the infections with *Cryptosporidium* sp. were single infections. Overall, 70.0% (7 out of 10) of *T. leonina* infections were single infections, while 31.8% (4 out of 13) of *T. mystax* infections were single infections. No mixed infections with both parasites, *T. leonina* and *T. mystax*, were detected.
In four samples from small felids (serval, leopard cat, caracal and a jungle cat), whereof three originated from private keepings, no parasites were found. No lungworm larvae were isolated using the Baermann-Wetzel technique in Felidae.

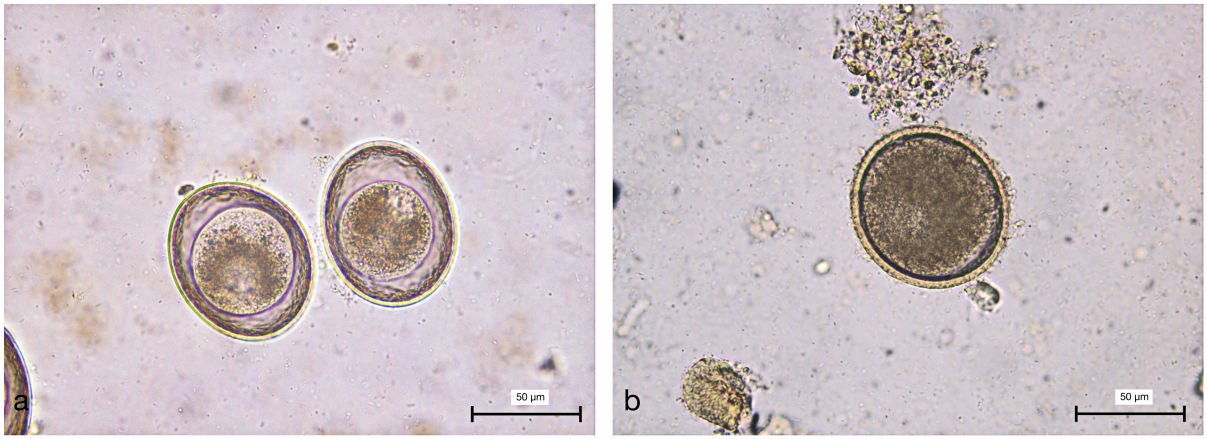

Figure 2: (a) *Toxascaris leonina* eggs in an individual sample of a lion *(Panthera leo),* size 76 µm x 64 µm and 77 µm x 62 µm; (b) *Toxocara mystax* egg in a bulk sample of two lynxes (*Lynx* sp.), size 77 µm x 71 µm.

5.1.2 Canidae

Most samples from Canidae originated from wolves or red foxes (17 out of 23). The most common parasite in Canidae was *Capillaria* sp. (n=11) followed by *Cryptosporidium* sp. (n=9), *T. canis* (n=6), hookworms (n=6) and *Isospora* (n=6). Other parasites were *G. duodenalis* (n=3) *A. vasorum* (n=2), *C. vulpis* (n=1), *Neospora caninum* or *Hammondia* sp. (n=1), *Heterakis* sp. (n=1) and *A. tetraptera* (n=1) (figure 3). The two latter are known to frequently occur as intestinal passages or to originate from environmental contamination (*Heterakis* sp. is a chicken parasite and *A. tetraptera* is a rodent parasite) (Deplazes, 2013). The red fox is the host species with the highest number of parasite species per sample (table 5). In a bulk sample from eight foxes six different parasite species were found. Most infections in Canidae were mixed infections (table 5). Only *Capillaria* sp., *G. duodenalis* (once each) and *Cryptosporidium* sp. (twice) occurred as single infections. *T. canis* occurred always together with other parasites, mostly *Cryptosporidium* sp. or *Capillaria* sp.

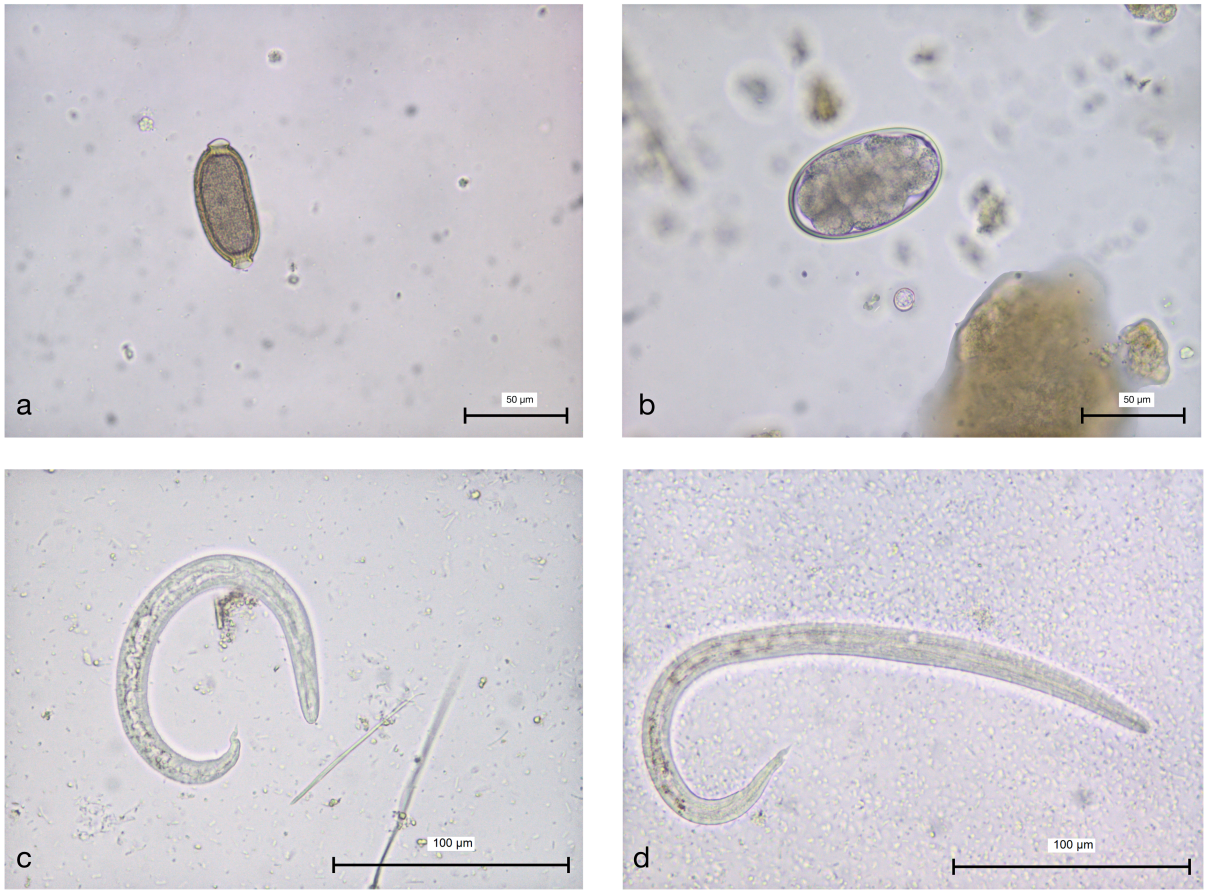

Figure 3: (a) *Capillaria* sp. egg in a bulk sample of two arctic foxes *(Vulpes lagopus)*, size 64 µm x 28 µm; (b) Hookworm egg in a bulk sample of two juvenile red foxes (*Vulpes vulpes*), size 79 µm x 48 µm and below: an unsporulated oocyst of *Neospora caninum* or *Hammondia* sp., size 11 µm x 10 µm; (c) first stage *Angiostrongylus vasorum* larva in a bulk sample of wolves *(Canis lupus)*, length 228 µm; (d) first stage *Angiostrongylus vasorum* larva in a bulk sample of red foxes, length 334µm.

5.1.3 Ursidae

Out of all samples from Ursidae (n=12), parasites were detected in brown bears *(Ursus arctos)* and American black bears *(Ursus americanus)* (table 4). In three samples from brown bears, Ascarididae eggs were found (figure 4). Further parasite species in Ursidae were *Crenosoma* sp. (n=3), *Capillaria* sp. (n=1) and *Cryptosporidium* sp. (n=1). The only mixed infection in Ursidae originated from a bulk sample from brown bears which was positive for Ascarididae and *Crenosoma* sp. (table 5).

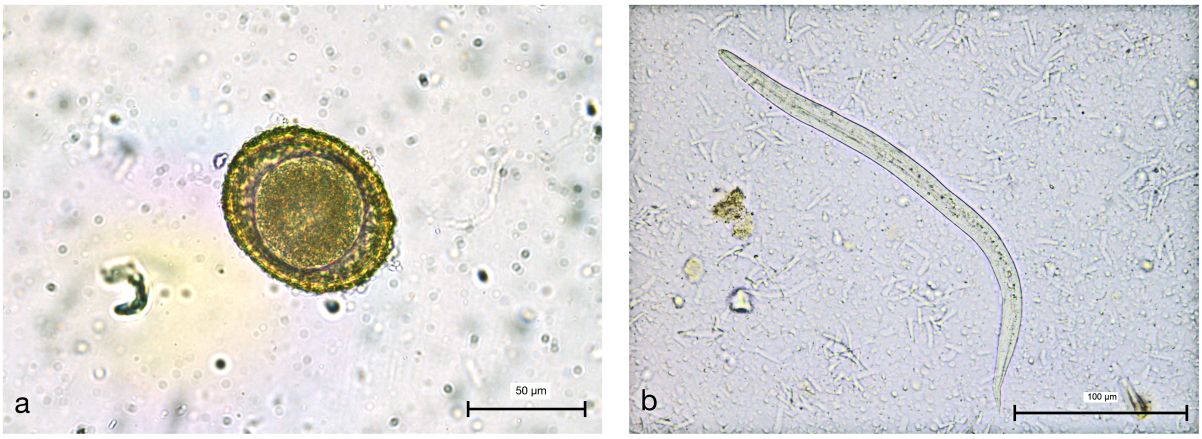

Figure 4: (a) unidentified Ascarididae egg in an individual sample of a brown bear *(Ursus arctos)*, size 77 µm x 63 µm; (b) *Crenosoma* sp. larva in a bulk sample of four brown bears *(Ursus arctos)*, length 295 µm.

5.1.4 Procyonidae

In total, 19 samples originated from Procyonidae and one from a group housing of Procyonidae with Canidae. Parasites were found in North American raccoons *(Procyon lotor)* and coatis (*Nasua* sp.) (table 4). The most common parasite in North American raccoons was *Cryptosporidium* sp. (n=4) followed by unidentified Ascarididae species (n=3), *Capillaria* sp. (n=2) and *Trichuris* sp. (n=1) (figure 5). In two samples from North American raccoons mixed infections (table 5) with *Capillaria* sp. and *Trichuris* sp. or *Capillaria* sp. and *Cryptosporidium* sp. respectively were observed. In coatis, only *Cryptosporidium* sp. (n=1) and *Trichuris* sp. (n=1) were identified.

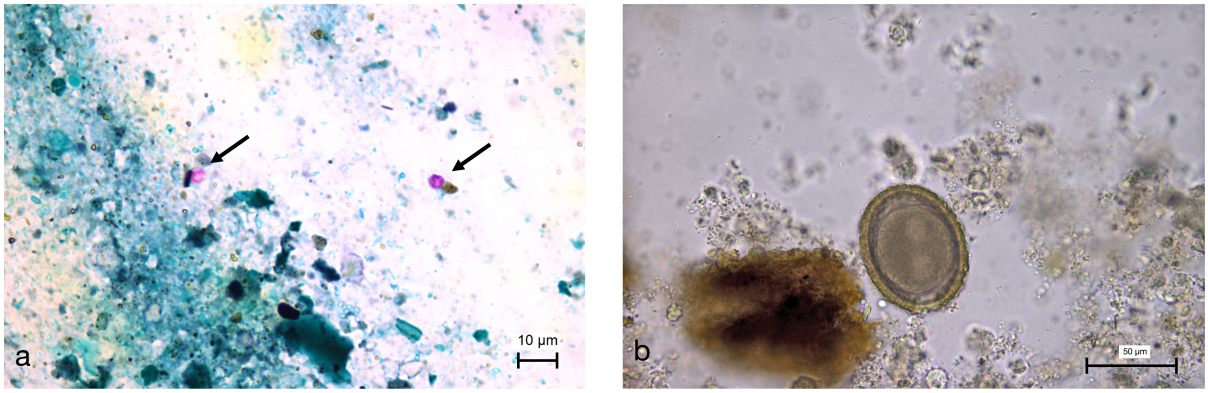

Figure 5: (a) Two *Cryptosporidium* sp. oocysts in a bulk sample of two adult North American raccoons *(Procyon lotor)*, 4 x 4µm each; (b) unidentified Ascarididae egg in a bulk sample of North American raccoons, size 72 µm x 57 µm.

5.1.5 Mustelidae

In Mustelidae, six out of 18 samples were positive for at least one parasite. As illustrated in table 5, most of them (66.6%) were positive for one parasite only. Remarkably, a single sample from a juvenile stone marten *(Martes foina)* was positive for four different parasites (hookworms, unidentified Ascarididae, *Capillaria* sp. and *Cryptosporidium* sp.).
The most common parasite in Mustelidae was *Capillaria* sp. (n=4) and *Cryptosporidium* sp. (n=2), followed by unidentified Ascarididae (n=1), hookworms (n=1), *A. tetraptera* (n=1) and *Crenosoma* sp. (n=1).
The sample from the European polecat *(Mustela putorius)* was the only sample from Mustelidae which was positive for lungworms by the Baermann-Wetzel technique.

5.1.6 Ailuridae

The only sample of the family Ailuridae was a bulk sample from two red pandas *(Ailurus fulgens)*. These animals were positive by the Baermann-Wetzel technique only and were infected with two different lungworm species (figure 6).

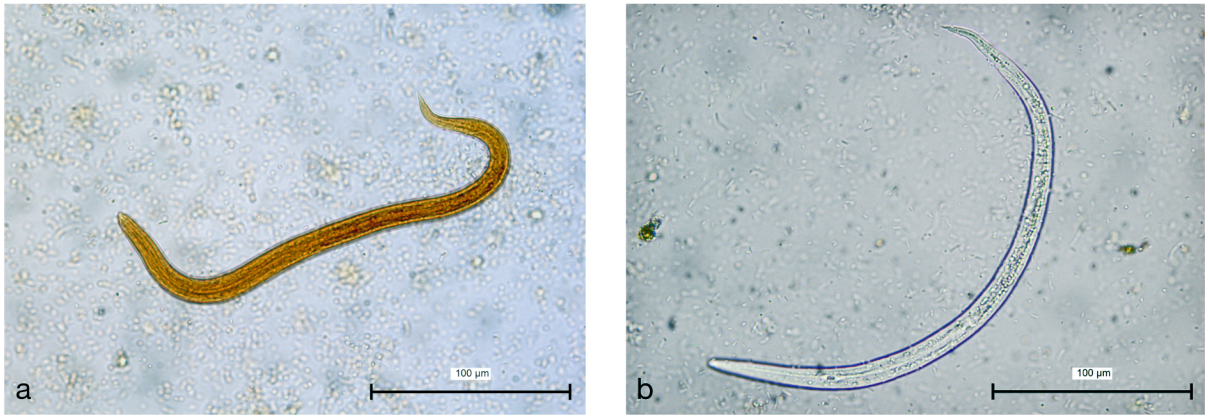

Figure 6: lungworm larvae found in the bulk sample of two red pandas *(Ailurus fulgens)*: (a) *Crenosoma* sp. larva, fixed with lugol, length 287 µm; (b) unidentified metastrongyloid L_1, length 320 µm.

5.1.7 Herpestidae

One sample originated from a group-housing of meerkats *(Suricata suricatta)* with yellow mongoose *(Cynictis penicillata)*. This sample was negative for any parasites species. In Herpestidae, parasites were found in meerkats only: in one sample *Trichuris* sp. was identified and in the other a mixed infection with *Capillaria* sp. and *Isospora* sp. was found.

5.1.8 Otariidae

The only species of the family Otariidae sampled were California sea lions *(Zalophus californianus)* which were negative by all four methods.

5.2 Excretion Intensity

Excretion intensity varied among the different parasites (table 6). Mostly, parasite detection was at low levels. Only in four samples, all containing Ascarididae, a very high excretion intensity was found. While 38.8% of Ascarididae positive samples were at high or very high levels, all *Capillaria* sp. infections were either in a very low, a low or moderate level. Thereby differences in excretion intensity depending on the host families were observed. The highest intensities of excretion were found in Felidae (mainly in cougars, lions and cheetahs). Out of all infections in Felidae, 25.5% (n=13) were either at high or very high levels, while in Canidae only 17.1% (n=41) were at high levels. A parasite species that was found in Felidae at very low levels only, were hookworms. Differences in excretion intensities were also found depending on the host species. For example the median excretion intensity in snow leopards *(Panthera uncia)* was moderate, while it was low in other leopard species *(Panthera pardus)*.

Table 6: Frequency of various excretion intensities in relation to parasite species observed by the combined sedimentation-flotation technique and Ziehl-Neelsen staining in 149 fecal samples of zoo carnivores in Switzerland.

	Toxascaris leonina	*Toxocara mystax*	*Toxocara canis*	unident. *Ascarididae*	*Capillaria* sp.	*Trichuris* sp.	hookworms	*Heterakis sp.*	*Aspiculuris tetraptera*	*Isospora* sp.	*Neospora/ Hammondia* sp.	*Crypto-sporidium* sp.	Total findings
(+)	1	2	3	2	11	4	3	-	2	3	-	2	33
+	2	3	-	-	7	1	4	2	-	5	-	8	32
++	2	3	-	4	8	-	1	2	-	3	1	9	33
+++	2	4	3	1	-	-	1	-	-	2	-	5	18
++++	3	1	-	-	-	-	-	-	-	-	-	n.a.	4
Total	10	13	6	7	26	5	9	4	2	13	1	24	120

Concerning *Cryptosporidium* sp. infections, it is remarkable that half of all samples with moderate or high levels (7/14) of excretion intensity originated from juveniles or enclosures with juveniles present, while only 3 out of 10 samples with very low or low excretion intensities originated from juveniles.
Lungworm larvae, identified by the Baermann-Wetzel technique, were calculated as larvae per gram of feces (table 7). The highest number of larvae were found in a single sample of a European polecat *(Mustela putorius)*.

Table 7: Lungworm larvae identified by the Baermann-Wetzel technique performed with overall 149 individual or bulked fecal samples of zoo carnivores in Switzerland and the number of larvae per gram of feces.

Host species	**Lungworm species**	**Number of larvae per gram feces**
Wolve (*Canis lupus)*	*Angiostrongylus vasorum*	6.8
	Crenosoma vulpis	0.1
Brown bear *(Ursus arctos)*	*Crenosoma* sp.	0.2
	Crenosoma sp.	1.1
American black bear *(Ursus americanus)*	*Crenosoma* sp.	0.1
Red fox *(Vulpes vulpes)*	*Angiostrongylus vasorum*	6.6
European polecat *(Mustela putorius)*	*Crenosoma* sp.	112.2
Red panda *(Ailurus fulgens)*	*Crenosoma* sp.	1.6
	unidentified metastrongyloid	1.6

5.3 Statistical analysis of risk factors

The null hypotheses were rejected for five different risk factors in six different parasites, as illustrated in table 8. For statistics, unidentified Ascarididae in Ursidae, Procyonidae and Mustelidae were taken together with *Toxocara* sp. infections in Felidae and Canidae, because they were not distinguished as two different species due to the morphology and size of their eggs, but due to the host species they were found in. For *Cryptosporidium* sp. and *Toxocara* sp./unidentified Ascarididae infections, more than one potential risk factor was identified.

Table 8: Significant correlations identifying potential risk factors for different parasite infections in zoo carnivores.

Potential risk factor	Parasite	Test used	p value	Conclusion
Juveniles present	*Cryptosporidium* sp.	Fishers exact test	0.000182	Juvenile animals or animals that live in enclosures with juveniles have a higher probability for being infected with *Cryptosporidium* sp. or *Toxocara* sp./unidentified Ascarididae
	Toxocara sp./unident. Ascarididae	Fishers exact test	0.012	
Origin of the animals	*Cryptosporidium* sp.	Fishers exact test	0.021	Wild born animals have a higher probability for being infected with *Cryptosporidium* sp. or *Toxocara* sp./unidentified Ascarididae
	Toxocara sp./unident. Ascarididae	Fishers exact test	0.021	
Clinical signs observed	*Cryptosporidium* sp.	Fishers exact test	0.047	Animals that are showing clinical signs* have a higher probability to be infected with *Cryptosporidium* sp., hookworms or *Angiostrongylus vasorum*.
	hookworms	Fishers exact test	0.034	
	Angiostrongylus vasorum	Fishers exact test	0.002	
Deworming strategy	*Toxascaris leonina*	Fishers exact test	0.01	Animals getting dewormed regularly without fecal examination have a higher probability for *Toxascaris leonina* infections.
Size of the enclosure	*Giardia duodenalis*	Fishers exact test	0.019	Animals that live in larger enclosures have a higher probability for *Giardia duodenalis* infections.

* Out of five samples, where animals were showing clinical signs, two showed slight diarrhea occasionally and three bulk samples originated from groups, where at least one of the animals was emaciated.

Number of animals in the enclosure

The number of animals living in an enclosure was not identified as a risk factor for infection with any parasite species in this investigation.

Juveniles present

A significant higher probability of infection with *Cryptosporidium* sp. was found in the group where juveniles were present. There were also significant more findings of *Toxocara* sp./unidentified Ascarididae in juvenile animals or in enclosures where juveniles were present.

Origin of the animals
A significant higher probability for infection with *Cryptosporidium* sp. and *Toxocara* sp./unidentified Ascarididae was found if sampled animals were born in the wild.

Clinical signs observed
All three samples from animals that showed emaciation were classified positive for C*ryptosporidium* sp. Furthermore, animals that showed clinical signs were statistically significant more often infected with hookworms or *Angiostrongylus vasorum*. Although no animal showed respiratory symptoms, it is noticeable that the only two samples which were positive for *Angiostrongylus vasorum* both originated from enclosures where at least one animal showed gastro-intestinal symptoms. In both cases, they showed low infections with other parasites (inter alia hookworms), too.

Time since last deworming
There was no correlation found between the period of time since last deworming and an infection with any parasite species.

Deworming strategy
There was a significantly higher prevalence of *Toxascaris leonina* infection in animals that were dewormed regularly without previous fecal examination.

Size of the e*nclosure*
A statistic significantly higher prevalence of *Giardia duodenalis* infections in animals housed in large enclosures was found.

6 Discussion

With a participation rate of 88.9% (24 out of 27) of all zoos in Switzerland, results can be considered as representative for carnivores kept in zoos in the country. Since multiple methods have been applied, we can assume that a considerable overview about gastrointestinal parasites and lungworms affecting zoo carnivores has been obtained. This is supported by a high diversity of parasite species that were documented compared to other studies (Bursee, 2015; Lim et al., 2008; Ramos, 2014).
In contrast to a previous investigation in Switzerland (Weber, 2009), additionally to the combined sedimentation-flotation technique, the Baermann-Wetzel technique and the Ziehl-Neelsen staining also a *Giardia* copro-antigen ELISA was performed. The number of zoos sampled in 2009 (n=23) versus 2017/18 is comparable. Three zoos that were sampled in 2009 have been closed since, or changed the husbandry of the animals in such a way that animal keepers do not enter animal enclosures anymore and sample collection was not possible. Though, four further zoos could were sampled, additionally to three private keepings.

The results of our investigation are mostly comparable to the parasitic situation described in a review about parasites in captive animals in European zoos (Panayotova-Pencheva, 2013). Panayotova-Pencheva summarized nematodes as the most frequently found parasite phylum in captive animals in European zoos, which confirms literature research (Atanaskova et al., 2011; Fagiolini et al., 2010; Roman et al., 2017) and supports our own investigation. We found nematodes in 42.3% of all samples. This prevalence is comparable to other prevalence rates (37.0% and 43.3% respectively) of similar investigations about zoo animals in eastern and southern Europe (Atanaskova et al., 2011; Fagiolini et al., 2010). Roma et al. (2017) reported nematodes in most carnivores sampled in a zoological park in Romania (prevalence 80.0%), while their prevalence for protozoan infections (20.0%) was similar to ours (26.8%). However, there are also data about zoo carnivores showing divergent results. Cordòn et al. (2008) explained the fact that they detected protozoan only by the „simplicity of their life cycle, the low infective dose, the short prepatent period and ability to survive in the environment."
In contrast to other investigations (Ahasan et al., 2010; Atanaskova et al., 2011; Bindke et al., 2017; Deshmukh et al., 2009; Javaregowda, 2016; Lim et al., 2008; Ramos, 2014), no cestodes or trematodes were detected in this study, as well as in the investigation from 2009 (Weber) and other reports (Ahasan et al., 2010; Fagiolini et al., 2010; Gurler et al., 2010; Roman et al., 2017; Singh et al., 2006). The presence of a cestode (*Echinococcus multilocularis*) was already reported in wild foxes that were trapped within the area of a zoo in Switzerland, which was also sampled for our investigation (Rehmann et al., 2005). Though, no zoo carnivore was reported positive for *E. multilocularis* by Rehmann et al. (Rehmann et al., 2005). In other studies, when cestodes were reported, they were often identified only sporadically (Ahasan et al., 2010; Atanaskova et al., 2011; Barutzki et al., 1985; Ramos, 2014). Other helminths such as *Strongyloides* sp. that were present in other zoos (Ahasan et al., 2010; Aviruppola et al., 2016; Fagiolini et al., 2010; Markowski, 2013; Varadharajan and Subramanian, 2003) were not identified in our study.
In a publication from Poland (Szafranska et al., 2010), no trematodes or cestodes were reported in captive Eurasian wolves (*Canis lupus lupus)*, while *Alaria alanta* (a trematode) presented as the most common parasite in wild Eurasian wolves in the same study. For differences between wild and captive animals, the availability of intermediate host can be made responsible for (Markowski, 2013; Szafranska et al., 2010). Parasites that need intermediate hosts (i.e. lungworms, *Neospora caninum*, *Capillaria* sp.) are possibly less present in zoo environments than parasites that can develop without intermediate hosts and which can lead to reinfections faster.
The excretion intensity in different parasites in the combined sedimentation-flotation technique or in Ziehl-Neelsen staining is not strictly comparable to the results of Weber (2009), because this author did

not noted specify criteria for the classification into a scale from one to four. Because the excretion intensity was not the purpose of our investigation, eggs and oocysts have not been counted exactly such as with McMaster technique as in other investigations (Aviruppola et al., 2016; Fagiolini et al., 2010; Singh et al., 2006).

6.1 Host families

6.1.1 Felidae

In accordance to the results of other studies, the highest prevalence of Ascarididae infection was found in Felidae. We identified either *T. mystax* (syn. *T. cati*) or *T. leonina* in 33.8% of all Felidae. In comparison, two investigations from Germany (Barutzki et al., 1985; Bursee, 2015) and one from Italy (Fagiolini et al., 2010) identified prevalences of Ascarididae infection in Felidae of 14.4%, 21.8% and 66.7%, respectively. Differences in prevalence can be explained partially because of different management conditions such as cleaning and deworming schedules (Fagiolini et al., 2010). In several studies, *T. leonina* was more prevalent than *T. mystax* (Barutzki et al., 1985; Bursee, 2015; Peng et al., 2016). Interestingly, in our investigation as well as in the work from Weber (2009), this was reverse. We also did not found mixed infections with *T. leonina* and *T. mystax* in contrast to the publications of Singh et al. (2006). In literature, mixed infections in lions and tigers are common. Mukarati et al. (2013) reported mixed infections in 93.3% of all examined lions, while Fagiolini et al. (2010) reported mixed infections in lions with *T. leonina* and hookworms in 30% of the examined samples. Javaregowta (2016) reported only 16% of examined lions to be infected with more than one parasite, but mixed infections in all tigers examined. Tigers are often reported to be infected with more than one parasite, such as *T. leonina* and *T. mystax* mixed infections (Peng et al., 2016), or *T. mystax* and *Cryptosporidium* sp. (Lim et al., 2008). In our investigation, no tiger was excreting more than one parasite.
Findings of Ascarididae in Felidae may differ between the animal species. While Peng et al. (Peng et al., 2016) reported *T. leonina* to be the most common Ascarididae in lions as well as in tigers, others reported *T. leonina* more frequently in lions and *T. mystax* more frequently in tigers (Barutzki et al., 1985; Fagiolini et al., 2010; Singh et al., 2006). This is also in accordance to our results: In lions *T. leonina* was detected in 6 out of 10 samples, while *T. mystax* was detected in one sample only. In tigers, only in 1 out of 11 samples Ascarididae *(T. mystax)* was identified.
In the publication from Singh et al. (2006) the excretion intensities were graded into a scale from + to ++++. Highest intensities were detected in lions *(Panthera leo)* and tigers *(Panthera tigris)* with *T. leonina* and *T. mystax*, respectively. Similar results were also reported by Bursee (2015). This author reported highest excretion intensities with Ascarididae in lions and cheetahs. In our study, these two species (additionally to a single sample from a cougar) were also the only species that showed excretion intensities (all with Ascarididae) at very high levels. In contrast, Bursee (2015) reported low excretion intensities with Ascarididae in tigers, which cannot be confirmed by our results since the only finding of Ascarididae in tigers was at high level.
Other parasites that were already detected in Felidae living in zoos (Darabus et al., 2014; Singh et al., 2006) were hookworms. In our investigation excretion intensity was always at very low levels. Over all host families, we identified hookworms in 77.8% of all cases at a very low to a low level. Another investigation (Fagiolini et al., 2010) found a higher excretion intensity with hookworms than with *T. leonina* in lions, which is exactly reverse to other publications (Singh et al., 2006) and to our own results, since no hookworms in lions were detected. In Felidae, hookworm eggs were found only in two samples from lynxes. Hookworms have rarely been reported in captive lynxes (Markowski, 2013), most probably due to rare lynx keepings in examined zoos outside Switzerland. Instead, hookworm

infections in other felids (lions, leopard, puma) are reported by Weber (2009) and also in other investigations (Deshmukh et al., 2009; Fagiolini et al., 2010; Gurler et al., 2010; Lim et al., 2008). However, since we did not perform further analyses such biomolecular examinations, the detected eggs could also originate from intestinal passages or from earth nematodes due to contamination during feces collection. The low excretion intensity in hookworms may support this possibility. However, zoo employees should consider that hookworms are potentially zoonotic and can cause larva migrans cutanea (Deplazes, 2013).

Another parasite which was detected in Felidae, but only in lynxes, was *Trichuris* sp. Findings of *Trichuris* sp. were already described in lynxes (*Lynx* sp.) from zoos in Germany (Markowski, 2013) and in wild lynxes in Switzerland (Schmidt-Posthaus et al., 2002). In accordance to these publications, we did not differentiate between different *Trichuris* species. Therefore, the isolated eggs may originate from intestinal passages. Prey animals, such as small ruminants, are frequently infected by *Trichuris* sp. (Deplazes, 2013). *Trichuris* infections in Felidae *(T. serrata, T. campanula, T. felis)* are rarely reported in Europe (Bowman, 2014; Deplazes, 2013) and rather unlikely.
A parasite that we often identified in Felidae was *Cryptosporidium* sp. High prevalences were detected in wildcats (40%) and lynxes (21%). Infection with *Cryptosporidium* sp. were reported in several previous publications and were detected in different carnivore species in captivity, from many animals of other orders (Fagiolini et al., 2010; Lim et al., 2008). In our study, we did not differentiated between *Cryptosporidium parvum*, the main cause for human cryptosporidiosis (Deplazes, 2013), and other species such as *C. felis*. It is known from further publications that *C. parvum* occurs in different carnivores such as leopards (Karanis et al., 2007), North American raccoons (Snyder, 1988), foxes (Hamnes et al., 2007) and even California sea lions (Deng et al., 2000).
Isospora sp. was found less frequently than in 2009 (Weber), where it was reported as the most common parasite (prevalence 19.2%). In Felidae prevalence of *Isospora* sp. oocysts was 16%, while in our investigation prevalence was 9% only. Like in our investigation, Weber (2009) did not differentiate between *Isospora* species from carnivore and coccidia oocysts of prey animals. Other investigations differentiated between different coccidian species by sporulation. (Cordòn et al., 2008; Darabus et al., 2014; Fagiolini et al., 2010). Thereby, Darabus et al. (2014) was able to identify *Eimeria* sp. in samples from lions, which are likely to be intestinal passages (Deplazes, 2013).
As already published by Beck et al. (2011), single Canidae and Felidae were positive for *Giardia duodenalis,* though many terrestrial mammals and even California sea lions are reported to be infected with *G. duodenalis* (Deng et al., 2000; Deplazes, 2013). In the zoo of Zagreb not only *G. duodenalis* but also *G. microti* was detected in two samples from Felidae (Beck et al., 2011). This was explained by the presence of rodents in the zoo. A contamination and cross reaction with *Giardia microti* cannot be ruled out in our investigation, but due to a high specificity (97-100%) of the ELISA used (Novitec®), a cross reaction with other parasites is very unlikely (Rosoff and Stibbs, 1986). In further publications, prevalences of *Giardia* sp. infections varied widely. Our prevalence of *G. duodenalis* infections in Felidae was 7.4% and 5.4% among all carnivore families. In the zoo of Zagreb (Beck et al., 2011) and in the Johannesburg zoological garden (Ramos, 2014) prevalence in carnivores was 57.1% (by immunofluorescence microscopy) and 41.2% (by sedimentation method, after sodium acetate-acetic acid-formalin fixation), respectively. While Bursee (2015) reported *Giardia* sp. in Felidae (with a *Giardia duodenalis* antigen rapid test) with a prevalence of 1.7% only, other investigations found no *Giardia* sp. in carnivores, using either commercial ELISA kits (Roman et al., 2017), immunochromatographic assay (Velante et al., 2017) or flotation methods only (Aviruppola et al., 2016; Cordòn et al., 2008).

6.1.2 Canidae

The most common parasite in Canidae was *Capillaria* sp., for highest prevalence (47%) out of all families was observed, as already reported by Weber (2009) (53%). However, as we did not distinguish the different *Capillaria* species, we cannot differentiate between infections and intestinal passages: several *Capillaria* species occur in poultry and other potential feed animals (Deplazes, 2013). With *Heterakis* sp., eggs of another poultry parasite were already identified in Canidae. *Capillaria* species that infect Canidae causing egg excretion in feces, are *C. putorii, C. aerophila* and *C. boehmi* (Deplazes, 2013; personal communication, Schnyder, M.). Findings of *C. aerophila* were already described in wild wolves in Poland, but no infection was reported in captive wolves in the same publication (Szafranska et al., 2010). In further investigations, findings of *Capillaria* sp. varied widely. Cordòn et al. (2008) did not identify *Capillaria* species in different carnivores in a zoological garden in Spain, while Bindke et al. (2017) reported *Capillaria* eggs in almost 20% of all samples from captive wolves in Germany, which is more comparable to our results (prevalence in wolves: 33.3%).
Hookworms were detected mainly in wolves and red foxes, in nearly equal prevalence as reported nine years ago (Weber, 2009). But in 2009, hookworms were found in animals from other, too. We did not differentiate between *Uncinaria* sp. and *Ancylostoma* sp. in our study, but in two publication about wild foxes in Switzerland, only *Uncinaria* sp. and no *Ancylostoma* sp. were reported (Hofer et al., 2000; Reperant et al., 2007). In literature, instead, *A. caninum* seems to be a very common parasite in captive wolves. In studies from Poland and Germany *A. caninum* was the most common parasite identified (Bindke et al., 2017; Szafranska et al., 2010). We identified hookworms and *Capillaria* sp. as the most common parasites in wolves. In wolves, hookworms were the parasites with the highest excretion intensities. This was reported by others, too (Szafranska et al., 2010): *Trichuris* sp. followed by *Toxocara* sp. were the only further parasites reported in captive wolves in Poland. While in our study *T. canis* was detected in wolves once (at very low excretion intensity), we found no *Trichuris* sp. in wolves as well as in any Canidae. Markowski (Markowski, 2013) also reported Ascarididae in wolves, though this author did not differentiate between different Ascarididae species. Compared to other carnivore species, Markowski (Markowski, 2013) reported higher prevalences of Ascarididae infections in lynxes than in wolves, which confirms our own results.
In a bulk sample of two juvenile red foxes, oocysts of *Neospora caninum* or *Hammondia* sp. were identified. The two juvenile foxes that excreted these oocysts were foundlings from the wild and were coinfected with four more parasites. One of the foxes was blind since its first day at the zoo. Although *Neospora caninum* and *Hammondia* sp. are known to produce brain and muscle cysts in dogs, this would rather result in paralysis or myopathy, and rarely blindness (Deplazes, 2013). In different carnivore species in Czeck and Slovak zoos, *Neospora caninum* was identified (Sedlak and Bartova, 2006). However, they did not examine fecal samples microscopically, but preformed immunofluorescence tests. Beck and Pantchev (2012) also reported findings of *Hammondia* sp. (as well as *Isospora* sp.) in fecal samples from foxes, although they did not specify if these foxes were wild animals or living in captivity.
Another protozoan that was found often in Canidae was *Cryptosporidium* sp. Except the Arctic fox *(Vulpes lagopus),* all Canidae species sampled were positive for *Cryptosporidium* sp. at least once. In red foxes *(Vulpes vulpes)*, prevalence was 62.5% in our investigation. A much lower prevalence (2.2%) was reported in wild red foxes in Norway (Hamnes et al., 2007).
In three samples form Canidae, first stage lungworm larvae (L_1) from *C. vulpis* as well as *A. vasorum* were detected. These findings are comparable to the results from 2009 (Weber), where *A. vasorum* in foxes and *C. vulpis* in wolves was reported. In the past, only few investigations were looking for lungworm infections in zoo carnivores (Bertelsen et al., 2010; Gurler et al., 2010; Markowski, 2013; Roman et al., 2017). Among them, only Bertelsen et al. (2010) reported several findings of different first

stage lungworm larvae. Markowski (2013) included samples from different North American carnivores in his study, but lungworm larvae were reported only in seals, which we did not sampled. Gurler et al. (2010) and Roman et al. (2017) reported the absence of lungworm larvae in all sample from carnivores examined with the Baermann-Wetzel technique. In contrast, Bindke et al. (2017) found *A. vasorum* L_1 with the sedimentation-flotation method, although this is not a sensitive method for lungworm larvae detection as the Baermann-Wetzel technique. Both, *A. vasorum* and *C. vulpis* are known to be endemic in central Europe (Barutzki and Schaper, 2009; Lurati et al., 2015). Performing ELISA, Lurati et al. (Lurati et al., 2015) reported *A. vasorum* in dogs over large areas of Switzerland. Both lungworms rely on intermediate hosts, i.e. snails and slugs, but other animals such as frogs and birds were discussed as possible intermediate or paratenic hosts too (Bolt et al., 1993; Mozzer and Lima, 2015). Since snails and slugs were likely identified as source of infection with *A. vasorum* in captive meerkats (Gillis-Germitsch et al., 2017), contamination of enclosures with intermediate hosts must be considered as source of infection in our investigation too.

6.1.3 Ursidae

In three samples from brown bears Ascarididae were identified. Thereby, *Baylisascaris* sp. as well as *Toxocara* sp. may be included (Rogers and Rogers, 1976). Although in our investigation the only Ursidae species that was found positive for Ascarididae was the brown bear, it is known that all bear species can get infected by *Baylisascaris* sp. (Perez et al., 2016; Sprent, 1968). Both, *Toxocara* sp. as well as *Baylisascaris* sp. are very important parasites, since toxocariasis is reported as one of the most frequent zoonotic helminth infection in humans worldwide (Magnaval et al., 2001). In humans, *Toxocara* spp. can cause visceral larva migrans, ocular larva migrans, neurological toxocariasis and covert toxocariasis. *Baylisascaris* sp. is feared for causing neural larva migrans in humans too (Deplazes, 2013; Kazacos et al., 2013). Due to the thick, sticky shell of Ascarididae eggs, not only feces but also contaminated enclosures may represent a potential source of infection for zoo employees and other animals in the same enclosure (Bindke et al., 2017; Bursee, 2015; Deplazes, 2013).
Another parasite that was found three times in Ursidae was *Crenosoma* sp. Weber (2009) already found *Crenosoma* sp. in brown bears twice. In our study, also in one sample of an American black bear, *Crenosoma* sp. L_1 were identified. *Crenosoma* sp. infections were also reported in wild black bears (Crum et al., 1978).
In Ursidae there was only one positive sample for *Cryptosporidium* sp. There, oocysts were at high levels in the samples of a female American black bear with her offspring. There might be a correlation between the fact that among ruminants calves are more susceptive for cryptosporidiosis than adult cows (Deplazes, 2013), and induce a high excretion intensity in our sample from the American black bear with her offspring. Compared to our study, an investigation from a zoo in Italy found a high prevalence (50%) of *Cryptosporidium* sp. infections in Asiatic black bears *(Ursus thibetanus)* (Fagiolini et al., 2010).

6.1.4 Procyonidae

In North American raccoons, *Cryptosporidium* sp. was the most common parasite. *Cryptosporidium* sp. infections were already reported in wild raccoons by Snyder (1988). There, all positive samples (prevalence 13%) were found in juvenile raccoons, while no adults were found positive. Compared to that publication, in our study one out of four *Cryptosporidium* sp. positive samples originated from an

enclosure with juvenile raccoons. One of the positive samples from adult raccoons originated from a group housing of raccoons and raccoon dogs, which are both known to be potentially infected with *Cryptosporidium* sp. (Matsubayashi et al., 2004; Snyder, 1988).
In North American raccoons also Ascarididae were identified, which may be *Baylisascaris* sp., as already described in the chapter about Ursidae. Different *Baylisascaris* species are reported in bears (*B. transfuga)*, badgers (*B. melis)* and other mammals (Sprent, 1968), but the most important *Baylisascaris* species is *Baylisascaris procyonis* of the North American raccoon, because of its high pathogenicity in humans (Beck, 2012). Not only humans can suffer from larva migrans, but *B. procyonis* is also the most common cause of clinical larva migrans in animals (Kazacos et al., 2013). We identified Ascarididae eggs in 19% of all samples from raccoons. This prevalence is much lower than the ones published by Kazacos (2008) in wild raccoons from the United States (prevalence 82%).

6.1.5 Mustelidae

In Mustelidae, we found *Crenosoma* sp. in a single sample of a European polecat *(Mustela putorius)*. To the author's knowledge, no studies about lungworms in captive polecats have been published yet. Torres et al. (2008) identified *C. melensis* with a prevalence of 17.65% in wild polecats *(Mustela putorius)* in south-western France, however there are other lungworms reported in different Mustelidae (Kollias and Fernandez-Moran, 2015; Martinez-Rondan et al., 2017), inter alia in wild Mustelidae in Switzerland (Akdesir et al., 2018). From the same polecat a follow-up examination was done eight month later, showing an even higher excretion of lungworm larvae (about 1'700 larvae per gram feces), although the polecat had been dewormed after the first finding. In contrast to the first examination not only *Crenosoma* sp., but also larvae similar to *Angiostrongylus* sp. were identified. Since it is already reported that Canidae do not represent the only definite host for *Angiostrongylus vasorum* (Gillis-Germitsch et al., 2017), further analyses would be required to characterize the larvae found. Despite intensive anthelmintic treatment, this European polecat died 1.5 month after the second examination due to a severe vasculitis wherefore a severe fungal infection was suspected at necropsy. Although a high infection of lungworms (mainly *Crenosoma* sp., but an infection with *Angiostrongylus* sp. could not be excluded), which was also documented during necropsy, is mentioned as a potential contributory factor (personal communication, Isler, S.).
In one sample from a stone marten *(Martes foina)*, Ascarididae eggs were detected, which may be *Baylisascaris* sp. as well as *Toxocara* sp. (Beck, 2012; Kollias and Fernandez-Moran, 2015).

6.1.6 Ailuridae

Lungworm infections in red pandas *(Ailurus fulgens)* were documented by Bertelsen et al. (2010), describing different lungworm larvae in fecal samples of this animal species from different European zoos. In that study the prevalence for shedding lungworm larvae was 35%; they were differentiated into three morphologically distinct types (*Crenosoma* sp. type, *A. vasorum* type and an unidentified metastrongyloid species). While they never found more than one type of L_1 in the same animal, we found *Crenosoma* sp. in the same sample with the unidentified metastrongyloid species.

6.1.7 Herpestidae

In wild Herpestidae (mongoose), *Trichuris* sp. infections were already reported in India (Acharjyo, 2004). Further parasites found in meerkats were *Capillaria* sp. and *Isospora* sp. *Capillaria* sp. in wild meerkats (Farhang-Azad and Schlitter, 1978) and coccidia infections in captive meerkats (El gayar et al., 2008; Weber, 2009) were already reported.

6.1.8 Otariidae

Although California sea lions are known to be susceptible for infections with different parasites such as *Uncinaria* sp., lungworms, *Cryptosporidium* sp. and *Giardia* sp. (Deng et al., 2000; Deplazes, 2013; Lyons et al., 2001), no parasites were identified in our study, however only one sample was examined.

6.2 Risk factors

We evaluated seven potential risk factors, whereof five (presence of juveniles, the origin of the animals, observation of clinical signs, the deworming strategy and the size of enclosure) showed statistically significant correlations with infection with different parasite species. In 1997, Malan et al. (1997) already described factors such as stress and overcrowding, resulting in significantly higher parasite burdens in wild animals. If risk factors were discussed in literature since, the examination was often limited on one specific risk factor only and did not consider several risk factors as in our investigation. Though, some investigations included further risk factors such as seasonality (Bindke et al., 2017; Bursee, 2015; Kumar et al., 2009; Panayotova-Pencheva, 2013; Peng et al., 2016), which we did not consider.
Many investigations about seasonal patterns in parasitic excretion are not easily transferable to Switzerland. Studies which identified positive correlations were either conducted with wild animals (Bryan et al., 2012) or were not carried out in central Europe, but in India (Kumar et al., 2009) or China (Peng et al., 2016), where seasons are shaped by the seasonal monsoon or other extreme weather conditions. Investigations that were conducted in central Europe did not found significant correlations between seasonal patterns and parasitic excretion (Bindke et al., 2017; Panayotova-Pencheva, 2013).

Juveniles present
Several investigations already looked for a correlation between age of the examined animals and parasitic infections. While Bursee (2015) reported no difference between juveniles and adult animals, Singh et al. (2006) reported juvenile tigers (< 18 month) excreting significantly more often parasites (mostly Ascarididae) than adults. They explained their results by a less developed immune system in young animals. In our investigation, juveniles showed a statistically significant higher excretion with *Toxocara* sp./unidentified Ascarididae species too, which is also reported in North American raccoons by Kazacos et al. (2008). These correlations between juvenile animals and *Toxocara* sp. is not unexpected, since juvenile animals get already infected prenatally (*T. canis)* or during the suckling period (*T. canis, T. mystax),* and after a prepatent period of at least 21 days these juveniles will excrete *Toxocara* sp. eggs. in feces. In *T. canis* the female does not even have to be patent, since hypobiotic larvae can get activated during pregnancy (Deplazes, 2013).
Opposite results were reported by Varadharajan and Subramaniam (2003). They reported adult animals to have more parasites than juveniles (< 1 year) but only in omnivores and herbivores. Modi et al. (1997) came to the same results, also in carnivores: they reported parasites in 57.18% of all samples from adult carnivores, compared to 18.75% in juveniles. A possible explanation for these findings could be better care and attention on juveniles and more frequent and/or prophylactic deworming (Varadharajan and Subramanian, 2003). Also differences in lifestyle and different housing systems of juvenile animals in zoos could play a role for different prevalences in parasitic infections.
In consideration of this results, it is not surprising that the prevalence of *Cryptosporidium* sp. infections was significantly higher in juveniles, since cryptosporidiosis in known to be a disease of juveniles and *Cryptosporidium* sp. are not eliminated with usual deworming treatments (Deplazes, 2013). In our study, 9 out of 24 *Cryptosporidium* sp. positive samples originated from juveniles or groups where juveniles were living in. In total, almost 50% of all samples from juveniles or from groups with contact to juveniles (n= 19) were positive for *Cryptosporidium* sp.

Clinical signs observed
There were only few animals showing clinical manifestations. As told from a zoo veterinarian, in some animals it is very difficult to observe symptoms of any disease. Apart from obvious diarrhea, other symptoms such as changes in behavior can be challenging to observe, for example in Mustelidae. If

health status was mentioned in publications, most of the time animals were classified as healthy (Beck et al., 2011; Ramos, 2014; Velante et al., 2017). In our investigation in *Cryptosporidium* sp., *Toxocara* sp./unidentified Ascarididae and *A. vasorum* positive animals a significant correlation between infection and clinical signs was found. Though, only gastro-intestinal symptoms, and no respiratory problems were observed in examined animals. The author of another publication about Ascarididae in different felids concluded that health problems associated with Ascarididae infections occurred only in a few individual cases (Bursee, 2015).

Size of the enclosure

The effect of the size of enclosures on the occurrence of parasitic infections was previously reported in a publication from Atanaskova et al. (2011). They divided the animals into three groups according to the type of enclosures (group one: indoor enclosures; group two: outdoor enclosures with open soil; group three: semi-open enclosures, where the animals were closed in cages during the cold seasons, but were free to go outside for the rest of the year). Group three, including wolves and brown bears, showed the lowest rate of infection. Because the deworming management in different groups varied too, Atanaskova et al. (2011) was not able to draw detailed conclusions. Nevertheless, it is still probable that type and size of the enclosure may have an influence on the parasitic burden of the animals, since the confined areas zoo animals live in are subject to high environmental contamination.

In wolves, a significant correlation between large enclosures and a higher number of hookworm egg detection was reported (Szafranska et al., 2010). This was not validated with our investigation as we found only a significant correlation of *G. duodenalis* infections with large enclosures. However, some other correlations must not be ignored. For example, a connection between lungworm infections in carnivores and water points in or around their enclosure is suggested, because lungworms such as *A. vasorum* and *Crenosoma* sp. rely on snails as intermediate hosts (Deplazes, 2013). Other parasites that do not rely on intermediate hosts, such as Ascarididae, may benefit from outdoor enclosures with open soil, since they are resistant to environmental influences (Deplazes, 2013).

Deworming strategy

The fact that we found a higher number of *T. leonina* infections in animals that are dewormed regularly (but without fecal examination) appears incoherent, and for instance it is published that the risk of *T. mystax* infection in cats drops significantly the more often cats are dewormed (Peng et al., 2016).

We should mention that *T. leonina* infections were most likely in lions. Lions and other Pantherinae (big cats) are more common in bigger zoos, which often have fixed deworming strategies, too. This may have led to the point that no *T. leonina* was found in zoos without regular deworming systems.

Ascarididae infections in general are reported to be under control in several zoos, but it seems very difficult to eliminate them (Atanaskova et al., 2011; Barutzki et al., 1985; Peng et al., 2016). Investigations in which repeated coproscopic analysis were performed showed that infections with Ascarididae could not get eliminated (Ahasan et al., 2010; Barutzki et al., 1985; Bursee, 2015). In contrast, Singh et al. (2006) reported that Ascarididae excretions in lions were stopped effectively seven days after treatment. However, reapparition of *T leonina* eggs in fecal samples was observed already 30 days post treatment. Therefore, it could be argued that in the other investigations (mentioned above) Ascarididae excretion was possibly eliminated too, but at time of retesting (three to six or even 12 month later) animals may have already been reinfected through environmental contamination with infectious stages.

In Germany, a significant correlation between anthelmintic treatment and a lower probability of hookworm egg detection in wolves was reported (Bindke et al., 2017), but could not be confirmed with our results due to small sample size.

Origin of the animals

In most of the studies animals were reported to be born in zoos (Aviruppola et al., 2016; Beck et al., 2011), or it was not differentiated between wild born animals and animals born in captivity. Because 12.0% of the animals sampled in our investigation were born in the wild, we differentiated between wild born animals and animals born in captivity. Apart from two animals, all wild born animals were likely born in Switzerland and were taken in as foundlings. Therefore, one can expect most carnivores in zoos in Switzerland to be infected with the same parasite spectrum that is common in central Europe (Bursee, 2015).

A possible explanation for the significant correlation between parasite infection with *Cryptosporidium* sp. and *Toxocara*/*Baylisascaris* sp. and the origin of the animals could be that 44.4% of all animals that were classified as „born in the wild" were juveniles, or bulk samples with juveniles in the enclosure (compared to 12.1% juveniles in animals that were born in captivity). *Cryptosporidium* sp. as well as *Toxocara*/Baylisascaris sp. infections were already reported to be more likely if juveniles are present in the enclosures (see „Juveniles present").

6.3 Limitations and conclusion

Because of the limitations given by extent of a master thesis and the large number of analysed samples, in contrast to other investigations all animals were examined only once and no continuous follow-up was possible. Another limitation may be that 75.2% of all analysed samples consisted of feces from one day only, although it is known that many parasites shed eggs, oocysts or larvae in batches (Deplazes, 2013). While most of the other investigations had the same limitations, Roman et al. (2017) managed it differently and examined feces from three consecutive days.
In addition, some animals were dewormed shortly before taking fecal samples. Though, no correlation between a short time since last deworming and a low prevalence for parasite infection could be drawn. Identification of eggs and oocysts was not always unambiguous. While *Heterakis* sp. and *Aspiculuris tetraptera* eggs were identified as intestinal passages or environmental contamination, it was not possible to make definitive statements on eggs from hookworms, *Capillaria* sp., *Trichuris* sp. and oocysts from *Isospora* sp. This would require further analyses.

In contrast to most of other previously performed investigations, it should be noted that we found numerous different parasite species. We examined a big sample size, and samples were collected from 27 different locations all over Switzerland. This raises the question whether different zoos may have individual spectra of parasite species. Fagiolini et al. (2010) examined animals (not only carnivores) from two different zoos in Italy and found the difference in helminth infection (overall animal orders) between the two zoos to be significant. However, in carnivores no significant difference between the two zoos was observed.
Geographical location is certainly another relevant factor for different results in similar investigations from other countries. Zoo animals get transported to other zoos across long distances, but transported animals should be placed in quarantine first (Fagiolini et al., 2010) and get adequate deworming. Otherwise this would represent an important way of parasite introduction (Atanaskova et al., 2011; Markowski, 2013). Fortunately, some parasites need appropriate climatic conditions for their successful establishment, but this fact can also lead to problems, for example in snow leopards *(Panthera uncia)*: In their natural environment, snow leopards probably have a low risk of parasitic infection. In warmer climate zones like central Europe, they may be more vulnerable to infectious diseases in general than other leopards *(Panthera pardus)* (Bursee, 2015; Worley, 1982). However, in an investigation from Bursee (2015) with 21 samples from different leopards, this assumption could not be confirmed. Because of the small sample size in our investigation (4 samples from snow leopards and 6 from other leopards), no statistically significant conclusion can be revealed. Though, it can be mentioned that parasites were found in every sample from snow leopards, while four out of six samples from other leopards were negative for any parasite. Further, *T. mystax*, *T. leonina* and *Cryptosporidium* sp. were found in snow leopards only and the average excretion intensity of parasitic infection was higher in snow leopards than in other leopards. Other leopards were positive for *Heterakis* sp., *Capillaria* sp. and *Isospora* sp., which may all represent intestinal passages only.

Comparing our results to those from other investigations, changes over time must be considered as well. Animals in zoos are no longer housed in cages like several years ago. They often live in outdoor enclosures with natural ground material which could represent perfect development conditions for diverse parasites (Bursee, 2015).
Therefore, it surprises that Vollrath (2010) could demonstrate a steady decrease of intestinal parasites between 1951 and 2001 on the basis of 522 samples from tigers kept in a zoo in Leipzig. Other investigations also evaluated changes over time and found similar (Bursee, 2015) but also contrary

(Markowski, 2013) results. The comparison of the two master theses shows that between 2009 (Weber) and 2017/18 the prevalences of samples that were positive in at least one of the three methods conducted in both investigations (combined sedimentation-flotation technique, Baermann-Wetzel technique and Ziehl-Neelsen staining) remained the same (51.0% in 2017/2018 and 51.4% in 2009). Despite equal prevalences for parasitic infections, more mixed infections were detected in the current study. In 2017/18, on average 0.87 different parasites were detected per sample (129 findings in 149 samples). In the investigation from Weber (2009) on average 0.75 different parasites were detected per sample (105 findings in 140 samples).

In conclusion, we can summarize that several different parasite species were found in carnivores in zoos in Switzerland in 2017/18, whereby various parasites are known to be zoonotic. The prevalence of infections is partially comparable to the situation in Switzerland nine years ago and to other investigations. Though, to the author's knowledge, our investigation was the first finding of some parasites that were reported in wild animals already (lungworms in a European polecat, *Cryptosporidium* sp. in American black bears). Additionally, some potential risk factors (juvenile animals, wild born animals, a regular deworming strategy without fecal examination, large enclosures and the observation of clinical signs) for parasitic infections were identified.

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The research was conducted in absence of any conflict of interest.

9 Annex

9.1 Zoos sampled

Abenteuerland Walter Zoo
9200 Gossau (SG)

Johns kleine Farm
3283 Kallnach (BE)

Knies Kinderzoo
8640 Rapperswil-Jona (SG)

La Garenne
1261 Le Vaud (VD)

Papiliorama
3210 Kerzers (FR)

Parc Challandes
1293 Bellevue (GE)

Plättli Zoo
8500 Frauenfeld (TG)

René Strickler's Raubtierpark
4553 Subingen (SO)

Siky Ranch
2746 Crémines (BE)

Tierpark Biel
2504 Biel-Bienne (BE)

Tierpark Dählhölzli
3005 Bern (BE)

Tierpark Goldau
6410 Goldau (SZ)

Tierpark Lange Erlen
4058 Basel (BS)

Toni's Zoo*
6023 Rothenburg (LU)

Wildnispark Zürich
8135 Langnau am Albis (ZH)

Wildpark Bruderhaus
8400 Winterthur (ZH)

Wildpark Peter und Paul
9010 St. Gallen (SG)

Zoo al Maglio
6983 Magliaso (TI)

Zoo Basel
4054 Basel (BS)

Zoo de Servion
1077 Servion (VD)

Zoo des Marécottes
1923 Les Marécottes (VS)

Zoo du Bois de Petit Château
2300 La Chaux-de-Fonds (NE)

Zoo Hasel
5236 Remigen (AG)

Zoo Zürich
8044 Zürich (ZH)

*only partially sampled, no further information about animals in this zoo.

9.2 Sample letter for the communication of the results to the zoos: in German and French

Resultate der Kotuntersuchung: Musterbeispiel
Datum der Probenerhebung:

Methoden

Kombinierte Sedimentation-Flotation
Dieses Verfahren wird angewendet, um ein breites Spektrum an Helmintheneiern (Wurmeier) oder Zysten von Protozoen (Einzeller) nachweisen zu können.

Baermann-Trichter
Mittels des Baermann-Trichters können noch lebende Lungenwurmlarven in Kotproben nachgewiesen werden. Diese Methode ist sehr sensitiv.

Modifizierte Ziehl-Neelsen Färbung
Diese Färbung wird durchgeführt, um Cryptosporidien Oocysten sensitiver nachzuweisen, als im kombinierten Sedimentation-Flotations-Verfahren.

Giardia-Antigen ELISA
Da die Test-kits idealerweise gleichzeitig mit einer grossen Anzahl Proben angewendet werden, wird dieser Test erst am Ende der Probenerhebung, mit eingefrorenen Kotproben aller Zoos durchgeführt. Die Resultate dieser Untersuchung werden Ihnen nachträglich mitgeteilt, voraussichtlich im Frühjahr 2018.

Proben

SP = Gehege-Sammelprobe; EP = individuelle Einzelprobe

Luchs (SP)
Wolf (SP)

Probe	Sedimentation-Flotation	Baermann-Trichter	Ziehl-Neelsen Färbung
Luchs (SP)	Toxocara mystax + Ancylostomatidae (+)	-	
Wolf (SP)	Ancylostomatidae + Capillaria sp. (+)	Angiostrongylus (68 Larven/10g Kot)	-

Befallsintensität: (+) sehr gering; + gering; ++ mittel; +++ hoch; ++++ sehr hoch;

Interpretation

Luchs

- **T*oxocara mystax***

  Toxocara mystax gehört zur Familie der Askariden, welche auch als „Spulwürmer“ bezeichnet werden. *Toxocara mystax* ist eine potentielle Zoonose. Wenn Menschen infektiöse, larvenhaltige Eiern aufnehmen, können die Larven in verschiedene Gewebe und Organe einwandern und dabei Schäden verursachen (Larva migrans visceralis oder ocularis).
  Die adulten Würmer leben im Dünndarm von Feliden. Diese scheiden über den Kot Eier aus, welche dank ihrer dicken Eihülle in feuchter Umgebung bis zu einem Jahr überleben können. Die Wirtstiere infizieren sich, indem sie diese Eier peroral aufnehmen. Infizierte Muttertiere können ihre Welpen auch direkt über die Milch mit *Toxocara mystax* infizieren.
  Bei einem sehr starken Befall mit *Toxocara mystax* kann es zu Erbrechen, breiigem Kot und Abmagerung kommen.

- ***Ancylostomatidae***

  Ancylostomatidae werden auch als Hakenwürmer bezeichnet. Für Carnivoren von Bedeutung sind die Gattungen *Ancylostoma* und *Uncinaria*. Diese sind potentielle Zoonosen, das heisst sie können auch den Menschen infizieren. Dabei kann es durch wandernde Larven zu Hautproblemen kommen.
  Caniden und Feliden können sich mit Hakenwürmer infizieren, wenn sie infektiöse Larvenstadien per os aufnehmen, befallene Zwischenwirte (wie z.B. Mäuse) fressen oder indem sich die Larven direkt durch die Haut in ihre Wirtstiere bohren. Bei *Ancylostoma caninum* können sich Welpen zusätzlich direkt über die Muttermilch infizieren.
  Meistens verläuft ein Hakenwurmbefall symptomlos, aber bei starkem Befall v.a bei Jungtieren kann es zu schleimigem oder blutigem Durchfall kommen.

Mögliche Therapie gegen *Toxocara mystax* und *Ancylostomatidae* Befall:

- Fenbendazol (Panacur®) 50mg/kg KGW, täglich während 3 Tagen
- Flubendazol (Flubenol®) 22mg/kg KGW, täglich während 3 Tage
- Milbemycinoxim (Milbemax®) 0.5mg/kg KGW p.o. einmalig

Zusätzlich ist es wichtig, auf gute Hygiene zu achten. Um die Umweltkontamination mit Eiern möglichst gering zu halten, sollte Kot regelmässig entfernt werden.

Wolf

- ***Ancylostomatidae***
  Siehe Luchs

- ***Capillaria* sp.**
  Capillaria sp. kommen bei vielen verschiedenen Tierarten vor und können in verschiedenen Organsystemen parasitieren. Dazu gehören die Harnblase, die Lunge, der Magen und der Dünndarm. Ein Befall mit *Capillaria* verläuft jedoch meist asymptomatisch.
  Wenn man *Capillaria* Eier im Kot von Carnivoren findet, muss dies nicht beweisend für eine Infektion sein. Wenn untersuchte Tiere zuvor einen infizierten Nager gefressen haben, können *Capillaria* Eier im Darm überleben und werden nach einer Magen-Darmpassage über den Kot ausgeschieden.
  Mögliche Therapie:
    - Mebendazol (Telmin®) 12.5mg/kg KGW, einmalig
    - Fenbendazol (Panacur®) 25mg/kg KGW, einmalig

- ***Angiostrongylus vasorum***
  Angiostrongylus vasorum ist ein Lungenwurm, der bei Caniden vorkommt. Er lebt in den Lungenarterien und im rechten Herzen. Seine Larven wandern durch die Lunge, die Luftröhre hinauf, werden abgeschluckt und anschliessend mit dem Kot ausgeschieden. Caniden infizieren sich, indem sie befallene Schnecken fressen, welche als Zwischenwirte benötigt werden und welche sich durch die Aufnahme von Erstlarvalstadien aus Canidenkot infiziert haben.
  Befallene Tiere können Symptome zeigen wie Husten, Lungenentzündung und weitere respiratorische Probleme, aber auch Blutungsstörungen oder unspezifische Symptome wie mangelnder Appetit und Abmagerung. Aus diesem Grund und um den Infektionsdruck durch die im Kot ausgeschiedenen Stadien zu senken, ist eine Behandlung empfohlen.
  Mögliche Therapie:
    - Fenbendazol (Panacur®) 25mg/kg KGW, täglich während 3 Wochen
    - Milbemycinoxim (Milbemax®) 0.5mg/kg KGW, 4x in wöchentlichem Abstand

Auch bei den Wölfen spielt regelmässige Kotentfernung eine wichtige Rolle. Zusätzlich sollte man wegen dem Befall mit Lungenwürmern eine gute Schneckenbekämpfung in Erwägung ziehen.

Danke für Ihre Teilnahme an diesem Projekt!

Résultates de l'analyse des selles: Modèle
Date de prélèvement d'un échantillon de selles:

Méthodes

Méthode combinée sédimentation-flottation
Cette méthode est usée pour prouver un large spectre des oeufs de ver et des oocystes protozoaires.

Méthode de Baermann
Avec la méthode de l'entonnoir de Baermann on peut prouver des larves vivantes de ver pulmonaire dans les excréments. Cette méthode est très sensitive.

Coloration de Ziehl-Neelsen modifié
Cette coloration est appliquée pour prouver des oocystes des Cryptosporidies, parce que elle est plus sensitive que la méthode combinée sédimentation-flottation.

Giardia-Antigen ELISA
A cause du test-kit on fait ce test idéalement en même temps avec un grand nombre d'échantillons. C'est pourquoi je ne fais ce test qu'à la fin de la période d'analyse, avec les échantillons gelés de touts les zoos. Je vais probablement vous communiquer le résultat de cette analyse qu'au printemps 2018.

Echantillon

EG = Echantillon groupé ; ES = Echantillon simple

Echantillon	Méthode combinée sédimentation-flottation	Méthode de Baermann	Coloration de Ziehl-Neelsen modifié
Loup (ES)	-	-	-
Lynx (EG)	-	-	-
Ours 0.1.1 (EG)	-	-	*Cryptosporidium* sp. +++
Ours 1.0.0 (ES)	-	*Crenosoma* sp. (1 larva dans 10g de selle)	-

Taux d'infestation: (+) très faible; + faible; ++ moyenne; +++ forte; ++++ très forte;

L'interprétation

Ours 1.0.0

- ***Crenosoma sp.***

  Crenosoma sp. est un nématode pulmonaire qui vit dans les bronches et dans la trachée et qui apparaît surtout chez les canoidea. Les larves passent la trachée, sont avalés et ensuite excrété. Les canoidea s'infectent en mangeant des escargots contaminés, les hôtes intermédiaires des larves.
  Si l'envahissement par les *Crenosoma* sp. est trop fort, les canoidea peuvent souffrir comme conséquence d'une pneumonie et/ou d'une toux.
  Une thérapie possible pour *Crenosoma* sp.:

  - Fenbendazol (Panacur®) 50mg par kg de poids corporel, par voie orale, une fois par jour et ceci pour trois jours.
  - Milbemycinoxim (Milbemax®) 0.5mg par kg de poids corporel, par voie orale et une seule fois.

Ours 0.1.1

- ***Cryptosporidium* sp.**

  Les *Cryptosporidium* sp. sont des protozoaires, qui se trouvent dans beaucoup de differentes espèces animales. L'homme peut être infecté lui aussi par des *Cryptosporidium* sp., mais normalement ce n'est pas un problème, non plus pour les animaux adultes. Seulement les animaux juvéniles peuvent souffrir de la diarrhée et de l'amaigrissement.
  Pour les carnivores il n'existe pas de thérapie contre *Cryptosporidium* sp. Si les animaux montrent des symptômes ou si l'envahissment est très fort, on ne peut que prendre soin d'une bonne hygiène, par exemple d'enlever régulièrement les excrément.

Merci beaucoup d'avoir participé à cette étude!

9.3 Figures of parasites

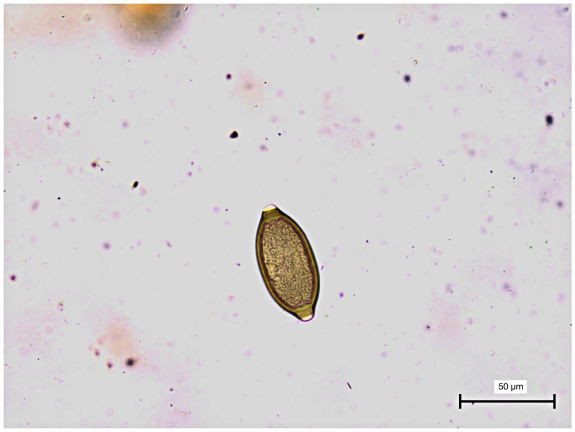

Figure 7: *Trichuris* sp. egg in a bulk sample of lynxes (*Lynx* sp.), size 64 µm x 30 µm.

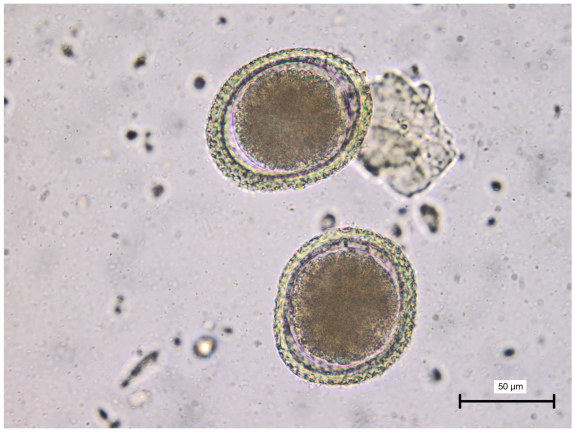

Figure 8: *Toxocara canis* eggs in a bulk sample of juvenile red foxes *(Vulpes vulpes)*, size 91 µm x 74 µm and 85 µm x 75 µm.

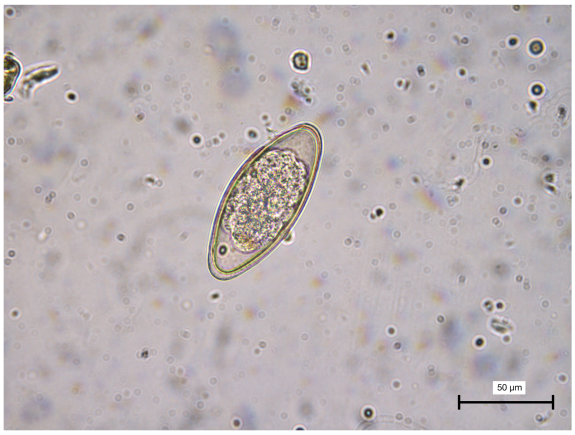

Figure 9: *Aspiculuris tetraptera* egg in a single sample of a Asian small-clawed otter *(Aonyx cinerea)*, size 96 µm x 41 µm.

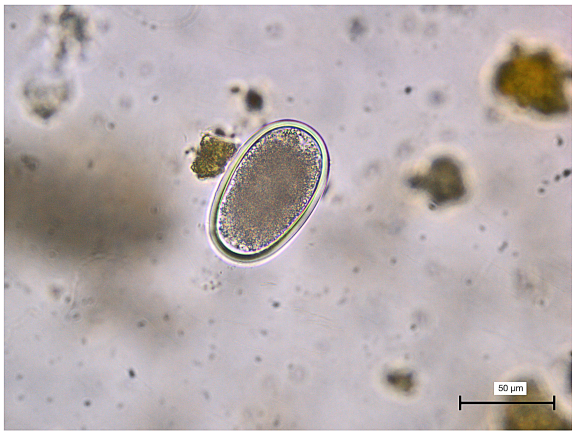

Figure 10: *Heterakis* sp. egg in a bulk sample of leopards *(Panthera pardus)*.

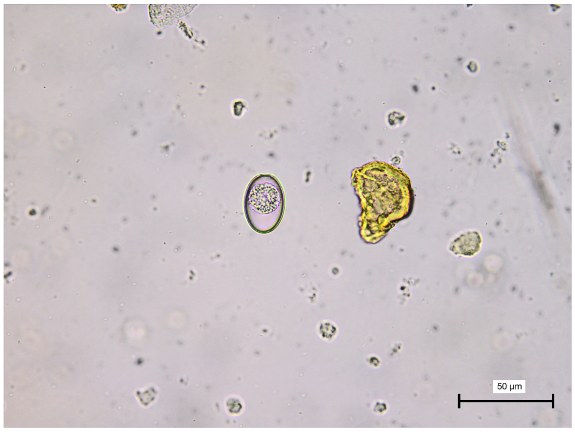

Figure 11: *Isospora* sp. oocyst in a single sample of a leopard *(Panthera pardus)*, size 33 µm x 22 µm.

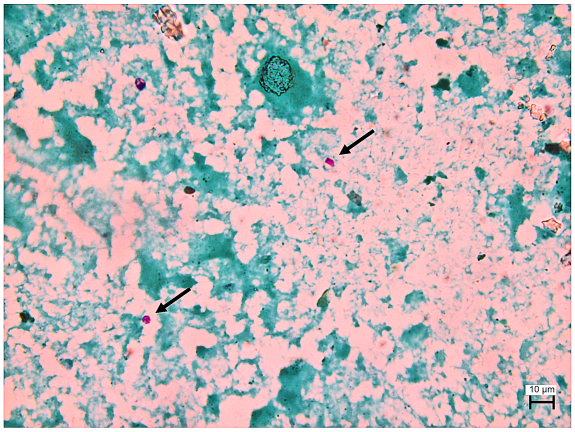

Figure 12: *Cryptosporidium* sp. oocysts in a bulk sample of snow leopards *(Panthera uncia)*, size 4 µm x 4 µm and 5 µm x 4 µm.

9.4 Eigenständigkeitserklärung

Ich erkläre hiermit,
- dass ich die vorliegende Arbeit ohne unlautere fremde Hilfe und ohne Verwendung anderer als der angegebenen Hilfsmittel verfasst habe,
- dass ich sämtliche verwendeten Quellen erwähnt und gemäss gängigen wissenschaftlichen Zitierregeln nach bestem Wissen und Gewissen korrekt zitiert habe.
- dass ich alle Originaldaten vollständig und wahrheitsgetreu wiedergegeben habe.

Riehen, 29.08.2018

Ronja Zuber