DOI: 10.2478/s11686-014-0249-8

Acta Parasitologica, 2014, 59(2), 343–353; ISSN 1230-2821

VERSITA

Parasitic infections detected by FLOTAC in zoo mammals from Warsaw, Poland

Gianpaolo Maesano, Michele Capasso, Davide Ianniello, Giuseppe Cringoli and Laura Rinaldi*

Unit of Parasitology and Parasitic Diseases, Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Via della Veterinaria 1, 80137, Naples, Italy,
CreMOPaR. Centro Regionale per il Monitoraggio delle Parassitosi. Strada Statale, Località Borgo Cioffi, 84025, Eboli (SA)

Abstract

The aim of this study was to estimate the occurrence of intestinal parasites in groups of mammals kept in the Warsaw zoological garden (Poland). 71 pools of fecal samples were analyzed using the FLOTAC techniques. 48% of animals were positive and 47% of positivities showed multiple infections. *Toxocara cati* (71.4%) was found in felines; marsupials were infected with Coccidia (90%). *Giardia* spp. (24.0%), *Blastocystis* spp. (12.3%), *Iodamoeba* spp. (10.0%), *Enterobius vermicularis* (6.0%) and *Entamoeba coli* (3.3%) were found in primates. Gastrointestinal strongyles (60.5%) were prevalent in ruminants which resulted positive also to Coccidia (Eimeria spp. = 50.0%), *Trichuris* spp. (25.0%) and *Nematodirus* (14.0%). *Strongyles* (34.0%) were the most frequent parasites in monogastric herbivores, followed by *Parascaris equorum* (17.0%). None of the animals showed any symptom associated with gastrointestinal parasitic infections. According to our results the need to prevent, diagnose, control, and treat intestinal parasitism trough specific control programs is mandatory for animal welfare in order to limit the spread of parasitic infections in animals and humans.

Keywords

FLOTAC, intestinal parasites, mammals, zoo

Introduction

Nowadays, Zoological Gardens exist to protect animal endangered species and to evaluate the need of protecting the biodiversity. It appears therefore necessary to gain an accurate and updated knowledge of every kind of disease that can affect wild and exotic animals when in captivity (Mitchell *et al.* 2009). Animals held in crowded cages and in limited environments for long time are more susceptible to parasitic infections. The diagnostic methods traditionally used are unable to detect low parasitic infections which are usually diagnosed only when massive and clinically detectable. In addition, the frequency of infections varies in relation to the type of breeding, prophylaxis and treatments; also hygiene of enclosures and of food, play a fundamental role for the development of endoparasites (Lim *et al.* 2008). Furthermore, the use of anthelmintics in sub-optimal dose or the frequent and continuous use of a single drug have contributed to the widespread development of drug-resistant helminthes (Shalaby Hatem 2013). The successful implementation of control programs against helminthes and protozoa depends on the availability of effective and sensitive methods for detection and monitoring of parasitic infections. Despite many *in vivo* and *in vitro* tests have been designed to limit the development of resistance in nematode populations, eventually they suffered to some degree from reliability, reproducibility, sensitivity and ease of interpretation (Taylor *et al.* 2002). The present cross-sectional copromicroscopic survey attempts to evaluate the occurrence of gastro-intestinal parasites in wild mammals (felines, hoofed mammals, marsupials and primates) kept in captivity, using FLOTAC (Cringoli *et al.* 2010) for the first time in zoo animals.

Materials and Methods

Study site and animals

The samples analyzed in the present study were collected at the Zoological Garden "Ogród Zoologiczny " in Warsaw (Poland), located in one of the parks next to the city center. The zoo extends over a 40 ha area and hosts a collection of over 4,000 autochthonous and exotic species of mammals, birds, reptiles, amphibians and fishes. Over the years, the zoo

*Corresponding author: lrinaldi@unina.it

Table I. Number of animals and pools examined, parassitological results (number of positives pools and %).

Species	N[a]	N [b]	Genus/Group of Helminthes N[b] pos (%)[c]		Genus/Group of Protozoa N[b] pos (%)			
Carnivores	8	5	Ascarids 2 (40.0%)	–	–	–	–	–
Jaguar (*Pantera onca*)	1	1	–	–	–	–	–	–
Lion (*Panthera leo*)	6	3	*Toxocara* 2 (66.7%)	–	–	–	–	–
Sumatra tiger (*Panthera tigris sumatrae*)	1	1	–	–	–	–	–	–
Marsupials	15	3	–	Coccidia 2 (66.7%)	–	–	–	–
Wallaby (*Macropus rufogriseus*)	6	1	–	–	–	–	–	–
Red kangaroo (*Macropus rufus*)	9	2	–	2 (100%)	–	–	–	–
Primates	100	20	Oxyurids 3 (15.0%)		*Giardia* 3 (15.0%)	*Blastocystis* 2(10.0%)	*Entamaeba coli* 3(15.0%)	*Iodamoeba* 1(5.0%)
Gorilla (*Gorilla gorilla*)	4	2	–	–	1 (50.0%)	1(50.0%)	–	–
Ring tailed lemur (*Lemur catta*)	5	2	–	–	–	–	–	–
Ruffed lemur (*Varecia variegata*)	5	1	–	–	1(100.0%)	1(100.0%)	1(100.0%)	1 (100.0%)
Black macaque (*Macaca nigra*)	5	1	–	–	–	–	–	–
Capuchin monkey (*Cebus apella*)	5	1	–	–	1(100.0%)	–	1(100.0%)	–
Diana monkey (*Cercopithecus diana*)	5	1	–	–	–	–	1(100.0%)	–
Squirrel monkey (*Saimiro sciureus*)	5	1	–	–	–	–	–	–
Allen's swamp monkey (*Allenopithecus nigroviridis*)	5	1	–	–	–	–	–	–
Chimpanzee (*Pan troglodites*)	61	11	*Enterobius* 3 (27.3%)	–	–	–	–	–

N[a] = number of animals examined. N[b] = number of pools examined. N[b] pos= number of positive pools (%)[c]= percentage of positive pools

Table II. Number of animals, pools and parasitological results (number of positives and %)in groups of ruminants

Species	N[a]	N [b]	Genus/Group of Helminthes N[b] pos (%)[c]			Genus/Group of Protozoa N[b] pos (%)
			Trichuris	GIN[d]	*Nematodirus*	Coccidia
Ruminants	114	29	6 (20.1%)	14(48.3%)	4(13.8%)	12 (41.4%)
Bongo(antelope) (*Tragelaphus eurycerus isaaci*)	6	1	–	1(100.0%)	–	1 (100.0%)
American buffalo (*Bison bison*)	9	2	–	1 (50.0%)	–	–
European buffalo (*Bison bonasus*	9	2	–	1(50.0%)	–	1 (50.0%)
Muskox (*Ovibos moschatus*)	5	1	–	–	–	–
Yak (*Bos grunniens*)	4	2	–	–	–	–
Camel (*Camelus bactrianus*)	9	2	2 (100.0%)	–	–	–
Pygmy goat (*Capra hircus hircus*)	5	1	1 (100.0%)	1(100.0%)	–	1 (100.0%)
Somali goat	10	2	1 (50.0%)	2 (100.0%)	–	2 (100.0%)
Sika deer (*Cervus Nippon*)	6	2	–	1 (50.0%)	–	–
Fallow deer (*Dama dama*)	11	3	1 (33.3%)	3 (100.0%)	–	–
Gazelle (*Oryx gazelle*)	3	1	–	–	–	–
Llama (*Lama glama*)	11	2	1 (50.0%)	2 (100.0%)	–	11 (100.0%)
Barbary sheep (*Ammotragus lervia*)	9	2	–	2 (100.0%)	2 (100.0%)	2 (100.0%)
Mountain sheep (*Ovis aries*)	2	1	–	–	1 (100.0%)	1 (100.0%)
Sitatunga antelope (*Tragelaphus spekei gratus*)	5	2	–	–	–	2 (100.0%)
Vicugna (*Vicugna vicugna*)	10	2	–	–	1 (50.0%)	1 (50.0%)

N[a] = number of animals examined. N[b] = number of pools examined. N[b] pos = number of positive pools (%)[c]= percentage of positive pools. GIN[d] = Gastrointestinal nematodes

has equipped with adequate facilities for animals that poorly tolerate the continental temperatures still ensuring in most cases adequate spaces similar to the environment of origin. Most exhibitions are organized to guarantee optimum usage of available space, avoiding as possible iron cages in order to reproduce the natural environment using dust, soil, wood, grass and endemic vegetation. At night felines, hippopotamus and other dangerous animals are taken into cages made of concrete with stonewalls and surrounded by iron railing. Those cages are cleared daily with high-pressure pipe water and in true-to-nature exhibits dung is removed at need. At the time of the study a moderate crowding was present in the stands of macaques, housed in temporary cages, pending the completion of the new facilities.

From March to June 2011, 71 composite samples (pools) of feces were collected from different animal species at the zoo by fresh deposit. The animals were divided into the following groups: carnivores, marsupial, primates, ruminants and monogastric herbivores. Table I, Table II and Table III list the animal species included in the study along with the number of subjects and samples analyzed.

Fecal sampling

The species in the zoo were sampled with the assistance of the animal's handlers. The sampling was conducted in early morning (7:00/7:30 A.M.) gathering the apical region of the feces deposited on the ground. Possible contamination was avoided

Table III. Number of animals, pools and parasitological results (number of positives and %)in groups of monogastric herbivores

Species	N[a]	N [b]		Genus/Group of Helminthes N[b] pos (%)[c]	Genus/Group of Protozoa N[b] pos (%)
			GIN[d]	Ascarids	
Monogastric herbivores	23	14	3 (21.4%)	1 (7.1%)	–
Donkey (*Equus asinus asinus*)	5	2	1 (50.0%)	–	–
Przewalsky horse (*Equus przewalskii*)	4	1	1 (100.0%)	1 (100.0%)[e]	–
African Elephant (*Loxodonta Africana*)	4	4	–	–	–
Hippopotamus (*Hippopotamus amphibious*)	1	1	–	–	–
Pony Shetland (*Equus caballus*)	5	2	–	–	–
Indian rhinoceros (*Rhinoceros unicornis*)	2	2	–	–	–
Zebra (*Equus burchelli boehmi*)	2	2	1 (50.0%)	–	–

N[a] = number of animals examined. N[b] = number of pools examined. N[b] pos = number of positive pools (%)[c]= percentage of positive pools. GIN[d] = Gastrointestinal nematodes. [e] = *Parascaris equorum.*

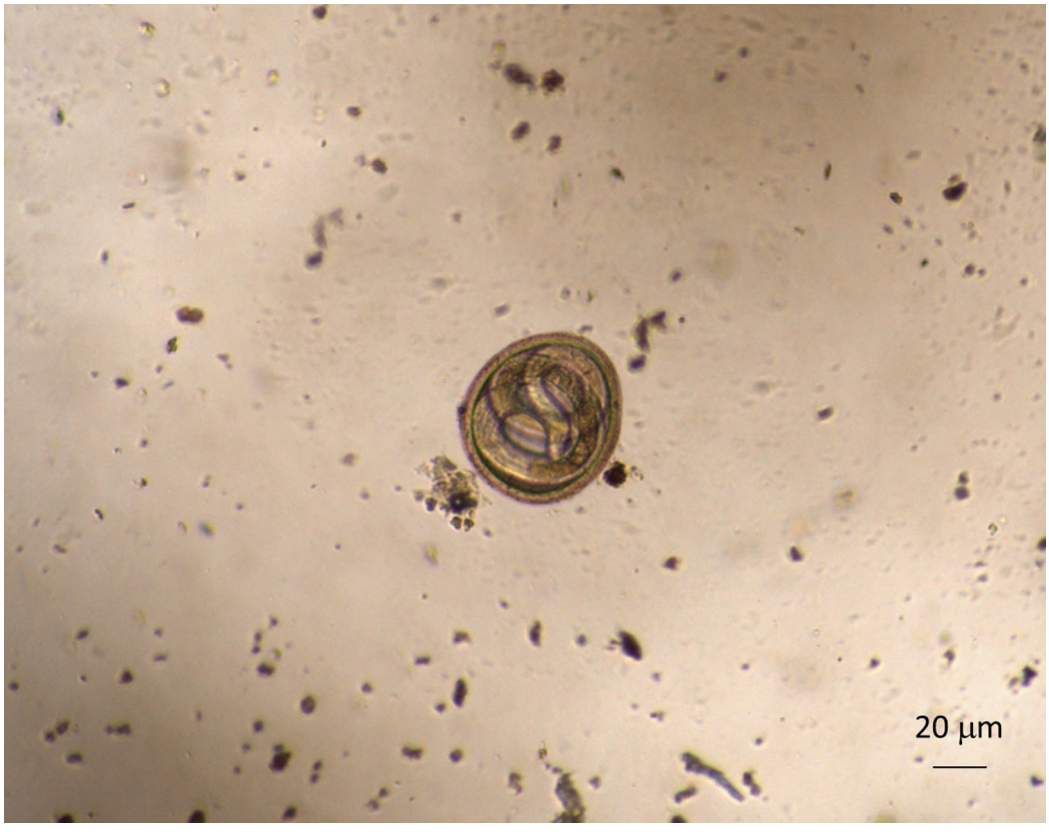

Fig. 1. Egg of *Toxocara cati* in a lion

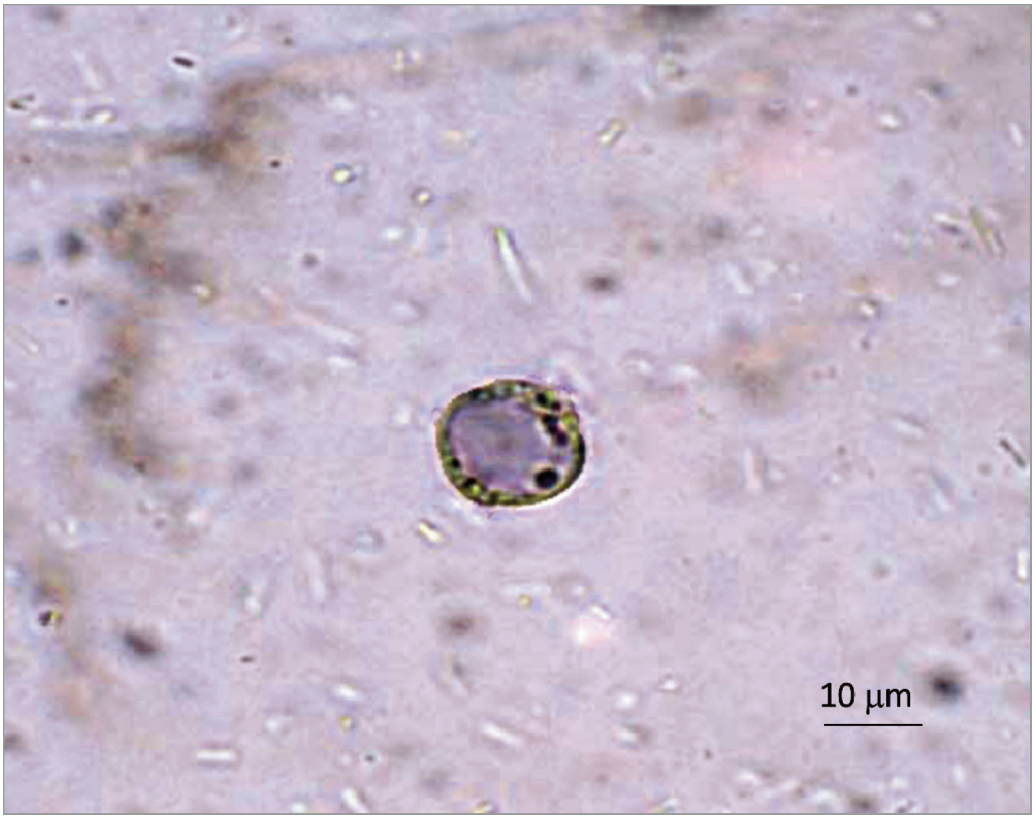

Fig. 2. Cyst of *Blastocystis* spp. in a gorilla

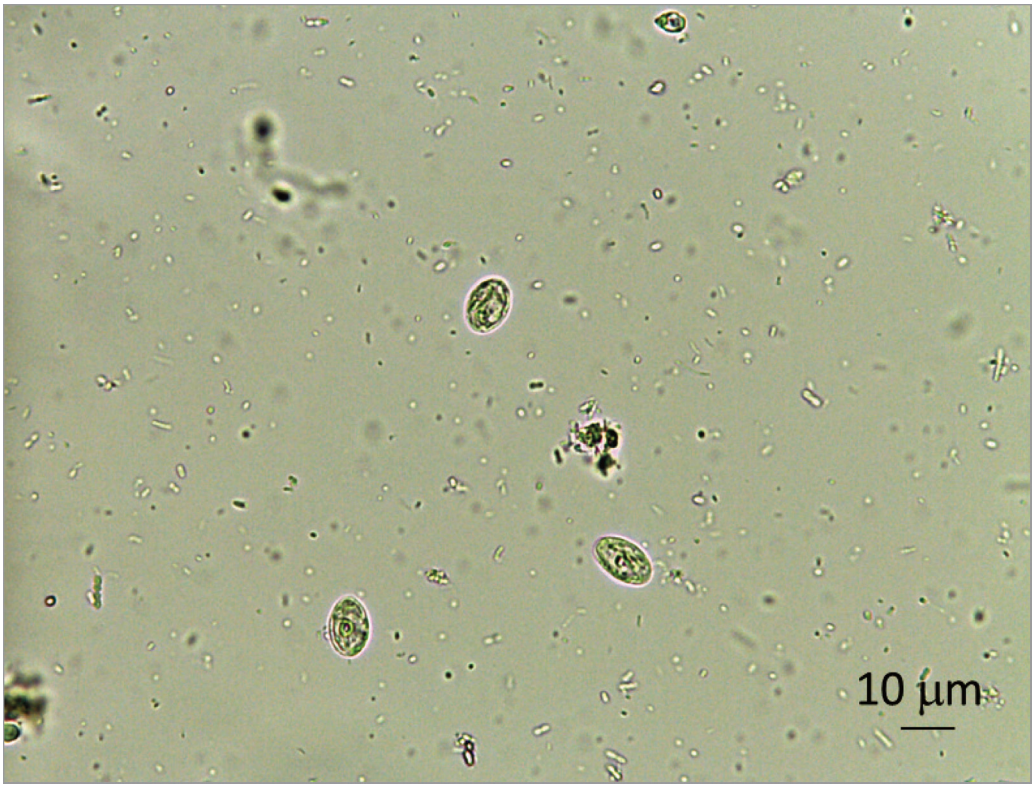

Fig. 3. Cysts of *Giardia* spp. in a red kangaroo

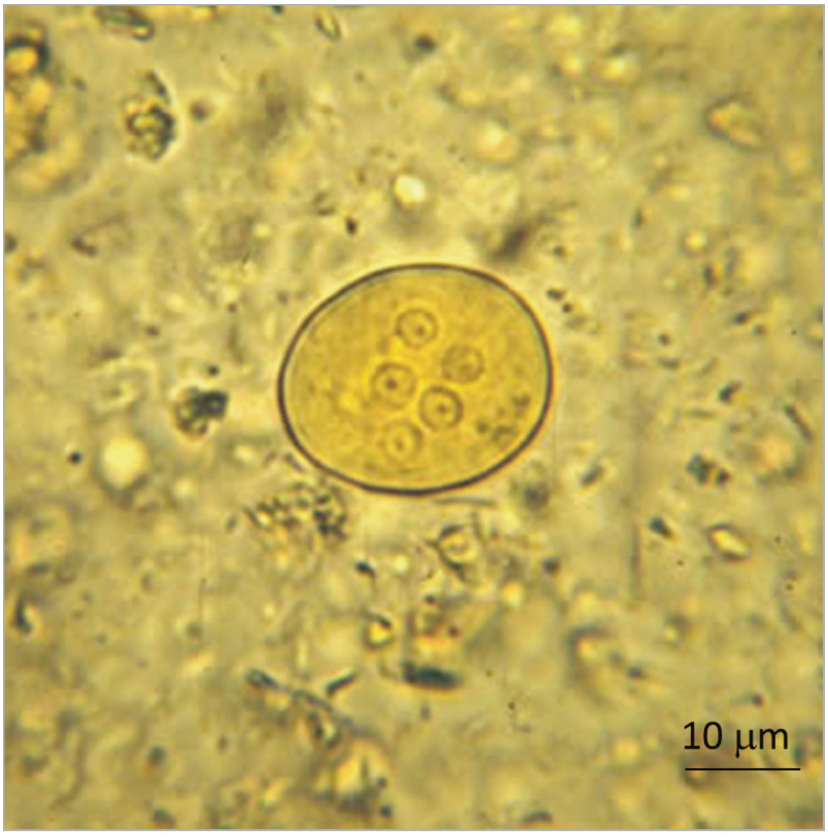

Fig. 4. Cystis of *Entamoeba coli* in a chimpanzee

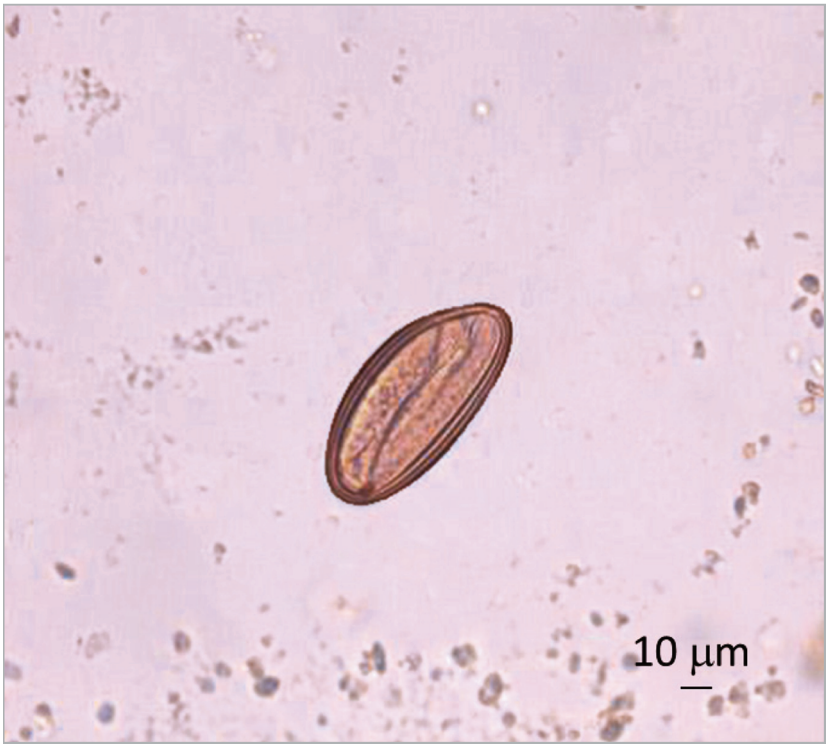

Fig. 5. Egg of *Enterobius vermicularis* in a chimpanzee

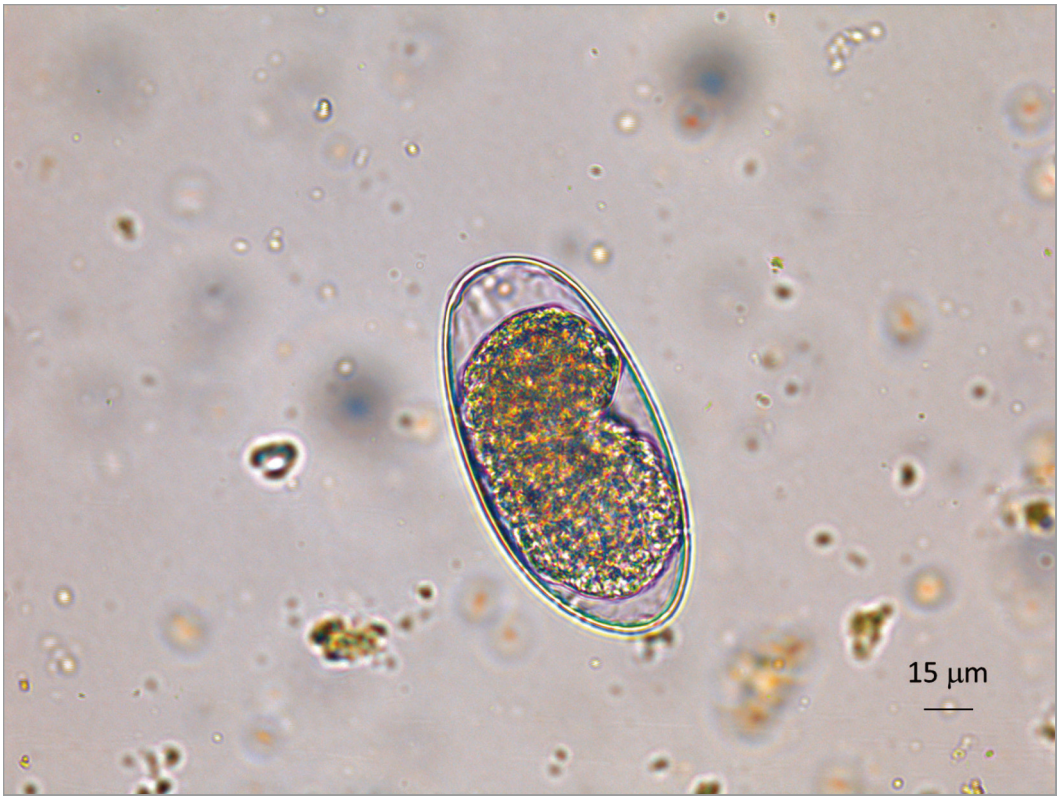

Fig. 6. Egg of gastrointestinal strongyles in a Pigmy goat

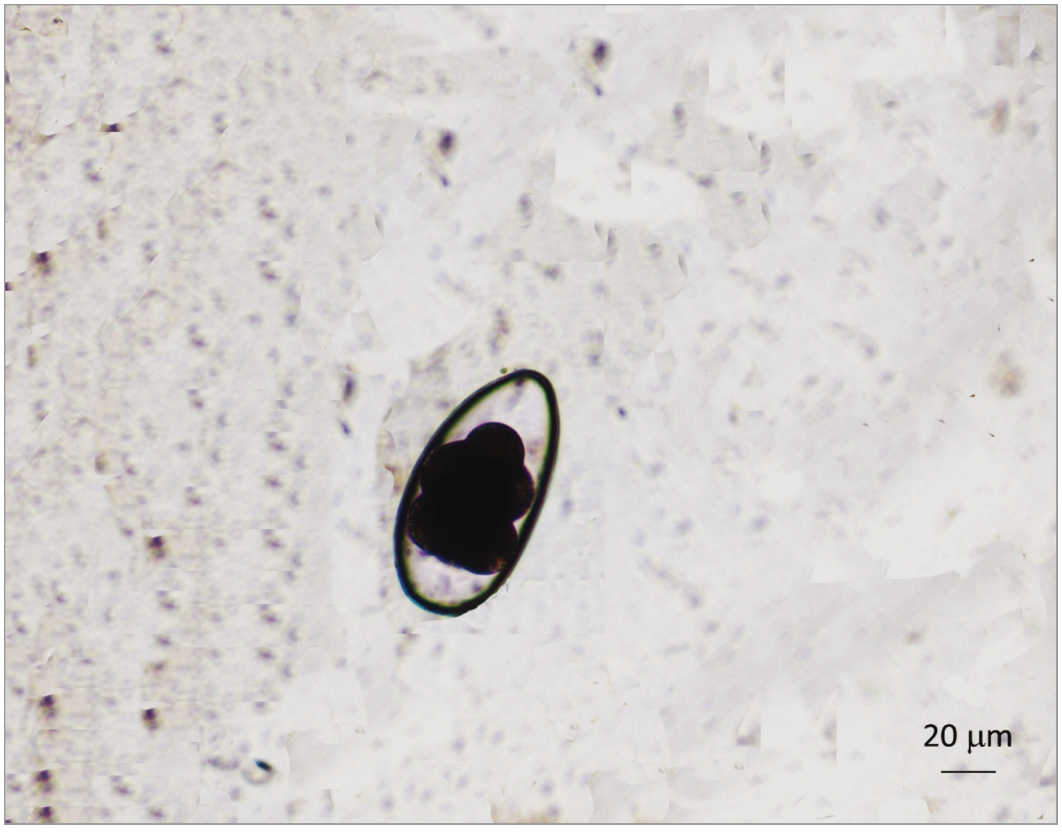

Fig. 7. Egg of *Nematodirus* in a Barbary sheep

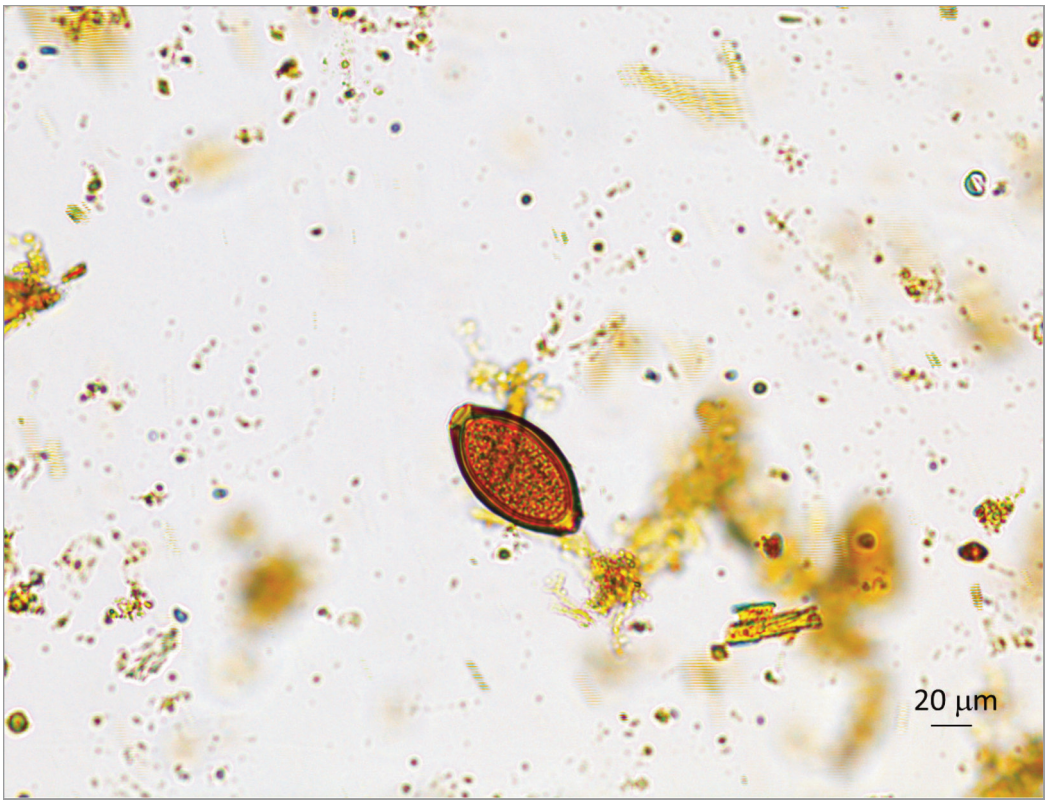

Fig. 8. Egg of *Trichuris* spp. in camel

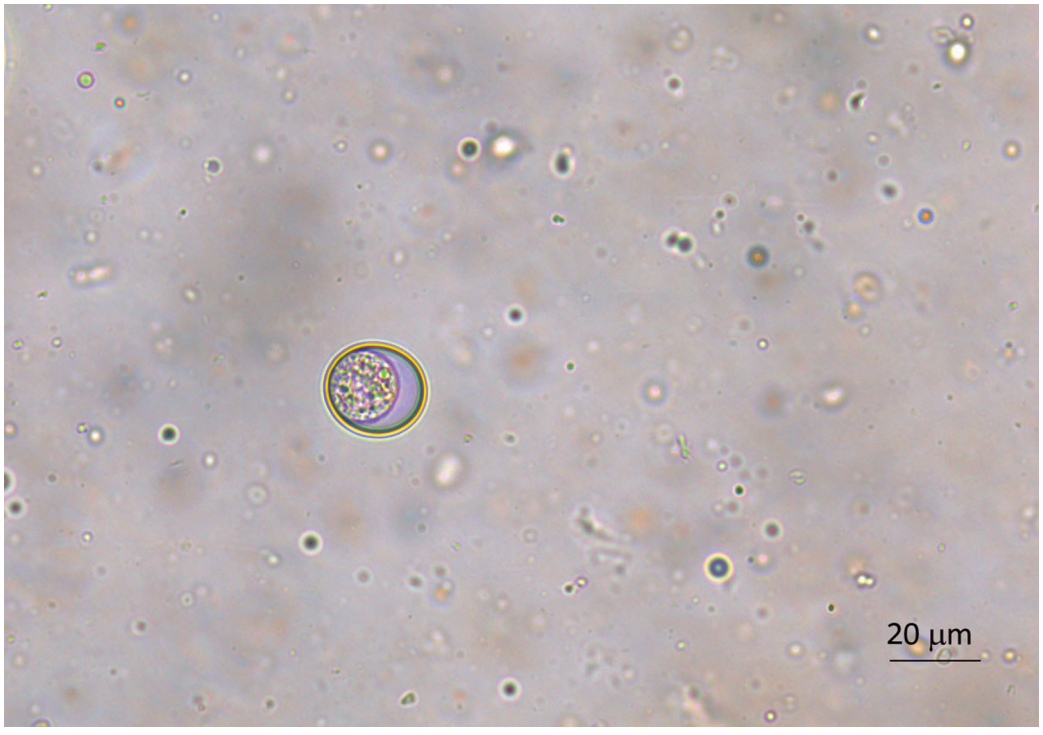

Fig. 9. Oocyst of *Coccidia* in a European bison

by collecting the feces by fresh deposit after the last cleaning of the enclosures. We managed to obtain a sample of secure correspondence with the animals housed individually. When animals were housed in groups, in order to obtain a representative sample, the fecal material was collected randomly in different areas of the enclosure and the amount of collected samples was linked to the number of the subjects present: a single pool was made out of five animals (e.g. 4 pools were collected to sample the feces of 20 animals). Species, number and sex of the animals were noted upon the sampling. In order to preserve the samples collected, 3 parts of fixative (formalin 5%) were added, obtaining a final dilution ratio of 1:4 (1 part of feces + 3 parts of fixative). Fixed fecal samples were kept at room temperature until transport to the laboratory where they were analyzed within 3 weeks.

Laboratory procedures

All samples collected were sent to the laboratories of CReMoPAR ("Centro Regionale Monitoraggio PARassitosi", located in Eboli, Italy) and then processed using the FLOTAC pellet technique (Cringoli *et al.* 2010).

This technique is performed for samples of unknown weight of fecal material. In these circumstances the weight of the fecal material can be obtained after weighting the sediment in the tube (pellet) after filtration and centrifugation of the fecal sample. Each sample was homogenized and filtered. Two 15 ml conic tubes were filled with the filtered suspension up to 6 ml and then centrifuged for 3 min at 1500 rpm (about 170 g). After centrifugation the supernatant was discarded and the two pellets (sediments) were weighted. Two different flotation solution were used to re-suspend the two pellets: FS2 (Sodium Chloride Solution) (specific gravity = 1200) and FS7 (Zinc Sulfate Solution) (specific gravity = 1350). Samples were again homogenized and each suspension was poured into the two flotation chambers of the FLOTAC apparatus. The FLOTAC was closed and centrifuged for 5 min at 1000 rpm (about 120 g); after centrifugation, the top parts of the flotation chambers were translated and each chamber was read under the microscope.

Different magnifications were used, 100x and 400x, respectively for the research of egg/larvae of helminths and cysts/oocysts of protozoa. The results were expressed in arithmetic mean of eggs/oocysts per gram (EPG/OPG) of faeces.

Results

Table 1, table 2 and table 3 show the positivities for each parasitic genus/groups in the different groups of animals and the percentage of positive pools. Samples positive to parasitic infections were 34 out of 71 pools (47.9%; 95% Confidence Interval (CI) = 36.0–60.0%) and most animals showed mono-parasitic infections (18), while 16 co-infections were found with 2 up to 4 different species from helminthes and protozoa. Both young animals and adults were analyzed but they did not show any symptom associated with gastrointestinal parasitic infections.

Helminthes

With regard to the nematoda, strongyles were the most frequent (48.0% of ruminants with 89 EPG; 21.0% of monogastric herbivores with 15 EPG) followed by *Toxocara cati* (40.0% of felines, 3 EPG) and the whipworms *Trichuris* spp. (20.1% of ruminants, 11 EPG). In addition, also the following roundworms were found: the pinworm *Enterobius vermicularis* (15.0% of primates, 10 EPG), *Nematodirus* (13.8% of ruminants, 58 EPG) and *Parascaris equorum* (7.1% of monogastric ruminants, 2064 EPG) .

Protozoa

Most samples were positive for coccidia (66.7% of marsupials with 13 OPG and 41.4% of ruminants with 132 OPG) followed by *Giardia* spp., *Entamoeba coli* and *Blastocystis* spp. (15.0%, 15.0% and 10.0% of primates, respectively).

Discussion

The present study found that nearly half of the examined samples (48%) were positive to at least one gastrointestinal parasitic species. The intestinal parasites identified in the present study were previously described by other authors in wild animals bred in captivity and some of these parasites are well-known pathogens for humans, particularly workers and visitors of the Zoo (Abe and Yasukawa 1996; Levecke *et al.* 2007; Munene *et al.* 1998). The accurate knowledge of the parasitic scenario in these animals will allow further understanding of the epidemiology of helminthes and protozoa in order to optimize the needed control strategies to guarantee animal health and welfare. The current trend is to keep under control parasitic infections through parasitological control programs. Their successful implementation is subject to their ease of application and mostly depends on the technique used, whose sensitivity and specificity, determines the effectiveness of these plans (Doenhoff *et al.* 2008; Taylor *et al.* 2002). The parasitological prevention plans could spread only if it is proven their effectiveness, allowing to control parasitic diseases, improving animal's health and optimizing resources. Regarding the need of using diagnostic techniques, which endorse the parasitological control plans, this is the first study where the FLOTAC techniques have been used for the diagnosis of gastrointestinal parasites in zoo animals. Actually the research of gastrointestinal parasites in zoo animals uses some of the routine procedures for coprology, i.e. the direct and indirect wet mount preparation or the McMaster technique (Levecke 2010; Lim *et al.* 2008; Pèrez Cordòn *et al.* 2008). The choice of using FLOTAC was dictated by the need to overcome the limitations of current methods used for the detection

of gastrointestinal parasites. The direct and indirect wet mount preparations have a lower sensitivity and accuracy caused by many human-variables concerning the modes of preparation and detection of parasites in the sample. The McMaster techniques (and variants) have an insufficient adaptability for low concentrations eggs of parasites (Cringoli *et al.* 2010).

The FLOTAC techniques allow looking for more parasites simultaneously in order to detect co-infections of both helminthes and/or protozoa, and allow to handle even the feces of primates which have a considerable fat content and would require an ether-based sedimentation (Munene *et al* 1998). The findings of the present survey showed that among primates infected there is a similar prevalence of protozoal and helminthic infections (15%). A study carried out in a zoo in Kenya on gastrointestinal parasites in primates obtained higher prevalence of helminthes (64.4%) compared to protozoa (17.1%), while another research conducted in the Zoological Gardens of Belgium on captive primates, found a higher prevalence of nematodes (36.5%) (Goossens *et al.* 2005; Munene *et al.* 1998). The clear differences are probably due to different types of management and housing, to the periods in which the studies were carried out but also to the use of different diagnostic techniques, as test properties vary significantly, particularly the sensitivity (Villers *et al.* 2008). A study on ruminants at the Dublin zoo (Ireland) found a prevalence of positive animals to gastrointestinal nematodes and in particular to *Trichuris* spp. in the camel and it was recorded, similarly to our results, that *Toxocara* spp. is the most common roundworm in carnivores, especially in big cats (Abe and Yasukawa 1996; Geraghty *et al.* 1982). The presence of *Blastocystis* spp. in captive primates has also been demonstrated by Lim and colleagues (2008) even if with a lower prevalence (2.1%). Some Authors have reported the possibility that these protozoa are transmitted to human beings or other animals as demonstrated in their study in which the "keepers" were found to be infected by *Blastocystis* spp. without showing any clinical symptom (Rajah *et al.* 1999). A study conducted on primates housed in Rome Biopark revealed worrying levels of *Giardia* spp. infection in Ring Tailed Lemurs (81.2%), obtaining similar results to previous researches carried out in other parts of Europe and to those of the present work (Berrilli *et al.* 2011; Levecke 2010; Levecke *et al.* 2007). Those protozoa, along with *Entamoeba* spp. appear very difficult to control and they are resistant to repeated treatments; for example, the veterinarians of the bio-park in Rome have unsuccessfully tried to eradicate *Giardia* spp. from a colony of ring-tailed lemurs through many specific treatments (Berrilli *et al.* 2011).

Conclusions

The results obtained with our study highlight the need of regular parasitological testing of wild captive animals. Moreover, the intestinal parasites found in this study are well known human pathogens and represent a potential source of zoonotic transmission, especially among animal handlers. This research emphasizes the need for the establishment of routine control programs that use a sensitive, accurate and precise methodology for qualitative and quantitative copromicroscopic analysis. Our work is the first parasitological study conducted on animals kept in a zoological garden using the FLOTAC techniques. This approach is particularly effective as it allows a very sensitive detection of parasitic infections, in order to be able to choose the best treatment avoiding drug resistance events and uneconomical behavior. A review of the main alternative methodologies for the detection of intestinal parasites, showed that each one of them lacks either sensitivity, reproducibility or reliability and that the FLOTAC techniques are a viable alternative to existing methods. The results obtained show that increasing the application of routine control plans is the way ahead, and their implementation in zoological gardens could drastically improve the way parasitic infection are treated, both from the clinical and logistic point of view.

Acknowledgements. The Authors would like to thank the director of the Warsaw Zoo for giving permission to collect necessary samples to conduct this work and especially the vets and keepers who have assisted the collection of samples.

References

Abe N., Yasukawa A. 1996 .Prevalence of *Toxocara* spp eggs in sandpits of parks in Osaka city, Japan, with notes on the prevention of egg contamination by fence construction. J. Vet. Med. Sci. 59: 79–80.

Berrilli F., Prisco C., Friedrich K.G., Di Verbo P., Di Cave D., De Liberato C. 2011. Giardia Duodenalis assemblages and *Entamoeba* species infecting non-human primates in an Italian zoological garden:zoonotic potential and management traits. Parasit. Vectors. 4: 199. DOI:10.1186/1756-3305-4-199.

Cringoli G., Rinaldi L., Maurelli M.P., Utzinger J. 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat. Protoc. 5: 503–514. DOI:10.1038/nprot.2009.235.

Doenhoff M.J., Cioli D., Utzinger J. 2008. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr. Opin. Infect. Dis. 21: 659–667. DOI: 10.1097/QCO.0b013e328318978f.

Geraghty V., Mooney J., Pike K. 1982. A study of parasitic infections in mammals and birds at the Dublin Zoological Garden. Vet. Res. Commun. 5: 343–348.

Goossens E., Dorny P., Boomker J., Vercammen F., Vercuysse J. 2005. A 12-month survey of the gastro-intestinal helminths of antelopes, gazelles and giraffids kept at two zoos in Belgium. Vet. Parasitol. 127: 303–312. DOI:10.1016/j.vetpar.2004.10.013.

Levecke B., Dorny P., Geurden T., Vercammen F., Vercruysse J. 2007. Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. Vet. Parasitol. 148: 236–246 DOI: 10.1016/j.vetpar.2007.06.020.

Levecke B. 2010. The importance of gastrointestinal protozoa in captive non-human primates. Ph.D. Dissertation, Ghent Univ. , Faculty of Veterinary Medicine, Merelbeke, Belgium. Pp. 22–30, 42–45, 72.

Lim Y.A.L., Ngui R., Shukri J., Rohela M., Mat Naim H.R. 2008. Intestinal parasites in various animals at a zoo in Malaysia. Vet. Parasitol. 157: 154–159.

Mitchell M.A., Tully T.N. Jr. 2009. Manual of Exotic Pet Practice. Saunders Elsevier. St. Louis.

Munene E., Otsyula M., Mbaabu D. , Mutahi M.T., Muriuki S.M.K. 1998. Helminth and protozoan gastro-intestinal parasites in captive and wild-trapped African non-human primates. Vet. Parasitol. 78, 1995–2201.

Pèrez Cordòn G., Hitos Prados A., Romero D., Sánchez Moreno M., Pontes A., Osuna A., Rosales M.J. 2008. Intestinal parasitism in the animals of the zoological garden "Pena Escrita" (Almunecar, Spain). Vet.Parasitol. 156: 302–309. DOI: 10.1016/j.vetpar.2008.05.023.

Rajah S., Suresh G.K., Vellayan J.W., Khairul Anuar M.A., Init I., Vennila R., Saminathan R. 1999. *Blastocystis* in animal's handlers. Parasitol. Res. 85: 1032–1033.

Shalaby Hatem A. 2013 Anthelmintics Resistance; How to Overcome it? Iran. J. Parasitol. 8: 18–32.

Taylor M.A., Hunt K.R., Goodyear K.L. 2002. Anthelmintic resistance detection methods. Vet.Parasitol. 103(3): 183–194 . DOI: 10.1016/S0304-4017(01)00604-5.

Villers L.M., Jang S.S., Lent C.L., Lewin-Koh S., Norosoarinaivo J. 2008. Survey and comparison of major intestinal flora in captive and wild ring-tailed lemur populations. Am. J. Primatol. 70: 175–184. DOI 10.1002/ajp.20482.

Received: January 13,2014
Revised: February 14, 2014
Accepted for publication: March 21, 2014