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# Intestinal parasites in primates: advances in diagnosis and control methods

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*Ai miei prof Laura Rinaldi e Giuseppe Cringoli*
*che hanno creduto in me e nella passione nel mio lavoro*

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## List of abbreviations

| | |
|---|---|
| AMU | All male unit |
| BZ | Benzimidazoles |
| CPG | Cysts per gram of faeces |
| CV | Coefficient of Variation |
| ELISA | Enzyme-linked immunosorbent assay |
| EPG | Eggs per gram of faeces |
| FBZ | Fenbendazole |
| FEC | Faecal egg count |
| FECR | Faecal egg count reduction |
| FECRT | Faecal egg count reduction test |
| FS | Flotation solution |
| FS2 | Satured sodium chloride |
| LPG | Larvae per gram of faeces |
| McM | McMaster |
| NHP | Non human Primate |
| OMU | One male unit |
| PCB | Printed Circuit Board |
| PCR | Polymerase Chain Reaction PE Parasitic Elements |
| PGE | Parasitic gastroenteritis |
| PRT | Positive Reinforcement Training |
| PV | Predictive Value |
| SD | Standard deviation |
| SG | Specific gravity |
| SOP | Standard operating procedures |
| WUI | Wildland urban interface |

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## Abstract

Non-human primates (NHP) are kept in human care from zoos, research laboratories, rescue centers and even as pet. In addition, free-range or feral primate species in several countries have close human interaction. Zoonotic diseases of NHP origin can occur during occupational exposure, hunting, consumption of contaminated food, vector exposure, and leisure activities (ecotourism), among others. Human and NHPs share many similarities, not only anatomically but also physiologically, which makes them both susceptible to many species-specific pathogens. NHP are valuable models for many human infectious diseases; therefore, staff can be exposed to many potential pathogens. Veterinary staff working with NHPs are exposed to zoonotic pathogens via bites, scratches, and accidental contact with body fluids. The zoonotic potential and the complexity of the different species required an advanced expertise in veterinary medicine. The agent of zoonotic disease of primates can be viruses, bacteria and parasites.
Animals reared in restricted environments are highly susceptible to gastrointestinal infection by helminths and protozoa and therefore zoos are characterized as being parasite rich environments. Successful implementation of control programs of these parasites in zoo environment depends upon precise and rapid diagnosing of gastrointestinal infections.

**Chapter 1** describes a field study conducted to demonstrate the role of the Mini-FLOTAC technique in combination with Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals, including NHP. Fecal samples were collected from 70 animals (10 samples of NHP) in four different zoos located in central and southern Italy. All the samples were analyzed using Mini-FLOTAC in combination with Fill-FLOTAC. Out of the 70 pooled samples examined, 80% (24/30) were positive for at least one parasite. Among the gastrointestinal nematodes, Strongyles were the most frequent (40%), followed by *Trichuris* spp. (23.3%), *Parascaris* spp. (13.3%) and *Capillaria* spp. (3.3%). Among the protozoa, *Blastocystis* spp., *Giardia* spp. and *Eimeria* spp. were detected in 6.6%, 3.3% and 3.3%, respectively. These results show that Mini-FLOTAC in combination with Fill-FLOTAC can be used, not only for rapidly diagnosing parasitic infections in zoo mammals, but also for monitoring control programs in which large numbers of fecal samples need to be examined rapidly and reliably.

**Chapter 2** reports the results of a study aimed at assessing how crop/pasture areas potentially may alter social behavior and health of wild geladas (*Theropithecus gelada*) frequenting the unprotected area of Kundi (Ethiopia). During January-May 2019 and December 2019-February 2020, data (via scan, focal animal sampling, and video analyses) were collected on direct human disturbance, external signs of pathologies and social behavior of 140 individuals from 14 one-male units and two all-male units. Animals experienced the highest level of human disturbance in crop areas. Individuals from the groups preferentially frequenting crop areas showed the highest prevalence of external signs of pathologies consistent with chemical and biological contamination (alopecia/abnormally swollen parts). A total number of 48 fecal samples were collected. Samples from frequent crop users contained the highest rates of protozoa and helminths as *Entamoeba histolytica/dispar*, *Giardia duodenalis* and hookworms, i.e. parasites common in human settlements of the Amhara region.

**Chapter 3** reports the results of a study aimed at comparing different techniques, i.e. FLOTAC, Mini-FLOTAC and classical parasitological techniques for detection of gastrointestinal parasites in neotropical non-human primates in Brazil. Fecal samples (n = 43) were collected from neotropical NHP at the Wild Animal Screening Center in Pernambuco, Brazil. Of all samples analyzed, 76.7% (33/43) detected the presence of gastrointestinal parasites in at least one of the techniques used. In particular, 9.3% (4/43) were positive through direct examination; followed by 23% (10/43) at sedimentation; 34.88% (15/43) at flotation; 48.83% (21/43) at Mini-FLOTAC, and 65.11% (28/43) at FLOTAC. In conclusion, FLOTAC and Mini-FLOTAC were the most efficient methods for diagnosing gastrointestinal parasites in neotropical non-human primates.

**Chapter 4** reports the results of a study aimed at evaluating the synergistic effect of fenbendazole and metronidazole for the treatment of *Giardia duodenalis* infection in different species of NHP housed in a zoological garden of southern Italy. Moreover, the study also aimed to better define the circulation of *G. duodenalis* zoonotic assemblages in NHP and the potential occurrence of zoonotic transmission between the staff (zookeepers, veterinarians) from the zoo and NHP. Briefly, six species belonging to four families (Lemuridae, Cercopithecidae, Atelidae, Hylobatidae) of NHP (N=23 animals) and

housed in six cages (CG) were identified as *Giardia*-positive and divided in two groups. Group F was treated with fenbendazole (50 mg/kg, orally, every 24 hours for 5 consecutive days) and Group M was treated with metronidazole (25 mg/kg, orally, twice a day for 5 consecutive days). After five days from the first round of therapy, all the animals were retreated by inverting the drugs in each group. At each sampling day (SDs -3-24) the faecal samples were tested for the presence of *Giardia* cysts using the FLOTAC technique. Moreover, multiple faecal tests for the antigen-detection of *Giardia* such as rapid enzyme immunoassay (ELISA) and direct immunofluorescence (IFA) were performed at each sampling point only on samples that resulted positive for *Giardia* cysts with FLOTAC. The efficacy of fenbendazole ranged from 30 to 67% on SDs 7-12 and 52-90% on SDs 19-24 after the second treatment with metronidazole. In the Group M, the efficacy of metronidazole range between 82-96% on SDs 7-12 and 98-100% on SDs 19-24 after the second treatment with fenbendazole. Overall, the synergistic effects of fenbendazole and metronidazole against the *Giardia* infection, in the Group M showed a statistically significant difference (P = 0.001) compared to the Group F. The overall k agreement between FLOTAC and IFA reached 0.858 (P=0.0001). In contrast, all the samples had a negative antigen result when using the rapid ELISA. At molecular analysis, six samples were confirmed positive for *Giardia* by nested PCR. Only two positive samples (CG1, CG2) were successful sequenced showing 100% of identity with Assemblage B (Accession number: MF095053), sub-assemblage BIV. *Cercopithecus mona* from CG2 resulted also positive for *Blastocystis* sp. The sample was successfully sequenced showing 100% of identity with Subtype ST1 (Accession number: MN338073). In addition, all the samples from the humans included in the study resulted negative for *Giardia* cysts. Overall, the study emphasizes the need for regular monitoring of *Giardia* infections in NHP housed in zoos by traditional diagnostic tools combined with molecular characterization of the parasite. In addition, in positive animals, control strategies should include an association of metronidazole and fenbendazole, in particular for the NHPs that are asymptomatic with marked persistence of cysts, in order to reduce the risk of *Giardia* infection for animals and humans in zoos.

**Chapter 5** is an overall discussion describing how parasites are common in both captive and wild NHP and can be present in both clinically normal and diseased animals. Surveys of captive and wild populations of NHP that live

near human populations have emphasized the potential impact of these organisms as zoonotic agents. Taken together, the data presented in this thesis showed a high occurrence of gastrointestinal parasites in captive animals, including zoonotic species, which may pose a risk to animal and human public health. Overall, the studies reported in this PhD thesis emphasize the need for traditional diagnostic tools combined with molecular characterization of parasites of NHP. Due to the impact of parasitic infections on the health and welfare of NHP and considering the zoonotic potential of some of them, it is strongly recommended to conduct regular parasitological screening and monitoring of these infections within the routine veterinary examinations in zoological. Furthermore, the efficacy of antiparasitic drugs should be assessed and combination of therapy are recommended to strategically control the risk of parasitic infections (and related diseases) in NHP.

## I. Primates in zoological gardens

In recent years, there has been a growing tendency to consider the last two decades as a new era in the management of zoo animals, and primates (Wallis et al., 1997). The great influx of scientific research into zoo primate management has been considered sufficient to coin the term "applied primatology" to indicate the developments in the sector beginning from the 1980s (Maple and Finlay et al., 1989). At the same time, a shift of zoos' interest from public entertainment to wildlife conservation has been universally reported (Rabb et al., 1994), reinforcing a clear-cut view of zoo history. A more thorough look at the specialized literature shows the existence of several areas of concern, ranging from nutrition to breeding programs and public response to new exhibit styles (Kaumanns et al., 1998/1999; Kaumanns et al., 2000; Hyson at el., 2000). In particular, there is often assumed to be a strong correlation between 'naturalistic' (generally meaning 'without bars') enclosures and improved animal welfare (Melfi and Feistenr et al., 2002). However, there is confusion between the public perception of how animals respond to 'naturalistic' habitats and the actual response of the animals themselves (Robison et al., 1999). Furthermore, neglecting the long history of primate keeping in zoos might cause the loss of precious knowledge of great utility in furthering current zoos' goals and animal welfare. This is particularly important as scientific knowledge is translated into legislation about zoos and animal welfare, but most studies on animal welfare and environmental enrichment originated in laboratories whose living standards are, on average, lower than in zoos (Buchanan and Smith et al., 2004). However, knowledge of the developments of some of the world's major zoos might furnish enough information about the general trends in primate management history. The foundation of the Menagerie at the *Jardin des Plantes* of Paris in 1793 is usually considered the birth date of scientifically-managed zoos, i.e. those directed by a scientific staff with the stated purpose of contributing to public education and acquiring knowledge of the animal world (Hancocks et al., 2001). In 1828 the Menagerie of the Zoological Society of London was founded. The great contribution to scientific knowledge achieved by these two early zoos is sharply in contrast with the recent introduction of science in primate management advocated by MAPLE and FINLAY'S (1989) definition of 'applied primatology'. Furthermore, a technical bulletin of the German zoo directors, *Der Zoologische Garten*, has been published ever since

1859, and technical innovations have been continuously introduced during the long history of zoos. Glass panels to separate apes from visitors, for example, were introduced in Frankfurt as early as 1871 (Rawlins et al., 1979). In Paris the original 'singerie' opened in 1837 consisted of a big rounded outdoor cage connected to several smaller indoor cages. In the 'singerie' of the Royal Turin Zoological Garden, ten small indoor cages were connected to the large, glass-covered outdoor cage (Maschietti et al., 1990), thus greatly limiting the opportunities of outdoor access to all primates, but in December 1871 hamadryas baboons were held outside in the carnivore section at -7°C (Gippoliti et al., 1997). In 1864 London Zoo opened a new glass-house style Monkey House with only indoor accommodation for the animals, an approach which subsequently became widespread in Europe at the time. The technical premise of such a choice was the unsuitability of the European climate for tropical animals, including primates. In 1875 Munster Zoo had a monkey house in which the animals could move as they pleased from outside to inside all the year round through specially designed metal doors (Hancocks et al., 1971). On empirical grounds, the importance of the climatic factor was also challenged by Carl Hagenbeck, a unique figure of animal trader, trainer and zoo man. With the aim of reducing costs and exhibiting animals in more natural settings, Hagenbeck was a strong supporter of the 'acclimation' of tropical animals to temperate climates. His monkey houses were simple, unheated buildings always provided with outdoor cages. In the period 1912-1913, Hagenbeck and his team created in Stellingen (Hamburg) and in Rome the first naturalistic, bar-less exhibits for primates. In Rome Zoo's original 'Monkey Village' (1912), Indian temples and living plants, including two hundred-year-old pine trees, were surrounded by a dry moat. The macaques, mainly *Macaca mulatta*, were kept outside year-round, and went to sleep high in the trees at night (Knottnerus-Meyer et al., 1925). Although this kind of open exhibit became widespread in zoos around the world, their use was restricted to the more robust, terrestrial baboons and macaques. Their utilization was often abandoned after problems encountered with diseases and social conflicts, such as those described in the pioneering work of Zuckerman (1932). Knowledge of problems encountered in those years is essential to understanding the rise of the modernistic-hygienist style in zoo architecture in the last Century. In 1932, for instance, a family of chimpanzees, including the first captive-bred one to be reared successfully in Europe, was killed by TB at the Rome Zoo (D'Alessandro and Gippoliti et al., 1996). Hygienic de-

velopments were positively accepted by Hediger, the founder of zoo biology, who emphasized the need for functionality in cage design instead of romantic attempts to recreate the wild (Hediger et al., 1950). Hediger greatly opposed another development in zoo management after World War II, i.e. the preparation of standard diets for zoo animals (Hediger et al., 1965). It is interesting to note that, while zoo design has now universally condemned the barren hygienic cage style, much less attention seems devoted to the effects on welfare and physiology of the now widespread commercial zoo diets, despite a few cautionary papers on the subject (Stolleret al., 1989; Schwitzer and Kaumanns et al., 2001). However, going back to exhibit design, "sanitary modernism" coupled with zoos' desire for "postage-stamp" style collections resulted often in highly restricted and deprived habitats for primates in zoos. Particular attention was devoted to apes' accommodation and care. Before World War II, they were often kept permanently indoors, with no access to water, all food boiled and peeled, and no bedding material was provided for nesting (Crandall et al., 1964; Stemmler-Morath et al., 1968). Yet Desmond Morris and Caroline Jarvis, while introducing a special section on Great Apes in the first volume of the *International Zoo Yearbook* in 1960 (a classical reference on scientific zoo-husbandry for almost half a century now) say "Methods of exhibiting and caring for great apes in captivity have improved tremendously during the past twenty years". In fact, several modern monkey or great ape houses were built in those years, often copying one another. New primate and ape houses made of glass, concrete and steel, sometimes without outdoor accommodation, were first opened in Philadelphia in 1958 and successively in Frankfurt, Berlin, Antwerp, Basle, Zurich etc. Apart from hygienic factors, there was often little improvement until the 1970s. Scientists made proposals to improve the situation of captive apes, particularly chimpanzees, as field work on these animals increased (Kortland et al., 1961; Reynolds et al., 1965). In the 1950s outside enclosures with water moats were created for great apes in continental-climate zoos, for instance at the Bronx Zoological Park, where the male gorilla Makoko drowned in 1951. This event inaugurated a list of tragedies still continuing in our days (Paterok et al., 2004), highlighting the conflict between aesthetically pleasant open exhibits and animal welfare. Chimpanzees generally achieved more success; in 1956 Chester moved its group onto islands surrounded by a water moat (Mottershead et al., 1960). In the Arnhem Zoo, a large colony of chimpanzees was assembled and a complex outdoor habitat, including living trees, created (Van

Hoff et al., 1973). In the sixties special houses were created to exhibit, through an inversion of the day-night regime, active nocturnal animals including primates (Morris et al., 1965). Although these 'night houses' are now losing popularity, the high artificiality of this kind of captive habitat suggests that more research should be directed toward the assessment of welfare among primates in zoos' night houses (Schulze et al., 1998). Interestingly, good results have been reported with owl monkeys of the genus *Aotus* kept in naturally lighted cages (Walter and Brown et al., 2004). Among the few early examples of more naturalistic habitats, gibbons were introduced to islands surrounded by water first by the French ornithologist Delacour in his own park at Clères in 1926 (Delacour et al., 1933) and later in 1934 at the new Paris Zoo in Vincennes and at Rome. For decades, however, many improvements in primate keeping were introduced by practitioners running their own private zoos, and thus far from the scientifically-managed collections. Foremost I would mention the late John Aspinall for having introduced so many crucial aspects in the management of zoo gorillas, mainly utilising available information from field studies and recognising the need to adopt an individualistic attitude in the care of these animals. His 'gorillariums' are nothing more than large cages able to hold a large social group of gorillas, with deep litter of oat straw and a roof open to the weather and cobwebbed with brachiating bars (Aspinall et al., 1986). Despite being one of the two most successful breeding colonies in the world, Aspinall's functionalist model has been largely ignored by the zoo world, now launched upon the landscape immersion architecture. A number of criticisms have been made of the old zoo exhibits by zoologists, psyhologists and landscape architects (Morris et al., 1964; Sommer et al., 1972; Coe et al., 1985). The rise of environmental issues in developed countries made still more evident the fact that, leaving apart objective welfare criteria for zoo animals, the message of hard architecture in zoos was one of anthropocentrism and superiority of humans over wildlife (Sommer et al., 1972). To reverse the situation, the landscape immersion approach was used extensively in the Seattle Zoo master plan developed by HAN- COCKS, JONES and COE in 1976 (Gold et al, 1997; Hyson et al., 2000). Later, a manifesto for a naturalistic way to present and manage animals in zoos appeared authored by the Seattle team of biologists (Hutchins et al., 1984). In the sixties, the Miami Monkey Jungle allowed the public access to a one and a half hectare tropical rain forest including several species of South American primates, such as the delicate

red uakari *Cacajao calvus* (Fontaine et al., 1977; Dumond et al., 1977). On the other hand is a fact that several of the costly large indoor 'tropical rain forest' facilities developed in the US since the eighties appear not substantially different from the old bare cages of the past, as the naturalistic experience (i.e. living plants) is mostly limited to the public pathway (Hancocks et al., 2001). This may be due to the extremely high construction costs of this kind of exhibit (Russell et al., 1977; West et al., 1997) and lack of space. Furthermore, even when spacious and naturalistic outdoor exhibits are available, animals may spend most of the twenty-four-hour day in smaller indoor holding cages that are almost perfect replicas of the old barren cages (Hancocks et al., 2001), and this may lead to stressful situations (Aureli and De Waal et al., 1997). Despite the importance of this aspect of the life of zoo animals, the issue does not appear to attract much scientific interest. However, a number of European institutions, such as the zoos of Apenheul (NL), Jersey (GB) and Rhene (GER), have been able to create fine outdoor naturalistic exhibits without the exaggerated costs of zoos in North America (Mager et al., 1986; Griede et al., 1986; Salzert et al., 1989; Redshaw and Mallison et al., 1991).

## II. Veterinary management and medical training

A program of veterinary care should be established and maintained under the supervision of a veterinary surgeon or practitioner. Routine examinations, including parasite checks, to be carried out and preventive medicine, including vaccination, to be administered at such intervals as may be recommended by a veterinarian.

Whenever possible, nonhuman primates should be trained, using positive reinforcement principles, to cooperate with routine husbandry. Nonhuman primates have significant cognitive abilities and training for cooperation in husbandry tasks. This can significantly reduce human and animal stress and the likelihood for injury or accidents to occur. When it is not possible to train animals to cooperate because of time or other constraints, physical and chemical methods of restraint may need to be considered.

Training animals to cooperate without the need for restraint devices or drugs is strongly encouraged and convenience alone must not dictate the method used for restraint. Whenever possible, cooperation by non-stressed, conscious animals yields the most relevant data for studies. Ideally, the veterinarian and researcher should work with a behaviorist and the training plan should have reasonable, sequential steps and attainable goals. The plan also should take into consideration the unique personality traits of each animal and their capacity to learn and remember tasks to condition them to be a willing worker. The plan should be based on receiving rewards (e.g. treats, juice, and other rewards) for the tasks learned and reproduced, and receiving no rewards for the lack of progress (i.e. positive reinforcement). Clicker training is designed to obtain desired behaviors by giving food and treats immediately after the sound of a clicker and has been used with very good success in primates (Wolf et al., 2012).

Animals may be trained to voluntarily cooperate for many procedures, and this should be considered for primates that will be maintained for extended periods of time. Chemical restraint, for example, ketamine (alone or in combination with dexmedetomidine/midazolam) may be considered when immobility is required (electrocardiography) or when the planned procedure has the potential to cause more than slight or momentary distress or discomfort (e.g., removal of skin sutures, etc.). Another consideration for using chemical restraint in primates is to ensure safety for personnel. Care should be taken to ensure that procedures involving chemical restraint are scheduled

with a maximal interval to avoid repeated fasting, inappetence, and associated weight loss; prolonged episodes of hypothermia, bloat, and other stressors associated with administration of sedative or anesthetic agents. Selection of the appropriate sedative or anesthesia regimen should be made in consultation with the veterinary personnel. Specific pharmacologic reversal agents should be used whenever possible to reduce duration of immobility and counteract potential side effects (hypothermia, bradycardia or hypotension). Primates must be monitored appropriately until they have completely recovered from immobilization. Ideally, animals recovering from anesthesia should be placed in a comfortable, warm, quiet area or space, preferably with restricted access to climbing apparatus, until they regain full physical coordination, to minimize potential associated injuries and trauma.

Animals are trained to present body parts with injuries, or areas that need to be monitored or rechecked. We can also train animals to cooperate with veterinary procedures such as auscultation, oral or ophthalmic examinations, and biological sample collection for various diagnostic tests or health monitoring. Once animals are trained to present various body parts, we can administer treatments in a manner that reduces stress while the animals remain in their home enclosures. Topical wound care and the initiation of complementary therapeutics are just some of the many possible applications. Animals can be trained also to receive drugs by humans.

The process of PRT promotes mental stimulation for the animals being trained. PRT has also been shown to help resolve social issues and facilitate animal introductions, and to reduce abnormal behaviors (Laule et al. 2003).

**Examinations**

Veterinarians can closely examine NHPs in their home enclosures with the use of PRT techniques. PRT enhances the level of trust among the animals, trainers, and veterinarians. This high level of trust enables us to closely visualize the animals. With close inspection, we can confirm continued good health or detect potential health issues early. Examinations often start by having the animals present various body parts. Using PRT techniques, animals are trained to present their chest for auscultation, which allows veterinarians to assess cardiorespiratory function. This is particularly beneficial when animals have a known disease process (e.g., cardiomyopathy, pneumonia, etc.). The trained behavior for auscultation enables us to monitor the disease state

without the risks or stress associated with anesthesia. Minimizing or avoiding anesthetic events for animals with compromised health status provides benefits in terms of overall health and the reduction of anesthesia-related stress. Training animals to open their mouths, or present ears and eyes, can also provide numerous health benefits. Veterinarians can assess dental disease, tooth fractures, or oral trauma when an animal is trained to open its mouth. We can also evaluate ears for possible infection or foreign bodies (generally sticks, rocks, or orange seeds) by doing an otoscopic exam. We can even assess eyes for possible trauma or ulceration with a thorough ophthalmic examination. Infections can be monitored by routine measurement of body temperature.

NHPs have also been trained to present for ultrasound examination. Using PRT, they can be desensitized to the ultrasound machine and the various probes. They are then trained to present either their chest for an echocardiogram to evaluate cardiac function, or their ventral abdomen for assessment of internal organs (Drews et al., 2011; Magden et al., 2015). In the past, all of these assessments have required sedation.

The procedures described here clearly indicate that NHPs can be trained to cooperatively participate in these types of health care-related behaviors.

NHPs can be trained using PRT techniques to voluntarily allow the collection of a variety of biological samples, which are crucial veterinary diagnostic tools. Through the collection of various biological samples, we can assess health status and begin appropriate treatments. We can obtain cultures from sites of infection (such as an infected wound), from nasal discharge, or from the back of the throat. These cultures help us to determine whether there is an active infection, to identify the infectious organism (if present), and allow us to start appropriate (antibiotic) treatment.

Collection of voluntary urine and semen samples is also possible in NHPs. Urine collection using positive reinforcement training has proven successful in common marmosets (*Callithrix jacchus*) and chimpanzees (*Pan troglodytes*) (Bassett et al. 2003; McKinley et al. 2003; Bloomsmith et al., 2015). Urine samples provide a noninvasive diagnostic test for a variety of possible health conditions. The veterinarian can assess the urine samples for possible urinary tract infections, monitor protein loss/renal disease, measure glucose in diabetic cases, or perform pregnancy tests (Laule et al., 1996; Schapiro et al., 2005; Lammey et al., 2011). Semen collection aids in the assessment of fertility or provides specimens for storage for future breeding purposes. While semen can be collected using a rectal probe to stimulate elec-

troejaculation in sedated animals (VandeVoort et al., 2004), we can avoid the stress and risks of anesthesia by training animals to voluntarily provide semen samples. This behavior is initially shaped by asking the animal to present its abdomen. As male chimpanzees often have erections during training sessions, the trainer must work to desensitize the animal to the artificial vagina (a PVC tube with penrose tubing) and coordinate thrusting direction to capture the ejaculate (Schapiro et al., 2005). In other NHP species, the animals can be trained to cooperate with direct penile electrostimulation, which does not require anesthesia (VandeVoort et al., 2004).

Blood sample collection is a particularly valuable trained behavior. Anesthetic events are associated with stress, and stress affects hematologic parameters. Therefore, the most accurate blood values can be obtained in samples that are collected without sedation (Lambeth et al., 2006). Both chimpanzees and rhesus macaques can be trained for voluntary venipuncture and blood collection (Lambeth et al., 2006; Coleman et al., 2008). The blood collected can be analyzed for general health status, to monitor disease progression, or to evaluate the success of treatments (i.e., infections treated with antibiotics). Capillary blood samples can also be obtained, and these smaller blood samples can be used to test blood glucose levels with a glucometer (Reamer et al., 2014). This is very valuable when providing the best care for diabetic animals. While many therapeutics are successfully administered orally or via injectable routes, there are some that have been more difficult to administer to non-sedated NHPs. Treatment of wounds is one of the most common veterinary interventions required by socially housed NHPs. Nonhuman primates are social animals with dynamic social relationships. Their interactions with one another occasionally result in wounding; however, the relatively low risk of injury is outweighed by the numerous benefits of social housing. If wounds do occur, we can optimize our treatment and minimize anesthesia, if animals have been trained to accept wound care in their home enclosures. Animals that are trained to present various body parts can also be trained to present wounds for topical treatments. When positive reinforcement is used, wounds can be with various cleansing solutions, and topical antibiotics or salves can be applied.

**Figure II.1: "**open mouth" behavior trained for assessment of the oral cavity of a female of western chimpanzee (*Pan troglodytes verus*).

**Figure II.2:** otoscopic examination trained using PRT techniques in an adult chimpanzee (*Pan troglodytes).*

**Figure II.3:** ophthalmic examination of an adult male chimpanzee (*Pan troglodytes*).

**Figure II.4:** injection in adult western chimpanzee (*Pan troglodytes versus*).

**Figure II.5:** Group of ring tailed lemur (*Lemur catta).*

**Figure II.6:** individual and direct food and drug administration for a pair of black-and-white ruffed lemur (*Varecia variegata*)

## III. Zoonosis of primates

## PARASITIC AGENTS

Parasites are common in both captive and wild NHP and can be present in both asymptomatic and diseased animals. Personnel working with NHP are at risk of acquiring parasitic diseases via contact with blood, feces, tissue and from the environment (Herwaldt et al., 2001; Pourrut et al., 2011). In addition, with the increase of international travel, immigrants, and immunocompromised people, parasitic infections need to be considered by physicians when consistent symptoms are present. Surveys of captive and wild populations of NHP that live near human populations have emphasized the potential impact of these organisms as zoonotic agents (Munene et al., 1998; Pourrut et al., 2011). Potential exposure routes include accidental needle sticks, scratches, bite wounds, skin exposure, and vector-borne transmission (Herwaldt et al., 2001). Potential intestinal parasites that may affect people working with NHP include *Strongyloides* spp*., Oesophagotomum apiostomum, Trichuris trichuria, Necator americanus, Ancylostoma duodenale* (Bush et al., Munene et al., 1998; Pourrut et al., 2011). Mixed-specie enclosures at zoologic institutions are a potential source of parasitic infection as some animals may serve as reservoirs of a parasite. In addition, helminth control in both zoologic institutions and laboratory settings is important because helminths may serve as not only reservoirs, but also vectors of parasites. *Baylisascaris procyonis*, the raccoon roundworm, which is a known zoonotic agent, has been reported in NHP with neurologic signs (Sato et al., 2005). Protozoal organisms that affect NHP also have the potential to cause zoonotic disease. Protozoal organisms can be transmitted via the fecal–oral route as well as via vectors (Cox-Singh et al., 2008; Galinski et al., 2009). *Plasmodium knowlesi*, a NHP protozoan transmitted by mosquitoes has been reported to cause disease and even death in humans (Cox-Singh et al., 2008). Other *Plasmodium* spp. that are present in NHP may have zoonotic potential and emphasizes the role of NHP as reservoirs (Galinski et al., 2009). NHP are carriers of several protozoal organisms in their feces such as *Giardia*, *Trichomonas,* and *Entoameba hystolitica* (Parker et al., 2007). Although zoonotic infection with these organisms is not common, there is still potential for exposure.

Another protozoa with zoonotic potential is *Trypanosoma cruzi*, the causative agent of Chagas disease (Carvalho et al., 2003). *T. cruzi* infection in humans

causes severe heart disease. Infection with *T. cruzi* has been reported in both captive and free-ranging NHP (Pung et al., 1998; Carvalho et al., 2003; Foshay et al., 2007). The organism is primarily transmitted by the triatomine bug, although transfusion with infected blood can also lead to exposure (Pung et al., 1998). The triatomine bug deposits fecal material with the parasite while taking a blood meal from the host. The animal or human then becomes infected when the fecal material enters the bite wound or crosses mucous membranes. NHP are not only affected by the disease, but also serve as reservoirs for the organism.Leishmaniasis, which is spread by sandflies, causes human infection in both cutaneous and chronic visceral form and has also been reported in NHP (Bush et al., 1977). A clinical case of *Leishmania* infection in a laboratory worker was caused by an accidental needle stick after handling an infected NHP.

The parasitic sarcodines are considered nonpathogenic except Dientamoeba, which sometimes can be pathogenic as *Entamoeba histolytica*, which can cause severe enteric disease in humans and nonhuman primate. *Entamoeba histolytica*, has a world-wide distribution and has been reported in New World monkeys (spider monkeys, cebus monkeys, woolly monkeys, howler monkeys, squirrel monkeys, and marmosets), Old World monkeys (rhesus monkeys, pig-tailed macaques, bonnet macaques, colobus monkeys, proboscis monkeys, African green monkeys, baboons, and langurs), and the great apes (gibbons, orangutans, and chimpanzees) (Deschiens et al., 1927; Hegner et al., 1934; Benson et al. 1955; Fine et al., 1961; Geiman et al., 1964; Kuntz et al., 1966; Dunn et al., 1968; Reardon et al., 1968; Kuntz et al., 1968 - 1969; Henderson et al., 1970; Kaufmann et al., 1970; Flynn et al., 1973; Geisel et al., 1975; Amyx et al. 1978; Muller et al., 1981; Frank et al., 1982; Sakikabara et al., 1982; Loomis et al., 1983; Palmieri et al., 1984).

Ameba organism reproduces by binary fission (Burrows et al., 1972; Flynn et al., 1973; Levine et al., 1973). Prior to producing the cyst form, the amebae become round and small. A cyst wall is formed; the nucleus divides twice, resulting in four small nuclei. These nuclei divide upon rupture of the cyst wall. Thus, each original organism separates into eight small amebae. Each of these in tum develops into a trophozoite. Infection with *E. histolytica* produces mild clinical signs or none at all. There is a great variability in virulence among strains of organisms (Burrow et al., 1982). *Entamoeba histolytica* usually lives in the intestinal lumen where it is nonpathogenic (Levine et al., 1970). Only when it invades the mucosa does it become pathogenic and may

lead to amebic dysentery (Levine et al., 1970). Clinically, affected animals show the following signs: lethargy, weakness, dehydration, anorexia, vomiting, and severe diarrhea that mayor may not be hemorrhagic or catarrhal. The gross and microscopic lesions associated with amebiasis in nonhuman primates have been described (Fremming et al., 1955; Miller et al., 1966; Bostom et al., 1968; Vickers et al., 1969;Flynn et al., 1973; King et al., 1976). At necropsy, a mild to severe necroulcerative colitis can be seen. *Entamoeba histolytica* trophozoites can be found in wet smears from material from the colon of clinically ill animals or from the colonic contents overlying the lesions seen at necropsy (King et al., 1976). Histologically, the colonic mucosa is necrotic and ulcerated down to the level of the muscularis mucosae; typical flask-shaped ulcers may be seen. These ulcers can be as small as a few millimeters or may become large and confluent and involve extensive areas of the colon. Trophozoites may be seen in or adjacent to the ulcers. Often, the host response is minimal unless secondary bacterial invasion has occurred (King et al., 1976; Shadduck et al., 1978). The diagnosis of amebiasis depends upon the microscopic recognition of the causative organisms in the feces or in intimate association with typical lesions. These organisms are also common as nonpathogenic commensals in the digestive tract of nonhuman primates. Their presence in the feces of animals with clinical signs is not definitive evidence that protozoa are the cause of the gastrointestinal disease (Flynn et al., 1973; Levine et al., 1973; Shadduck et al., 1978). Wet-mount preparations may be used to examine the feces for trophozoites. This requires a fresh sample that must be placed immediately in a saline or buffer solution and examined while the preparation is still warm. The movement of the organisms can be seen. *E. histolytica* makes the most obvious kinds of progressive movement of all the intestinal amebae.

Sarcocystosis is a disease caused by coccidian parasites commonly classified in the genus *Sarcocystis.* The cystic phase of this parasite has been described in skeletal muscle fibers and occasionally in cardiac or smooth muscle fibers in a wide variety of animals throughout the world (Eisenstein et al., 1956; Baker et al., 1972; King et al., 1976; Levine et al., 1976; Levine et al., 1977). These cysts are common in the skeletal muscle of the tongue or the esophageal muscle of many nonhuman primates. *Sarcocystis kortei* and *S. nesbitti* have been described in the rhesus monkey, and other unnamed species have been reported in both Old and New World monkeys.

*Babesia* has been reported from Old World monkeys (mangabeys, guenons,

macaques, and baboons). (Hill et al., 1950; Baker et al., 1972; Flynn et al., 1973) and New World monkeys (marmosets) (Hill et al., 1953). Its distribution and incidence in nature is unknown (Flynn et al., 1973; Loeb et al., 1978). The complete life cycle is unknown, but ticks are thought to be the biological vectors (Baker et al., 1972; Flynn et al., 1972; Loeb et al., 1978). This babesial parasite is considered to be only slightly pathogenic in normal intact monkeys but can result in severe anemia and death after splenectomy. Marked poikilocytosis and anisocytosis are associated with the anemia (Gamma et al., 1950; Baker et al., 1972; Flynn et al., 1972; Loeb et al., 1978). *Babesia pitheci* organisms are pyriform in shape and measure 2-6 p.m long. Round, elliptical, oval, lanceolate, and ameboid stages have been also observed in peripheral blood smears (Baker et al., 1972; Flynn et al., 1973). The second babesia-like organism, *Entopolypoides macaci*, is a mildly pathogenic hemosporozoal parasite that has been described in Old World monkeys (cynomolgus monkeys, rhesus monkeys, baboons, and guenons) and great apes (chimpanzees). (Dubin et al., 1947; Belding et al., 1965; Kim et al., 1968; Pucak et al., 1972; Gleason et al., 1974; Shadduck et al., 1978). This organism does not have true pyriform stages, but early ring-shaped stages and ameboid stages with polypoid projections of cytoplasm similar to the true *Babesia* species have been seen. *Entopolypoides macaci* is smaller than *Babesia* and *Plasmodium* species parasites and is morphologically distinct. Parasitized erythrocytes are not enlarged, and pigment is not formed (Loeb et al., 1978). Fever, monocytosis, and anemia have been reported in parasitized nonhuman primates; however, infection with *E. macaci* appears to have little effect on the host (Flynn et al., 1973; Loeb et al., 1978). Chronic, latent infections are known to occur, and splenectomy or immunosuppression will result in recurrence of the parasitemia and a marked increase in the intensity of hemolytic anemia and icterus. Under these conditions, the disease may be fatal (Shadduck et al., 1978). There are indications that this organism is common in nonhuman primates (Loeb et al., 1978). Diagnosis of these two organisms depends on the demonstration and identification of the parasites within the host's erythrocytes (Flynn et al., 1973; Shadduck et al., 1978). There is no public health significance associated with either *B. pitheci* or *E. macaci* infections because neither parasite has been reported in humans (Flynn et al., 1978).

*Balantidium coli* has been reported in a number of nonhuman primate species including New World monkeys (howler monkeys, spider monkeys, and cebus

monkeys), Old World monkeys (rhesus monkeys, cynomolgus monkeys, and baboons), and great apes (orangutans, chimpanzees, and gorillas). The organism is usually nonpathogenic and is a common inhabitant of the cecum of nonhuman primates (Van Riper et al., 1966; Kunt et al., 1968; Myers et al., 1968; Prine et al., 1968; Reardon et al., 1968; Burrows et al., 1972; Butler et al., 1973; King et al.; 1976). Some have been reported to be symptomless carriers. *Balantidium coli* trophozoites are large, ovoid structures with a heavily ciliated outer surface (Auebach et al., 1953; Krascheninnikow et al., 1958; Flynn et al., 1973; Stadduck et al., 1978). This form measures 30-150 x 25-120 micrometers. Internal structures consist of a macronucleus and micronucleus, two contractile vacuoles, and numerous food vacuoles. Cyst forms are spherical to ovoid and measure 40-60 micrometers in diameter. Reproduction occurs by conjugation or by transverse binary fission. Infection occurs through ingestion of trophozoites or cysts. Infection with *B. coli* can cause severe ulcerative enterocolitis that can be fatal in great apes. Signs of clinically ill animals are weight loss, anorexia, muscle weakness, lethargy, watery diarrhea, tenesmus, and rectal prolapse (Burrows et al., 1972; Teare et al., 1982). At necropsy, lesions may resemble those seen in amebiasis and may consist primarily of an ulcerative colitis (Burrows et al., 1972; King et al., 1976). Histologically, the ulcers may be large and may extend down to the muscularis mucosae. There may be an accompanying lymphocytic infiltrate and, in time, coagulation necrosis and hemorrhage (Flynn et al., 1973; Stadduck et al., 1978). Typical large *B. coli* organisms can be seen in masses associated with lesions in the tissues or in capillaries, lymphatics, or regional lymph nodes (Burrows et al., 1972; Flynn et al., 1973; Shadduck et al., 1978). Diagnosis depends on identification of the characteristic *B. coli* organisms associated with the typical colonic lesions (Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). Their presence as secondary invaders to a primary disease caused by other microorganisms should always be considered and must be ruled out (Flynn et al., 1973). *Balantidium coli* may cause diarrhea in humans; therefore, care should be taken in handling captive nonhuman primates to avoid infection (Flynn et al., 1973).

*Giardia duodenalis* (syn. *Giardia lamblia, Giardia intestinalis*) is a ubiquitous enteric flagellated protozoan of global importance that infects a wide range of hosts, i.e. >40 animal species, including humans. It is a common leading cause of infection known as giardiosis (or giardiasis) and infects up to ~28.2 million people worldwide, with 500,000 new cases every year (Ryan

et al., 2019). *Giardia duodenalis* has been frequently identified as pathogen in non-human primates (NHP) (Levecke et al., 2009; Koster et al., 2022). The prevalence of *G. duodenalis* in NHP kept in zoos in different European countries ranged between 6% and 70% in studies conducted in Belgium (Levecke et al., 2007), Croatia (Beck et al., 2011), Poland (Maesano et al., 2014), Slovakia (Mravcova et al., 2021), Spain and Brazil (Martinez et al., 2011; David et al., 2014). A recent study conducted in six European zoological gardens (located in France, Germany, and Spain) reported a 18.1% prevalence of *G. duodenalis* with the presence of both Assemblages A and B (Koster et al., 2022). The Assemblages of *G. duodenalis* found in the NHP in Italy are mainly A and B, with the dominant Assemblage B (Berrilli et al., 2011; Cacciò et al., 2008). Giardiosis in NHP causes diarrhea and slow growth, especially in juvenile animals (Karim et al.., 2015). However, few studies have demonstrated an association between the presence of *G. duodenalis* infection and the occurrence of clinical manifestations (Sestak et al., 2015), strongly suggesting that the pathogenic role of *G. duodenalis* in captive NHP is limited (Koster et al., 2022). Nevertheless, NHP play an important role as reservoirs of zoonotic *Giardia* infections in the zoological gardens (Kowalewski et al., 2011; Einarsson et al., 2016; Koster et al., 2022; , 19-21).

Strongyloidiasis is a disease results from infection by the parasitic members of the genus *Strongyloides*. These small nematodes are prevalent in most tropical and subtropical areas, but their occurrence in the temperate zones is sporadic. Several species have been reported to affect nonhuman primates: *Strongyloides cebus* has been found in New World monkeys (cebus monkeys, woolly monkeys, spider monkeys, squirrel monkeys and marmosets). *Strongyloides fulleborni* in Old World monkeys and great apes (rhesus monkeys, cynomolgus monkeys,guenons, baboons, and chimpanzees) and *Strongyloides stercoralis* and *Strongyloides sp.* in Old World monkeys (patas monkeys) and great apes (gibbons, chimpanzees, gorillas, and orangutans). Only adult females and larvae are found in the gastrointestinal tract of the host animal. Migrating larvae can be found in the lungs and other parenchymatous organs. Parasitic males have never been described (Flynn et al., 1973). The life cycle of *Strongyloides sp.* is complex and consists of both parasitic and free-living generations (Chandler et al., 1961; Flynn et al. 1973). The reader is referred to parasitology texts and referenced papers for a detailed discussion of this unique life cycle (Chang et al., 1957; Soulsby et al., 1965; Little get al., 1966; Flynn et al., 1973; Shadduck et al., 1978). A vari-

ation in this life cycle, known as autoinfection, is a direct reinvasion of the host animal by filariform larvae that have developed during passage through the lower intestinal tract (De Paoli et al., 1978). This phenomenon results in hyperinfection of the infected host and is most responsible for sustained infections that result in clinical disease, severe damage to affected organs, and death (Brown et al., 1958; DePaoli et al., 1978; Flynn et al., 1973). There is also evidence of intrauterine or transcolostral transmission (Moncol et al 1966; Flynn et al., 1973). The disease in gibbons has been reported in detail. Diarrhea, which may be hemorrhagic or mucoid, is the most common clinical sign described in infected animals (De Paoli et al., 1978). Other common clinical signs are dermatitis, urticaria, anorexia, depression, listlessness, debilitation, vomiting, emaciation, reduced growth rate, dehydration, constipation, dyspnea, cough, prostration, and death. Paralytic ileus is described in infected gibbons (DePaoli et al., 1978). Gross lesions consist of catarrhal to hemorrhagic or necrotizing enterocolitis (DePaoli et al., 1978; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). There may be a secondary peritonitis associated with the enterocolitis (Flynn et al., 1973; Jones et al., 1983). Pulmonary hemorrhage is the most common lesion outside the digestive tract (Flynn et al., 1973; King et al., 1976; DePaoli et al., 1978; Shadduck et al., 1978).. Histologic examination of the small intestine of the infected animal shows a multifocal erosive and ulcerative enteritis caused by adults, eggs, and rhabditiform larvae (DePaoli et al., 1978). The mucosa contains numerous parasites, most of which are in intraepithelial tunnels or lumina of intestinal glands. These lesions may be infiltrated by neutrophils. Mononuclear cells and an occasional eosinophil can be seen in the lamina propria. Intestinal villi are short and blunt, and in severe infections bridging and loss of villi are seen (DePaoli et al., 1978). In cases where autoinfection has occurred, changes in the small and large intestines in response to invasion by the filariform larvae range from a mild inflammatory cell response to severe, acute, or granulomatous or necrotizing enterocolitis. Larval invasion of the submucosal and serosal lymphatics results in a severe granulomatous endolymphangitis (DePaoli et al., 1978). These changes are associated with various degrees of lymphatic obstruction and submucosal and serosal edema, fibrosis, or both. In the lungs, acute multifocal or diffuse hemorrhage is most common. Larval granulomas may be seen over the surface of the pleura. Filariform larvae also are seen in many tissues throughout the body, most commonly in the lymph nodes and liver (DePaoli et al., 1978). Fatal

strongyloidiasis has been described in lowland gorillas and chimpanzees (Penner et al., 1981).

This condition may be diagnosed by identification of typical larvae in the stool; by clinical signs; or by demonstration of parasitic adult females, eggs, and larvae at necropsy or at histologic examination (Flynn et al., 1973). Strongyloidiasis in nonhuman primate colonies is considered a potential public health problem.

Pinworms are small nematode parasites inhabit the colon and cecum of nonhuman primate hosts. Genera described in nonhuman primates are *Trypanoxyuris* and *Oxyuronema* species found in New World monkeys (Inglis et al., 1965; Dunn et al., 1968; King et al., 1976). *Enterobius vermicularis* and other *Enterobius* species, found in Old World monkeys and great apes; *Enterobius anthropopitheci* in the chimpanzee and several species in prosimian primates (Chabaud et al., 1958 and 1961; Berghe Van den et al., 1963). These parasites are considered cosmopolitan in geographic distribution but are more prevalent in temperate and cold climates. The life cycle is direct. Most reports of oxyuriasis in nonhuman primates state that these infections are essentially innocuous (Orihel et al., 1972; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). Clinical signs usually are limited to anal pruritis and irritation that may lead to self-mutilation, restlessness, and increased aggressiveness. Heavy pinworm infections are reported to be common in chimpanzees, and their coprophagic habits make constant reinfection inevitable (Rewell et al., 1948). Fatal cases of enterobiasis have been reported in chimpanzees (Schmidt et al., 1970; Keeling et al., 1974; Schmidt et al., 1978; Holmes et al., 1980), characterized by extensive ulcerative enterocolitis, peritonitis, and necrogranulomatous lymphadenitis involving the mesenteric lymphnodes. Numerous parasites with the morphologic characteristics of *Enterobius vermicularis* were associated with these lesions. There is also an early report of the death of a red spider monkey caused by an overwhelming pinworm infection (Kreis et al., 1931). Multiple intestinal polyps associated with immature male oxyurid parasites have been described in a male chimpanzee (Toft et al., 1976). The gross and histologic characteristics of these lesions were identical to those produced by *Nochtianochti* in the stomach and esophagus of Old World primates. It was thought that the lesion resulted from hypersensitivity to oxyurid infection in an aberrant host.

Adult oxyurids may be seen emerging from the anus. Perianal swabs or cellophane tape also can be used to recover the typical ellipsoid, asymmetrical

pinworm eggs (Faust et al., 1968; Flynn et al.,1973). Naturally infected non-human primates may be sources of infection in humans. Also, captive primates can acquire *E. vermicularis* infection from humans and then can act as reservoirs to reinfect them (Flynn et al, 1973).

*Oesophagostomum* is a parasite known commonly as nodular worms and are considered to be the most common nematode parasite found in Old World monkeys and great apes (Berghe Van den et al., 1968; Reardon et al., 1968; McClure et al., 1971; Flynn et al. 1973; Shadduck et al., 1978; Kennard et al., 1981). They have been described in baboons, mangabeys, guenons, macaques, chimpanzees, and gorillas. At least 11 different species have been proposed but not clearly defined (Yamashita et al., 1963; Oribel et al., 1970). The species mentioned most frequently are *O. apiostomum, O. bijurcim, O. aculeatum*, and *O. stephanostomum* (Faust et al., 1968; Flynn et al., 1973; Levine et al., 1976). Infected monkeys usually are asymptomatic, and light infections usually go unrecognized (Ruch et al., 1959; Flynn et al., 1973; Shadduck et al., 1978). Monkeys with severe infections may show general unthriftiness and debilitation characterized by increased weight loss and diarrhea; the mortality rate increases for this group (Ruch et al., 1959; Flynn et al., 1973; Shadduck et al., 1978). Lesions seen at necropsy consist of the typical oesophagostomum nodules, which are elevated, smooth 2-4 mm in diameter, and firm. They are seen most frequently on the serosal surface of the large intestine and cecum and in the mesentery supporting these organs but also in ectopic sites, such as the peritoneal wall, mesentery of the small intestine, omentum, kidney, liver, lungs, or diaphragm. The nodules may be black or brown if there is associated hemorrhage; older nodules usually are white because of caseation of the contents. Viable worms may be seen in relatively young nodules; usually, however, the parasite is dead and surrounded by a mass of caseous debris. Older nodules may contain foci of mineralization (Habermann et al., 1958; Flynn et al., 1973; Shadduck et al., 1978). The parasite and cell detritus usually are surrounded by a mantle of chronic inflammatory cells, mainly macrophages with scattered eosinophils and lymphocytes and plasma cells. Foreign-body giant cells sometimes are present in the cellular exudate. A fibrous capsule of various degrees of thickness and maturity, depending on the age of the nodule, surrounds the centrally located necroinflammatory mass. Sometimes ulcers form in the colonic mucosa at the point where the larval penetration occurred, and a migratory tract filled with inflammatory exudate connects the nodule in the wall with the intestinal

lumen (Habermann et al., 1958; Flynn et al., 1973; Shadduck et al., 1978). *Oesophagostomum* infection can be diagnosed by identifying the eggs in the feces. A problem arises, however, because the eggs of the different *Oesophaostomum* species cannot be differentiated from one another and also are indistinguishable from those of Temidens and other hookworm species. Occasionally, adults are passed and can be identified. The postmortem diagnosis is based on typical nodular lesions or identification of adults, or both (Flynn et al., 1973; Shadduck et al., 1978). This parasite has been reported to infect humans and therefore should be considered to have zoonotic potential (Haaf et al., 1964; Flynn et al, 1973).

*Ancylostoma duodenale* and *Necator americanus* are recorded occasionally in nonhuman primates, including monkeys, mandrills, baboons, gibbons, chimpanzees, and gorillas. Reports of their presence in South American monkeys are rare (Orihel et al., 1972). These parasites have a direct life cycle. Clinical signs associated with heavy hookworm infection in nonhuman primates are similar to those produced by these parasites in humans and other animals and include anemia, eosinophilia, "pot-belly" dyspnea on exertion, and a general debilitation (Flynn et al., 1973; Ruch et al., 1959). Necropsy findings have included a general pallor of all tissues. The mucosa of the small intestine was thickened by a chronic inflammatory reaction. Small hemorrhages were seen throughout the intestinal mucosa, and large numbers of hookworms were attached to the mucosa (Hamerton et al., 1941-1942). The diagnosis of hookworm disease is based on finding eggs in the feces or mature worms in the bowel at necropsy (Flynn et al., 1973). Since hookworm eggs are morphologically identical to those of several species of strongyles that also infect nonhuman primates, diagnosis based on the eggs alone should be viewed with caution (Orihel et al., 1972).

Because humans are the normal definitive host for these parasites, infected captive primates should be handled with caution (Flynn et al., 1973).

Molineiasis is a disease caused by trichostrongyles in the genus *Molineus*. These are small, slender, pale red worms that inhabit the upper digestive tract, duodenum, and sometimes the pyloric region of the stomach of nonhuman primates. Occasionally, they may involve the pancreas and mesentery. They are always found lying on the mucosa, never attached. Geographically, they are distributed throughout Central and South America, with one species occurring in Africa (Dunn et al., 1961 and 1968; Flynn et al., 1973). Species described in nonhuman primates include *M. vexillarius* in marmosets; *M. ele-*

*gans* in squirrel monkeys, cebus monkeys, and howler monkeys; *M. torulosus* in cebus monkeys, squirrel monkeys, and owl monkeys. *Molineus torulosus* is the only species reported to be a specific pathogen (Lapin et al., 1960; Brack et al., 1973; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). The life cycle and method of transmission of these parasites are unknown. Diagnosis rests upon the identification of typical eggs in the feces or the presence of adult worms associated with typical lesions in the digestive tract (Flynn et al., 1973; Levine et al., 1976; Orihel et al., 1971).

Infection with *M. torulosus* has been reported to cause hemorrhagic or ulcerative enteritis, sometimes associated with diverticula of the intestinal wall (Brack et al., 1973; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). Serosal nodules that involved the upper portion of the small intestine have been seen in capuchin monkeys (Brack et al., 1973). These nodules communicated with the intestinal lumen through I-mm reddish brown ulcers. Histologically, the nodules were composed of an intense granulomatous inflammatory response surrounded by a rim of proliferating fibrous connective tissue. The central portion contained a mass of nematode parasites and their eggs surrounded by eosinophilic debris. Neutrophils, histiocytes, and other chronic inflammatory cells were present adjacent to the worms. Chronic pancreatitis also was seen, with worms and eggs in inflamed pancreatic ducts. Nothing is known of the public health significance of this parasite.

Ascariasis is  a disease results from infection with members of the genus *Ascaris*, commonly known as roundworms. They are a common finding in the intestinal tract of non-human primates. The specimens that have been recovered are reported to be indistinguishable from *Ascaris lumbricoides* in humans (Dunn et al., 1962; Yamashita et al., 1963). Both Old World monkeys and great apes have been reported to beinfected. The life cycle of this parasite is direct.

Although roundworm infection in nonhuman primates is thought to be relatively innocuous and oflittle clinical significance (McClure et al., 1971; Orihel et al., 1972), fatal cases of ascariasis have been reported in both monkeys and great apes (Habermann et al., 1957; Stam et al., 1960).

Death in the great apes was thought to be due to the presence of many worms, blockage of the bowel, and migration of the worms into the bile duct and liver. Diagnosis of ascariasis is based on the presence of typical eggs in the feces or adults in the digestive tract at necropsy. Since the ascarids reported in nonhuman primates are morphologically identicalto *A. lumbricoides* in humans, cross-infection from infected animals to humans is possible. We could find no

reports that documented such an occurrence; nevertheless, infected nonhuman primates should be considered a potential zoonotic threat and should be handled accordingly.

*Trichospirura leptostoma* is a spirurid nematode that parasitizes the pancreatic ducts of several species of New World monkeys including marmosets, squirrel monkeys, and owl monkeys. Geographic distribution is confined to Central and South America (Cosgrove et al., 1968 and 1970; Smith et al., 1967 and 1969; Orihel et al., 1971 and 1972; Flynn et al., 1973; King et al., 1976).

Male and female adult parasites measure up to 15 mm and 120 mm, respectively. The eggs are medium in size and are typically spirurid in that they are thick shelled and contain a larva (Lillie et al., 1947). The complete life cycle is unknown (Flynn et al., 1973; King et al., 1976).The parasite usually is found incidentally on histological examination. Infection usually causes little tissue destruction or inflammatory reaction. Tissue response apparently varies in proportion to the number of parasites present (King et al., 1976; Orihel et al., 1972). Chronic pancreatitis in association with the worms has been described in marmosets (*Callithrix sp*.) (Smith et al., 1969; Orihel et al., 1972). Acute pancreatitis in owl monkeys consisting of a patchy granulocytic interstitial infiltrate adjacent to intralobular ducts was thought to be associated with leakage of retained pancreatic secretions. Larger ducts containing cross sections of worms also contained granulocytes (Orihel et al., 1971 and 1972). *Pterygodermatites nycticebi*, is a spirurid nematode that has been reported from prosimians (slow loris), New World monkeys (tamarins and marmosets), and the great apes (gibbons) (Lindquist et al., 1980). Several reports in members of the family Callitrichidae refer to this parasite by a synonym, *Rictularia nycticebi*. The life cycle of *P. nycticebi* is indirect with cockroaches serving as intermediate hosts.

Morbidity and mortality associated with infection of *P. nycticebi* have been reported in golden lion tamarins (*Leontopithecus rosalia*). Clinical signs in heavily infected animals included extreme weakness, passage of watery diarrhea that contained the adult parasites, anemia, leukopenia, and hypoproteinemia. At necropsy, masses of *P. nycticebi* parasites were found throughout the gastrointestinal tract. Histopathologically, the anterior ends of the adult worms were embedded in the mucosa of the small intestine. Larvae were seen deeper in the submucosa. In a few cases, worms were seen in the tunica muscularis and the pancreatic ducts. There was severe clubbing of the small intestinal villi and randomly located foci composed of a necrotic pseudomembrane containing

spirurid eggs, numerous yeasts, and pseudohyphae consistent with *Candida* sp. Diagnosis depends upon demonstrating and identifying the characteristic spirurid eggs, adult worms, or larvae in the feces, in the gastrointestinal tract at necropsy, or in histopathological slide preparations.

Nothing is known about the public health significance of this parasite. Methods of control should be directed against the cockroach intermediate host through reducing populations and preventing consumption by susceptible hosts.

Physalopteriasis is a disease is caused by infection by members of the genus *Physaloptera*. Four species of physalopterids have been reported to occur in the upper gastrointestinal tract of nonhuman primates. *Physaloptera tumefaciens* is common in the stomach of Asian macaques. *Physaloptera dilatata* is found in the stomach of New World monkeys (titi monkeys, bearded sakis, and marmosets). *Abbreviata caucasica* has been found in the esophagus, stomach, and small intestine of the rhesus monkey, baboon, and orangutan. *Abbreviata poicilometra* has been found in the stomach ofmangabeys and guenons (Flynn et al., 1973; Slaughter et al., 1969). The life cycle of the physalopterids is indirect; an arthropod intermediate host is required. The entire life cycle is not completely understood, and a second intermediate or paratenic host may be necessary (FLynn et al., 1973; Soulsby et al., 1965). Lesions result from the attachment of the worms to the wall of the affected organ. Gastritis, esophagitis, enteritis, erosion, and ulceration of the mucosa at the point of attachment are seen at necropsy (Flynn et al., 1973). Hyperplastic gastric lesions and perforation of the stomach wall associated with *Physaloptera* sp. infection in cynomolgus monkeys have been described (Sakakibara et al., 1981). Diagnosis depends upon identification of the ova in the feces or the presence of adult worms attached to the mucosa of the upper digestive tract (Flynn et al., 1973). The public health aspects of these parasites are unknown.

Capillariasis is a disease results from infection with the cosmopolitan trichurid parasite *Capillaria hepatica*. It has been reported in the liver of a wide variety of mammalian hosts throughout the world including New World monkeys (squirrel monkeys, cebus monkeys, and spider monkeys), Old World monkeys (rhesus monkeys), and great apes (chimpanzee).

The anterior portion of these parasites is more slender than the posterior, but it is not as pronounced as in the whipworms. The eggs have bipolar opercula, and the shell contains many small perforations giving it a striated appearance. This feature is unique and is used to distinguish ova of *C. hepatica* from those of

other trichurids (Flynn et al., 1973; Orihel et al., 1972; Shadduck et al., 1978). The life cycle is direct and unique. Adult worms are found only in the hepatic parenchyma, and eggs are retained within the liver until the host dies or is killed. The eggs must be liberated from the liver either by decomposition of the original host or by passage through a predator or scavenger. Ingestion of infected liver tissue produces only spurious passage of the eggs in the feces. To become infective, the eggs must undergo embryonation under aerobic conditions. Infection occurs when embryonated eggs are ingested. The liver of infected animals reveals randomly placed white or yellow patches or nodules over the surface. Histopathologically, these foci are composed of adult C hepatica and masses of eggs that are surrounded and infiltrated by proliferating fibrous connective tissue, chronic inflammatory cells, and foreign body giant cells. These lesions are ultimately converted to scar tissue, and the liver becomes cirrhotic. Fatal hepatitis has been reported in infected nonhuman primates (Fiennes et al., 1967; Orihel et al., 1972; Flynn et al., 1973; Shadduck et al., 1978). Neither eggs or adult parasites will be found in the feces; therefore, diagnosis depends on demonstration and identification of the typical eggs and/or worms through liver biopsy or at necropsy (Flynn et al., 1973; Shadduck et al., 1978). This parasite is pathogenic for humans, but because of the unusual life cycle of C hepatica, infective nonhuman primates do not constitute a public health menace for persons caring for or working with them (Faust et al., 1968; Flynn et al., 1973). *Anatrichosoma cutaneum or Anatrichosoma cynomolgi* have been described from both Asian and African Old World nonhuman primates (rhesus monkeys, cynomolgus monkeys, patas monkeys, vervets, talapoin monkeys, mangabeys, and baboons). (Chitwood et al., 1958; Allen et al., 1960; Faust et al., 1968; Reardon et al., 1968; Oribel et al., 1970; Migaki et al., 1971; Flynn et al., 1973; Kessler et al., 1982) and great apes (gibbons), (Breznock et al., 1975).

The adult worms are small and slender. The eggs are large, barrel shaped, have bipolar opercula, and unlike *Trichuris* and *Capillaria* contain a larva (Orihel et al., 1972; Flynn et al., 1973).

The entire life cycle and method of transmission are not known, but the cycle is thought probably to be direct. The female worms migrate through the stratified layers of squamous epithelium forming tunnels in which the embryonated eggs are deposited. These tunnels are composed of epithelial cells and maintain their integrity. They are sloughed with the superficial keratin layers of the squamous epithelium and accumulate on the mucosal surface of the nares.

Eggs are excreted from the host in the nasal secretions and less often in the feces (Allen et al., 1960; Orihel et al., 1970; Flynn et al., 1973). The original report of this parasite in nonhuman primates was from skin lesions on the extremities. Grossly, these lesions had the appearance of white, serpentine tracks on the palms and/or soles of the hands and feet (Orihel et al., 1972). Since then, they have been reported only from the stratified squamous epithelium of the external nares. Infection of the nares does not produce serious disease and is usually subclinical but is considered to be common in susceptible animals. Histopathologically, the affected epithelium is diffusely hyperplastic and parakeratotic, and there is a mild inflammatory infiltrate composed of leukocytes and plasma cells in the underlying lamina propria. Diagnosis in the living animal can be made through the use of nasal mucosal scrapings or swabs that will reveal the characteristic eggs. In the dead animal, finding of the parasite in microscopic slides of the mucosa is considered to be diagnostic (Allen et al., 1960; Flynn et al. 1973; Orihel et al., 1972; Shadduck et al., 1978). This parasite has been reported in humans, where it causes a type of creeping eruption. Even though infection in humans is considered to be uncommon, those personnel who work with and care for nonhuman primates should handle those species known to be infected or susceptible to infection with proper caution. Tapeworm that have been described in the intestinal tract of nonhuman primates, including prosimians, New and Old World monkeys, and great apes. Life cycles for all the genera listed, except one, are indirect and require an arthropod intermediate host for completion of their cycle. *Hymenolepis nana* can complete its life cycle either through direct or indirect means (Flynn et al., 1973; King et al., 1976). Although these parasites may be present in large numbers, clinical disease or enteric lesions are seldom associated with tapeworm infection. Diagnosis depends on the identification of characteristic eggs in the feces, passing of proglottids of adult worms, or the recovery of adult worms at necropsy (Flynn et al., 1973).

Some tapeworm genera (*Hymenolepis, Raillietina, Bertiella*) rarely affect humans. Proper precautions in handling captive nonhuman primates, good personal hygiene by the caretakers, and care in disposing of bedding and feces of infected animals should be stressed in order to rule out accidental transfer of infection to humans (Flynn et al., 1973).

## LARVAL CESTODIASIS

Nonhuman primates may serve as intermediate hosts for several species of tapeworm parasites and thus develop various larval forms of these parasites in their somatic tissues. The cestode larvae are classified as solid and bladder forms. The solid larvae are represented by the sparganum. The bladder larvae consist of cysticercus, coenurus, hydatid, and tetrathyridium.

### *Sparganosis*

This term denotes infection with the elongate, nonspecific plerocercoid larvae of cestodes in the order Pseudophylloidea (King et al, 1976; Shadduck et al., 1978).The adult tapeworms belong to the genera *Diphylobothrium* and *Spirometra*, which are intestinal parasites of various carnivores, birds, and reptiles (Myers et al., 1972). Spargana have been described in New World monkeys (squirrel monkeys and marmosets) (Dunn et al., 1963 and 1968; Cosgrove et al., 1968; Flynn et al., 1973; King et al., 1976), Old World monkeys (rhesus monkeys, cynomolgus monkeys, vervets, baboons, and talapoin monkeys), and prosimians (tree shrew),(Schmidt et al., 1977). These larvae are solid with a scolex that contains a pseudosucker. They are white, ribbon-like, and of variable size and motility. They resemble the adult except they lack proglotids and mature genitalia. *Spargana* can vary from a few millimeters to several centimeters in length (Myers et al., 1972; Shadduck et al., 1978; Toft et al., 1980). In nonhuman primates, *Spargana* may be found in any part of the body: in retroperitoneal tissues, in abdominal or pleural cavities, or in subcutaneous and muscular tissues. They are commonly encased by a connective tissue capsule, and they do not incite much of an inflammatory response unless they die. These degenerating larvae may cause local inflammation and edema. Most infections in nonhuman primates are usually asymptomatic, and their presence is considered to be an incidental finding at necropsy (Myers et al., 1972; Shadduck et al., 1978). Diagnosis can be made in the live animal by radiography, which may reveal calcified nodules. Also, one may palpate mobile nodules in the subcutaneous tissue with localized edema. In the dead animal, diagnosis is made through demonstration and identification of the characteristic *Spargana* larvae either grossly at necropsy or microscopically in histopathologic specimens (Myers et al. 1972; Shadduck et al., 1978).

***Cystricercosis***

This condition is the result of infection with the larval form of various members of the family Taeniidae. Adult tapeworms of this family commonly parasitize birds and mammals (Mayers et al., 1972; King et al., 1976). Cystericerci have been described in New World monkeys (squirrel monkeys and marmosets) (King et al., 1976), Old World monkeys (rhesus monkeys, baboons, mangabeys, patas monkeys, langurs, and vervets), (Graham et al., 1960; Mayers et al., 1965 and 1972; Fiennes et al., 1967; Vickers et al., 1968; Flynn et al., 1973), great apes (gibbons and chimpanzees) (Yamashita et al., 1963; Mayers et al., 1972; Sagartz et al., 1974), and prosimians (lemur), (Mayers et al., 1972). Cysticerci are oval, translucent cysts that contain a single invaginated scolex with four suckers. In those species that have them, a circle of hooks is present (Mayers et al., 1972; King et al., 1976; Toft et al., 1980). These cysts may be found in the abdominal or thoracic cavities, muscle, subcutaneous tissue, and central nervous system. Usually there is very little host inflammatory reaction to the presence of viable cysts. As the cysts enlarge, there may be compression of adjacent tissues. Dead cysts will provoke an intense chronic inflammatory reaction (Vickers et al., 1968; Myers et al., 1972; King et al., 1976). Symptoms in nonhuman primates are directly related to the tissue in which the cysticercus develops and the number present. Involvement of the central nervous system can produce neurological disorders, but this appears to be less of a problem in infected nonhuman primates than in cerebral cysticercosis in the human patient (Vickers et al., 1968; Myers et al., 1972). Diagnosis depends upon the finding of the characteristic bladder-shaped structure in the tissues. Identification of the specific species involved is based on the characteristic hook size and structure (Myers et al., 1972).

***Coenurosis***

This condition is the result of infection with the larval form of the tapeworms *Taenia multiceps* or *Taenia serialis*, which are intestinal cestodes of dogs and related carnivores (Flynn et al., 1973; King et al., 1976). Coenurosis has been reported in Old World monkeys (macaques, vervets, gelada baboon, and other baboons), (Fain et al., 1956; Kuntz et al., 1967, Clarck et al., 1969; Mayers et al., 1972; Flynn et al., 1973) and prosimians (lemur), (Mayers et al., 1972). The coenurus is a polycephalid larval form that produces both internal and external daughter cysts. The inner layer of the cyst wall is composed of ger-

minal epithelium from which numerous scolices develop. Coenuri have been described in the subcutaneous tissues, peritoneal cavity, liver, brain, and other organs of affected nonhuman primates. Clinical signs and histopathology depend on the number of coenuri present and their location. In general, infection in nonhuman primates has produced minimal symptoms and lesions ( Mayers et al., 1972). However, in those cases where there is involvement of the central nervous system, typical neurological symptoms are observed (Mayers et al., 1972). Diagnosis can be made by radiography or the finding of a tumor-like mass in the subcutaneous tissues. Identification of the species of cestode is based on the hook structure of the scolex (Flynn et al., 1973; Mayers et al., 1972).

***Hydatidosis***

This disease, also known as echinococcosis, is the result of infection by the larval stage of cestode parasites in the genus *Echinococcus*. The adult tapeworms are found in the intestinal tract of dogs, wolves, bush dogs, other members of the canine family, and related carnivores (Mayers et al., 1972; Flynn et al., 1973; King et al., 1976). Hydatid cysts caused by *E. granulosus* have been described from a number of Old World monkeys (guenons, colobus monkey, mangabeys, mandrills, rhesus monkeys, other macaques, Celebes ape, and baboons), (Dissanaike et al., 1958; Summers et al., 1960; Healy et al., 1963; Myers et al., 1965 and 1970; Powers et al., 1966; Crosby et al., 1969; Houser et al., 1971; Flynn et al., 1973; Boever et al., 1975; Palotay et al., 1975; Eisenbrandt et al., 1978; Parker et al., 1979). New World monkeys (marmoset),(King et al., 1976), great apes (chimpanzee, gorilla, and orangutan),(Bernstein et al., 1972; Gardner et al., 1978; O'Grady et al., 1982), and prosimians (galago and lemurs), (Flynn et al., 1973; Palotay et al., 1975). Recently, hydatid cysts from the tapeworm *E. vogeli* have been reported from a group of young great apes (gorillas, orangutans, and chimpanzees),(Howard et al., 1980). Hydatid cysts are large, unilocular cysts. The inner layer of the cyst wall is composed of germinal epithelium from which numerous brood capsules develop. Multiple scolices then develop from the wall of the brood capsule. The cyst wall of *E. granulosa* is characteristically laminated and composed of a thick hyaline material (Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978; Toft et al., 1980). Hydatid cysts may be located in the abdominal cavity, liver, lungs, subcutis, or throughout the body (Dissanaike

et al., 1958; Powers et al., 1966; Crosby et al., 1968; Ilievski et al., 1969; Myers et al., 1970 and 1972; Flynn et al., 1973; King et al., 1976; Shadduck et al. 1978). The size of the cyst and the amount of involvement and host reaction depends on its age and the location within the host. Abdominal distension or localized subcutaneous swellings are sometimes seen, but usually the presence of the cysts causes no clinical signs or ill effects and they are found incidentally at necropsy (Summers et al., 1960; Healy et al., 1963; Crosby et al., 1968; Lapage et al., 1968; Myers et al., 1972; Flynn et al., 1973; Shadduck et al., 1978). The gross appearance of the cyst is that of a varying sized, spherical mass, usually in the liver, but it may sometimes be embedded in the lungs or be free in the abdominal cavity. Rupture of pulmonary hydatid cysts and resulting anaphylactic shock have been suggested as the cause of death in several cases of echinococcosis in nonhuman primates. Free scolices from ruptured cysts can implant in other tissues and produce additional cysts (Mayers et al., 1972). The diagosis of hydatidosis is usually not made until after the cyst reaches considerable size. Symptoms may mimic a neoplasm. Radiographs can be helpful in detecting the presence of pulmonary or calcified hepatic cysts. Specific identification is based on the finding of detached scolices, or daughter cysts, in the cyst fluid. The hook is considered characteristic for the genus. If scolices are not present, the his to morphology of the cyst wall can be used as identifying criteria (Kagan et al., 1959; Crosby et al., 1968; Myers et al., 1972; Flynn et al., 1973; shadduck et al., 1978). Abdominal ultrasonic scanning has been used successfully in diagnosing echinococcosis in gorillas (O'Grady et al., 1982).

***Tetrathyridiosis***

This condition results from infection with the larval stage of cestode parasites in the genus *Mesocestoides*. The adult tapeworms of this genus parasitize various birds and mammals. It has been described in Old World monkeys (rhesus monkeys, guenons, cynomolgus monkeys, and baboons) (Graham et al., 1960; Reardon et al., 1968; Myers et al., 1972; Flynn et al., 1973; Reid et al., 1976) and great apes (gibbon), but its occurrence in nonhuman primates is considered to be uncommon or even rare (Flynn et al., 1973). The tetrathyridial larva is flat and has an extremely contractile body. They may be confused with spargana. The anterior end is knotlike and contains an invaginated holdfast apparatus with four suckers. Length can vary from 2 to

70 mm, depending on the species of cestode and species of host. The tetrathyridum is proglotted shaped in the monkey. These larvae usually are found free in the serous cavities of the body or are found encysted in various tissues. *Tetrathyridum* evokes little host response and is usually considered to be an incidental finding in nonhuman primates.

Diagnosis depends on demonstration and identification of the characteristic larval form in the body cavities or encysted in the host tissues. Larval cestodes in nonhuman primates are of little public health importance to humans because infection can occur only by ingestion of the larval form. Of more importance to both humans and captive nonhuman primates is the possible ingestionof eggs passed by the infected definitive host. For this reason, feces from domestic and feral canids should be handled and disposed of with extreme care. Control of these parasites can only be accomplished through programs aimed at eliminating them from the definitive host (Flynn et al., 1973).

## ACANTHOCEPHALIASIS

This disease in nonhuman primates is most frequently the result of infection with acanthocephalan parasites in the genus *Prosthenorchis*. These parasites are distributed throughout Central and South America and have been reported in a variety of New World monkeys. Prosimians, Old World primates, and great apes can become infected under laboratory or captive conditions. The species involved are *P. elegans*, which inhabits the cecum or colon, and *P. spirula*, which favors the terminal ileum (Dunn et al., 1963 and 1968; Richart et al., 1963; Middleton et al., 1966; Nelson et al., 1966; Gamer et al., 1967; Deinhardt et al., 1969; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978; MacKenzie et al., 1979; Nielsen et al., 1980).

The life cycle is indirect with cockroaches and beetles acting as the intermediate hosts. Diagnosis depends upon identification of the characteristic thick-walled eggs or, more rarely, the worm itself in the feces. Conventional fecal flotation methods are ineffective as a means of demonstrating the eggs of these worms; fecal smears or sedimentation techniques must be used. At necropsy the finding of typical “thorny-headed worms” attached to the intestinal mucosa is considered diagnostic (Deinhardt et al., 1969; Schmidt et al., 1972; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). No distinctive symptoms accompany infection with acanthocephalans. Suspected

cases must be confirmed by diagnostic methods. Clinical signs vary depending upon the severity of the infection. Diarrhea, anorexia, debilitation, and death all have been associated with acanthocephaliasis in New World monkeys. In cases of massive infection, there is often cachexia caused by secondary complications and perhaps pain, sometimes of sudden onset; death follows rapidly. Most often the parasite does not contribute directly to the animal's death, but rather produces lesions that allow secondary pathogens to become established, resulting in debilitation and the ultimate demise of the host (Deinhardt et al., 1969; Moore et al., 1970; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). Attachment of the proboscis of these parasites to the intestinal mucosa causes a pronounced, usually severe, granulomatous inflammatory response, and the nodules formed usually can be seen from the serosal surface. The proboscis often penetrates the mucosa and invades the muscular layers of the intestinal wall. If complete penetration of the intestinal wall occurs, a fatal peritonitis result. Adult parasites sometimes are found in the abdominal cavity. Severe infections can cause mechanical blockage of the intestinal tract, intussusception, or rectal prolapse. Under these circumstances, infected animals will be depressed and pass bright red blood and scanty feces (Dunn et al., 1963; Middleton et al., 1966; Nelson et al., 1966; Deinhardt et al., 1967 and 1969; Gamer et al., 1967; Schmidt et al., 1972; Flynn et al., 1973; King et al., 1976; Shadduck et al., 1978). Histologically, a chronic, active inflammatory response is seen, with ulcers of the mucosa and granuloma and abscess formation in the intestinal wall associated with penetration of the proboscis and the resulting destruction of existing tissues. A focal suppurative to fibrinopurulent serositis also may be present in areas where the parasites approach penetration or actually rupture the intestinal wall. A hepatic abscess and granulomatous myositis (diaphragm) associated with migration of an unidentified acanthocephalan has been reported from an adult bushbaby (*Galago crassicaudatus*), (Brown et al., 1969). Infection with these parasites has not been reported in humans (Flynn et al., 1973).

## IV. Coprological techniques

The Coproscopy (from the Greek words *κόπρος* = faeces and *-σκοπία* = examen), i.e. the analysis of faecal samples for the presence of parasites (adult or part of them) and/or parasitic elements (PE) (i.e.. eggs, larvae, oocysts and cysts) is the most widely used diagnostic procedure in veterinary parasitology (Cringoli et al., 2004). This is the so-called coproscopy *sensu stricto*, instead, coproscopy *sensu lato* is the detection of antigens and/or DNA in faecal samples by immunological (e.g. ELISA) or molecular (e.g. (q)PCR) methods. Copromicroscopic techniques can be either qualitative, providing only the presence/assence of parasites or quantitative, providing also the number of parasitic elements (PEs= eggs, larvae, oocysts and cysts) per gram of faeces (i.e. EPG/LPG/OPG and CPG). After foundation of copromicroscopy by C.J. Davaine in 1857, several copromicroscopic techniques (and devices) have been developed, such as the qualitative techniques direct simple flotation in tube (Fulleborn et al., 1921) and direct centrifugal flotation method (Lane et al., 1924) or the quantitative Stoll dilution technique (Stoll et al., 1923, 1930), McMaster method (Gordon and Whitlock et al., 1939), Wisconsin flotation (Cox and Todd et al., 1962) and FLOTAC and Mini-FLOTAC techniques (Cringoli et al., 2010, 2017). Morevover, qualitative and/or quantitative techniques can be performed using an enrichment solution (e.g. sedimentation, flotation) or not (e.g. direct smear), in order to better concentrate the PE and to separate the PE from faecal debris. When tap water is added to faeces, PEs sediment at the bottom of the medium (i.e. sedimentation technique), whilst, when a flotation solution (FS) is added, PEs float at the top of the medium (i.e. flotation technique). Diagnosis of gastrointestinal nematodes (GIN) in mammals, still relies mainly on qualitative techniques, even if quantitative techniques, also called faecal egg count (FEC) are very important to determine anthelmintic efficacy/resistance through the faecal egg count reduction test (FECRT) (Vercruysse et al., 2018).

Several variants of these above mentioned techniques, are available in different manuals of diagnostic veterinary parasitology (MAFF et al., 1986; Thienpont et al., 1986; Foreyt et al., 2001; Hendrix et al., 2006; Zajac and Conboy et al., 2012). The simplest diagnostic technique was the direct smear, but provided false negative results (Cringoli et al., 2017). One of the first modifications to the faecal smear was the use of sedimentation to concentrate PEs (Rivas et al., 1928). It should be noted that, in livestock species, sedimentation techniques

are considered of less use (and time-consuming) to detect GIN eggs, whereas they are very useful for recovering heavy and operculated eggs (e.g. eggs of rumen and liver flukes, Paramphistomidae and *Fasciola hepatica*) that do not reliably float or are distorted by the effect of flotation solution (FS) (Dryden et al., 2005).

**Flotation techniques and flotation solutions**

The methods most frequently used to recover GIN eggs in ruminant faeces are based on flotation.

The PEs float in a FS with a specificity gravity higher (Koutz et al., 1941; Ballweber et al., 2014). Most of the FSs used in coprology are saturated and are made by adding a measured amount of salt or sugar (or a combination of them depending on the FS) to a specific amount of water to produce a solution with the desired specific gravity (SG). After preparing any FS, it is mandatory to check the SG with a hydrometer, recognizing that the SG of the saturated solution will vary depending on ambient temperature.

It should be noted that the choice of FS is important but does not receive sufficient consideration by the scientific community, despite the substantial effect that the FS can have on the diagnostic performance of any flotation technique (Cringoli et al., 2004, 2017). Usually, in the manuals of diagnostic parasitology only the specific gravity is reported for FS. It is commonly believed that the efficiency of a FS in terms of the capacity to bring eggs to float increases as the specific gravity of the FS increases. However, parasitic eggs should not be considered "inert elements" (Cringoli et al., 2004, 2017). Instead, interactions between the elements within a floating faecal suspension (e.g., FS components, eggs and residues of the host alimentation) might be complex and new research is needed to elucidate potential interactions between these elements.

As a rule of thumb, it is noteworthy that:

- Different FSs with the same s.g. do not produce the same results with respect to the same PE, even when the same technique is used.
- A given FS, which might be highly efficient with respect to a given PE, using a given technique, does not produce the same results if the technique is changed.
- A given FS, which is efficient with respect to a given PE, using a given technique on a fresh fecal sample, does not produce the same results if the method of fecal preservation changes (e.g., frozen, preserved in formalin

or SAF, or in other fixatives).
- It may happen that a given FS, which is efficient with respect to a given PE, using a given technique, does not produce the same results if the diet of the host changes.

It follows that when a copromicroscopic technique based on flotation is employed, each PE must be considered independently with respect to the FS, the technique and the method of fecal preservation used. What is known for a given PE cannot be readily translated to a 'similar' PE, or to the same PE when the technique or the fecal preservation method changes. Therefore, especially calibration of FEC techniques, to determine the optimal FS and fecal preservation method for an accurate diagnosis of parasitic elements, is a challenging topic of research (Rinaldi et al. 2011) performed a calibration for GIN detection in sheep and suggested that the best FS is sodium chloride (NaCl) with a low specific gravity (1.200).

**Flotation solution**
Sodium chloride (specific gravity 1.200)

**Recipe**
1 - Combine 1000 ml of warm water and about 500 grams of salt until no more salt goes into solution and the excess settles on the bottom of the container.
2 - Dissolve the salt in the water by stirring on a magnetic stirrer.
3 - To ensure that the solution is fully saturated, it should be allowed to stand overnight at room temperature. If the remaining salt crystals dissolve overnight, more can be added to ensure that the solution is saturated.
4 - Check the s.g. with a hydrometer, recognizing that the s.g. of saturated solution will vary slightly with environmental temperature.

In literature, many diagnostic techniques, using different FSs were developed (Fulleborn et al., 1921; Stoll et al., 1923, 1930; Gordon and Whitlock et al., 1939; Whitlock et al., 1941; Eigenfeld and Schlesinger et al., 1944; Seghetti et al., 1950; Mayhew et al., 1962; Slocombe et al, 1973; Rossanigo and Gruner et al., 1991; Presland et al., 2005; Cringoli et al., 2004; Cringoli et al., 2017). The flotation in tube is the simplest flotation method. The faecal material is mixed with a FS into a tube. Then, a coverslip is placed on the surface of the tube and after 15 minutes the coverslip is removed to examine it under the

microscope (MAFF et al., 1986). The main limit of this technique is that when the coverslip is removed from the top of the faecal suspension tube and then placed on a microscope slide, not all the floated PEs adhere to the underside of the coverslip. For these reasons flotation in centrifuge techniques were developed, e.g. Clayton- Lane, Wisconsin, Cornell-Wisconsin etc.

## Faecal egg count (FEC) techniques

Copromicroscopic diagnosis is usually performed by quantitative (FEC) techniques. All FEC techniques are based on the microscopic examination of an aliquot of faecal suspension from a known volume of faecal sample (Nicholls and Obendorf et al., 1994). The results are expressed like number of Pes (eggs, larvae, oocysts and cysts) per gram of faeces (i.e., EPG, LPG, OPG and CPG). Below are reported the main FEC techniques used for GIN detection in ruminants. For each method in Table 1.2 are reported diagnostic and technical performances (e.g. analytic sensitivity, accuracy and precision in assessing FECs, timing and ease of use), strengths and limitations (Cringoli et al., 2017).

**Table 1.2**. Parasite intensity (minimum, mean, and maximum) of eggs/oocysts/cysts per gram (EPG/OPG/CPG) of feces detected in zoo mammals in central and southern Italy by Mini-FLOTAC combined with fill FLOTAC techniques.

| Parasitic Group/Genus | No. pos. (%) | Mini-FLOTAC | | |
|---|---|---|---|---|
| | | Intensity (EPG/OPG/CPG*) | | |
| | | Min | Mean | Max |
| **Helminths** | | | | |
| **GI strongyles** | 12 (17) | 5 | 78 | 370 |
| ***Trichuris*** | 7 (10) | 5 | 20 | 40 |
| ***Parascaris*** | 4 (6) | 5 | 10 | 15 |
| ***Capillaria*** | 1 (1) | 0 | 8 | 10 |
| ***Nematodirus*** | 1 (1) | 0 | 4 | 4 |
| **Protozoa** | | | | |
| ***Eimeria*** | 1 (1) | 0 | 35 | 35 |
| ***Blastocystis*** | 2 (3) | ++ | + | + |
| ***Entamoeba coli*** | 1 (1) | - | + | + |
| ***Giardia*** | 1 (1) | - | + | + |

***CPG:** + = 1 cyst per microscopic fielf; ++ = 3 cyst per microscopic fielf.

**Stoll technique**

The first FEC technique was developed in 1923 by Stoll which published the paper entitled "Investigations on the control of hookworm disease. An effective method of counting hookworm eggs in faeces" (Stoll et al., 1923). In the Stoll dilution egg-counting technique, a diluent was added to the faecal sample and a known aliquot was withdrawn, so the eggs per gram of faeces (EPG) were determined using an appropriate dilution factor. In 1930 Stoll published a paper "On Methods of Counting Nematode Ova in Sheep Dung" developing the quantitative faecal egg count also in veterinary medicine (Stoll et al., 1930).

**Cornell-Wisconsin technique**

The modified Cornell-Wisconsin technique (Egwang and Slocombe et al., 1981, 1982) is based on flotation in centrifuge and eggs are recovered by means of adding a cover slide to the meniscus of the flotation solution. (Figure IV.1). This method has an analytic sensitivity of 1 EPG. However, when the number of eggs is high, inefficiencies may arise due to the lack of precision in the egg counting procedures owing to different factors as the possible loosing of some material during centrifugation, and the absence of a grid on

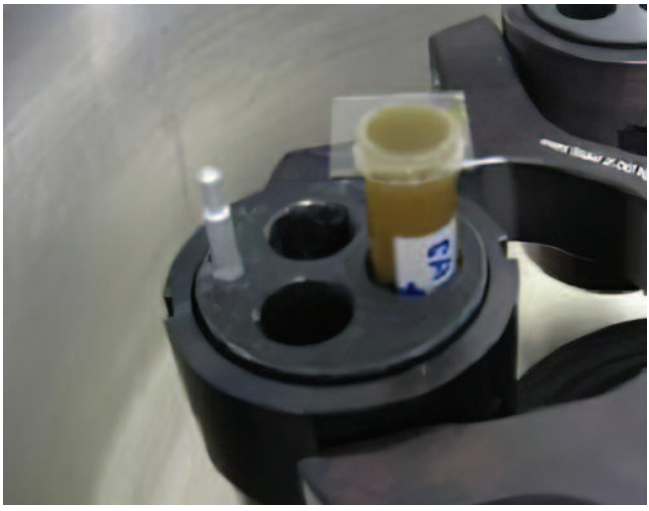

**Figure IV.1:** Flotation in centrifuge (Cornell-Wisconsin technique).

the coverslip (Cringoli et al., 2010; Levecke et al., 2012).

**McMaster technique**

The McMaster technique (Figure IV.2), developed and improved at the McMaster laboratory of the University of Sidney (Gordon and Whitlock et al., 1939; Whitlock et al., 1948), and whose name derives from one of the great benefactors in veterinary research in Australia, the McMaster family (Gordon et al., 1980), is the most universally used technique for estimating the number of helminth eggs in faeces (Rossanigo and Gruner et al., 1991; Nicholls and Obendorf et al., 1994). For decades, numerous modifications of this method have been described (Whitlock et al., 1948; Roberts and O'Sullivan et al., 1951; Levine et al., 1960; Raynaud et al., 1970), and most teaching and research institutions apply their own modifications to existing protocols (Kassai et al., 1999). Many of these modifications make use of different FSs, sample dilutions and counting procedures, which achieve varying analytic sensitivities (Cringoli et al., 2004; Roeber et al., 2013a,b). There are at least three variants of the McMaster technique (MAFF et al., 1986) with different analytic sensitivities: 50 EPG for the "modified McMaster method" and the "modified and further improved McMaster method" or 10 EPG in the case of the "special modification of the McMaster method" (MAFF et al., 1986). Although this technique is used in many laboratories, however in different studies showed a low sensitivity, accuracy and precision (Cringoli et al., 2017). Moreover, as reported in Cringoli et al., 2004, this method tends to overestimate the GIN, due to the tendency of eggs, during the flotation, to concentrate in the center of the McMaster slide.

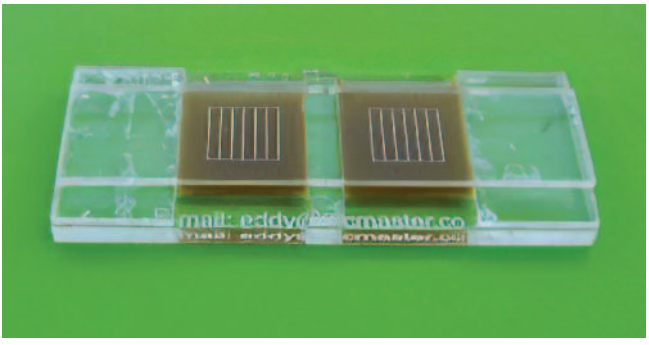

**Figure IV.2:** McMaster chamber.

**FECPAK technique**

FECPAK (Figure IV.3) was developed in New Zealand to provide a simple "on farm" method for GIN egg counting to make decisions on the need to treat or to determine whether anthelmintics are effective. It is a larger version of the McMaster slide, having a higher analytic sensitivity (usually 10-30 EPG). The apparatus has two flotation chambers and the total volume under the grids is of 1ml. The use of such a system requires a significant level of cooperation by farmers and adequate training to ensure that correct diagnoses are made (McCoy et al., 2005). Moreover, this technique is very expensive and it takes a lot of time to be performed.

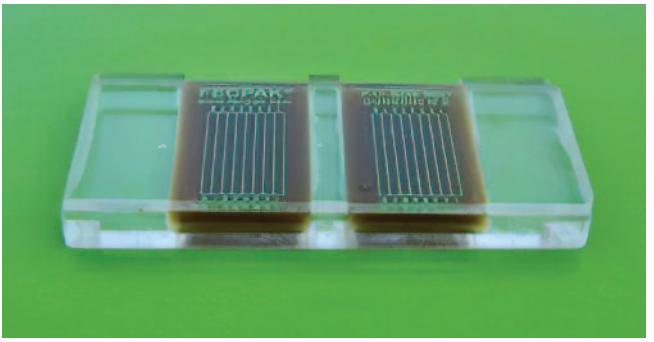

**Figure IV.3:** FECPAK chamber.

**FLOTAC and Mini-FLOTAC techniques**

The FLOTAC techniques are based on the centrifugal flotation of a fecal sample suspension and subsequent translation of the apical portion of the floating suspension. The FLOTAC apparatus is a cylindrical-shaped device made of polycarbonate amorphous thermoplastic. This material has been chosen because of excellent light transmission, high heat resistance, robustness (can be washed and re-used many times) and high-dimensional stability. The FLOTAC apparatus comprises three physical components, namely the base, the translation disc and the reading disc. There are two 5- ml flotation chambers, which are designed for optimal examination of large fecal sample suspensions in each flotation chamber (total volume = 10 ml). There are five accessories, namely the screw, the key, the bottom, the centrifuge adapter and the microscope

adapter. These accessories essential for proper functioning of the FLOTAC apparatus during centrifugation and subsequent examination under a microscope. There are two versions of the FLOTAC apparatus: FLOTAC-100, which permits a maximum magnification of ×100, and FLOTAC-400, which permits a maximum magnification of ×400. FLOTAC-400 is a further development and improvement over FLOTAC-100, as it allows microscopic diagnosis at a four fold higher magnification compared with FLOTAC-100, which is necessary for the detection of intestinal protozoa. FLOTAC-100, however, is still recommended for the diagnosis of helminth eggs and larvae, and for teaching purposes, because the reading disc is considerably thicker and hence more robust than the one used in FLOTAC-400, and because the flotation chambers can be filled more easily.
The FLOTAC device can be used with three techniques (basic, dual and double), which are variants of a single technique but with different applications.

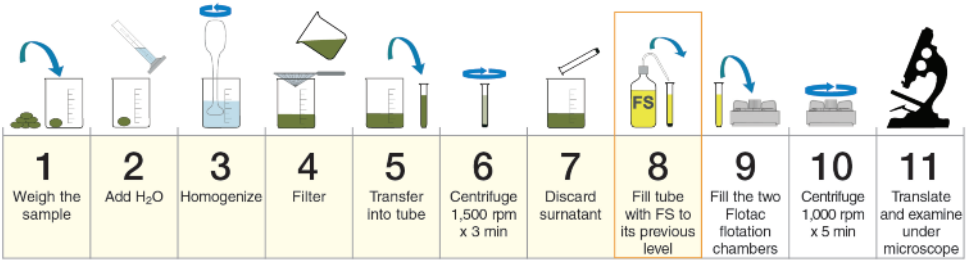


**Figure IV.4:** The steps of the FLOTAC technique.

The basic steps of the FLOTAC technique are showed in Figure IV.4.
The FLOTAC basic technique (analytic sensitivity = 1 EPG) uses a single FS and the reference units are the two flotation chambers (total volume 10 ml, corresponding to 1 g of feces). The FLOTAC dual technique (analytic sensitivity = 2 EPG) is based on the use of two different FSs that have complementary specific gravities and are used in parallel on the same fecal sample. It is suggested for a wide-ranged copromicroscopic diagnosis (GIN, lungworms, trematoda). With the FLOTAC dual technique, the reference unit is the single flotation chamber (volume 5 ml; corresponding to 0.5 g of feces). The FLOTAC double technique (analytic sensitivity = 2 EPG) is based on the simultaneous examination of two different fecal samples from two dif-

ferent hosts using a single FLOTAC apparatus. With this technique, the two fecal samples are each assigned to its own single flotation chamber, using the same FS. With the FLOTAC double technique, the reference unit is the single flotation chamber (volume 5 ml; corresponding to 0.5 g of feces).
A main limitation of FLOTAC is considered the centrifugation steps of the sample with a specific device, equipment that is often not available in all laboratories. To overcome these limitations, under the "FLOTAC strategy" of improving the quality of copromicroscopic diagnosis, a new simplified tool has been developed, i.e. the Mini-FLOTAC, that is a logical evolution of the FLOTAC techniques having an analytic sensitivity of 5 EPG (Cringoli et al., 2017). It is a easy-to-use and low cost method, which does not require any expensive equipment (i.e. centrifugation requirement) or energy source, so to be comfortably used to perform FECs (Cringoli et al., 2017) allowing easy transfer and very simple application. The Mini-FLOTAC apparatus is a disk-shaped device made of polycarbonate amorphous thermoplastic, with an excellent light transmission, heat resistance, robustness, high- dimensional stability, and good electrical insulation properties and it is composed by a base and a reading disk (physical components) and a key and a microscope adaptor (accessories) (Cringoli et al., 2017). There are two 1- ml flotation chambers, which are designed for optimal examination of fecal sample suspensions in each flotation chamber (total volume = 2 ml). The basic steps of

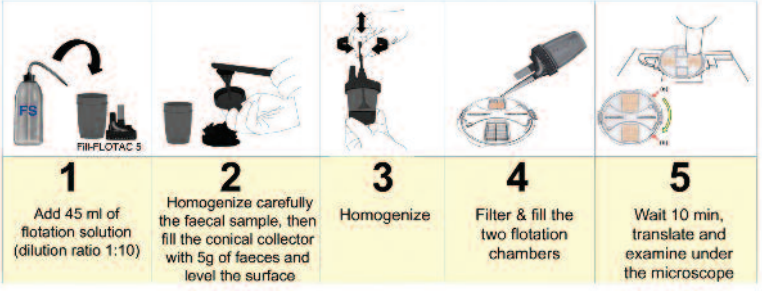


**Figure IV.5:** The steps of the Mini-FLOTAC technique

the Mini-FLOTAC techniques are showed in the Figure IV.5.
The Mini-FLOTAC permits a maximum magnification of 400× and can be very useful also for detection of intestinal protozoa and for the recognition of the details for the speciation of lungworms.

It is recommendable to combine Mini-FLOTAC with Fill-FLOTAC, a disposable sampling kit, which consists of a container, a collector (2 or 5 gr of feces) and a filter (Figure IV.6). FLOTAC, Mini-FLOTAC and Fill-FLOTAC are showed in Figure IV.7. There is a cone on the bottom of the graduated container, which permits homogenization of samples within the closed system of the container. On the top of the lid, there are two holes with screw caps: one central (with a large screw cap) for the collector/homogenizer pole and one lateral (with a small screw cap) for passage of filtered samples. The upper end (handle) of the collector/homogenizer pole is slightly thicker, whereas the lower part is conical and fits over the cone on the bottom of the graduated container. The conical end of the collector/homogenizer pole has a volume of either 2 g (Fill-FLOTAC 2) or 5 g (Fill-FLOTAC 5). As the name suggests, this part of the Fill-FLOTAC allows the collection and homogenization of the fecal sample in the Fill-FLOTAC container before laboratory processing. The filter is in the lower part of the lid. The thin plastic layer is perforated with 250-µm holes to ensure an optimal filtration of the fecal suspension.
The Fill-FLOTAC facilitates the performance of the first four consecutive steps of the Mini-FLOTAC technique, i.e. sample collection and weighing, homogenisation, filtration and filling. Morevoer, it is available a commercial kit, called “Mini-FLOTAC portable kit 200 tests” (Figure IV.8) that is very useful for the copromicroscopic diagnosis directly on the field.

**Figure IV.6:** The components of the Fill-FLOTAC.

**Figure IV.7:** (a) Mini-FLOTAC (b) FLOTAC and (c) Fill-FLOTAC apparatus.


**Figure IV.8:** The components of the "Mini-FLOTAC portable kit 200 tests": (1) Salt for flotation Solution; (2) Tank; (3) Wooden spatula (n = 200); (4) Mini-FLOTAC (n = 4); (5) Fill-FLOTAC (n= 4); (6) Tap; (7) Microscope adaptor (n = 2); (8) Instructions; (9) Devices to disassembly Fill-FLOTAC; (10) Tips for Fill-FLOTAC.

## V. References

Addessi E, Stammati M, Sabbatini G, Visalberghi E, 2005. How tufted capuchin monkeys (*Cebus apella*) rank monkey chow in relation to other foods. *Anim Welf* 14, 215-222.

Allemandi & C. Melfi V, Feistner ATC, 2002. A comparison of the activity budgets of wild and captive Sulawesi crested black macaques (*Macaca nigra*). *Anim Welf* 11, 213-222.

Allen AM (1960) Occurrence of the nematode, Anatrichosoma cutaneum, in the nasal mucosae of Macaca mulatta monkeys. Amer J Vet Res 21:389-392.

Amyx HL, Asher DM, Nash TE, Gibbs CJ Jr, Gajdusek DC (1978) Hepatic amebiasis in spider monkeys. Amer J Trop Med Hyg 27:888-891.

Anderson JR, Chamove AS, 1984. Allowing captive primates to forage. In: *Standards in animal management*. The Universities Federation for *Anim Welf*, 253-256.

Aspinall J, 1986. The Howletts gorilla bands. In: Benirschke K (ed.): *Primates. The road to self-sustaining populations*. *Springer-Verlag*, 465-470.

Auerbach E (1953) A study of Balantidium coli Stein, 1863 in relation to cytology and behavior in culture. J Morphol 93 :405-445.

Aureli F, de Waal F, 1997. Inhibition of social behavior in chimpanzees under high- density conditions. *Am J Primat* 41, 213-228.

Bailey C, Mansfield K, 2010. Emerging and reemerging infectious diseases of nonhuman primates in the laboratory setting. Vet Pathol 47, 462-81.

Baillie SR, Sutherland WJ, Freeman SN, Gregory RD, Paradis E, 2000. Consequen- ces of large-scale processes for the conservation of bird populations. *J Appl Ecol* 37, 88-102.

Baker JR (1972) Protozoa of tissues and blood (other than the Haemosporina). In: Fiennes RNT-W (ed) Pathology of simian primates. Part II. Infections and parasitic diseases. S Karger, Basel, pp 29-56.

Banish LD, Sims R, Sack D, et al., 1993. Prevalence of shigellosis and other enteric pathogens in a zoologic collection of primates. J Am Vet Med Assoc 203, 126-32.

Barman S, Chatterjee S, Chowdhury G, et al., 2010. Plasmid-mediated streptomycin and sulfamethoxazole resistance in Shigella flexneri 3a. Int J Antimicrob Agents 36, 348-51.

Beck R, Sprong H, Bata I, Lucinger S, Pozio E, Cacciò S. (2011) Prevalence

and molecular typing of Giardia spp. in captive mammals at the zoo of Zagreb, Croatia. Vet Parasitol (2011) 175(1–2): 40–46.

Belding DL (1965) Textbook of parasitology, 3rd ed. Appleton-Century-Crofts, New York.

Bennett BT, Abee CR, Henrickson R, 1995. Nonhuman primates in biomedical research.San Diego: Academic Press.

Benson RE, Fremming BD, Young RJ (1955) Care and management of chimpanzees at the Radiobio-logical Laboratory of the University of Texas and the United States Air Force, School Aviation Med. US Air Force Rept 55-48.

Berghe van den L, Chardome M, Peel E (1963) Trypanosomes of the African lemurs,Perodicticus potto ibeanus and Galago demidovi thomasi. J Protowol 10: 133-135.

BernsteinJ] (1972) An epizootic of hydatid disease in captive apes. J Zoo Anim Med 3:16-20.

Blancou J, Chomel BB, Belotto A, et al., 2005. Emerging or re-emerging bacterial zoonoses: factors of emergence, surveillance and control. *Vet Res* 36, 507-22.

Bland Sutton JB, 1884. Observations on rickets in wild animals. *J Anat Physiol* 18, 363-397.

Boever WJ, Britt J (1975) Hydatid disease in a mandrill baboon. JAVMA 167:619-621.

Bostrom RE, Ferrell JF, Martin JE (1968) Simian amebiasis with lesions simulating human amebic dysentery. Abstr 51 19th Ann Meeting Arner Assoc Lab Anim Sci,Las Vegas.

Brack M, Myers BJ, Kuntz RE (1973) Pathogenic properties of Molineus torulosus in capuchin mon-keys, Cebus apella. Lab Anim Sci 23:360-365.

Britt A, 1998. Encouraging natural feeding in captive-bred black and white ruffed lemurs (*Varecia variegata variegata*). *Zoo Biol* 17, 379-392.

Britt A, Welch C, Katz A, 2001. The impact of *Cryptoprocta ferox* on the *Varecia v. variegata* reinforcement project at Betampona. *Lemur News* 6, 35-37.

Brown RJ (1969) Acanthocephalan myositis in a bushbaby. JAVMA 155:1141-1143.

Buchanan-Smith HM, Prescott MJ, Cross NJ, 2004. What factors should determine cage sizes for primates in the laboratory? *Anim Welf* 13, S197-201.

## References


Butler TM (1973) The chimpanzee. In: Selected topics in laboratory animal medicine.Vol XVI: Aeromedical Review 1-73. USAF Sch Aerospace Med, Aerospace Med Div (AFSC), Brooks AFB, Texas, pp 1-81.

Burrows RB (1972) Protozoa of the intestinal tract. In: Fiennes RNT-W (ed) Pathology of simian primates. Part II. Infectious and parasitic diseases. S Karger, Basel, pp 2-28.

Cacciò SM, Ryan U. Molecular epidemiology of giardiasis. Mol Biochem Parasitol. (2008) 160:75-80. doi: 10.1016/j.molbiopara.2008.04.006.

Cacciò SM, Beck R, Lalle M, Marinculic A, Pozio E: Multilocus genotyping of Giardia duodenalis reveals striking differences between assemblages A and B. Int J Parasitol (2008) 38:1523-1531.

Caprioli A, Morabito S, Brugere H, et al., 2005. Enterohaemorrhagic Escherichia coli: emerging issues on virulence and modes of transmission. Vet Res 36, 289-311.

Carvalho CM, Andrade MC, Xavier SS, et al., 2003. Chronic Chagas' disease in rhesus monkeys (Macaca mulatta): evaluation of parasitemia, serology, electrocardiography, echocardiography, and radiology. Am J Trop Med Hyg 68, 683-91.

Catherine's Island, Georgia, USA. J Zoo Wildl Med 1998; 29, 25-30.

Castro MI, Beck BB, Kleiman DG, Ruiz-Miranda CR, Rosenberg AL, 1998. Environmental enrichment in a reintroduction program for golden lion tamarin (*Leontopithecus rosalia*). In: Shepherdson DJ, Mellen JD, Hutchins M (eds.): *Second Nature Environmental enrichment for captive animals*. Smithsonian, 113-128

Chabaud AG, Petter AJ (1958) Les nematodes parasites de lemuriens Malgaches. I. Mem Inst Sci Madagascar Ser A 12:139-158. Cited by Inglis WG, Dunn FL (1963) The occurrence of Lemuricola (Nematoda:Oxyurinae) in Malaya: with the description of a new species. Z Parasitenkunde 23:354-359.

Chabaud AG, Petter AJ, Golvan YJ (1961) Les nematodes parasites de lemuriens Malgaches. ITI. Collection recoltee par M et Mme Francis Petter. Ann Parasit Hum Comp 36: 113-126. Cited by In-glis WG, Dunn FL (1963) The occurrence of Lemuricola (Nematoda:Oxyurinae) in Malaya: with the description of a new species. Z Parasitenkunde 23 :354-359.

Chang PCH, Graham GL (1957) Parasitism, parthenogenesis and polyploidy: the life cycle of Stron-gyloides papillosus. J Parasitol Suppl43: 13.

Chang TR, Forthman DL, Maple TL, 1999. Comparison of confined mandrill

(*Mandrillus sphinx*) behaviour in traditional and "ecologically representative" exhibits. *Zoo Biol* 18, 163-176.

Chitwood MB, Smith WN (1958) A redescription of Anatrichosoma cynomolgi Smith and Chitwood 1954. Proc Helminthol Soc, Washington, DC 25:112-117.

Choi YK, Simon MA, Kim DY, et al., 1999. Fatal measles virus infection in Japanese macaques (*Macaca fuscata*). Vet Pathol 36, 594-600.

Chosewood LC, Wilson DE, Centers for Disease Control and Prevention (US), National Institutes of Health (US), 2009. Biosafety in microbiological and biomedical labora- tories. 5th edition. Washington (DC): US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health.

Clark JD (1969) Coenurosis in a Gelada baboon (Theropithecus gelada). JAVMA 155:1258-1263.

Coe, JC, 1985. Design and perception: making the zoo experience real. *Zoo Biol* 4, 197-208.

Cosgrove GE, Humanson G, Lushbaugh CC (1970) Trichospirura leptostoma, a nematode of the pancreatic ducts of marmosets (Saguinus spp.). JAVMA 157: 696-698.

Cosgrove GE, Nelson B, Gengozian N (1968) Helminth parasites of the tamarin, Saguinus fuscicollis. Lab Anim Care 18: 654-656.

Cox-Singh J, Davis TM, Lee KS, et al., 2008. Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis, 46, 165-71.

Crandall LS, 1964. *The management of wild mammals in captivity*. Chicago, The University of Chicago Press.

Crosby WM, Ivey MH, Shaffer WL, Holmes DD (1968) Echinococcus cysts in the Savannah ba-boon. Lab Anim Care 18:395-397.

David EB, Patti M, Coradi ST, Oliveira-Sequeira, TCG, Ribolla PEM, & Guimaraes S. Molecular typing of Giardia duodenalis isolates from non-human primates housed in a brazilian zoo. Revista Do Instituto De Medicina Tropical De São Paulo (2014) 56 (1), 49–54 doi.org/10.1590/S0036-46652014000100007.

Dawkins MS, 1998. Evolution and animal welfare. *Quart Rev Biol* 73, 305-328. D'Alessandro A, Gippoliti S, 1996. Le scimmie antropomorfe del Giardino Zoologico di Roma: storia e prospettive. *Museol Sci* 13, 23-37.

De Paoli A, Johnsen DO (1978) Fatal strongyloidiasis in gibbons (Bylobates

lar). Vet Pathol 15:31-39.

De Vleeschouwer K, Leus K, Van Elsaker L, 2003. Stability of breeding and non- breeding groups of golden-headed lion tamarins (*Leontopithecus chrysomelas*). *Anim Welf* 12, 251-268.

De Vleeschouwer K, Van Elsaker L, Heistermann M, Leus K, 2000. An evaluation of the suitability of contraceptive methods in golden-headed lion tamarin (*Leontopithecus chrysomelas*), with emphasis on melengestrol acetate (MGA) implants: (II) Endocrinological and behavioural effects. *Anim Welf* 9, 251-271.

Deinhardt F, Holmes AW, Devine J, Deinhardt Jean (1969) Marmosets as laboratory animals. IV. The microbiology of laboratory kept marmosets. Lab Anim Care 17:48-70.

Deinhardt JB, Devine J, Passovoy M, Pohlman R, Deinhardt F (1967) Marmosets as laboratory ani-mals. I. Care of marmosets in the laboratory. Pathology and outline of statistical evaluation of data. Lab Anim Care 17: 11-29

Delacour J, 1933. On the Indochinese gibbons (*Hylobates concolor*). *J Mamm* 14:

Deschiens REA (1927) Sur les protozoaires intestinaux des singes. Bull Soc Path Exot 20:19-23. Cit-ed by Miller MJ, Bray RS (1966) Entamoeba histolytica infections in the chimpanzee (Pan satyrus). J ParasitoI52:386-388.

Dissanaike AS (1958) On hydatid infection in a Ceylon toque monkey Macaca sinica. Ceylon Vet 7:33-35.

Doherty JG, 1991. The exhibition and management of geladas in the Baboon Re- serve at the New York Zoological Park. *Proc Ann Conf Am Assoc Zool Parks Aquaria,* 599-605.

Doran DM, McNeilage A, 1998. Gorilla ecology and behavior. *Evol Anthrop* 6, 120- 131.

Dunn FL (1961) Molineus vexillarius sp. n. (Nematoda:Trichostrongylidae) from a Peruvian primate Tamarinus nigricollis (Spix, 1823). J ParasitoI47:953-956.

Dunn FL (1963) Acanthocephalans and cestodes of South American monkeys and marmosets. J Pa-rasitoI49:717-722.

Dunn FL (1968) The parasites of Saimiri in the context of Platyrrhine parasitism. In: Rosenblum LA, Cooper RW (eds) The squirrel monkey. Academic, New York, pp 31-68.

Dunn FL, Greer WE (1962) Nematodes resembling Ascaris lumbricoides L.,

1758, from a Malayan gibbon, Hylobates agilis F. Cuvier, 1821. J Parasito148: 150.

DuPont HL, Levine MM, Hornick RB, et al., 1989. Inoculum size in shigellosis and implications for expected mode of transmission. J Infect Dis 159, 1126-8.

Einarsson E, Ma'ayeh S, Svard SG. An up-date on Giardia and giardiasis. Curr. Opin. Microbiol. 2016; 34:47–52. 10.1016/j.mib.2016.07.019

Eisenstein R, Innes JRM (1956) Sarcosporidiosis in man and animals. Vet Rev Annot 2:61-78.

Eisenbrandt DL, Floering DA, David TD, McKee AE (1978) Scanning electron microscopy of a cry-ofractured hydatid cyst. In: Scanning electron microscopy, vol II. SEM, AMF O'Hare, IL, pp 229-233.

Faust EC, Beaver PC, Jung RC (1968) Animal agents and vectors of human disease, 3rd ed. Lea & Febiger, Philadphia.

Fineg J, Britz WE Jr, Cook JE, Edwards RH (1961) Clinical observations and methods used in the treatment of young chimpanzees. Air Force Missile Development Center, Holloman Air Force Base, New Mexico, Report Number AFMCDTR- 6l-l2.

Fiennes RNT-W (1967) Zoonoses of primates. The epidemiology and ecology of simian diseases in relation to man. Weidenfeld and Nicolson, London.

Flynn RJ (1973) Parasites oflaboratory animals. Iowa State University Press, Ames, IA.

Fontaine R, Dumond FV, 1977. The red ouakari in a seminatural environment: potentials for propagation and study. In: Prince Rainer III of Monaco, Bourne GA, (eds.): *Primate Conservation*. Academic Press: 167-236.

Forthman Quick DL, 1984. An integrative approach to environmental engineering in zoos. *Zoo Biol* 3, 65-77.

Fowler ME, Miller RE, 2008. Zoo and wild animal medicine: current therapy. 6th edition. St. Louis: Saunders/Elsevier.

Fox JG, 2002. Laboratory animal medicine. San Diego: Academic Press.

Fox JG,2002 Laboratory animal medicine. 2nd edition. Amsterdam: Academic Press.

Frank H (1982) Pathology of amebiasis in leaf monkeys (Colobidae). Proc 24th Int Symp Dis Zoo Anim, pp 321-326.

Freedman DO, MacLean JD, Viloria JB, 1987. A case of laboratory acquired

Leishmania donovani infection; evidence for primary lymphatic dissemination. Trans R Soc Trop Med Hyg 81, 118-9.

Fremming BD, Vogel FS, Benson RE, Young RJ (1955) A fatal case of amebiasis with liver ab-scesses and ulcerative colitis in a chimpanzee. JAVMA 126:406-407.Geiman QM (1964) Shigellosis, amebiasis, and simian malaria. Lab Anim Care 14:441-454.

Galinski MR, Barnwell JW, 2009. Monkey malaria kills four humans. Trends Parasitol 25, 200-4.

Gamer E, Hemrick R, Rudiger H (1967) Multiple helminth infections in cinnamonringtailed monkeys (Cebus albifrons). Lab Anim Care 17:310-315.

Gamham PCC (1950) Blood parasites of East African vertebrates with a brief description of exoeryth-rocytic schizogony in Plasmodium pitmani. Parasitology 40:328-337.

Gardner MB, Esra G, Cain MJ, Rossman S, Johnson C (1978) Myelomonocytic leukemia in an orangutan. Vet PatholI5:667-770.

Gardner MB, Luciw PA, 2008. Macaque models of human infectious disease. Ilar J 49, 220-5.

Geisel O, Krampitz HE, Willaert E (1975) Invasive amoebiasis caused by Entamoeba histolytica in a douc langur (Pygathrix nemaeus L. 1771). Berlin Muench Tieraerztl Woch 88:52-55.

Gippoliti S, 1997. A contribution to the history of zoos in Italy up to the second world war. *Int Zoo News* 44, 458-465.Primate Report 73, July 2006

Gippoliti S, 2006. Report 73, July 2006 : History of Primates in Zoos.

Gippoliti S, Speranza L, 2005. Incrementare la rilevanza biologica e di conservazione degli zoo: evoluzione dei criteri espositivi e gestione dei primati. *Museol Sci* 20, 159-174.

Glatston AR, Geilvoet-Soeteman E, Hora-Pecek E, van Hooff JARAM, 1984. The influence of the zoo environment on social behavior of groups of cotton-topped tamarins, *Saguinus oedipus oedipus*. *Zoo Biol* 3, 241-253.

Gold KC, 1997. The conservation role of primate exhibits in the zoo. In: Wallis J (ed.): *Primate conservation: the role of zoological parks*. American Society of Primatologists, 43-61.

Guarner J, Johnson BJ, Paddock CD, et al., 2004. Monkeypox transmission and pathogenesis in prairie dogs. Emerg Infect Dis 10, 426-31.

Graham GL (1960) Parasitism in monkeys. Ann NY Acad Sci 85:842-860

Habermann RT, Williams FP Jr (1958) The identification and control of helminths in laboratory ani-mals. J Nat! Cancer Inst 20:979-1009.Hegner

RW (1934) Intestinal protozoa of chimpanzees. Amer J Hyg 19:480-501.
Hamerton AE (1941-1942) Report on the deaths occurring in the Society's gardens during 1939-1940. Proc Zool Soc London 111:151-184.
Hancocks D, 1971. *Animals and architecture*. Praeger, New York.
Hancocks D, 2001a. A different nature. The paradoxical world of zoos and their un- certain future. Berkeley, University of California Press.
Hancocks D, 2001b. Is there a place in the world for zoos? In: *State of the animals: 2001*. Humane Society Press, 137-144.
Hardie SM, Prescott MJ, Buchanan-Smith HM, 2003. Ten years of tamarin mixed- species troops at Belfast Zoological Gardens. *Primate Report* 65, 21-38.
Healy GR, Hayes NR (1963) Hydatid disease in rhesus monkeys. J Parasitol49: 837.
Hediger H, 1950. Wild animals in captivity: An outline of the biology of zoological gardens. London, Butterworth.
Hediger H, 1965. Diet of animals in captivity. *Int Zoo Yb* 5, 37-57
Hediger H, 1982. Zoo biology; retrospect and prospect. *Zoo Biol* 1, 85-86.
Henderson JD Jr, Webster WS, Bullock BC, Lehner NDM, Clarkson TB (1970) Naturally occurring lesions seen at necropsy in eight woolly monkeys (Lagothrix sp.). Lab Anim Care 20:1087-1097.
Herwaldt BL, 2001. Laboratory-acquired parasitic infections from accidental exposures. Clin Microbiol Rev 14, 659-88.
Hill WCO (1953) Report of the Society's prosector for the year 1952. Proc Zool Soc London 123:227-251.
Hosey GR, 2005. How does the zoo environment affect the behaviour of captive pri- mates? *Appl Anim Behav Sci* 90, 107-129.
Houser WD, Paik SK (1971) Hydatid disease in a macaque. JAVMA 159:1574-1577.
Howard EB, Gendron AP (1980) &hinococcus vogeli infection in higher primates at the Los Angeles zoo. In: Montali RJ, Migaki G (eds) The comparative pathology of zoo animals. Smithsonian Institu-tion Press, Washington, DC, pp 379-382.
Huemer HP, Larcher C, Czedik-Eysenberg T, et al., 2002. Fatal infection of a pet monkey with Human herpesvirus. Emerg Infect Dis 8, 639-42.
Huff JL, Barry PA, 2003. B-virus (Cercopithecine herpesvirus 1) infection in humans and macaques: potential for zoonotic disease. Emerg Infect Dis 9, 246-50.

## References


Hutchins M, Conway W, 1995. Beyond Noah's Ark: the evolving role of modern zoological parks and aquariums in field conservation. *Int Zoo Yb* 34, 117-130.

Hutchins M, Hancocks D, Crockett C, 1984. Naturalistic solutions to the behavioral problems of captive primates. *Zoolog Garten* 54, 28-42.

Huxley E, 1981. Whipsnade. Captive breeding for survival. London, Collins. Hyson J (2000): Institute of Laboratory Animal Resources (US). Committee on Occupational Safety and Health in Research Animal Facilities. Occupational health and safety in the care and use of research animals. Washington (DC): National Academy Press, 1997.

Ilievski V, Esber H (1969) Hydatid disease in a rhesus monkey. Lab Anim Care 19: 199-204.

Inglis WG, Cosgrove GE (1965) The pinworm parasites (Nematoda:Oxyuridae) of the Hapalidae (Mammalia:Primates). Parasitology 55:731-737.

Jensen K, Alvarado-Ramy F, Gonzalez-Martinez J, et al., 2004. B-virus and free-ranging macaques, Puerto Rico. Emerg Infect Dis 10, 494-6.

Jones-Engel L, Engel GA, Schillaci MA, et al., 2006. Considering human-primate transmission of measles virus through the prism of risk analysis. Am J Primatol 68, 868-79.

Jones TC, Hunt RD (1983) Diseases caused by parasitic helminths and arthropods.vVeterinary pa-thology, 5th ed. Lea & Febiger, Philadelphia, pp 778-879.

Kagan IG, Allain DS, Norman L (1959) An evaluation ofthe hemagglutination and flocculation tests in the diagnosis of Echinococcus disease. Amer Trop Med Hyg 8:51-55.

Kahnv LH, 2006. Confrontingzoonoses, link in human and veterinary medicine. EmergInfect Dis 12, 556-61.

Karim MR, Wang R, Yu F, Li T, Dong H, Li D, Zhang L, Li J, Jian F, Zhang S. Multi-locus analysis of Giardia duodenalis from nonhuman primates kept in zoos in China: geographical segregation and host adaptation of assemblage B isolates. Infect Genet Evol (2015) 30: 82–88.

Kaufmann AF, Morris G, Richardson JR, Healy G, Kaplan W (1970) A survey ofnewly arrived South American monkeys for potential human pathogens. In: Primate zoonoses surveillance. Report No. 1. Centers for Disease Control, Atlanta, GA.

Kaumanns W, Gansloßer U, Beiyner A (eds): *Zoo animal nutrition.* Filander, 91-106.

## References


Kaumanns W, Hampe K, Schwitzer C, Stahl D, 2000. Primate nutrition: towards an integrated approach. In: Nijboer J, Hatt JM,

Kaumanns W, Schmid W, Schwitzer C, Husung A, Knogge C, 2001. The European population of lion-tailed macaques (*Macaca silenus*): status and problems. *Primate Report* 59, 65-75.

Khabbaz RF, Heneine W, George JR, et al., 1994. Brief report: infection of a laboratory worker with simian immunodeficiency virus. N Engl J Med 330, 172-7.

Khan AS, 2009. Simian foamy virus infection in humans: prevalence and management. Expert Rev Anti Infect Ther 7, 569-80.

Kennard MA (1981) Abnormal findings in 246 consecutive autopsies on monkeys. Yale J BioI Med 13:701-712.

Kennedy FM, Astbury J, Needham JR, et al., 1993. Shigellosis due to occupational contact with non-human primates. Epidemiol Infect 110, 247-51.

Kessler MJ (1982) Nasal and cutaneous anatrichosomiasis in the free-ranging rhesus monkeys (Macaca mulatta) of CayoSantiago. Amer J PrimatoI3:55-60.

Kessler MJ, Berard JD, Rawlins RG, et al., 2006. Tetanus antibody titers and duration of immunity to clinical tetanus infections in free-ranging rhesus monkeys (Macaca mulatta). Am J Primatol 68, 725-31.

King NW Jr (1976) Synopsis of the pathology of new world monkeys. In: First interAmerican con-ference on conservation and utilization of American nonhuman primates in biomedical research. Sci-entific Publ No 317, Pan American Health Organization, Pan American Sanitary Bureau Regional Of-fice of the WHO, Washington, DC, pp 169-198.

Knottnerus-Meyer T, 1925. *Nel Giardino Zoologico*. Rome, Maglione e Strini. Kortland A, (1961. Can lessons from the wild improve the lot of captive chimpanzee? Int Zoo Yb 2, 76-80.

Kondgen S, Kuhl H, N'Goran PK, et al., 2008. Pandemic human viruses cause decline of endangered great apes. Curr Biol 18, 260-4.38

Köster PC, Martínez-Nevado E, González A, Abelló-Poveda MT, Fernández-Bellon H, de la Riva-Fraga M, Marquet B, Guéry JP, Knauf-Witzens T, Weigold A, Dashti A, Bailo B, Imaña E, Muadica AS, González-Barrio D, Ponce-Gordo F, Calero-Bernal R, Carmena D. Intestinal Protists in Captive Non-human Primates and Their Handlers in Six European Zoological Gardens. Molecular Evidence of Zoonotic Transmission. Front Vet Sci. (2022 ) 8:819887. doi: 10.3389/fvets.2021.819887.

Krascheninnikow S, Wenrich DH (1958) Some observations on the morphology and division of Bal-antidium coli and Balantidium caviae (?). J Protozool 5: 196-202.

Kreis HA (1932) A new pathogenic nematode of the family Oxyuroidea, Oxyuronema atelophora n. g., n. sp in the red spider monkey, Ateles geoffroyi. J Parasitol 18:295-302.

Kuntz RE, Myers BJ (1966) Parasites of baboons (Papio doguera Pucheran, 1856) captured in Ken-ya and Tanzania, East Africa. Primates 7:27-32.

Kuntz RE, Myers BJ (1967) Primate cysticercosis: Taenia hydatigena in Kenya vervets (Cerco-pithecus aethiops Linnaeus, 1758) and Taiwan macaques (Macaca cyclopis, Swinhoe, 1864). Prima-tes 8:83-88.

Kuntz RE, Myers BJ (1969) Parasitic protozoa, commensals and helminths of chimpanzees imported from the Republic of the Congo. Proc 2nd Int Congr Primat, Atlanta GA, 1968, vol 3. S Karger, Ba-sel/New York, pp 184-190.

Kuntz RE, Myers BJ, Bergner JF Jr, Armstrong DE (1968) Parasites and commensals of the Taiwan macaque (Macaca CYclopis Swinhoe, 1862). Formosan Sci 22:120-136.

Lapage G (1968) Veterinary parasitology. Oliver and Boyd, Edinburgh/London.

Lerche NW, Switzer WM, Yee JL, et al., 2001. Evidence of infection with simian type D retrovirus in persons occupationally exposed to nonhuman primates. J Virol 75, 1783-9.

Levecke B, Dorny P, Geurden T, Vercammen F, Vercruysse J. Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. Vet Parasitol (2007) 148(3): 236–246.

Levecke B, Geldhof P, Claerebout E, Dorny P, Vercammen F, Cacciò SM, et al. Molecular character-isation of Giardia duodenalis in captive non-human primates reveals mixed assemblage Aand B in-fections and novel polymorphisms. Int. J. Parasit. (2009) 39:1595–601.

Levine ND (1970) Protozoan parasites of nonhuman primates as zoonotic agents. Lab Anim Care 20:377-382.

Levine ND (1976) Nematode parasites of domestic animals and of man. Burgess, Minneapolis.

Levine ND (1977) Nomenclature of Sarcocystis in the ox and sheep and of fecal coccidia of the dog and cat. J Parasitogy I63:36-51.

Lillie RD (1947) Reactions of various parasitic organisms in tissues to the

Bauer Feulgen Gram and Gram-Weigert methods. J Lab Clin Med 32:76-88.

Lindburg DG, Coe J, 1995. Ark design update: primate needs and requirements. In: Gibbons EF, Durrant BS, Demarest J, (eds.): *Conservation of endangered spe- cies in captivity*. State University of New York, 553-570.

Lindquist WD, Bieletzki J, Allison S (1980) Pterygodermatites sp. (Nematode: Rictulariidae) from primates in Topeka, Kansas Zoo. Proc Helminthol Soc Washington, DC 47:224-227.

Little KA, Sommer V, 2002. Change of enclosure in langur monkey: implications for the evaluation of environmental enrichment. *Zoo Biol* 21, 549-559.

Little MD (1966) Comparative morphology of six species of Strongyloides (Nematoda) and redefini-tion of the genus. J ParasitoI52:69-84.

Loeb WF, Bannerman RM, Rininger BF, Johnson AJ (1978) Hematologic disorders. In: Benirschke K, Gamer FM, Jones TC (eds) Pathology of laboratory animals, chapter 11, vol I. Springer-Verlag, New York, pp 1000-1021, 1032-1050.

Loomis MR, Britt JO (1983) An epizootic of Entamoeba histolytica in colobus monkeys. Amer As-soc Zoo Vet Ann Proc, p 10.

Loomis MR, Britt JO, Gendron AP, Holshuh HJ, Howard EB (1983) Hepatic and gastric amebiasis in black and white colobus monkeys. JAVMA 183:1188-1191.

MacKenzie PS (1979) Pathogenicity, identification and treatment of Prosthenorchis elegans infesta-tion in squirrel monkeys (Saimiri sciureus). Prim SuppI4:5-7

Maesano G, Capasso M, Ianniello D, Cringoli G, Rinaldi L. Parasitic infections detected by FLOTAC in zoo mammals from Warsaw, Poland. Acta Parasitol (2014) :343-53. doi: 10.2478/s11686-014-0249-8.

Mager WB, Griede T, 1996. Using outside areas for tropical primates in the north- ern hemisphere: Callithricidae, *Saimiri* and *Gorilla.* In: Benirschke K (ed.): *Primates. The road to self-sustaining populations*. Springer-Verlag, 471-477.

Mangili G, 1970. Untersuchungen über die ernährung exotischer tiere mit pellets aus abgestimmten nährstoffen. *Zoolog. Garten* 39, 174-182.

Maple TL, 1993. *Zoo man. Inside the zoo revolution*. Atlanta, Longstreet.

Maple TL, Finlay TW, 1987. Post-Occupancy Evaluation in the zoo. *Appl Anim Behav Sci* 18, 5-18.

Maple TL, Finlay TW, 1989. Applied primatology in the modern zoo. *Zoo Biol Suppl* 1, 101-116.

Martínez-Díaz RA, Sansano-Maestre J, del Carmen Martínez-Herrero M, Ponce-Gordo F, Gómez-Muñoz MT. Occurrence and genetic characterization of Giardia duodenalis from captive nonhuman primates by multilocus sequence analysis. Parasitol Res (2011) 109: 539–544.

Maschietti G, Muti M, Passerin D'Entrèves P, 1990. *Giardini zoologici. Vicende storico-politiche degli zoo torinesi (1851-1989)*. Turin, Umberto

Matz-Rensing K, Floto A, Schrod A, et al., 2007. Epizootic of tularemia in an outdoor housed group of cynomolgus monkeys (*Macaca fascicularis*). Vet Pathol 44, 327-34.

McClure HM, Guilloud NB (1971) Comparative pathology of the chimpanzee. In:Bourne GH (ed) The chimpanzee, vol 4. Behavior, growth and pathology of chimpanzees. University Park Press, Bal-timore, pp 103-272.

Mellen J, Sevenich MacPhee M, 2001. Philosophy of environmental enrichment: past, present and future.

Meng XJ, 2010. Hepatitis E virus: animal reservoirs and zoonotic risk. Vet Microbiol 140, 256-65.

Middleton CC (1966) Acanthocephala (Prosthenorchis elegans) infection in squirrel monkeys (Saimiri sciureus). Lab Anim Dig 2:16-17.

Migaki G, Seibold HR, Wolf RH, Gamer FM (1971) Pathologic conditions in the patas monkey. JAVMA 159:549-556.

Miller MJ, Bray RS (1966) Entamoeba histolytica infections in the chimpanzee (Pan satyrus). J Para-sitol 52:386-388.

Minette HP, 1966. Leptospirosis in primates other than man. Am J Trop Med Hyg 15, 190-8.

Mitchell G, Obradovich S, Herring F, Dowd B, Tromborg C, 1991. Threats to observers, keepers, visitors, and others by zoo mangabeys (*Cercocebus galeritus chrysogaster*). *Primates* 32, 515-522.

Monath TP, 2001. Yellow fever: an update. Lancet Infect Dis 1, 11-20.

Moncol DJ, Batte EG (1966) Transcolostral infection of newborn pigs with Strongyloides ransomi. Vet Med 61:583-586.

Montali RJ, Connolly BM, Armstrong DL, et al., 1995. Pathology and immunohistochemistry of callitrichid hepatitis, an emerging disease of captive New World primates caused by lymphocytic choriomeningitis virus. Am J Pathol 147, 1441-9.

Morris D, 1964. The response of animals to a restricted environment. *Symp*

*Zool Soc Lond* 13, 99-118.
Morris D, 1965. Experimental nocturnal house at London Zoo. *Int Zoo Yb* 5, 240- 242.
Mottershead GS, 1960. Experiments with a chimpanzee colony at Chester Zoo. *Int Zoo Yb* 1, 18-20.
Mravcová K, Štrkolcová G, Mucha R, Goldová M. Zoonotic assemblages of Giardia duodenalis in captive non-human primates from the largest zoo in Slovakia. J Parasit Dis. (2021) 45:302-305. doi: 10.1007/s12639-020-01324-3.
Muller R, Ruedi D (1981) Gastric amebiasis in a proboscis monkey (Nasalis larvatus). ACTA Zool Pathol Antverp 76:9-16.
Munene E, Otsyula M, Mbaabu DA, et al., 1998. Helminth and protozoan gastrointestinal tract parasites in captive and wild-trapped African non-human primates. Vet Parasitol 78, 195-201.
Murphy HW, Miller M, Ramer J, et al., 2006. Implications of simian retroviruses for captive primate population management and the occupational safety of primate handlers. J Zoo Wildl Med 37, 219-33.
Myers BJ, Kuntz RE (1965) A checklist of parasites reported for the baboon. Primates 6: 137-194.
Myers BJ (1972) Echinococcosis, coenurosis, cysticercosis, sparganosis, etc. In:Fiennes RNT-W (ed) Pathology of simian primates. Part II. Infectious and parasitic diseases. S Karger, Basel, pp 124-143. [608].
Myers BJ, Kuntz RE, Vice TE, Kim CS (1970) Natural infection of Echinococcus granulosus (Batsch, 1786) Rudolph, 1805 in the Kenya baboon (Papio sp.). Lab Anim Care 20:283-286.
Nayar GP, Crawshaw GJ, Neufeld JL, 1979. Tularemia in a group of nonhuman primates. J Am Vet Med Assoc 175, 962-3.
Nelson B, Cogrove GE, Gengozian N (1966) Diseases of an imported primate Tamarinus nigricollis. Lab Anim Care 16:255-275.
Newberry RC, 1995. Environmental enrichment: increasing the biological relevance of captive environments. *Appl Anim Behav Sci* 44, 229-243.
Niyogi SK, 2005. Shigellosis. J Microbiol 43, 133-43
Nizeyi JB, Innocent RB, Erume J, et al., 2001. Campylobacteriosis, salmonellosis, and shigellosis in free-ranging human-habituated mountain gorillas of Uganda. J Wildl Dis 37, 239-44.
O'Grady JP, Yeager CH, Esra GN, Thomas W (1982) Ultrasonic evaluation of echinococcosis in four lowland gorillas. JAVMA 181:1348-1350.

## References


Orihel RE, Seibold HR (1971) Trichospirurosis in South American monkeys. J ParasitoI57:1366-1368.

Orihel RE, Seibold HR (1972) Nematodes of the bowel and tissues. In: Fiennes RNT-W (ed) Patholo-gy of simian primates. Part II. Infectious and parasitic diseases. S Karger, Basel, pp 76-103.

Orihel TC (1970) The helminth parasites of nonhuman primates and man. Lab AnimvCare 20:395-401.

Palmieri JR, Dalgard DW, Conner DH (1984) Gastric amebiasis in a silvered leaf monkey. JAVMA 185:1374-1375.

Palotay JL, Uno H (1975) Hydatid disease in four nonhuman primates. JAVMA 167:615-618.

Parker GA, Gilmore CJ, Roberts CR (1979) Diagnostic exercise. Lab Anim Sci 29:457-458.

Parker S, Nuara A, Buller RM, et al., 2007. Human monkeypox: an emerging zoonotic disease. Future Microbiol, 2, 17-34.

Parnell RJ, Buchanan-Smith HM, 2001. An unusual social display by gorillas. *Nature* 412, 294.

Paterok O, 2004. Gorillas in Germany. A review on the last ten years and an outlook to the future 31.12.1993-31.12.2003. Available at www.leszoos-danslemonde.com

Pavio N, Meng XJ, Renou C, 2010. Zoonotic hepatitis E: animal reservoirs and emerging risks. Vet Res 41, 46.

Pedersen AB, Davies TJ, 2009. Cross-species pathogen transmission and disease emergence in primates. Ecohealth 6, 496-508.

Penner LR (1981) Concerning threadworm (Strongyloides stercoralis) in great apes-lowland gorillas (Gorilla gorilla) and chimpanzees (Pan troglodytes). J Zoo Anim Med 12:128-13l.

Pennington H. Escherichia coli O157. Lancet 23, 376, 1428-35.

Perolat P, Poingt JP, Vie JC, et al., 1992. Occurrence of severe leptospirosis in a breeding colony of squirrel monkeys. Am J Trop Med Hyg,46, 538-45.

Pourrut X, Diffo JL, Somo RM, et al., 2011. Prevalence of gastrointestinal parasites in primate bushmeat and pets in Cameroon. Vet Parasitol 10,175, 187-91.

Price EC, 1997. Group instability following cessation of breeding in marmosets and tamarins. *Dodo J Durrell Wildlife Conserv Trust* 33, 157-158.

Prine JR (1968) Pancreatic flukes and amoebic colitis in a gorilla. Abst 50

19th Ann Meeting Amer Assoc Lab Animal Sci, Las Vegas, 1968.

Pung OJ, Spratt J, Clark CG, et al., 1998. *Trypanosoma cruzi* infection of free-ranging lion-tailed macaques (Macaca silenus) and ring-tailed lemurs (Lemur catta) on St. Catherine's Island, Georgia, USA. J Zoo Wildl Med 29, 25-30.

Rabin LA, 2003. Maintaining behavioural diversity in captivity for conservation: natural behaviour management. *Anim Welf* 12, 85-94.

Rawlins C, 1979. Frankfurt. In: Zuckerman L (ed.) *Great zoos of the world*. Weiden- feld & Nicolson, 75-82.

Reardon LV, Rininger BF (1968) A survey of parasites in laboratory animals. Lab Anim Care 18:577-580.

Redshaw ME, Mallinson JJC, 1991. Learning from the wild: improving the well-be- ing of captive primates. *Dodo J Durrell Wildlife Conserv Trust* 27, 18-26.

Reid WA, Reardon MJ (1976) Mesocestoides in the baboon and its development in laboratory ani-mals. J Med Primato15:345-352.

Rewell RE (1948) Diseases of tropical origin in captive wild animals. Trans Roy SocTrop Med Hyg 42:17-36.

Reynolds V, Reynolds F, 1965. The natural environment and behaviour of chimpanzees *Pan troglodytes schweinfurthi* and suggestions for their care in zoos. *Int Zoo Yb* 5, 141-144.

Richart R, Benirschke K (1963) Causes of death in a colony of marmoset monkeys. J Path Bacteriol 86:221-223.

Rifakis PM, Benitez JA, De-la-Paz-Pineda J, et al., 2006. Epizootics of yellow fever in Venezuela (2004-2005): an emerging zoonotic disease. Ann N Y Acad Sci 1081, 57-60.

Ritz N, Curtis N, Buttery J, et al., 2009. Monkey bites in travelers: should we think of herpes B virus? Pediatr Emerg Care 25, 529-31.

Ruch TC (1959) Diseases of laboratory primates. WB Saunders, Philadelphia.

Sakakibara I (1981) Naturally occurring diseases in cynomolgus monkeys. Jap J Med Sci BioI 34:263-267.

Sakakibara I, Sugimoto Y, Koyama T, Honjo S (1982) Natural transmission of Entamoeba histolytica from mother cynomolgus monkeys (Macaca fascicularis) to their newborn infants under indoor rear-ing conditions. Exper Anim 31: 135- 138.

Sestak K, Merritt CK, Borda J, Saylor E, Schwamberger SR, Cogswell F, Di-

dier ES, Didier PJ, Plauche G, Bohm RP, Aye PP, Alexa P, Ward RL, Lackner AA. Infectious agent and immune re-sponse characteristics of chronic enterocolitis in captive rhesus macaques. Infect Immun (2003) 71:4079-86. doi: 10.1128/IAI.71.7.4079-4086.2003

Sato H, Une Y, Kawakami S, et al., 2005. Fatal Baylisascaris larva migrans in a colony of Japanese macaques kept by a safari-style zoo in Japan. J Parasitol 91, 716-19.

Schmidt GD, File S (1977) Tupaiataenia quentini gen. et sp. n. (Anoplocephalidae:Linstowiinae) and other tapeworms from the common tree shrew, Tupaia glis. J Parasitol 63:473-475.

Schmidt RE (1978) Systemic pathology of chimpanzees. J Med Primatol 7 :274-318.

Schmidt RE, Prine JR (1970) Severe enterobiasis in a chimpanzee. Path Vet 7:56-59.

Schou S, Hansen AK, 2000. Marburg and Ebola virus infections in laboratory non-human primates: a literature review. Comp Med 50, 108-23.

Schwitzer C, Kaumanns W, 2003. Foraging patterns of free-ranging and captive primates - implications for captive feeding regimes. In: Fidgett AL, Clauss M, Gansloßer U, Hatt J-M, Nijboer J (eds.): *Zoo Animal Nutrition II*. Filander Verlag, 247-265.

Sestak K, Merritt CK, Borda J, et al., 2003. Infectious agent and immune response characteristics of chronic enterocolitis in captive rhesus macaques. Infect Immun 71, 4079-86.

Shadduck JA, Pakes SP (1978) Protozoal and metazoal diseases. In: Benirschke K, Garner FM, Jones TC (eds) Pathology of laboratory animals, chapter 17, vol II. Springer-Verlag, New York, pp 1587-1696.

Shephersdon DJ, 2003. Environmental enrichment: past, present and future. *Int Zoo Yb* 38, 118-124.

Slaughter U, Bostrom RE (1969) Physalopterid (Abbreviata poicilometra) infection in a sooty manga-bey monkey. Lab Anim Care 19:235-236

Smith A, Lindburg DG, Vehrencamp S, 1989. Effects of food preparation on feeding behavior of lion-tailed macaques. *Zoo Biol* 8, 57-65.

Smith WN, Chitwood MB (1967) Trichospirura leptostoma gen et sp n (Nematoda: Thelazioidea) from the pancreatic ducts of the white-eared marmoset, Callithrix jacchus. J Parasito153:1270-1272. 807.

Smith WN, Levy BM (1969) The effects of Trichospirura leptostoma on the pancreas of Callithrixjac-chus. Program and Abstracts No 7, p 45, 44th

Annual Meeting Amer Soc Path, Washington, DC 1969.
Sommer R, 1972. What do we learn at the zoo? *Nat Hist* 81, 26-27, 86-87.
Song B, Javanbakht H, Perron M, et al., 2005. Retrovirus restriction by TRIM5alpha variants from Old World and New World primates. J Virol 79, 3930-7.
Soulsby EJL (1965) Textbook of veterinary clinical parasitology, vol. Helminths. FA Davis, Phila-delphia.
Springer DA, Phillippi-Falkenstein K, Smith G, 2009. Retrospective analysis of wound characteristics and tetanus development in captive macaques. J Zoo Wildl Med 40, 95-102.
Starn AB (1960) Fatal ascaridosis in a dwarf chimpanzee. Ann Parasitol Hum Comp 35:675.
Stemmler-Morath C, 1968. Ultimate responsability rests with the keeper.
Summers WA (1960) A case of hydatid disease in the rhesus monkey (Macaca mulatta). Allied Vet 31:141-143.
Szonyi B, Agudelo-Florez P, Ramirez M, et al., 2011. An outbreak of severe leptospirosis in capuchin (Cebus) monkeys. Vet J 188, 237-9.
Teare JA, Loomis MR (1982) Epizootic of balantidiasis in lowland gorillas. JAVMA181:1345-1347.
Toft JD II, Ekstrom ME (1980) Identification of metazoan parasites in tissue sections. In: Montali RJ, Migaki G (eds) The comparative pathology of zoo animals. Smithsonian Institution Press, Washing-ton, DC, pp 369-378.
Toft JD II, Schmidt RE, DePaoli A (1976) Intestinal polyposis associated with oxyurid parasites in a chimpanzee (Pan troglodytes). J Med Primatol 5:360-364.
Trevejo RT, Barr MC, Robinson RA, 2005. Important emerging bacterial zoonotic infections affecting the immunocompromised. Vet Res 36, 493-506.
Tribe GW, Fleming MP, 1983. Biphasic enteritis in imported cynomolgus (Macaca fascicularis) monkeys infected with Shigella, Salmonella and Campylobacter species. Lab Anim 17, 65-9.
Twenhafel NA, Alves DA, Purcell BK, 2009. Pathology of inhalational Francisella tularensis spp. tularensis SCHU S4 infection in African green monkeys (*Chlorocebus aethiops*). Vet Pathol 46, 698-706.
Une Y, Mori T, 2007. Tuberculosis as a zoonosis from a veterinary perspective. Comp Immunol Microbiol Infect Dis 30, 415-25. US Centers for Disease Control and Prevention. Available at: www.cdc.gov/tb/statistics/default.htm. Accessed May 12, 2011.

## References


Update: Ebola-related filovirus infection in nonhuman primates and interim guidelines for handling nonhuman primates during transit and quarantine. MMWR Morb Mortal Wkly Rep 1990, 39, 22-4.

Update: multistate outbreak of monkeypox - Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. MMWR Morb Mortal Wkly Rep 52, 642-6.

US Centers for Disease Control and Prevention, 2011. Available at: www.cdc.gov/tb/ statistics/default.htm. Accessed May 12, 2011.

Van Riper DC, Day PW, Finey J, Prince JR (1966) Intestinal parasites of recently imported chimpan-zees. Lab Anim Care 16:360-362.

Vickers JH, Penner LR (1968) Cysticercosis in four rhesus brains. JAVMA 153:868-871.

Voevodin A, Samilchuk E, Schatzl H, et al., 1996. Interspecies transmission of macaque simian T-cell leukemia/lymphoma virus type 1 in baboons resulted in an outbreak of malignant lymphoma. J Virol 70, 1633-9.

Waitt C, Buchanan-Smith HM, 2001. What time is feeding? How delays and anticipation of feeding schedules affect stump-tailed macaque behavior. *Appl Anim Behav Sci* 75, 75-85.

Wallis J, 2006 (ed.): *Primate conservation: the role of zoological parks*. American Society of Primatologists: 1-27.70 Primate Report 73, July 2006

Wallis J, Lee DR, 1999. Primate conservation: the prevention of disease transmission. *Int J Primat* 20, 803-826.

Walter O, Brown C, 2004. The one and only nocturnal monkey: *Aotus*. *EAZA News* 45, 26.

Wang SM, MaJC, HaoZY, et al. Surveillance of shigellosis by real-time PCR suggests underestimation of shigellosis prevalence by culture-based methods in a population of rural China. J Infect 61, 471-5.

Webster D, 2000. The setting up of a public walk-through mixed lemur exhibit. *Int Zoo News* 47, 483-491.

Willy ME, Woodward RA, Thornton VB, et al, 1999. Management of a measles outbreak among Old World nonhuman primates. Lab Anim Sci 49, 42- 8.

Wolfe ND, Escalante AA, Karesh WB, et al., 1998. Wild primate populations in emerging infectious disease research: the missing link? Emerg Infect Dis 4,149-58.

Wolfe ND, Dunavan CP, Diamond J, 2007 Origins of major human infectious diseases. Nature 447, 279-83.

## References


Woodford MH, Butynski TM, Karesh WB, 2002. Habituating the great apes: the disease risks. *Oryx* 36, 153-160.

Woolhouse ME, Gowtage-Sequeria S, 2005. Host range and emerging and reemerging pathogens. Emerg Infect Dis 11, 1842-7.

World Health Organization, 2010. Available at: www.who.int. Accessed December12, 2010.

World Health Organization, 2011. Xenotransplantation: guidance on infectious disease prevention and management. Available at: http://whqlibdoc.who.int/hq/1998/ WHO_EMC_ZOO_98.1.pdf. Accessed May 12, 2011.

Wormell D, Brayshaw M, 2000. The design and redevelopment of New World pri- mate accomodation at Jersey Zoo: a naturalistic approach. *Dodo J Durrell Wildlife Conserv Trust* 36, 9-19.

Wrangham RW, 1980. An ecological model of female-bonded primate groups. *Behaviour* 753 262-300.

Yamashita J (1963) Ecological relationships between parasites and primates. I. Helminth parasites and primates. Primates 4: 1-96.

Zheng H, Wolfe ND, Sintasath DM, et al., 2010. Emergence of a novel and highly divergent HTLV-3 in a primate hunter in Cameroon. Virology 5, 401, 137-45.

Zimmermann A, Feistner ATC, 1996. Effects of feeding enrichment on ruffed le- murs *Varecia variegata variegata* and *Varecia v. rubra*. *Dodo J Durrell Wildlife Conserv Trust* 32, 67-75.

Zuckerman S, 1932. The social life of monkeys and apes. Routledge & Kegan, Lon- don.

Zumpe D, Silberman MS, Michael RP, 1980. Unusual outbreak of tuberculosis due to *Mycobacterium bovis* in a closed colony of rhesus monkeys (Macaca mulatta). Lab Anim Sci 30, 237-40.

# Chapter 1

## Use of Mini-FLOTAC and Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals

**Capasso M**, Maurelli MP, Ianniello D, Alves LC, Amadesi A, Laricchiuta P, Silvestre P, Campolo M, Cringoli G, Rinaldi L., 2019. Use of Mini-FLOTAC and Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals. Rev Bras Parasitol Vet 28(1), 168-171. doi: 10.1590/S1984-296120180087.

## 1.1 Abstract

Animals reared in restricted environments are highly susceptible to gastrointestinal infection by helminths and protozoa and therefore zoos are characterized as being parasite-rich environments. Successful implementation of control programs of these parasites in zoo environment depends upon precise and rapid diagnosing of gastrointestinal infections. The aim of this study was to demonstrate the role of the Mini-FLOTAC technique in combination with Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals. Fecal samples were collected from 70 animals in four different zoos located in central and southern Italy. All the samples were analyzed using Mini-FLOTAC in combination with Fill-FLOTAC. Out of the 70 pooled samples examined, 80% (24/30) were positive for at least one parasite. Among the gastrointestinal nematodes, Strongyles were the most frequent (40%), followed by *Trichuris* spp. (23.3%), *Parascaris* spp. (13.3%) and *Capillaria* spp. (3.3%). Among the protozoa, *Blastocystis* spp., *Giardia* spp. and *Eimeria* spp. were detected in 6.6%, 3.3% and 3.3%, respectively. These results show that Mini-FLOTAC in combination with Fill-FLOTAC can be used, not only for rapidly diagnosing parasitic infections in zoo mammals, but also for monitoring control programs in which large numbers of fecal samples need to be examined rapidly and reliably.

## 1.2 Introduction

Currently, there are few reports on occurrences of gastrointestinal (GI) parasites in zoo mammals. Some of these studies only reported the local incidence and prevalence of infection in captivity. These animals infected by GI parasites are usually asymptomatic even when the parasite burden is heavy (Sachs et al., 1968). However, parasitic diseases constitute one of the major problems causing morbidity and mortality among them (Geraghtyet al., 1982; Varadharajan & Kandasamy et al., 2000; Doenhoff et al., 2008; Lim et al., 2008).

Diagnosing of GI parasites is based on detection of eggs, larvae cysts and oocysts in stool samples through microscopic examination (Ten Hove et al., 2009). These samples may have been obtained by using different parasitological techniques, such as direct examination, spontaneous sedimentation, centrifugation in ethyl acetate, centrifugal sedimentation in formalin-ether, centrifugal flotation with zinc sulfate, centrifugal flotation techniques with sucrose or flotation with sodium chloride. Thus, there are great divergences between the methods for diagnosing GI parasites, particularly with regard to sensitivity/specificity, cost and the time required for each method.

The Mini-FLOTAC technique was developed since 2013 as a novel direct method for diagnosing intestinal parasitic infections. Mini-FLOTAC attempts to address the challenge of using modern technology matched with high sensitivity and affordability. This procedure does not require any centrifugation step or expensive equipment; it can be performed on both fresh and fixed stool samples; and it requires less than 15 minutes of preparation before microscopic analysis (Cringoli et al., 2017).

Some degree of prophylactic anthelmintic treatment is necessary for many species of zoo mammals kept in captivity (Bais et al., 2017). Parasite control is usually done at least twice a year or, alternatively, only if the fecal examination is positive. Therefore, the aim of this study was to demonstrate the role of the Mini-FLOTAC technique in combination with Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals.

## 1.3 Materials and Methods

This study was conducted at four zoos: two located in central Italy, Aprilia (41°35'40"N; 12°39'15"E) and Lanciano (42°13'37"N; 14°23'24"E); and two in southern Italy, Naples (40°51'22"N; 14°14'47"E) and Pesco Sannita (41°13'57"N; 14°48'40"E). In these zoos, exhibits are organized to ensure optimal usage of the available space and the natural environment is reproduced by creating habitats with dust, soil, wood, grass and endemic vegetation. The cages are cleaned daily using high-pressure piped water and dung is removed regularly. At the time of our study, non-human primates were kept under moderately crowded conditions, in temporary cages, pending the completion of new facilities in one of the zoos. At each institution, the zoo veterinarian is responsible for preventive health programs, which include health monitoring, parasite control and vaccination. Parasitological investigations are conducted at least twice per year, and animals are treated with antiparasitic drugs based on parasitological outcomes. Furthermore, the zoos are subject to the European Union Council Directive 92/65/EEC (Balai Directive), which outlines annual disease surveillance plans for approved establishments, along with animal transfer and quarantine procedures.

From June to August 2016, 70 fresh composite samples (pools) were collected by means of Fill-FLOTAC from animals in five mammal orders (Artiodactyla, Hyracoidea, Perissodactyla, Primates and Rodentia) at the zoos of Aprilia (8 pools), Lanciano (17 pools), Naples (16 pools) and Pesco Sannita (29 pools). The species in each zoo were sampled with the assistance of the animals' keepers. Sampling was conducted in the early morning using Fill-FLOTAC and the apical region of the feces deposited on the ground was collected. Each pool was composed of 2 g from each individual fecal sample (Fagiolini et al., 2010).

The Mini-FLOTAC technique was performed at the zoo facilities and two different flotation solutions were used: FS2 (sodium chloride, specific gravity [SG] = 1.200; AppliChem, Brentwood, MO, USA) and FS7 (Zinc Sulfate, SG = 1.350; AppliChem). Magnifications of 100x and 400x were used to identify helminth eggs and protozoan cysts/oocysts. The results were expressed as the arithmetic mean number of eggs/oocysts/cysts per gram (EPG/OPG/CPG) of feces.

## 1.4 Results

Mini-FLOTAC was shown to be a simple and rapid technique for detecting the number of eggs/cysts/oocysts per gram of feces. Among the 70 pools analyzed, 80% (24/30; 95% confidence interval [CI] = 30-54%) were positive for at least one parasite (Table1.1).

Parasite intensity express in eggs, larvae, cysts and oocyst per gram (EPG, LPG, CPG and OPG) of feces detected in zoo mammals are showed in Table 2.1.

Higher prevalence of parasites was detected in the zoos in central Italy (Lanciano 47% and Aprilia 37.5%) than in the zoos in southern Italy (Naples 37.5% and Pesco Sannita 20.1%).

Several genera of helminths and protozoa were detected in mammals at the four zoos. Among the nematodes, GI strongyles were the most frequent (40%), followed by *Trichuris* spp. (23.3%), *Parascaris* spp. (13.3%) and *Capillaria* spp. (3.3%). With regard to protozoa, most of the samples were positive for *Blastocystis* spp. (6.6%), followed by *Giardia* spp. (3.3%) and *Eimeria* spp. (3.3%).

**Table 1.1:** Parasitological stool test results, according to mammal orders, in the central and southern Italian zoos.

| Order of zoo mammals | Pools examined | Positive pools | (%) |
|---|---|---|---|
| Artiodactyla | 31 | 11 | 35.4 |
| Carnivora | 9 | 0 | 0 |
| Chiropthera | 1 | 0 | 0 |
| Diprodotondia | 4 | 0 | 0 |
| Hyracoidea | 1 | 0 | 0 |
| Perissodactyla | 9 | 6 | 66.6 |
| Primates | 10 | 5 | 50 |
| Proboscidea | 1 | 0 | 0 |
| Rodentia | 4 | 2 | 50 |
| **Total** | **70** | **24** | **34.3** |

**Table 1.2:** Parasite intensity (minimum, mean, and maximum) of eggs/oocysts/cysts per gram (EPG/OPG/CPG) of feces detected in zoo mammals in central and southern Italy by Mini-FLOTAC combined with fill FLOTAC techniques.

| Parasitic Group/Genus | No.pos. (%) | Mini-FLOTAC Intensity (EPG/OPG/CPG*) | | |
|---|---|---|---|---|
| | | Min | Mean | Max |
| Helminths | | | | |
| GI strongyles | 12 (17) | 5 | 78 | 370 |
| *Trichuris* | 7 (10) | 5 | 20 | 40 |
| *Parascaris* | 4 (6) | 5 | 10 | 15 |
| *Capillaria* | 1 (1) | 0 | 8 | 10 |
| *Nematodirus* | 1 (1) | 0 | 4 | 4 |
| Protozoa | | | | |
| *Eimeria* | 1 (1) | 0 | 35 | 35 |
| *Blastocystis* | 2 (3) | ++ | + | + |
| *Entamoeba coli* | 1 (1) | - | + | + |
| *Giardia* | 1 (1) | - | + | + |

*CPG: + = 1 cyst per microscopic field; ++ = 3 cysts per microscopic field.

Widespread presence of GI parasites was confirmed in the four zoos. Helminthic infections were more common than protozoan infections in all the mammal orders examined and this was concordant with the findings from two other Italian zoos located in Apulia and Tuscany, where the overall prevalence of parasitic infection was 61.5% (96/156) by flotation test using a low-specific-gravity solution, sedimentation and a modified McMaster technique (Fagiolini et al., 2010).

All of the gastrointestinal parasites identified in our study were described previously in zoo animals by other authors, and these parasites are known to be pathogenic to both animals and humans (animal handlers and zoo visitors) (Fontenot et al., 2008; Fagiolini et al., 2010; Tahas & Diakou et al., 2013; Maesano et al., 2014; Nosal et al., 2016; Cringoli et al., 2017). Gastrointestinal parasites of zoo mammals include zoonotic species and therefore their presence raises public health concerns, particularly *Blastocystis* spp. and *Giardia* spp. (Goossens et al., 2005; Levecke et al., 2007).

Some authors have reported the possibility that these protozoa may be transmitted to humans or other animals (Rajah Salim et al., 1999). Transmission

between animals and humans in association with clinical outbreaks among animal keepers has been reported in various studies (Miller et al., 2004; Levecke et al., 2007; Berrilli et al., 2011). The presence of zoonotic parasites emphasizes the need to use rapid copro-microscopic techniques and specific devices that protect the operator.

This was the first study in which Mini-FLOTAC in combination with Fill-FLOTAC was used for rapidly diagnosing parasitic infections in zoo mammals. Currently, to make diagnoses of GI parasites in zoo animals, routine coprological procedures such as direct and indirect wet-mount preparations or the McMaster method are used (Malan et al., 1997; Perez Cordon et al., 2008; Levecke et al., 2010). Achieving accurate rapid diagnoses is a critical point in enabling control over parasitic infections.

In conclusion, Mini-FLOTAC in combination with Fill-FLOTAC was shown to be user-friendly and safe, with a wide diagnostic range (protozoa and helminths). These features are particularly useful for monitoring and control programs in which large numbers of fecal samples need to be processed rapidly and safely.

In this study, we showed the ability of the Mini-FLOTAC technique to detect GI parasites in different species of zoo mammals. The different flotation solutions used in this study appeared to increase the detected recovery rates for eggs, cysts and oocysts in all samples. This is a big advantage in comparison with other copro-microscopic methods, in which only one solution is used to perform the tests.

Mini-FLOTAC in combination with Fill-FLOTAC ensured a high level of safety for the operator. In fact, Fill-FLOTAC equipped with a collector/homogenizer allows sampling and processing of fecal samples without coming into contact with them. It operates as a closed system and therefore not only provides protection for the operator but also enables the possibility of sample preservation in formalin for subsequent processing.

Furthermore, the present study highlighted the presence of parasitic elements in samples from zoo mammals despite regular diagnosis.

## 1.5 References

Bais B, Tak L, Mahla S, 2017. Study of preventive health measures for wildlife in captivity: a review of management approaches. *Int J Avian Wildl Biol* 2(3), 73-75.

Ballweber, L., Beugnet, F., Marchiondo, A.A., Payne, P.A., 2014. American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use-Is there really one best technique? Vet. Parasitol. 204(1).

Berrilli F, Prisco C, Friedrich KG, Di Cerbo 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(1) 199. http://dx.doi.org/10.1186/1756-3305-4-199. PMid:21988762.

Cox, DD, Todd, AC, 1962. Survey of gastrointestinal parasitism in Wisconsin dairy cattle. J Am Vet Med Assoc 141, 706-709.

Cringoli G, Maurelli MP, Levecke B, Bosco A, Vercruysse J, Utzinger J et al., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Protoc. 12, 1723–32.

Cringoli G, Rinaldi L, Maurelli MP, Utzinger J, 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 5, 503-515.

Cringoli G, Rinaldi L, Veneziano V, Capelli G, Scala A, 2004. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. Vet Parasitol 123, 121-131.

Dryden MW, Payne PA, Ridley R, Smith V, 2005. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet Ther 5, 15-28.

Egwang TG, Slocombe JO, 1981. Efficiency and sensitivity of techniques for recovering nematode eggs from bovine feces. Can J Comp Med 45, 243-248.

Egwang TG, Slocombe JO, 1982. Evaluation of the Cornell-Wisconsin centrifugal flotation technique for recovering trichostrongylid eggs from bovine feces. Can J Comp Med 46, 133-137.

Eigenfeld DD, Schlesinger CJ, 1944. An improved flotation method for the

recovery of ova from feces. J Am Vet Med Assoc 104, 26.
Foreyt WJ, 2001. Veterinary Parasitology Reference Manual, 5th ed. Ames, Iowa State University Press, pp. 236.
Gordon HM, Whitlock HV, 1939. A new technique for counting nematode eggs in sheep faeces. J Counc Sci Ind Res 12, 50-52.
Hendrix CM, Robinson E, 2016. Diagnostic Parasitology for VeterinaryTechnicians, fifth ed. Elsevier Mosby, St. Louis, MO, pp. 480.
Hungerford TG, 1990. Diseases of Livestock, nineth ed. MacGraw-Hill Medical, Sydney, Australia.
Koutz RR, 1941. A comparison of flotation solutions in the detection of parasite ova in feces. Am J Vet Res 2, 95–100.
Lane C, 1924. The mass diagnosis of anlylostome infestation. (Parts II toVII.). Trans R Soc Trop Med Hyg 17, 407–436.
Levecke B, Rinaldi L, Charlier J, Maurelli MP, Bosco A, Vercruysse J, Cringoli G, 2012. The bias, accuracy and precision of faecal egg count reduction test results in cattle using McMaster, Cornell-Wisconsin and FLOTAC egg counting methods. Vet Parasitol 188, 194-199.
Levine ND, Mehra KN, Clark DT, Aves IJ, 1960. A comparison of nematode egg counting techniques for cattle and sheep feces. Am J Vet Res 21, 511-515.
MAFF, 1986. Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationary Office, London, UK. 20-27.
Mayhew RL, 1962. Studies on bovine gastrointestinal parasites. XXVI. A flotation method for the recovery of parasitic eggs using cane sugar. Trans Am Microsc Soc 81, 264–267.
McCoy MA, Edgar HW, Kenny J, Gordon AW, Dawson LE, Carson AF, 2005. Evaluation of on-farm faecal egg counting in sheep. Vet Rec 156, 21-23.
Nicholls J, Obendorf DL, 1994. Application of a composite faecal egg count procedure in diagnostic parasitology. Vet Parasitol 52, 337-342.
Presland SL, Morgan ER, Coles GC, 2005. Counting nematode eggs inequine faecal samples. Vet Rec 156, 208–210.
Raynaud JP, 1970. Etude de l'efficacite d'une technique de coproscopie quantitative pour le diagnostic de routine et le controle des infestations parasitaires des bovins, ovins, equins et porcins. Ann Parasitol Hum Comp 45, 321-342.
Rinaldi L, Coles GC, Maurelli MP, Musella V, Cringoli G, 2011. Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. Vet Parasitol 11, 345-52.

## References


Rinaldi L, Cringoli G, 2014. Exploring the interface between diagnostics and maps of neglected parasitic diseases. Parasitol 28, 1-8.

Roeber F, Jex AJ, Gasser RB, 2013a. Next-generation molecular- diagnostic tools for gastrointestinal nematodes of livestock, with an emphasis on small ruminants: a turning point? Adv. Parasitol. 83, 267- 333.

Roeber F, Jex AJ, Gasser RB, 2013b. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - an Australian perspective. Parasit. Vectors 6, 153.

Roberts FH, O'Sullivan PJ, Riek RF, 1951. The significance of faecal egg counts in the diagnosis of parasitic gastro-enteritis of cattle. Aust Vet J 27, 16-18.

Rossanigo CE, Gruner L, 1991. Accuracy of two methods for counting eggs of sheep nematode parasites. Vet Parasitol 39, 115-121.

Seghetti L, 1950. An improved method of mixing fecal suspensions for nematode egg counts. Proc Helminthol Soc Wash 17, 26–27.

Slocombe JOD, 1973. Parasitisms in domesticated animals in Ontario I. Ontario Veterinary College records 1965–70. Can Vet J 14, 36–42.

Stoll NR, 1923. Investigations on the control of hookworm disease. XV. An effective method of counting hookworm eggs in faeces. Am J Epidemiol 3, 59-70.

Stoll NR, 1930. On Methods of counting Nematode Ova in Sheep Dung. Parasitol 22, 116-136.

Thienpont D, Rochette F, Vanparijs OFJ, 1986. Diagnosing helminthiasis by coprological examination, 2nd ed. Janssen Research Foundation, Beerse.

Vercruysse J, Charlier J, Van Dijk J, Morgan ER, Geary T, von Samson-Himmelstjerna G, Claerebout E, 2018. Control of helminth ruminant infections by 2030. Parasitol. 145, 1655–1664.

Whitlock HV, 1948. Some modifications of the McMaster helminth egg-counting technique and apparatus. J Counc Sci Ind Res 21, 177-180.

Zajac AM, 2006. Gastrointestinal Nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. Vet. Clin. North Am. Food Anim. Pract. 22, 529-541.

Zajac AM, Conboy GA, 2012. Veterinary Clinical Parasitology 8th ed. Blackwell Publishing, Ames, Iowa, pp. 368.

# Chapter 2

## Wild geladas (*Theropithecus gelada*) in crops more than in pasture areas reduce aggression and affiliation

Caselli M, Zanoli A, Dagradi C, Gallo A, Yazezew D, Tadesse A, **Capasso M**, Ianniello D, Rinaldi L, Palagi E, Norscia I., 2021. Wild geladas (*Theropithecus gelada)* in crops-more than in pasture areas-reduce aggression and affiliation. Primates 62(4), 571-584. doi: 10.1007/s10329-021-00916-8.

## 2.1 Introduction

The growing expansion of human settlement (Koh and Wilcove 2008) is causing changes in wildlife behavior due to a forced coexistence of wildlife and humans (Sih et al., 2011). Previous investigations report behavioral changes in different taxa (reptiles: Batabyal et al., 2017; birds: Blumstein et al., 2005; Belton et al., 2018). Nonhuman primates (hereafter primates) are no exception and are particularly affected because approximately 30% of the existing species live in proximity to human settlements and rely on anthropic land cover for their maintenance activities (McLennan et al., 2017; Galán-Acedo et al., 2019).

Various types of human–primate interfaces, including tourist-provisioned sites, temples, urban settlements, and agricultural fields (Kaburu et al., 2019; Balasubramaniam et al., 2020; Jaman and Huffman, 2013), are described in the literature. Agricultural areas can have a particularly strong impact on primate behavior (Arroyo-Rodríguez and Fahrig, 2014) because crops are often associated with close human settlements (Minta et al., 2018). They can include patches with clumped, high quality and palatable resources, leading to high-risk crop foraging by primates (Riley et al., 2013). Hill (2018) proposed two hypotheses to explain crop foraging: the *crops as fallback foods hypothesis*, according to which primates would feed on crops when wild resources are scarce, and the *crop foraging as an optimizing strategy hypothesis*, according to which the high risk associated with crop foraging would be compensated by an increase in nutritional intake, with consequent benefits for reproductive potential.

One of the main risks that primates face when frequenting areas in which humans are present, including agricultural fields, is related to direct or indirect pathogen transmission among humans, livestock, and primates (Goldberg et al., 2007; Krief et al., 2010). Such transmission can include gastrointestinal parasites, such as protozoans in *Gorilla gorilla gorilla* (*Giardia intestinalis*; Sak et al., 2013), several nematode species in *Papio* spp. (Hahn et al., 2003), and, if wild or domestic canids are present, the cestode *Taenia serialis* in *Theropithecus gelada* and other primates (Schneider-Crease et al., 2017; Chanove et al., 2019).

The health of wild primates can also be impacted when their home ranges include agriculture land and herbicides and other chemical pollutants are used on crop fields (Garabrant and Philbert, 2002). For example, 2,4-dichloro-

phenoxyacetic acid, frequently used for weed control (de Castro Marcato et al., 2017), has been associated with the presence of alopecia (e.g. in dogs, Charles et al., 1996), tumors (in humans, Anthony and Saleh et al., 2013), and reproductive problems (e.g. in chimpanzees and olive baboon, Krief et al., 2017). We urgently need more evidence on the possible harm due to the ingestion of herbicides and pesticides.

Finally, different types of human–primate interfaces may variably influence primate social behavior (Chowdhury et al., 2020) found that in chacma baboons, *Papio ursinus*, social grooming decreased in anthropogenic areas. Other studies were mostly focused on macaques. For example, in peri-urban areas, *Macaca radiata* showed reduced grooming effort due to interaction with both visitors and local residents (Balasubramaniam et al., 2020). In temple areas, depending on the level of human–monkey interaction, *Macaca mulatta* can reduce social grooming considerably (Kaburu et al., 2019), but in urban areas they can increase groom- ing and play compared to rural areas (Jaman and Huffman et al., 2013). The time spent grooming in *Macaca fascicularis* varies depending on whether the interaction with humans is moderate or high (Marty et al., 2019). The social behavior of primate groups frequenting agricultural lands may be particularly affected for at least three reasons. First, the measures used by humans to protect their crops, such as chasing, throwing objects, or even shooting at animals (Osborn and Hill et al., 2005), can disrupt primate behavior (McKinney et al., 2015; McLennan et al., 2017). Second, the high-quality, concentrated resources found in agricultural lands can lead to reduced affiliation and increased overt competition (Jaman and Huffman et al., 2013; Arseneau-Robar et al., 2016). Third, time budget trade-offs can come into play, as in agricultural areas primates might be constrained by time linked to a higher risk of being herded by humans that monitor them to keep them away (Priston et al., 2012; Chowdhury et al., 2020). Based on this framework, our goal was to contribute to a better understanding of how different human/primate interfaces can affect the health and social behavior of nonhuman primates. Specifically, we investigated whether the relative use of two different human–primate interfaces, namely agriculture and pasture, affected the health and the social behavior of a population of wild geladas (*Theropithecus gelada*), a primate species endemic to Ethiopia. Geladas are group-living, terrestrial, and mostly herbivorous; consequently, part of their natural plant food species is shared with livestock (Fashing et al., 2014). Moreover, the products of cultivated plants (e.g. *Era-*

*grostis tef*) are also highly attractive to geladas, which can approach human settlements and enter crop fields in search of food (Abu et al., 2018). Based on the observation that primates frequenting crops can be exposed to direct (e.g. active chasing: Osborn and Hill, 2005) and indirect human disturbance (Garabrant and Philbert et al., 2002; Nunn et al., 2006), we predicted that the geladas using the crop area the most would be exposed to more frequent direct human disturbance (prediction 1a), higher risk of developing pathologies (prediction 1b), and increased risk of infection by parasites typical of human settlements (prediction 1c).

Geladas live in a multi-level society whose basic unit is the one-male/multi-female unit (hereafter, OMU) (Dunbar and Dunbar et al., 1975; Zinner et al. 2018). An OMU generally comprises one adult male, several adult females, and their offspring. Bachelor groups, separate from OMUs, are called all-male units (hereafter, AMU). OMUs and AMUs can form teams, bands and, at a larger level, herds, which can include hundreds of individuals (Dunbar and Dunbar et al., 1975; Snyder Mackler et al., 2012; Zinner et al., 2018). High-intensity sporadic aggression is observed when a male tries to take over a group or to claim a territory (Beehner and Bergman et al., 2008). However, the absence of a strict reproductive season and the control of a single male over a group of females largely reduces inter-male competition over females (Dunbar and Dunbar et al., 1975). Moreover, groups are characterized by extremely high tolerance levels (Dunbar and Dunbar et al., 1975). As a result, gelada societies are characterized by low rates of inter- and intra-group (OMUs/AMUs) aggression and high levels of affiliative social grooming between group members (Dunbar and Dunbar et al., 1975; Mancini and Palagi et al., 2009). Because human interference and resource competition in primates can lead to decreased affiliation (Jaman and Huffman et al., 2013) and increased aggressive patterns (Arseneau-Robar et al., 2016; Thatcher et al., 2019), both of which can jeopardize group cohesion and social stability, we predicted that geladas would spend less time grooming (prediction 2a) and engage in aggression of higher intensity when in the crop area compared to the pasture area (prediction 2b).

## 2.2 Methods

### *2.2.1 Study site and subjects*

This study was conducted with a population of wild geladas frequenting the Kundi plateau, in the Wof-Washa area (Ethiopia, Amhara region, N9°40.402' E39°45.060'; altitude (min–max): 3370–3592 m). We followed the subjects from January to May 2019 and from December 2019 to February 2020, spanning the dry and the beginning of the small rainy season, on a daily basis, five days per week (excluding days with heavy rain or mist), from around 9:30 to 17:00 (for a total of 94 full days and a total of 658 h). We considered that the small rainy season (Yazezew et al., 2020) had started when the rain set in for three consecutive days. The late dry and early wet periods often including the post-harvesting phase can be key periods of nutritional need, possibly associated with crop raiding by geladas searching for crop food remains and seeds (Hirvonen et al., 2016; Dunbar et al., 1977).

Surrounded by cliffs, the Kundi plateau (26 ha) is characterized by crop (about 12 ha) and pasture areas (about 14 ha), which have the same visibility conditions. In this study, we defined "crop area" as the agriculture fields (including human settlements) and the zone within 300 linear meters from the closest house or cultivated land. This criterion allowed for cultivated land, houses, domestic animal shelters, and passage zones from crop to crop or from crop to houses to be included in the "crop area". We defined "pasture area" as the grassland without human settlements and cultivated fields, where livestock (horses, goats, sheep, donkeys, and cows) grazed during the day, led by shepherds. During the study period, animals spent 77.083 ± 14.360 (mean ± SE) and 276.458 ± 23.500 (mean ± SE) non-consecutive minutes per day in the crop and pasture areas, respectively. Gelada groups were free to move down the cliffs from the plateau. In the first month of the study, a subset of groups frequenting the Kundi plateau were habituated and surveyed by four to six researchers (EP, IN, MaC, AZ, CD, AG). Group size, sex ratio, age ratio, and natural markers of the central male and/or other individuals (as detailed below) were used to identify gelada groups (one-male unit; OMU/all-male unit; AMU), based on Dunbar and Dunbar (1975) criteria. This process required around one month and was facilitated by video-recording of the groups. We were able to survey 14 OMUs and two AMUs and counted 27 adult males, 79 adult females, 60 subadult in-

dividuals, 35 juveniles, and 65 infants (31 late, 21 early, 13 black). The number of groups present on the plateau on a daily basis was 8.706 ± SE 0.950 (mean ± SE).
Individual discrimination was achieved for 140 subjects (excluding infants) by considering long-lasting distinctive features (including sex, size, permanent scars, deformations, and particular shapes of the red chest area in adults; Dunbar and Dunbar, 1975). Such features were identified during field observations or via video recordings during and after the field data collection.

*2.2.2 Field data collection*

Each day four observers (MaC, AZ, CD, AG) went on the Kundi plateau and split into two groups to search for the gelada groups toward the top and the bottom of the plateau, respectively. The group composition of observers changed every week, following a rotation schedule. One observer (videographer) recorded the videos and the other assisted the videographer by vocally recording the ongoing activities and the subjects involved in the behavior. Not all of the identified gelada groups were present on the highland every day. Thus, on each day (after the end of the habituation period) data were collected on the visible and recognizable groups, giving priority to the less commonly observed groups when multiple groups were present to reduce observation imbalance and ensure sufficient data collection for all groups.
We conducted scan sampling (Altmann et al., 1974) live (not on video) at 10-min intervals on the recognized, visible groups present on the plateau each day. We gathered a mean of 304.357 ± SE 43.879 scans per group covering the whole daily observation period. Multiple groups could be present in a scan. Whenever possible, we recorded for the purpose of this study (i) group identity, (ii) GPS position based on the central male position (Garmin GPS Map 64), and (iii) the percentage of individuals foraging.
Data on direct human/gelada interactions (e.g. chasing animals, throwing stones, sticks; see table S1 for a detailed description, video MPEG-1) were collected via an all occurrences sampling method (Altmann et al., 1974) to gather data on each possible episode.
On the recognizable groups, we also collected data via two video cameras (Panasonic HC-V180, full-HD, 50 fps, optical zoom 50x) for a total of 120 h of videos. We gathered a mean of 8.071 ± SE 1.336 video hours per group

and a mean of 2.128 ± SE 0.198 video hours per subject, spreading the observational effort across morning and afternoon.
Grooming videos were collected via 10-min focal sampling (Altmann et al., 1974), with the focal subject being selected on the basis of the criteria explained above (giving priority to visible, recognizable, and less observed subjects). If the grooming continued, the recording went on until the end of the grooming session to allow analyses on grooming duration. This rule was applied to all dyads, and extra video duration (after 10 min) was considered only to calculate grooming duration (normalized as explained in the behavioral data section). The videos including grooming lasted on average 11.502 ± SE 0.686 min and involved 22 adult males (belonging to both OMUs and AMUs), 30 adult females, 5 immature males, and 2 immature females.
Owing to the tolerant nature of the study species, aggressive encounters are known to be infrequent (Bergman, 2010; Dunbar, 2014). Hence, data on aggressive events were collected via all-occurrences sampling (Altmann et al., 1974). Cameras were always kept on, on the clearly visible groups. While the videographer recorded the scene, the assistant would describe the aggressive event aloud to also gather data on what happened off-screen if necessary. At least three aggressive events per group were recorded, involving 23 adult males, 61 adult females, 29 immature males, and 10 immature females. The observed aggressions occurred to displace individuals from a foraging spot.

### *2.2.3 Health, disturbance data and operational definitions*

We calculated how frequently the OMUs + AMUs ($N = 16$) were present in the crop area by considering the number of scans in which each group was inside the crop area normalized over the total scans per group. The group position was assessed via GPS coordinates, referring to the alpha-males. We then separated the groups into two categories ("frequent crop users" and "infrequent crop users"), depending on whether the frequencies fell above or below the median frequency of the proportion of scans per group recorded in crops (median = 0.189; range = 0.020–0.340). Then, we considered the number of events of direct human disturbance (e.g. humans chasing geladas using stones, dogs, sticks, shooting for frequent and infrequent crop users, normalized over the total scans per group in each area (i.e. crop vs. pasture). On the basis of photos and videos, the individuals (adults and immatures)

were considered as bearing external signs of pathology when they showed at least one of the following external signs: abnormal swelling on trunk, limbs, and/or neck, probably related to *Taenia serialis* infection, as it has been found in other gelada populations (Ohsawa and Dunbar et al., 1984; Nguyen et al., 2015; Schneider Crease et al., 2017) and alopecia, defined as hair loss either diffuse or patchy, in areas where the loss could not be caused by infant clinging. The external signs of pathologies were considered for males and two categories of females (lactating and non-lactating) due to the effect that lactation can have on the immune system (Wang et al., 2016). Depending on the group they belonged to, individuals were assigned to either frequent or infrequent crop user groups.

### *2.2.4 Behavioral data*

We determined the daily frequency of foraging in the pasture and crop areas by considering the number of scans in which at least 10% of animals were foraging in either area normalized on the total number of daily scans per area. Data on grooming were extracted from videos using the focal animal sampling (Altmann et al., 1974). To calculate grooming duration, we considered a grooming session as started when one of the two individuals began cleaning the fur of the other, and as finished when grooming was interrupted for at least 10 s (Mancini and Palagi et al., 2009). We recorded (i) groomer and grooming receiver identities, (ii) age class of both individuals (adult or immature), (iii) sex class (male or female), (iv) time spent grooming, and (v) area where grooming took place (pasture or crop). Because the observation time varied across dyads, for each dyad we divided the daily time spent grooming by the focal daily observation time of that dyad (normalized data). The aggressive events were extracted from video and audio recorded information, following an all occurrences method (Altmann et al., 1974) on the observable groups. For each aggressive event, we recorded the following data: (i) the identity of the aggressor (individual that initiated the first agonistic pattern) and the identity of the recipient (the individual that received the first aggressive pattern), (ii) age class (adult or immature), (iii) sex class (male or female), (iv) intensity of aggression, i.e. mild (chasing or chasing attempt without contact between opponents) or strong (chasing with contact between opponents), (v) whether aggression was intra or inter group, and (vi) the area where the aggression took place (pasture or crop). We recorded a

total of 114 aggressive events, with a minimum of three aggressive events per group. All videos were analyzed via the free software VLC 3.0.6 (©VideoLAN) by MaC and AG (Cohen's value for inter-observer reliability calculated on 10% of the total grooming/aggressive events ≥ 0.75).

### 2.3 Fecal sample collection and parasitological analyses

We collected 48 fresh fecal samples (preserved in 10% formalin) from 48 unique individuals during observations and identified the samples as from individuals in the frequent or infrequent crop user group. The number of gastrointestinal parasitic elements (egg/larva/oocyst/cyst)/g of feces was determined using the FLOTAC pellet dual technique (Cringoli et al., 2010). This protocol is a multivalent, quali/quantitative copromicroscopic method for detecting parasitic elements (eggs, larvae, oocysts, and cysts) in animal fecal samples, with an analytical sensitivity of one parasitic element per gram of feces (EPG/LPG/OPG/CPG). The pellet technique is performed for samples with unknown fecal material weight, so the weight of the fecal material can be obtained after weighing the sediment in the tube (pellet) after filtration and centrifugation of the fecal sample. These steps are very important for discriminating between parasites and pseudoparasites, considering that the identification of parasites in fecal samples is often complicated by the high fiber content of the animal diet, as well as the common presence of pollen, plant tissue, flowers, and invertebrate frag- ments (accidentally ingested with the plants), all of which can be misclassified as parasitic structures (Alvarado-Villalobos et al., 2017).

Two different flotation solutions were used to detect the gastrointestinal parasites: FS2 (sodium chloride solution, specific gravity = 1200) and FS7 (zinc sulfate solution, specific gravity = 1350). Different magnifications were used, ×100 and ×400, respectively, for the study of egg/larvae of helminths and cysts/oocysts of protozoa.

The diagnostic technique described above does not allow the identification at the species/assemblage level, so it was not possible to measure the specific richness.

## 2.4 Results and Discussion

In the fecal samples of both frequent and infrequent crop users we found *Giardia intestinalis* (detected for the first time in a wild gelada population; mean ± $SE_{infrequent\_users}$ = 1480.00 ± 851.66; mean ± $SE_{frequent\_users}$ = 386.38 ± SE 198.37), Ancylostomatidae (mean ± $SE_{infrequent\_users}$ = 231.45 ± 63.75; mean ± $SE_{frequent\_users}$ = 249.68 ± 67.47), *Chilomastix* spp. (mean ± $SE_{infrequent\_users}$ = 36.14 ± 10.43; mean ± $SE_{frequent\_users}$ = 30.32 ± 19.08), *Endolimax nana* (mean ± $SE_{infrequent\_users}$ = 22.21 ± 6.05; mean ± $SE_{frequent\_users}$ = 18.63 ± 3.09), and *Entamoeba histolytica/dispar* (mean ± $SE_{infrequent\_users}$ = 1.31 ± 0.73; mean ± $SE_{frequent\_users}$ 21.47 ± 12.99). We found that the number of parasitic elements/g of *Entamoeba histolytica/ dispar* was significantly greater in frequent crop users compared to infrequent crop users (exact Mann–Whitney: $N_{infrequent\_users}$ = 29, $N_{frequent\_users}$ = 19, U = 128.50, P < 0.001). There was, however, no significant difference between frequent and infrequent crop users in the number of parasitic elements/g (i.e. egg/larva/oocyst/cyst) of Ancylostomatidae, *Chilomastix* spp., *Endolimax nana* or *Giardia intestinalis* (exact Mann–Whitney: $N_{infrequent\_users}$ = 29, $N_{frequent\_users}$ = 19; Ancylostomatidae: U=262.00, P=0.776; *Chilomastix* spp.: U = 223.50, P = 0.207; *Endolimax nana*: U = 241.00, P = 0.443; *Giardia intestinalis*: U = 243.50; P = 0.500).
Our results are consistent with the hypothesis that crop area can be challenging to wild geladas, because frequent crop users were more exposed to direct human disturbance (in line with prediction 1a) and a waterborne parasite (i.e. *Entamoeba histolytica/dispar*; in partial agreement with prediction 1c), and showed more signs of external pathologies (i.e. alopecia and abnormal swelling), in line with prediction 1b. According to previous studies on geladas and other primates, the observed external signs of pathology were compatible with the presence of ectoparasites (i.e. alopecia) or endoparasites (i.e. abnormal swelling) possibly shared with livestock and humans (Toft et al., 1986; Schneider-Crease et al. 2017). Throat swelling and alopecia may also be symptoms of iodine deficiency, which is common in the human population living in the Amhara region of Ethiopia, where this study took place (Abuye and Berhane et al., 2007). These two pathology signs have also been observed in captive geladas (Borst et al. 1972). Similar symptoms may be caused by thyroid-disrupting chemical contaminants, including those used in agriculture (Maliszewska-Kordybach and Smreczak et al., 1998; Rolland et al., 2000). In particular, the 2,4-dichlorophenoxyacetic acid used in the study area as

herbicide (pers. obs.) has been reported to cause tumors in humans (Anthony and Saleh et al., 2013) and alopecia in dogs (Charles et al. 1996). Currently there is a lack of information on whether iodine deficiency and the above mentioned herbicide are also responsible for external signs of pathology in geladas. Hence, the causality of alopecia remains unclear, whereas swelling is most likely the result of infection with *Taenia* spp. (*Taenia serialis* in wild geladas) reported for other gelada populations, with canids being the primary host of this tapeworm (Ohsawa and Dunbar et al., 1984; Nguyen et al. 2015; Schneider-Crease et al. 2017). In the study area, domestic dogs were present mostly around houses and crops, but both domestic and stray dogs may have roamed crop- and pastureland, which might explain why the area had only a small to medium effect on the presence of external signs of pathology. Even if we cannot confirm the presence of *Taenia serialis* in our study population from a biological point of view (to confirm the presence of this parasite, it is necessary to analyze urine samples; Schneider-Crease et al. 2017), the presence of abnormal swelling may be a predictor of the presence of this parasite. Indeed, *Taenia serialis* develops in the hypodermal musculature, causing abnormal swelling, and at the end of its development process the parasite perforates the skin and exits, causing suppurating masses (Ohsawa et al., 1979). Once all the mass is purged, the swelling disappears (Dunbar et al., 1980). On the other hand, the fact that apart from parasites, other factors specifically associated with farming may be linked to abnormal swelling and alopecia might explain why the effect of the area on the presence of external signs of pathologies was nevertheless significant. A diagnosis could not be performed on biological samples; therefore none of these possibilities can be ruled out. The fact that the external signs of pathology were significantly more frequent in non-lactating adult females than in lactating females (Fig. 2b) might be related to the immunological properties of oxytocin, produced during lactation to regulate milk production (Wang et al., 2016). On the contrary, testosterone in males can weaken the immune system, potentially explaining the more frequent signs of pathology in adult males than adult females (Roberts et al. 2004; Weisman et al. 2014; Muller et al., 2017). Another, non exclusive explanation is that females with abnormal swelling may be in poorer health conditions and therefore less able to reproduce (Nguyen et al. 2015). The effect of sex, although significant, was small to moderate, possibly because various factors, together or separately, can cause alopecia and abnormal swelling (including parasites and chemical pollutants as described above).

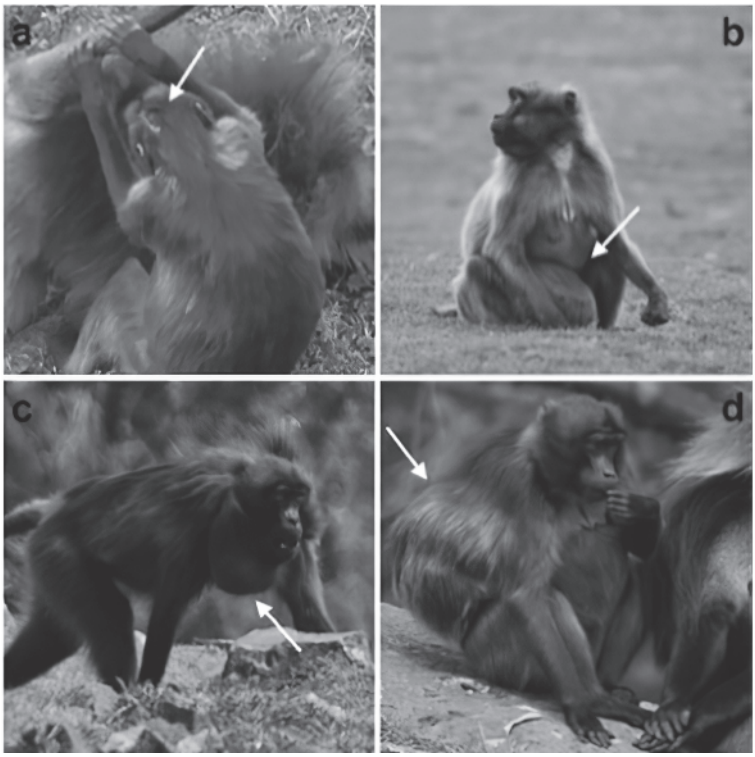


**Fig. 2.1** Pathologies observed in the geladas from the Kundi plateau: (**a**) adult female with alopecia, (**b**-**c**) adult female with abnormal swelling, (**d**) adult female with both alopecia and swelling. Photos by: Ivan Norscia, Alessandro Gallo, Carlo Dagradi

The trend observed in the increase of the external signs of pathology in adults is in line with previous studies on geladas (Nguyen et al. 2015; Schneider-Crease et al. 2017). The higher frequency of these signs in adult than in immature subjects could be related to parasite accumulation and/ or higher stress levels. Adult subjects are more affected by social and environmental stress than immatures, causing a decrease in their immune system and making them more susceptible to parasitic infections (Muehlenbein and Bribiescas et al., 2005).

We also found the presence of a wide range of gastrointestinal parasites (Nematoda and Protozoa) in gelada fecal samples. Most of the parasites detected showed no differences between frequent and infrequent crop users. However, we found that *Entamoeba histolytica/dispar* was highest in the feces of the frequent crop users. This result may be linked to the especially high contamination levels by *E. histolytica* reported for the Amhara region around human settlements, compared to other regions of Ethiopia (Aiemjoy et al. 2017; Zemene and Shiferaw et al., 2018). In addition to indirect human disturbance (prevalence of external pathology signs and highest fecal parasite load), direct human disturbance was also high in the crop area. As a matter of fact, in the crop area, geladas were most likely to be chased away. This may have negative implications for gelada welfare. In other species, for example, it has been found that human/primate interactions (or even proximity) can be detrimental to health due to decreased feeding efficiency (related to increased vigilance for human aggression) and increased stress levels related to interactions with or threats by humans (Behie et al. 2010; Maréchal et al. 2011; Jaimez et al. 2012; Shutt et al. 2014; Chowdhury et al. 2020).

In summary, the first block of results suggests that agricultural activities close to human settlements can have a strong impact on wild gelada health. Frequenting agricultural areas may allow access to concentrated, high-quality resources (Strum et al., 1994; Osborn and Hill et al., 2005; Riley et al., 2013), but in the long term, crop foraging can have negative consequences on gelada health due to both direct and indirect disturbance. Further analyses on fecal samples collected from individuals showing external signs of pathologies could enable the identification of the possible direct link between the observed signs and parasite infections.

## 2.4 References

Abu K, Mekonnen A, Bekele A, Fashing PJ, 2018. Diet and activity patterns of Arsi geladas in low-elevation disturbed habitat south of the Rift Valley at Indetu, Ethiopia. Primates 59(2), 153–161. https://doi.org/10.1007/s10329-017-0640-9.

Abuye C, Berhane Y, 2007. The goitre rate, its association with repro- ductive failure, and the knowledge of iodine deficiency disorders (IDD) among women in Ethiopia: cross-section community based study. BMC Public Health 7(1):316. https://doi.org/10.1186/ 1471-2458-7-316.

Aiemjoy K, Gebresillasie S, Stoller NE, Shiferaw A, Tadesse Z, Chan- yalew M, Keenan JD, 2017. Epidemiology of soil-transmitted helminth and intestinal protozoan infections in preschool-aged children in the Amhara region of Ethiopia. Am J Trop Med Hyg 96(4), 866–872. https://doi.org/10.4269/ajtmh.16-0800.

Altmann J, 1974. Observational study of behavior: sampling methods. Behaviour 49:227–267. https://doi.org/10.1163/156853974X 00534

Alvarado-Villalobos MA, Cringoli G, Maurelli MP, Cambou A, Rinaldi L, Barbachano-Guerrero A, Serio-Silva JC, 2017. Flotation techniques (FLOTAC and mini-FLOTAC) for detecting gastrointestinal parasites in howler monkeys. Parasitevectors 10(1):586. https://doi.org/10.1186/s13071-017-2532-7

Anthony KP, Saleh MA, 2013. Free radical scavenging and antioxidant activities of silymarin components. Antioxidants 2(4), 398–407. https://doi.org/10.3390/antiox2040398

Arroyo-Rodríguez V, Fahrig L, 2014. Why is a landscape perspective important in studies of primates? Am J Primatol 76(10), 901–909. https://doi.org/10.1002/ ajp.22282

Arseneau-Robar TJM, Müller E, Taucher AL, Van Schaik CP, Wil- lems EP, 2016. Male food defence as a by-product of intersexual cooperation in a non-human primate. Sci Rep 6(1), 1–7. https:// doi.org/10.1038/srep35800

Balasubramaniam KN, Marty PR, Arlet ME, Beisner BA, Kaburu SS, Bliss-Moreau E, McCowan B, 2020. Impact of anthropogenic factors on affiliative behaviors among bonnet macaques. Am J Phys Anthropol 171(4), 704–717. https://doi.org/10.1002/ajpa.24013

Barr DJ, Levy R, Scheepers C, Tily HJ, 2013. Random effects structure for confirmatory hypothesis testing: keep it maximal. J Mem Lang 68, 255–

278. https://doi.org/10.1016/j.jml.2012.11.001
Batabyal A, Balakrishna S, Thaker M, 2017. A multivariate approach to understanding shifts in escape strategies of urban lizards. Behav Ecol Sociobiol 71(5), 83. https://doi.org/10.1007/ s00265-017-2307-3
Bates D, Mächler M, Bolker B, Walker S, 2015. Fitting linear mixed- effects models using lme4. J Stat Softw 67, 1–48. https://doi.org/10.18637/jss.v067.i01
Beehner JC, Bergman TJ, 2008. Infant mortality following male takeo- vers in wild geladas. Am J Primatol 70(12), 1152–1159. https://doi.org/10.1002/ajp.20614
Behie AM, Pavelka MS, Chapman CA, 2010. Sources of variation in fecal cortisol levels in howler monkeys in Belize. Am J Primatol 72(7), 600–606. https://doi.org/10.1002/ajp.20813
Belton LE, Cameron EZ, Dalerum F, 2018. Social networks of spotted hyaenas in areas of contrasting human activity and infrastructure. Anim Behav 135, 13–23. https://doi.org/10.1016/j.anbehav.2017.10.027
Bergman TJ, 2010. Experimental evidence for limited vocal recog- nition in a wild primate: implications for the social complexity hypothesis. Proc R Soc B 277(1696), 3045–3053. https://doi.org/ 10.1098/rspb.2010.0580
Blumstein DT, Fernández-Juricic E, Zollner PA, Garity SC, 2005. Inter-specific variation in avian responses to human disturbance. J Appl Ecol 42(5), 943–953. https://doi.org/10.1111/j.1365-2664. 2005.01071.x
Borst GH, Vroege C, Poelma FG, Zwart PP, Strik WJ, Peters JC, 1972. Pathological findings on animals in the Royal Zoological Gardens of the Rotterdam Zoo during the years 1963, 1964 and 1965. Acta Zol Pathol Antverp 56, 3–20
Bretz F, Hothorn T, Westfall P, 2010. Multiple comparisons using R. Chapman and Hall/CRC Press, Florida, Boca Raton
Bros WE, Cowell BC, 1987. A technique for optimizing sample size (replication). J Exp Mar Biol Ecol 114(1), 63–71. https://doi.org/ 10.1016/0022-0981(87)90140-7
Chanove E, Ionicaˇ AM, Hochman D, Berchtold F, Gherman CM, Mihalca AD, 2019. Severe coenurosis caused by larvae of *Taenia serialis* in an olive baboon (*Papio anubis*) in Benin. Int J Parasitol 9:134–138. https://doi.org/10.1016/j.ijppaw. 2019.04.008
Charles JM, Dalgard DW, Cunny HC, Wilson RD, Bus JS, 1996. Comparative subchronic and chronic dietary toxicity studies on 2, 4-dichlorophe-

noxyacetic acid, amine, and ester in the dog. Toxicol Sci 29(1):78–85. https://doi.org/ 10.1093/toxsci/29.1.78

Chowdhury S, Brown J, Swedell L, 2020. Anthropogenic effects on the physiology and behaviour of chacma baboons in the Cape Peninsula of South Africa. Cons Physiol 8(1), coaa066

Cringoli G, Rinaldi L, Maurelli MP, Utzinger J, 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Pro- toc 5(3), 503–515. https://doi.org/10.1038/ nprot.2009.235

de Castro Marcato AC, de Souza CP, Fontanetti CS, 2017. Herbicide 2, 4-D: a review of Toxicity on non-target organisms. Water Air Soil Pollut 228(3), 120. https://doi.org/10.1007/s11270-017-3301-0

de la Torre S, Snowdon CT, Bejarano M, 2000. Effects of human activities on wild pygmy marmosets in Ecuadorian Amazonia. Biol Conserv 94(2), 153–163. https://doi.org/10.1016/S0006- 3207(99)00183-4

Dobson AJ, 2002. An introduction to generalized linear models, 2nd edn. Chapman and Hall/CRC Press, Florida, Boca Raton

Dunbar RIM, 1977. The gelada baboon: status and conservation. In: Rainier of Monaco, Bourne GH (eds) Primate conservation. Academic Press, New York, pp 363–383

Dunbar RIM, 1980. Demographic and life history variables of a popu- lation of gelada baboons (*Theropithecus gelada*). J Anim Ecol 49(2), 485–506

Dunbar RIM, 1991. Functional significance of social grooming in primates. Folia Primatol 57(3), 121–131. https://doi.org/10.1159/ 000156574

Dunbar RIM, 1992. Time: a hidden constraint on the behavioural ecol- ogy of baboons. Behav Ecol Sociobiol 31(1), 35–49. https://doi. org/10.1007 /BF00167814

Dunbar RIM, 2014. Reproductive decisions: an economic analysis of gelada baboon social strategies. Princeton University Press, Princeton

Dunbar RIM, Dunbar P, 1975. Social dynamics of gelada baboons. In: Kuhn H, Luckett WP, Noback CR, Schultz AH, Starck D, Szalay FS (eds) Contributions to primatology, S. Karger, Basel, pp 1–157

Estienne V, Mundry R, Kühl HS, Boesch C, 2017. Exploitation of underground bee nests by three sympatric consumers in Loango National Park, Gabon. Biotropica 49(1), 101–109. https://doi.org/ 10.1111/btp.12354

Fashin PJ, Nguyen N, Venkataraman VV, Kerby JT, 2014. Gelada feed- ing ecology in an intact ecosystem at Guassa, Ethiopia: variabil- ity over time

and implications for theropith and hominin dietary evolution. Am J Phys Anthropol 155(1), 1–16. https://doi.org/10. 1002/ajpa.22559

Forstmeier W, Schielzeth H, 2011. Cryptic multiple hypotheses test- ing in linear models: overestimated effect sizes and the winner's curse. Behav Ecol Sociobiol 65(1), 47–55. https://doi.org/10.1007/ s00265-010-1038-5

Galán-Acedo C, Arroyo-Rodríguez V, Andresen E, Arregoitia LV, Vega E, Peres CA, Ewers RM, 2019. The conservation value of human-modified landscapes for the world's primates. Nat Commun 10(1), 152. https://doi.org/10.1038/ s41467-018-08139-0

Garabrant DH, Philbert MA, 2002. Review of 2, 4-dichlorophenoxy- acetic acid (2, 4-D) epidemiology and toxicology. Crit Rev Toxicol 32(4), 233–257. https://doi.org/10.1080/20024091064237

Goldberg TL, Gillespie TR, Rwego IB, Wheeler E, Estoff EL, Chap- man CA, 2007. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tour- ism in western Uganda. Biol Conserv 135(4), 511–517. https://doi.org/10.1016/j.biocon.2006.10.048

Hahn NE, Proulx D, Muruthi PM, Alberts S, Altmann J, 2003. Gastro- intestinal parasites in free-ranging Kenyan baboons (*Papio cynocephalus* and *P. anubis*). Int J Primatol 24(2), 271–279. https://doi.org/10.1023/A:1023092915171

Hill CM, 2018. Crop foraging, crop losses, and crop raid- ing. Annu Rev Anthropol. https://doi.org/10.1146/annur ev-anthro-102317-050022

Hirvonen K, Taffesse AS, Hassen IW, 2016. Seasonality and house- hold diets in Ethiopia. Public Health Nutr 19(10), 1723–1730. https://doi.org/10.1017/ S1368980015003237

Jaimez NA, Bribiescas RG, Aronsen GP, Anestis SA, Watts DP, 2012. Urinary cortisol levels of gray-cheeked mangabeys are higher in disturbed compared to undisturbed forest areas in Kibale National Park, Uganda. Anim Conserv 15(3), 242–247. https://doi.org/10. 1111/j.1469-1795.2011.00508.x

Jaman MF, Huffman MA, 2013. The effect of urban and rural habitats and resource type on activity budgets of commensal rhesus macaques (*Macaca mulatta*) in Bangladesh. Primates 54(1), 49-59. https://doi.org/10.1007/s10329-012-0330-6

Judge PG, de Waal FB, 1993. Conflict avoidance among rhesus mon- keys:

coping with short-term crowding. Anim Behav 46(2), 221– 232. https://doi.org/10.1006/anbe.1993.1184

Kaburu SS, Marty PR, Beisner B, Balasubramaniam KN, Bliss-Moreau E, Kaur K, McCowan B, 2019. Rates of human–macaque inter- actions affect grooming behavior among urban-dwelling rhesus macaques (*Macaca mulatta*). Am J Phys Anthropol 168(1), 92– 103. https://doi.org/10.1002 /ajpa.23722

Kalin NH, Shelton SE, 2003. Nonhuman primate models to study anxi- ety, emotion regulation, and psychopathology. Ann N Y Acad Sci 1008(1), 189–200. https://doi.org/10.1196/annals.1301.021

Koh LP, Wilcove DS, ,2008. Is oil palm agriculture really destroying tropical biodiversity? Conserv Lett 1(2), 60–64. https://doi.org/10.1111/j.1755-263X. 2008.00011.x

Krief S, Berny P, Gumisiriza F, Gross R, Demeneix B, Fini JB, Wasswa J, 2017. Agricultural expansion as risk to endangered wildlife: pesticide exposure in wild chimpanzees and baboons displaying facial dysplasia. Sci Tot Environ 598:647–656. https://doi.org/10. 1016/j.scitotenv.2017.04.113

Krief S, Vermeulen B, Lafosse S, Kasenene JM, Nieguitsila A, Berthelemy M, Guillot J, 2010. Nodular worm infection in wild chimpanzees in Western Uganda: a risk for human health? PLOS Negl Trop Dis 4(3), e630. https://doi.org/10.1371/ journal.pntd. 0000630

le Roux A, Snyder-Mackler N, Roberts EK, Beehner JC, Bergman TJ, 2013. Evidence for tactical concealment in a wild primate. Nat Commun 4(1), 1–6. https://doi.org/10.1038/ncomms2468

Lee PC, 1984. Ecological constraints on the social development of vervet monkeys. Behaviour 91(4), 245–261. https://doi.org/10. 1163/156853984 X00092

Majolo B, van Lavieren E, Maréchal L, MacLarnon A, Marvin G, Qarro M, Semple S, 2013. Out of Asia: the singular case of the Barbary macaque. The macaque connection. Springer, New York, pp 167–183. https://doi.org/10.1007/978-1-4614-3967-7_11

Maliszewska-Kordybach B, Smreczak B, 1998. Polycyclic aromatic hydrocarbons (PAH) in agricultural soils in eastern Poland. Toxi- col Environ Chem 66(1–4), 53–58. https://doi.org/10.1080/02772 249809358583

Mancini G, Palagi E, 2009. Play and social dynamics in a captive herd of gelada baboons (*Theropithecus gelada*). Behav Process 82(3), 286–292. https://doi.org/10.1016/j.beproc.2009.07.007

Maréchal L, Semple S, Majolo B, Qarro M, Heistermann M, MacLar- non A, 2011. Impacts of tourism on anxiety and physiologi- cal stress levels in wild male Barbary macaques. Biol Conserv 144(9), 2188–2193. https://doi.org/10.1016/j.biocon.2011.05.010

Marty PR, Beisner B, Kaburu SS, Balasubramaniam K, Bliss-Moreau E, Ruppert N, McCowan B, 2019. Time constraints imposed by anthropogenic environments alter social behaviour in longtailed macaques. Anim Behav 150, 157–165. https://doi.org/10.1016/j. anbehav.2019.02.010

McKinney T, 2015. A classification system for describing anthropo- genic influence on nonhuman primate populations. Am J Primatol 77(7), 715–726. https://doi.org/10.1002/ajp.22395

McLennan MR, Spagnoletti N, Hockings KJ, 2017. The implications of primate behavioral flexibility for sustainable human–primate coexistence in anthropogenic habitats. Int J Primatol 38(2), 105-121. https://doi.org/10.1007/s10764-017-9962-0

Minta M, Kibret K, Thorne P, Nigussie T, Nigatu L, 2018. Land use and land cover dynamics in Dendi-Jeldu hilly-mountainous areas in the central Ethiopian highlands. Geoderma 314, 27–36. https:// doi.org/10.1016/j.geoderma.2017.10.035

Muehlenbein MP, Ancrenaz M, Sakong R, Ambu L, Prall S, Fuller G, Raghanti MA, 2012. Ape conservation physiology: fecal glucocorticoid responses in wild *Pongo pygmaeus* morio fol- lowing human visitation. PLoS ONE 7(3), e33357. https://doi. org/10.1371/journal.pone.0033357

Muehlenbein MP, Bribiescas RG, 2005. Testosterone-mediated immune functions and male life histories. Am J Hum Biol 17(5), 527–558. https://doi.org/10.1002/ajhb.20419

Muller MN, 2017. Testosterone and reproductive effort in male primates. Horm Behav 91, 36–51. https://doi.org/10.1016/j.yhbeh. 2016.09.001

Mundry R, Fischer J, 1998. Use of statistical programs for nonpara- metric tests of small samples often leads to incorrect P values: examples from animal behaviour. Anim Behav 56(1), 256–259. https://doi.org/10.1006/anbe.1998.0756

Nguyen N, Fashing PJ, Boyd DA, Barry TS, Burke RJ, Goodale CB, Miller CM, 2015. Fitness impacts of tapeworm parasitism on wild gelada monkeys at Guassa, Ethiopia. Am J Primatol 77(5), 579–594. https://doi.org/10.1002/ajp.22379

Norscia I, Carrai V, Borgognini-Tarli SM, 2006. Influence of dry season and

food quality and quantity on behavior and feed- ing strategy of *Propithecus verreauxi* in Kirindy, Madagas- car. Int J Primatol 27(4), 1001–1022. https://doi.org/10.1007/ s10764-006-9056-x

Nunn C, Altizer S, Altizer SM, 2006. Infectious diseases in primates: behavior, ecology and evolution. Oxford University Press, Oxford. https:// doi.org/10.1093/ acprof:oso/9780198565 857.003.0005

Ohsawa H, 1979. The local Gelada population and environment of the Gich area. In: Kawai M (ed) Ecological and sociological studies of Gelada baboons (Contributions to Primatology, vol 16). Karger, Basel, pp 4–45

Ohsawa H, Dunbar RIM, 1984. Variations in the demographic struc- ture and dynamics of gelada baboon populations. Behav Ecol Sociobiol 15(3), 231–240. https://doi.org/10.1007/BF00292980

Osborn FV, Hill CM, 2005. Techniques to reduce crop loss: human and technical dimensions in Africa. Conserv Biol Ser 9:72

Priston NE, Wyper RM, Lee PC, 2012. Buton macaques (*Macaca ochreata brunnescens*): crops, conflict, and behavior on farms. Am J Primatol 74(1), 29–36. https://doi.org/10.1002/ajp.21003

R Core Team, 2018. R: A Language and Environment for Statisti- cal Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org

Riley EP, Tolbert B, Farida WR, 2013. Nutritional content explains the attractiveness of cacao to crop raiding Tonkean macaques. Curr Zool 59(2), 160–169. https://doi.org/10.1093/czoolo/59.2.160

Roberts ML, Buchanan KL, Evans MR, 2004. Testing the immuno- competence handicap hypothesis: a review of the evidence. Anim Behav 68(2), 227–239. https://doi.org/10.1016/j.anbehav.2004.05. 001

Rolland RM, 2000. A review of chemically-induced alterations in thyroid and vitamin A status from field studies of wildlife and fish. J Wildl Dis 36(4), 615–635. https://doi.org/10.7589/0090-3558- 36.4.615

Sak B, Petrzelkova KJ, Kvetonova D, Mynarova A, Shutt KA, Poma- jbikova K, Kvac M, 2013. Long-term monitoring of micro- sporidia, *Cryptosporidium* and *Giardia* infections in western Lowland Gorillas (*Gorilla gorilla gorilla*) at different stages of habituation in Dzanga Sangha Protected Areas, Central African Republic. PLoS ONE 8(8), 71840

Schneider-Crease I, Griffin RH, Gomery MA, Bergman TJ, Beehner JC (2017) High mortality associated with tapeworm parasitism in geladas (*Theropithecus gelada*) in the Simien Mountains National Park, Ethiopia.

Am J Primatol 79(9), e22684. https://doi.org/10. 1002/ajp.22684
Shutt K, Heistermann M, Kasim A, Todd A, Kalousova B, Profosouva I, Setchell JM, 2014. Effects of habituation, research and ecotour- ism on faecal glucocorticoid metabolites in wild western lowland gorillas: Implications for conservation management. Biol Conserv 172, 72–79. https://doi.org/10.1016/j.biocon. 2014.02.014
Siegel S, Castellan NJ Jr, 1988. Nonparametric statistics for the behavioral sciences, 2nd edn. Mcgraw-Hill Book Company, New York. https://doi.org/10.1177/ 0146621689013002 12
Sih A, Ferrari MC, Harris DJ, 2011. Evolution and behavioural responses to human-induced rapid environmental change. Evol Appl 4(2), 367–387. https://doi.org/10.1111/j.1752-4571.2010. 00166.x
Snyder-Mackler N, Beehner JC, Bergman TJ, 2012. Defining higher levels in the multilevel societies of geladas (*Theropithecus gelada*). Int J Primatol 33(5), 1054–1068. https://doi.org/10.1007/ s10764-012-9584-5
Southwick CH, Siddioi MF, Farooqui MY, Pal BC, 1976. Effects of artificial feeding on aggressive behaviour of rhesus monkeys in India. Anim Behav 24(1), 11–15. https://doi.org/10.1016/S0003- 3472(76)80093-0
Strum SC, 1994. Prospects for management of primate pests. Rev D'écol 49, 295–306
Thatcher HR, Downs CT, Koyama NF, 2019. Anthropogenic influ- ences on the time budgets of urban vervet monkeys. Landsc Urban Plan 181, 38–44. https://doi.org/10.1016/j.landurbplan.2018.09. 014
Toft JD, 1986. The pathoparasitology of nonhuman primates: a review. primates. Springer, New York, pp 571–679. https://doi.org/10. 1007/978-1-4612-4918-4_45
Wang YF, 2016. Center role of the oxytocin-secreting system in neuroendocrinimmune network revisited. J Clin Exp Neuroimmunol 1(102), 10–4172
Warren Y, 2009. Crop-raiding baboons (*Papio anubis*) and defensive farmers: a West African perspective. West Afr J Appl Ecol. https://doi.org/10.4314/wajae.v 14i1.44705
Weisman O, Zagoory-Sharon O, Feldman R, 2014. Oxytocin administration, salivary testosterone, and father–infant social behavior. Prog Neuropsychopharmacol Biol Psychiatry 49, 47–52. https:// doi.org/10.1016/j.pnpbp.2013.11.006
Wittig RM, Boesch C, 2003. "Decision-making" in conflicts of wild chim-

panzees (*Pan troglodytes*): an extension of the Relational Model. Behav Ecol Sociobiol 54(5), 491–504. https://doi.org/10. 1007/s00265-003-0654-8

Wrangham RW, 1974. Artificial feeding of chimpanzees and baboons in their natural habitat. Anim Behav 22(1), 83–93. https://doi.org/10.1016/S0003-3472(74) 80056-4

Yazezew D, Bekele A, Ibrahim H, 2020. Activity Budget and Feed- ing Ecology of Geladas (*Theropithecus gelada obscurus*) around Abogedam Church West of Debre Berhan Town, Ethiopia. Sci World J. https://doi.org/ 10.1155/2020/ 9829834

Zemene T, Shiferaw MB, 2018. Prevalence of intestinal parasitic infections in children under the age of 5 years attending the Debre Birhan referral hospital, North Shoa, Ethiopia. BMC Res Notes 11(1), 58. https:// doi.org/10.1186/s13104-018-3166-3

Zinner D, Atickem A, Beehner JC, Bekele A, Bergman TJ, Burke R, Roos C, 2018. Phylogeography, mitochondrial DNA diversity, and demographic history of geladas (*Theropithecus gelada*). PLoS ONE 13(8):e0202303. https://doi.org/ 10.1371/journal.pone.02023

# Chapter 3

## Comparison of FLOTAC, Mini-FLOTAC and classical parasitological techniques for detection of gastrointestinal parasites in neotropical non-human primates (NHP) in Brazil

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## 3.1 Abstract

**Background:** Endoparasites are quite commonly present in wild animals, particularly in non-human primates. Detection of gastrointestinal parasites in these animals has usually been performed using classical methods, but sometimes its low sensitivity is responsible for false-negative results. Therefore, the aim of this study was to compare the FLOTAC, Mini-FLOTAC and classical parasitological techniques for detection of gastrointestinal parasites in neotropical non-human primates in Brazil.

**Results:** Fecal samples (n = 43) were collected from neotropical non-human primates at the Wild Animal Screening Center in Pernambuco, Brazil. Of all samples analyzed, 76.7% (33/43) detected the presence of gastrointestinal parasites in at least one of the techniques used. In particular, 9.30% (4/43) were positive through direct examination; followed by 23% (10/43) at sedimentation; 34.88% (15/43) at flotation; 48.83% (21/43) at Mini-FLOTAC, and 65.11% (28/43) at FLOTAC.

**Conclusion:** In conclusion, FLOTAC and Mini-FLOTAC were the most efficient methods for diagnosing gastrointestinal parasites in neotropical non-human primates.

## 3.2 Introduction

Although *non-human primates* (NHPs) are found in many environments around the world, anthropic action has given rise to major threats to these species. These disturbances are affecting species diversity in natural environments (Sousa WP et al., 1984). Some degree of ecological change including agroindustry development, deforestation, livestock, mining and construction of dams and highways in these habitats (Estrada et al., 2017) may cause a change at the wildland-*urban interface* (WUI), such that transmission of or exposure to pathogens is favored among these animals (Hassell et al., 2016). Thus, NHPs can be affected by different species of parasites, some of them of medical concern such as *Toxoplasma gondii* (Molina et al., 2014), *Trypanosoma* spp. (Minuzzi-Souza et al., 2016) and *Leishmania* spp. (Karim et al., 2014). On the other hand, several intestinal parasites causing only asymptomatic or mild disorders can occur in NHPs (Karim et al., 2014; Kouassi et al., 2015; Li et al., 2015). These include *Cryptosporidium* spp*., Giardia* spp. (David et al., 2014), and a several of species of helminths (Alcantara et al., 2016; McLennan et al., 2017). Detection of gastrointestinal parasites in NHPs has usually been performed using classical methods such as flotation (Willis et al., 1921), centrifugal flotation (Faust et al., 1939), spontaneous sedimentation (Hoffman et al., 1934) or direct examination (Garcia et al., 2001). Despite low cost and easy execution, these techniques present low sensitivity, which can lead to false negative diagnoses (De Carli et al., 2011). A new technique known as FLOTAC has been developed and proposed for diagnosing gastrointestinal parasites in animals and humans (Cringoli et al., 2010). In several studies, it has been shown to have high sensitivity, specificity and accuracy when compared with other methods. On the other hand, the Mini-FLOTAC technique has been developed since 2013 as a novel direct method for diagnosing intestinal parasitic infections. Mini-FLOTAC attempts to address the challenge of using modern technology and matching high sensitivity with affordability (Cringoli et al., 2013). Accordingly, the present study aimed to assess the performance of the FLOTAC, Mini-FLOTAC and classical parasitological techniques for diagnosing gastrointestinal parasites in neotropical primates.

## 3.3 Methods

### *3.3.1 Ethics statement*

This study was conducted in accordance with the licenses granted by the Research *Ethics Committee* for*Animal use* of the *Federal Rural University of Pernambuco* (*CEUA*-UFRPE), Brazil (license number 18/2018), and by the *Biodiversity Authorization and Information System* (*SISBIO*) (license number 60975-1).

### *3.3.2 Animals and area of study*

Fecal samples (n = 43) were collected from two neotropical NHP species: 16 from *common marmosets* (*Callithrix jacchus*) and 27 from capuchin monkeys (*Sapajus libidinosus*). The animals were being kept at the Pernambuco *Wild Animal Screening Center* (CETAS-Tangará), which is located in the Guabiraba district of Recife, Pernambuco (7° 56' 49'' S and 34° 58' 48'' W). All the NHPs were kept in individual or paired pens, and were fed basically on leaves, fruits and extruded feed.

### *3.3.3 Samples and laboratory processing*

Fresh fecal samples from the NHPs were collected on clean paper that was placed under each pen for a period of 24 hours. This procedure was repeated for two days to form a pool of stool samples from each animal. Each of these pooled samples was collected into a plastic vials and maintained in isothermal boxes (4°C) until laboratory processing. Data about each animal were recorded in individual clinical charts. Samples were analyzed using five different techniques (i.e., direct examination using Lugol's iodine staining; Willis-Mollay flotation technique (Willis et al., 1921), spontaneous sedimentation of Hoffman, Pons, and Janer (Hoffman et al., 1934), FLOTAC (Cringoli et al., 2010), and Mini-FLOTAC (Cringoli et al., 2013) for detection of eggs, larvae, cysts and/or oocysts. The FLOTAC and Mini-FLOTAC techniques were performed using two flotation solutions (saturated sodium chloride 1.200 and zinc sulfate 1.350). The Willis-Mollay flotation technique was used as the *gold standard test.* All methods were performed in accordance with the instructions reported in the original description of each technique.

### 3.4 Data analysis

Data were analyzed through the chi-square or Fisher's exact test was used. The significance level was taken to be 5% (Zar et al., 1999). In addition, Kappa concordance analysis was used to compare the results. The kappa values were interpreted in accordance (Landis et al., 1977). The sensitivity, specificity, positive predictive value (+PV), negative predictive value (−PV), accuracy, true estimated prevalence, and incorrect classification were determined based on the Willis technique as the gold standard. The InStat software (Graphad et al 2018) was used to calculate all the parameters. The concordance calculations were performed through the website http://www.winepi.net/uk/index.

### 3.5 Results

Out of all samples analyzed 76.7% (33/43) scored positive for the presence immature forms of gastrointestinal parasites in at least one technique employed. In particular, 50% (8/16) and 77.77% (21/27) of *C. jacchus* and *S. libidinosus* were positive, respectively (p = 0.062). The infection rates observed through the different methodologies were 9.30% (4/43) using direct examination; 23.25% (10/43) using Hoffman's method; 34.88% (15/43) using the Willis technique; 48.83% (21/43) using by Mini-FLOTAC; and 65.11% (28/43) using FLOTAC (Table 1). All samples of *C. jacchus* were negative at direct examination, Hoffman's method and Willis-Mollay flotation technique.

*Ancylostoma* spp. and *Strongyloides* spp. were the species most frequently detected, regardless of the method used. *Oesophagostomum* spp. was detected in *S. libidinosus* by using the FLOTAC and Mini-FLOTAC technique, whereas *Platynosomum* spp. and oocysts of *Isospora* spp. were detected by the FLOTAC in *C. jacchus.*

Values of sensitivity, specificity, real prevalence, estimated prevalence, positive predictive value (+ PV), negative predictive value (- PV), accuracy and incorrect classification are shown in Table 3.2.

**Table 3.1:** Infections and co-infections by gastrointestinal parasites in Neotropical Primates in captivity

| Techniques | Parasites | Positivity (%; n/N) |
|---|---|---|
| **Direct Examinatiom** | *Ancylostoma* spp. | 75% (03/4) |
| | *Strongyloides* spp. | 75% (03/4) |
| | *Ancylostoma* spp. + *Strongyloides* spp. | 75% (03/4) |
| ***Hoffman*** | *Ancylostoma* spp. | 100% (10/10) |
| | *Strongyloides* spp. | 30% (3/10) |
| | *Ancylostoma* spp. + *Strongyloides* spp. | 30% (3/10 |
| **Willis** | *Ancylostoma* spp. | 100% (15/15) |
| | *Strongyloides* spp. | 46,66% (7/15) |
| | *Ancylostoma* spp. + *Strongyloides* spp. | 40% (6/15) |
| **Mini-FLOTAC** | Ascaridade | 4,46% (1/21) |
| | *Oesophagostomum* spp. | 19,04% (4/21) |
| | *Strongyloides* spp. | 57,14% (12/21) |
| | *Ancylostoma* spp. | 90,47% (19/21) |
| | *Ancylostoma* spp. + *Strongyloides* spp. | 47,6% (10/21) |
| | *Ancylostoma* spp. + *Strongyloides* spp. + *Oesophagostomum* spp. | 19,04% (4/21) |
| **FLOTAC** | *Isospora* spp. | 3,57% (1/28) |
| | *Platynossomun* spp. | 3,57% (1/28) |
| | *Toxocara* spp. | 3,57% (1/28) |
| | *Strongyloides* spp. | 3,57% (14/28) |
| | *Oesophagostomum* spp. | 7,14% (2/28) |
| | *Ancylostoma* spp. | 85,71% (24/28) |
| | *Ancylostoma* spp. + *Isospora* spp. | 3,5% (1/28) |
| | *Ancylostoma* spp. + *Strongyloides* spp. + *Oesophagostomum* spp. | 7,1% (2/28) |
| | *Ancylostoma* spp. + *Strongyloides* spp. | 42,8% (12/28) |

**Table 3.2:** Evaluation of the techniques used in relation to the Willis technique as a gold standard in the diagnosis of gastrointestinal parasites in Neotropical Primates in captivity.

| | Parameters (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Technique / Parasite | Sensitivity | True prevalence | Predictive value (+) | Predictive Value (-) | Accuracy | Incorrect classification | Kappa | P value |
| **Exame Direto** | | | | | | | | |
| *Ancylostoma* spp. | 20,0 | 100,0 | 100,0 | 70,0 | 72,1 | 27,9 | 0,246 | 0,036 |
| *Strongyloides* spp. | 16,7 | 94,6 | 33,3 | 87,5 | 83,7 | 16,3 | 0,142 | 0,370 |
| **Hoffman** | | | | | | | | |
| *Ancylostoma* spp. | 60,0 | 96,4 | 90,0 | 81,8 | 83,7 | 16,3 | 0,612 | 0,000 |
| *Strongyloides* spp. | 0,0 | 91,9 | 0,0 | 85,0 | 79,1 | 20,9 | 0,000 | 0,629 |
| **Mini-FLOTAC** | | | | | | | | |
| *Ancylostoma* spp. | 80,0 | 78,6 | 66,7 | 88,0 | 79,1 | 20,9 | 0,560 | 0,000 |
| *Strongyloides* spp. | 66,7 | 78,4 | 33,3 | 93,5 | 76,7 | 23,3 | 0,317 | 0,041 |
| *Ascaridade* | 0,0 | 97,7 | 0,0 | 100,0, | 97,7 | 2,3 | 0,000 | 0,760 |
| *Oesophagostomum* spp. | 0,0 | 83,7 | 0,0 | 100,0 | 83,7 | 16,3 | 0,000 | 0,000 |
| **FLOTAC** | | | | | | | | |
| *Ancylostoma* spp. | 100,0 | 60,7 | 57,7 | 100,0 | 74,4 | 25,6 | 0,519 | 0,000 |
| *Strongyloides* spp. | 100,0 | 64,9 | 31,6 | 100,0 | 69,8 | 30,2 | 0,340 | 0,004 |
| *Isospora* spp. | 0,0 | 97,7 | 0,0 | 100,0, | 97,7 | 2,3 | 0,000 | 0,760 |
| *Platynossoma* spp. | 0,0 | 97,7 | 0,0 | 100,0, | 97,7 | 2,3 | 0,000 | 0,752 |
| *Oesophagostomum* spp. | 0,0 | 97,7 | 0,0 | 100,0, | 97,7 | 2,3 | 0,000 | 0,042 |
| *Toxocara* spp. | 0,0 | 97,7 | 0,0 | 100,0, | 97,7 | 2,3 | 0,000 | 0,760 |

## 3.6 Discussion

This study assessed for the first time five different copromicroscopic techniques to diagnosing gastrointestinal parasites in NHP from *Brazil.* The overall frequency (76.7%) herein obtained was similar to observed in previous studies where a frequency of 74.20% was reported in *M. mulatta* (Adhikari et al., 2018). Conversely, it was lower than data (92.3%) reported for *Nasalis larvatus* (Klaus et al., 2017). The findings of this study demonstrated that NHPs are commonly affected by gastrointestinal parasites, but frequently without clinical manifestations (Karim et al., 2014; Kouassi et al., 2015; Li et al., 2015).

A wide diversity of nematodes have been retrieved in NHPs throughout the world (Li et al., 2015; Solorzano-Garcia et al., 2017). From an epidemiological perspective, data of the present study are important since some nematode species herein detected (e.g., *Ancylostoma* spp., *Strongyloides* spp. and *Ascaris*) present a zoonotic aspect. It has already been demonstrated that hookworms may be shared between humans and NHPs that cohabit (Hasegawa et al., 2014). Therefore, this finding is important particularly for zoo workers that are in close contact with NHPs, and consequently are more exposed to the risk through the contact with feces.

FLOTAC and Mini-FLOTAC techniques were the most efficient methods for diagnosing gastrointestinal parasites, particularly *Oesophagostomum* spp. eggs in *S. libidinosus*and *Platynosomum* spp. eggsand *Isospora* spp. oocysts in *C. jacchus*. It is important to note that factors such as age, genetic, parasite load and NHP species may influence the parasitological results (Pozo-Montuy et al., 2006; Souza-Dantas et al., 2007; Dias et al., 2015).

Recently, the Mini-FLOTAC technique has been recommended using the Mini-FLOTAC technique for detection of gastrointestinal parasites in the howler monkey, especially if many samples are analyzed (Alvarado-Villalobos et al., 2017). This technique has high sensitivity when compared with classical methods (e.g., EPG counts), thus enabling qualitative and quantitative analyses of parasite load without the need of specialized devices.

In conclusion, the FLOTAC and Mini-FLOTAC were the most efficient methods for diagnosing gastrointestinal parasites in *C. jacchus* and *S. libidinosus*; therefore it is recommended its use in surveys performed in NHPs since are techniques with high sensitivity and accuracy.

## 3.7 References

Adhikari P, Dhakal P, 2018. Prevalence of gastro-intestinal parasites of rhesus macaque (*Macaca mulatta* Zimmermann, 1780) and hanuman langur (*Semnopithecus entellus* Dufresne, 1797) in Devghat, Chitwan, Nepal. J Inst Sci Technol 22, 12-18. doi: 10.3126 / jist.v22i2.19590.

Alcântara DS, Mendonça IL, Fernandes neto VP, Carniel PG, Pessoa FB, 2016. Estudo coproparasitologico da espécie *Cebus libidinosus* (macaco-prego) Arq Bras Med Vet Zootec 68, 1609-1612. doi.org/10.1590/1678-4162-9102.

Alvarado-Villalobos MA, Cringoli G, Maurelli MP, 2017. Flotation techniques (FLOTAC and Mini-FLOTAC) for detecting gastrointestinal parasites in howler monkeys. *Parasite Vector* 10, 1-8. doi: 10.1186/ s13071-017-2532.

Cringoli G, Rinaldi L, Maurelli MP, Utzinger J, 2010. FLOTAC: new multivalente techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nat Protoc* 5, 503-515.doi: 10.1038 / nprot.2009.235

Cringoli G, Rinaldi L, Albonico M, Bergquist R, Utzinger J et al., 2013. Geospatial (s)tools: integration of advanced epidemiological sampling and novel diagnostics. *Geospatial Health* 7, 399-404.doi.org/10.4081/gh. 2013.97

David EB, Patti M, Coradi ST, Oliveira-Sequeira TCG, Ribolla PEM, Guimarães S, 2014. Molecular typing of Giardia duodenalis isolates from nonhuman primates housed in a Brazilian zoo. Rev Inst Med Trop *Sao Paulo* 56, 49-54.doi: 10.1590 / S0036-46652014000100007

De Carli GA, 2011. Diagnóstico laboratorial das parasitoses humanas, métodos e técnicas. Rio de Janeiro: Medsi, pp 455-459.

De Souza-Dantas LM, Bastos OPM, Brener B, 2007. Técnica de centrífugo-flutuação com sulfato de zinco no diagnóstico de helmintos gastrintestinais de gatos domésticos. Cienc Rural 37: 904-906. doi.org/10.1590/S0103-84782007000300051

Dias PAD, Rangel-Negrín A, 2015. Diets of howler monkeys. In: Kowalewski M, Garber P, Cortés-Ortiz L, Urbani B, Youlatos D, editors. Howler monkeys. Developments in primatology: progress and prospects. New York, NY: Springer. pp 21-56.

Estrada A, Garber PA, Rylands AB, Roos C, Fernandez-Duque E, Di Fiore A

et al., 2017. Impending extinction crisis of the world's primates: Why primates matter. *S*cience Advances, *Science Advances,* 3: 1:e1600946.doi: 10.1126/sciadv.1600946.

Faust EC, D'Antoni JS, Odom VA, 1938. Critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in feces. I. Preliminary communication. Am J Trop Med Hyg 1-18, 169-183.doi.org/10.4269/ajtmh.1938.s1-18.169

Garcia LS, 2001. Diagnostic medical parasitology, 4th ed. ASM press, Washington, DC, pp 723.

Graphpad Software, INC.http://www.winepi.net/uk/index. Accessed June 5, 2018.

Hasegawa H, Modrý D, Kitagawa M, Shutt KA, Todd A, Kalousová B et al., 2014. Humans and Great Apes Cohabiting the Forest Ecosystem in Central African Republic Harbour the Same Hookworms. *PLoS* Negl TropDis 8: 1-11.doi.org/10.1371/journal. pntd.0002715

Hassell JM, Bego M, Ward MJ, 2017. Urbanization and Disease Emergence: Dynamics at the Wildlife–Livestock–Human Interface. *Trends* Ecol Evol 32, 55-67. doi:10.1016 / j. tree.2016.09.012.

Hoffman WA, Pons JA, Janer JL, 1934. The sedimentation-concentration method in schistosomiasis mansoni. *J Public Health* 9, 281-98.

Karim MR, Zhang S, Jian F, Li J, Zhou C, Zhang, L et al., 2014. Multilocus typing of *Cryptosporidium* spp. and *Giardia duodenalis* from non-human primates in China. *Int J* Parasitol *Parasites* 44, 1039-1047. doi.org/10.1016/j.ijpara.2014.07.006

Klaus A, Zimmermann E, Roper KM, 2017. Co-infection patterns of intestinal parasites in arboreal primates (proboscis monkeys, *Nasalis larvatus*) in Borneo. *Int J* Parasitol *Parasites* Wildl 6:320–329.

Kouassi RYW, Mcgraw SW, Yao PK, Abou-Bacar A, Brunet J, Pesson B et al., 2015. Diversity and prevalence of gastrointestinal parasites in seven non-human primates of the Taï National Park, Côte d'Ivoire. Parasite 22, 1- 12.doi.org/10.1051/parasite/ 201500

Landis JR, Koch GG, 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159-174.

Li J, Qi M, Chang Y, Wang R, Li T, Dong H, Zhang L, 2015. Molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in captive wildlife at Zhengzhou Zoo, China. J Eukaryot Microbiol 62, 833 – 839.doi.org/10.1111/jeu.12269

## References


Malta MC, 2010. Naturally acquired visceral leishmaniasis in non-human primates in Brazil. Vet Parasitol 7, 169, 193. doi.org/10.1016/j.vetpar. 2009.12.016.

Mclennan MR, Hasegawa H, Bardi M, 2017. Gastrointestinal parasite infections and selfmedication in wild chimpanzees surviving in degraded forest fragments within na agricultural landscape mosaic in Uganda. PLoS ONE, 12, 1-29. doi.org/10.1371/ journal.pone.0180431.

Minuzzi-Souza TTC, Nitz N, Knox MB, 2016. Vector-borne transmission of *Trypanosoma cruzi* among captiveNeotropical primates in a Brazilian zoo. Parasites & Vectors 9, 1-6.doi.org/10.1016/j.vetpar.2009.12.016.

Molina CV, Catão-Dias, JL, Ferreira JS, Vasconcellos AS, Gennari SM, Valle RR et al., 2014. Sero-*epidemiological survey* for *brucellosis*, *leptospirosis*, and *toxoplasmosis* in free-ranging *Alouatta caraya* and *Callithrix penicillata* from *São Paulo State*, *Brazil*. *J Med* Primatol 43:197-201.doi.org/10.1111/jmp.12112.

Pozo-Montuy G, Serio-Silva JC, 2006. Comportamiento alimentario de monos aulladores negros (*Alouatta pigraLawrence*, Cebidae) en hábitat fragmentado en Balancán, Tabasco, México. *Acta* Zool Mex 22, 53-66.

Solorzano-García B, Pérez-POnce de Leon G, 2017. Helminth parasites of howler and spider monkeys in Mexico: Insights into molecular diagnostic methods and their importance for zoonotic diseases and host conservation. Int J Parasitol Parasites Wildl 6:76-84.doi.org/10.1016/j.ijppaw.2017. 04.001

Sousa WP, 1984. The role of disturbance in natural communities. Annu Rev Ecol Evol Syst, 15, 353-391.

Willis HH, 1921. A simple levitation method for the detection of hookworm ova. Med J Aust 2, 375-376.

Zar JH, 1999. Biostatistical analysis. 4ª ed. New Jersey, Prentice-Hall, Inc. p 663.

# Chapter 4

## Single and synergistic effects of fenbendazole and metronidazole against subclinical infection by *Giardia duodenalis* in non-human primates in a zoological garden in southern Italy

**Capasso M,** Ciuca L, Guadano Procesi I, Zinno F, Berrilli F, Cringoli G, Rinaldi L., 2022. Single and synergistic effects of fenbendazole and metronidazole against subclinical infection by *Giardia duodenalis* in non-human primates in a zoological garden in southern Italy. Frontiers in Veterinary Science, section Parasitology, in press.

## 4.1 Abstract

The aim of this study was to assess the single and synergistic effects of fenbendazole (Fenb) and metronidazole (Metro) for the treatment of *Giardia duodenalis* infection in different species of non human primates (NHP) housed in a zoological garden of southern Italy. Moreover, the study also aimed to better define the circulation of *G. duodenalis* zoonotic assemblages in NHP and the potential occurrence of zoonotic transmission between the staff from the zoo and NHP. Briefly, six species belonging to four families (Lemuridae, Cercopithecidae, Atelidae, Hylobatidae) of NHP and housed in six cages (CG) were identified as *Giardia*-positive, and divided in two groups. Group F (N=16 animals) was treated with Fenb (50 mg/kg, every 24 hours for 5 consecutive days) and Group M (N=7 animals) was treated with Metro (25 mg/kg, twice a day for 5 consecutive days). After the first round of therapy, all the animals were retreated for five days by inverting the drugs in each group. At each sampling day (SDs -3-24) the samples were tested for the presence of *Giardia* cysts using the FLOTAC technique. Multiple faecal tests for the antigen-detection of *Giardia* such as rapid ELISA and direct immunofluorescence (IFA) were performed at each sampling point only on samples that resulted positive for *Giardia* cysts with FLOTAC. The efficacy of Fenb ranged from 30 to 67% and for Metro ranged between 82-96%. The results showed the synergistic effects of Metro and Fenb (98-100%) over the combination of fenbendazole and metronidazole (52-90%) against the infection by *Giardia* in NHPs. The overall k agreement between FLOTAC and IFA reached 0.858 (P=0.0001). In contrast, all the samples had a negative antigen result when using ELISA. At molecular analysis, six samples were confirmed positive for *Giardia* by nested PCR. Only two positive samples were successful sequenced showing 100% of identity with Assemblage B. All the samples from the humans included in the study resulted negative for *Giardia* cysts. Overall, the study emphasizes the need for regular monitoring of *Giardia* infections in NHP housed in zoos by traditional diagnostic tools combined with molecular characterization of the parasite.

## 4.2 Introduction

*Giardia duodenalis* (syn. *Giardia lamblia, Giardia intestinalis*) is a ubiquitous enteric flagellated protozoan of global importance that infects a wide range of hosts, i.e. >40 animal species, including humans. It is a common leading cause of infection known as giardiosis (or giardiasis) and infects up to ~28.2 million people worldwide, with 500,000 new cases every year (Ryan et al., 2019). *Giardia duodenalis* has been frequently identified as pathogen in non-human primates (NHP) (Levecke et al., 2009; Koster et al., 2022). The prevalence of *G. duodenalis* in NHP kept in zoos in different European countries ranged between 6% and 70% in studies conducted in Belgium (Leveke et al., 2007), Croatia (Beck et al., 2011), Poland (Maesano et al., 2014), Slovakia (Mravcoca et al., 2021), Spain and Brazil (Martinez-Diaz et al., 2011). A recent study conducted in six European zoological gardens (located in France, Germany, and Spain) reported a 18.1% prevalence of *G. duodenalis* with the presence of both Assemblages A and B (Koster et al., 2022). In Italy, the presence of *G. duodenalis* has been reported in NHP kept in the Bioparco in Rome with a prevalence of 47.0% in *Lemur catta* (Berrilli et al., 2011), and recently also in NHP kept in four zoos in central and southern Italy with an overall prevalence of 3.3% (Capasso et al., 2019) whereas the parasite was not reported in captive cynomolgus macaques (*Macaca fascicularis*) imported from registered breeding facilities in China (Zanzani et al., 2015). The Assemblages of *G. duodenalis* found in the NHP in Italy are mainly A and B, with the dominant Assemblage B (Cacciò et al., 2008; Berrilli et al., 2011). Giardiosis in NHP causes diarrhea and slow growth, especially in juvenile animals (Karim et al., 2015). However, few studies have demonstrated an association between the presence of *G. duodenalis* infection and the occurrence of clinical manifestations, strongly suggesting that the pathogenic role of *G. duodenalis* in captive NHP is limited (Koster et al., 2022). Nevertheless, NHP play an important role as reservoirs of zoonotic *Giardia* infections in the zoological gardens (Kowalewski et al., 2011; Einarsson et al., 2016; Koster et al., 2022). The most common therapy used against giardiosis in veterinary medicine is based on metronidazole and fenbendazole (Plumb et al., 2002). Metronidazole, including tinidazole, is used as single dose in the treatment of giardiosis in humans with high rates of healing (about 90%) and low com-

plications (Phung et al., 2005; Petri et al., 2005). Additionally, tinidazole has been used in the treatment of giardiosis in Barbary macaque (Kramer et al., 2009). Though *Giardia* is a common parasite of zoonotic relevance in NHP, a limited number of field studies have been conducted on the efficacy of antiparasitic treatments in NHP. Both fenbendazole and metronidazole are recommended as therapy for protozoa infections in NHP (Parrott et al., 2020; Ortega-Pierres et al., 2020). The aim of this study was to evaluate the single and synergistic effects of fenbendazole and metronidazole for the treatment of *G. duodenalis* subclinical infection in different species of NHP housed in a zoological garden in southern Italy. Moreover, the study also aimed to better define the circulation of *G. duodenalis* zoonotic assemblages in NHP and the potential occurrence of zoonotic transmission between the staff (zookeepers, veterinarians) from the zoo and NHP.

## 4.3 Materials and methods

### *4.3.1 Animals and housing*

The study was conducted in a zoological garden located in the Benevento province of southern Italy (Pesco Sannita, 41°13'57"N; 14°48'40"E). Husbandry in this zoo is based on the EAZA Best Practice Guidelines for each species or similar, providing the best possible care with good levels of welfare and with sanitary safety for animals, staff and visitors. Employees and visiting staff working with NHP wore personal protective equipment when in contact with the animals or their faecal material (Capasso et al., 2019).

This study was performed under the annual preventive medicine plan of the facility recognised by the National/International Regulations for the EU Zoo Directive (DL73/2005 and 92/65 CEE). The Zoo includes a variety of 115 species, housing over 500 animals and is in continuous expansion. Currently, there are 42 NHP belonging to 11 species of the following families: Lemuridae, Cercopithecidae, Atelidae, Hylobatidae, and Cebidae. Animals' food consists in specific commercial food combined with daily fresh fruit, vegetables, seeds, eggs and/or mealworms. Fresh water is provided daily *ad libitum* in polycarbonate water bottles. The cages are cleaned three times a week and disinfected every two weeks.

*4.3.2 Parasitological screening-NHP and humans (staff from* Delle Maitine *Zoo)*
All the non-human primates from the zoo were firstly screened for identification of *G. duodenalis*. Faecal samples (pools) were collected from 11 cages (N= 42 subjects of NHP belonging to 11 species) and subjected to copromicroscopic analysis using the FLOTAC technique as detailed below (Cringoli et al., 2010; Pepe et al., 2019). Six cages (CG) out of 11 (N=23 animals) resulted positive for *Giardia* cysts (mean CPG of *Giardia* in each cage: 300 CPG in CG1; 1200 CPG in CG2; 240 CPG in CG3; 2280 CPG in CG4; 950 CPG in CG5; 273 CPG in CPG6). One of the cages was positive also for *Blastocystis* sp.. All the NHP that resulted positive for *Giardia* cysts were included in the treatment groups. In addition, all the zookeepers (N=4) and veterinarians (N=2) from the zoo were tested for detection of *Giardia* cysts using the same technique used for NHP (Pepe et al., 2019). All the human stool samples resulted negative for *Giardia* cysts.

*4.3.3 Laboratory analysis*
Faecal samples analyzed at each sampling point were represented by pools (5-10 g) collected from the inner core of the cages. Analyses were performed within 24 h of sampling. At each sampling day the faecal samples were tested for the presence of *Giardia* cysts using the FLOTAC technique based on zinc sulfate (specific gravity = 1.350) flotation solution with a detection limit of 1 cyst per gram (CPG) of feces (Cringoli et al., 2010; Pepe et al., 2019). Moreover, multiple faecal tests for the antigen-detection of *Giardia* such as rapid enzyme immunoassay (Remel Xpect *Giardia/Cryptosporidium*, Thermo Fisher) and direct immunofluorescence (MeriFluor *Giardia/Cryptosporidium*, Biosciense) were performed at each point of the study only on samples that resulted positive for *Giardia* cysts with FLOTAC (Cringoli et al., 2010).

*4.3.4 Molecular analysis and sequencing*
Genomic DNA was extracted from six faecal samples of NHP (pools from each cage) using the QIAamp DNA Stool Mini Kit (Qiagen, Italy) following manufacturer's instructions. To identify *G. duodenalis* the TPI fragment was amplified by nested PCR using the protocol described by Sulaiman et al. (2003) (Sulaiman et al., 2003). Briefly, for the primary PCR, a PCR product of 605 bp was amplified by using primers AL3543 [5′-AAATIATGCCT-

GCTCGTCG-3′] and AL3546 [5′-CAAACCTTITCCGCAAACC-3′]. For the secondary PCR, a fragment of 530 bp was amplified by using 2.5 µL of primary PCR reaction and primers AL3544 [5′-CCCTTCATCGGIGGTAACTT-3′] and AL3545 [5′-GTGGCCACCACICCCGTGCC-3′]. Molecular analyses were carried out by amplifying a 18S rRNA fragment for *Blastocystis* sp. The specific primer BhRDr (GAGCTTTT-TAACTGCAACAACG) and the broad-specificity eukaryote-specific primer RD5 (ATCTGGTT-GATCCT-GCCAGT) were used in a standard PCR reaction with Taq DNA polymerase (BIOTAQ, Bioline, UK) using the protocol described by Stephanie et al. (2006) (Scicluna et al., 2006).
PCR products were analyzed by agarose gel electrophoresis and visualized after ethidium bromide staining. Subsequently, all the secondary PCR products were sent for sequencing to the Bio-Fab Research, Rome, Italy. Sequences for each amplified region were compared to those previously published in GenBank database. Identities at assemblage/subtype level were verified using the Basic Local Alignment Search Tool (BLAST). Sequences were submitted to GenBank under Accession Number ON246260-ON246261 for *G. duodenalis* TPI locus and ON215732 for *Blastocystis* 18S rRNA fragment.

*4.3.5 Study design and Treatment-groups*

The study design is summarised in Table 1. Six species belonging to four families (Lemuridae, Cercopithecidae, Atelidae, Hylobatidae) of NHP (N=23 animals) and housed in six cages (CG) were identified as *Giardia*-positive, and divided in two groups: the Group F treated with fenbendazole, (Panacur®, 2.5%, Intervet Italia Srl; 50 mg/kg, orally, every 24 hours for 5 consecutive days) and the Group M treated with metronidazole (ERADIA®, Virbac Italia Srl; 25 mg/kg, orally, twice a day for 5 consecutive days). After five days from the first round of therapy, all the animals were retreated for five days by inverting the drugs in each group, as follows: metronidazole (25 mg/kg, orally, every 24 hours for 5 consecutive days) in the Group F and fenbendazole (50 mg/kg, orally, twice a day for 5 consecutive days) in the Group M. The treatment groups were allocated based on the distance and position of the cages in the zoo where the NHP were housed according to the species. Specifically, the Group F included three cages housing the following species of NHP each: 12 males of *Lemur catta* (ring-tailed lemur) in CG1; one male and one female of *Cercopithecus mona* (mona monkey) in CG2 and two fe-

males of *Alouatta caraya* (black howler) in CG3. Group M included three CG with the following species of NHP: one male and one female of *Nomascus concolor* (black crested gibbon) in CG4; one male and one female of *Colobus Guereza* (mantled guereza) in CG5; and two females and one male of *Semnopithecus entellus* (gray langur) in CG6. For ethical reasons, no untreated control-group of animals was available. All the animals were monitored for the presence of *Giardia* cysts and *Giardia*-antigen for 24 study days (SD) as follows: before treatment (SD3-SD1), during the first treatment (T1) (SD2-SD6); during post-treatment-T1 (SD7-SD13); during the second treatment (T2) (SD14-SD18) and post-treatments (SD19-SD24). All the animals included in the study were healthy animals as determined by a physical examination performed before (3 to 1 days) the beginning of the study. Moreover, all the animals received physical examinations by a veterinarian during the treatments and on the last day of the trial.

*4.3.6 Drug-administration*

The medicated meal was obtained using specific "meatballs" consisting of a moistened mixture of primate-pellets, honey, yogurt or chopped fruit, which easily allowed the administration of the drugs to the animals. Noteworthy, this type of drug administration allowed the veterinarians to apply the correct amount of the drug for each animal (individually) according to their weight.

*4.3.7 Treatment efficacy*

Treatment efficacy was evaluated based on *Giardia* cyst per gram of faeces (CPG) on SDs 7-12 and SDs 19-24 for both groups (33).

$$\%\ Efficacy = \frac{Mean\ CPG\ SD\ Pre\text{-}T - Mean\ CPG\ SD\ Post\text{-}T}{Mean\ CPG\ SD\ Pre\text{-}T} \times 100$$

CPG = cysts per gram faeces

*4.3.8 Statistical Analysis*

Statistical analysis was performed using Windows SPSS® (version 17.0). The non-parametric Mann-Whitney U test was used to determine the level of significant difference between groups of treatment (F, M). Moreover, Kappa (k) statistic was employed to determine the strength of agreement between FLOTAC/IFA and FLOTAC/rapid ELISA, using the following criteria: ≤0.2 = poor; 0.21-0.40 = fair; 0.41-0.60 = moderate, 0.61-0.80 = good and ³0.80 = very good (34). The level of significance was set at a p-value of 0.05.

## 4.4 Results

The values of *Giardia* CPG in both groups (F and M) and for each SD are shown in Tables 2 and 3. Tables 4 and 5 shows data of mean *Giardia* CPG and efficacies (%) of fenbendazole and metronidazole treatments calculated at different post-treatment days. Briefly, in the Group F, the results of the parasitological analyses on the SD1 (pre-treatment) revealed a mean value of 2160 CPG of *Giardia* and on SD 13, after the first treatment, the mean value was 1257 CPG. In the Group M, on SD1, the mean value of *Giardia* CPG was 2427 and after the first treatment with metronidazole, on SD 13, the mean value was reduced to 227 CPG. However, after the second treatment in both groups, by using metronidazole in the Group F and fenbendazole in the Group B, the mean of CPG decreased significantly, in particular in the Group M (Tables 4, 5). Moreover, the results of mean CPG of *Giardia* for each cage after six days of treatment with each single drug used in the study was the following: post-treatment with fenbendazole/metronidazole in the Group F (n=261/6 mean CPG in the CG1, n=752/0 mean CPG in the CG2, n= 5200/945 mean CPG in the CG3); post-treatment with metronidazole/fenbendazole in the Group M (n=368/1 mean CPG in the CG4, n=162/15 mean CPG in the CG5, n=334/11 mean CPG in the CG6). The efficacy of fenbendazole ranged from 30 to 67% on SDs 7-12 and 52-90% on SDs 19-24 after the second treatment with metronidazole. In the Group M, the efficacy of metronidazole range between 82-96% on SDs 7-12 and 98-100% on SDs 19-24 after the second treatment with fenbendazole. Overall, the synergistic effects of fenbendazole and metronidazole against the *Giardia* infection, in

the Group M showed a statistically significant difference (P = 0.001) compared to the Group F. All the subjects included in both groups F and M continued to eliminate *Giardia* cysts after the first treatment with either fenbendazole or metronidazole. Instead, all the subjects from the cages CG1 and CG3 remained *Giardia*-negative and the only subjects from CG2 remained *Giardia*-positive after the second treatment with metronidazole in the Group F. Furthermore, all the subjects from Group M remained *Giardia*-negative after the second treatment with fenbendazole, at the end of the study (SD 24). In addition, the subjects from CG2 and CG6 (*Cercopithecus mona* and *Semnopithecus entellus*) presented co-infections with other parasites such as *Trichuris* sp. and *Blastocystis* sp.
Moreover, all the faecal samples from each sampling point resulted negative at the antigen-detection of *Giardia* using the rapid enzyme immunoassay comparing with direct immunofluorescence which resulted all positive. The overall k agreement between FLOTAC and IFA was 0.858 (P=0.0001). Analyses were not carried out comparing FLOTAC with the rapid ELISA, given that no sample resulted positive with the ELISA test.
All six samples were confirmed positive for *Giardia* by nested PCR. Only two positive samples (CG1, CG2) were successful sequenced showing 100% of identity (498/498; 100% query coverage) with Assemblage B (Accession number: MF095053). *Cercopithecus mona* from CG2 also resulted positive for *Blastocystis* sp.; the sample was successfully sequenced showing 100% of identity (579/579; 100% query coverage) with Subtype ST1 (Accession number: MN338073).

## 4.5 Discussion

Little is known about the occurrence and genetic diversity of *Giardia duodenalis* in non-human primates (NHP); however, giardiosis has been described in the following species of NHP: squirrel monkeys, rhesus macaques, lemuridae and marmosets (Potkay et al., 1992; Hamlen et al., 1994; Kramer et al., 2009; Berrilli et al., 2011).
Though *Giardia* is a common parasite in zoo-housed primates, few studies have evaluated the treatment options for giardiosis in NHP. One study reported the efficacy of tinidazole in Barbary macaque (Kramer et al.,

2009) and another study assessed whether oral administration of metronidazole dissolved in drinking water would be successful to eliminate *Giardia* cysts in rhesus macaques (Labberton et al., 2013). Our field study evaluated the efficacy of metronidazole and fenbendazole and assessed the synergistic effect of the two drugs against *G. duodenalis* infection in different species of NHP housed in a zoological garden in southern Italy. This study revealed a high and persistent detection of *Giardia* cysts in various species of NHP including, *Lemur catta, Cercopithecus mona, Alouatta caraya, Nomascus concolor, Colobus guereza* and *Semnopithecus entellus*. These results obtained are consistent with other similar studies (Leveke et al., 2007; Beck et al., 2011; Berrili et al., 2011; Martinez-Diaz et al., 2011). The occurrence of *Giardia* was reported in NPH species that were not well-documented until now, such as mona monkeys, black howler, black crested gibbon, mantled guereza and gray langur.

The results on the efficacy of fenbendazole *vs* metronidazole and their synergistic effect on *Giardia* infection underlined that almost all the animals became negative when combining fenbendazole at 50 mg/kg sid for five days with metronidazole at 25 mg/kg bid for five days. The only group of animals that remained positive for *Giardia* cysts at the end of the study was the one belonging to the Cercopithecidae family (the pair of one female and male of mona monkeys). In addition, they had the highest infection rate due to the variation in cyst shedding per days. Our data further indicate that pooling serial fecal samples from multiple colony animals likely would identify the presence of *Giardia* in a colony.

In the present study, *Giardia* infection in NHPs was not associated with clinical signs of diarrhea. These findings contrast with several studies in humans, in which 60% to 70% of infected persons show signs of diarrhea (Hashan et al., 2020). Instead, the condition observed in the NHPs seems to be similar to the chronic asymptomatic carrier state that occurs companion animals (Chon et al., 2005; Ciuca et al., 2021). This condition of subclinical *Giardia* infection indicates that NHPs may represent important reservoirs and serve as a source of zoonotic infection for other animals.

This study provided an unexpected finding concerning the diagnostic tools for antigen-detection of *Giardia*. In fact, all the samples resulted positive with FLOTAC were confirmed with the gold standard IFA, assessing a very high agreement between the two techniques (Pepe et al.,

2019). However, the present study yielded discordant results when using the rapid test (Remel *Giardia/Cryptosporidium*) on *Giardia* positive samples. In fact, all the faecal samples from each point of the study that were positive at FLOTAC and IFA, had a negative antigen result with the rapid test used. These findings are not in agreement with several studies that reported the rapid enzyme immunoassays as a precise tool for detecting *Giardia* in fecal specimens with test sensitivities and specificities that have approached 100% (Maraha et al., 2000; Weitzel et al., 2006). However, future work should include investigations regarding the combined factors that may result in *Giardia*-antigenic stimulation of the intestinal tract in NHP.

The molecular analysis revealed Assemblages A and B in all samples tested positive for *Giardia*-DNA. *Giardia* zoonotic Assemblages A and B have been already described in NHP in many studies, including in some species of NHPs from a zoological garden in Rome, Italy (Thomoson et al., 2000; Vitazkova et al., 2006; Berrilli et al., 2011). The potential zoonotic *G. duodenalis* Assemblage B was identified in two groups of NHP (*Lemur catta* and *Cercopithecus mona*). These findings suggest that ring-tailed lemurs, as already has been reported, and mona-monkeys may be asymptomatic carriers of *G. duodenalis* and a higher parasitic load might occur in these species of NHP held in walk-through enclosures (Fomsgaard et al., 2020). Moreover, all the animals (*Cercophitecus mona*) from CG2 were the only animals that remained positive for *Giardia*, until the end of the study, after two treatments of five days each with fenbendazole and metronidazole. We cannot rule-out reinfection, due to the prepatent period of *Giardia*, that can be as short as five days, but on the other side, the host susceptibility of the NHPs for *Giardia* infection can be as well incriminated for the persistence of the *Giardia* cysts. However, the increase of the number of *Giardia* cysts shed by the two animals from CG2, after the treatment, for e.g Day 9 (N=10000 CPG) and Day 10 (N=12000 CPG) has occured in other studies as well (Montoya et al., 2008; Bowmann et al., 2009). It is important to note that the shedding of the mean CPG of *Giardia* from CG2 in the Group F, resulted in a high value (N=5200 CPG) post-treatment with fenbendazole comparing with a low value (N=945 CPG) post-treatment with metronidazole in the same group.

In addition to the presence of *G. duodenalis*, the other parasites identified in this study were *Trichuris* sp. and *Blastocystis* sp. that could cause serious gastrointestinal enteritis in NHPs (Hamlen et al., 1994; Kalishman et al., 1996; Koster et al., 2022).

To our knowledge this is the first study that showed the efficacy of fenbendazole that ranged from 30 to 67% on SDs 7-12 and the efficacy of metronidazole that ranged between 82-96% on SDs 7-12 against the subclinical infection by *Giardia* in NHPs. Moreover, the final output revealed the synergistic effects of metronidazole and fenbendazole (98-100%) over the combination of fenbendazole and metronidazole (52-90%) against the infection by *Giardia* in NHPs. Also, tinidazole can be a good therapeutic strategy for giardiosis in NHPs, as it has been already demonstrated in marmosets (Kramer et al., 2009), however future work should include the evaluation of the efficacy of this drug in other species of NHP. Indeed, the evaluation of a health profile markers of pre-and post-treated animals, would have underlined the safety of the drugs used in this study.

The results of this study showed that *G. duodenalis* is a common parasite in NHP in southern Italy. The findings of this study enriched the list of host species susceptibility of NHPs to *G. duodenalis* from Italy, by adding other species of NHPs such as *Cercopithecus mona, Nomascus concolor, Colobus guereza, Semnopithecus entellus, Alouatta caraya*, that were either not studied or tested negative for *Giardia* cysts in previous studies. Furthermore, the substantial variation in the number of *Giardia* cysts per gram shed in various days of the study could be explained by the differences in susceptibility in the host species of NHPs or by the continuous exposure with cysts from the environment. Indeed, a more effective sampling strategy, would have improved the overall output of the study regarding the epidemiological data obtained, for example collecting individual faecal samples and not pooled samples from the cages as performed in the present study.

For *Blastocystis* sp. detection, as reported by Koster et al. (2022) ST1 in non-human primates in zoological gardens was uniquely documented from Spain, despite being ST1 the most prevalent genotype worldwide. This trend may be justified by the lack of molecular data regarding *Blastocystis* sp. isolated from zoological institutes (Koster et al., 2022).

**Table 1.** Study design

| | |
|---|---|
| **Treatment-Groups** | **Group F (Fenbendazole):** 50mg/kg fenbendazole, for 5 consecutive days+metronidazole 25mg/kg, twice a day for 5 consecutive days<br><br>**Group M (Metronidazole):** 25mg/kg metronidazole, twice a day for 5 consecutive days+50mg/kg fenbendazole, for 5 consecutive |
| **No. animals per Group** | Group F:16<br><br>Group M:7 |
| **Cages (CG) per Group** | Group F: CG1; CG2; CG3<br><br>Group M: CG4; CG5; CG6 |
| **Study Days (SD) and activities** | SD-3-SD1(screening for Giardia cysts; physical examinations; selection treatment-groups)<br><br>SD2-SD6 (treatments (T1) fenbendazole/metronidazole - Groups F/M)<br><br>SD14-SD18 (treatment (T2) metronidazole/fenbendazole- Groups F/M) |
| **Laboratory analysis** | **SD-3-SD24**<br>Flotation test (FLOTAC technique (Cringoli et al., 2010)<br><br>Rapid ELISA -Remel Xpect, *Giardia/Cryptosporidium* (Thermo Fisher)<br><br>Direct immunofluorescent assay (IFA) -Merifluor *Giardia/Cryptosporidium* (Bioscense) |

**Table 2.** Results of *Giardia* CPG for all the animals in each cage (CG1, CG2, CG3) during the entire study (pre-treatment and post-treatments-T1 and T2) for Group F.

| Group F | T I (Fenbendazole) | | | | | | | | | | | | T II (Fenbendazole) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CPG-DAYS | | | | | | | | | | | | | | | | | | | | | | | |
| | Pre-T | **T0** | Post-T | | | | | | | | | | Pre-T | **T0** | Post-T | | | | | | | | | |
| | 1 | **2** | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | **14** | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| **CG1** | 3600 | **0** | 10 | 0 | 10 | 240 | 416 | 240 | 0 | 72 | 0 | 840 | 600 | **0** | 180 | 72 | 0 | 0 | 0 | 25 | 0 | 12 | 0 | 0 |
| **CG2** | 2400 | **960** | 1200 | 2880 | 9600 | 3800 | 1800 | 4800 | 10000 | 12000 | 1400 | 1400 | 2800 | **1568** | 680 | 2000 | 480 | 890 | 1800 | 460 | 890 | 360 | 600 | 1560 |
| **CG3** | 480 | **120** | NE | 120 | 240 | 180 | 1200 | 760 | 530 | 0 | 924 | 1100 | 370 | **0** | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

T0- first day of treatment; NE-Not examined; Giardia CPG- the total number of cysts per gram eliminated by all the animals from each cage.

**Table 3.** Results of *Giardia* CPG for all the animals in each cage (CG4, CG5, CG6) during the entire study (pre-treatment and post-treatments-T1 and T2) for Group M.

| Group M | T I (Fenbendazole) | | | | | | | | | T II (Fenbendazole) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CPG-DAYS | | | | | | | | | | | | | | | | | | | | | | | |
| | Pre-T | **T0** | Post-T | | | | | | | | | | Pre-T | **T0** | Post-T | | | | | | | | | |
| | 1 | **2** | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | **14** | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| **CG4** | 4560 | **1600** | 480 | 120 | 60 | 10 | 270 | 96 | 720 | 84 | 498 | 538 | 300 | **418** | 294 | 174 | 70 | 0 | 0 | 8 | 0 | 0 | 0 | 0 |
| **CG5** | 1900 | **1200** | 600 | 240 | 0 | 0 | 4 | 30 | 0 | 360 | 250 | 330 | 250 | **340** | 366 | 228 | 132 | 54 | 32 | 50 | 6 | 0 | 0 | 0 |
| **CG6** | 820 | **400** | 220 | 72 | 140 | 560 | 840 | 120 | 240 | 0 | 376 | 426 | 130 | **290** | 412 | 314 | 98 | 20 | 0 | 18 | 40 | 10 | 0 | 0 |

T0- first day of treatment; Giardia CPG- the total number of cysts per gram eliminated by all the animals from each cage.

**Table 4**. Results of mean *Giardia* CPG and efficacy (%) of the treatments performed in Group F, on SD 7-12 and SD 19-20

| **Group F** | | |
|---|---|---|
| **Pre-T (mean CPG=2160)** | | |
| **Study Days post-T (T1) Fenbendazole** | Mean CPG | **% Efficacy** |
| **7** | 1500 | 30.6 |
| **8** | 1933 | 11.7 |
| **9** | 3510 | 0 |
| **10** | 4024 | 0 |
| **11** | 708 | 67.2 |
| **12** | 1113 | 48.5 |
| **Pre-T (mean CPG=1257)** | | |
| **Study Days post-T (T2) Metronidazole** | Mean CPG | **% Efficacy** |
| **19** | 600 | 52.2 |
| **20** | 162 | 87.1 |
| **21** | 297 | 76.4 |
| **22** | 124 | 90.1 |
| **23** | 200 | 84.1 |
| **24** | 520 | 58.6 |

**Table 5**. Results of mean Giardia CPG and efficacy (%) of the treatments performed in Group M, on SD 7-12 and SD 19-24

| **Group M** | | |
|---|---|---|
| **Pre-T (mean CPG=2427)** | | |
| **Study Days post-T (T1) Metronidazole** | Mean CPG | **% Efficacy** |
| **7** | 371 | 84.7 |
| **8** | 82 | 96.6 |
| **9** | 320 | 86.8 |
| **10** | 148 | 93.9 |
| **11** | 374 | 84.6 |
| **12** | 431 | 82.2 |
| **Pre-T (mean CPG=227)** | | |
| **Study Days post-T (T2) Fenbendazole** | Mean CPG | **% Efficacy** |
| **19** | 11 | 95.1 |
| **20** | 25 | 89 |
| **21** | 15 | 93.4 |
| **22** | 0 | 98.7 |
| **23** | 0 | 100 |
| **24** | 0 | 100 |

Zookeepers and veterinarian staff resulted negative to the parasitological exams. However, the monitoring of a potential zoonotic transmission must continue to be a priority, possibly with a temporal continuity in sampling and through an increased number of recovered samples. This would enable to assess any potential transmission risk between staff, visitors and NHPs.
Overall, the study emphasizes the need for traditional diagnostic tools combined with molecular characterization of the parasite and for the elimination of the infection by using an association of metronidazole and fenbendazole, in particular for the NHPs that are asymptomatic with marked persistence of cysts, in order to better assess and reduce the zoonotic risk of *Giardia* infection. Regarding the detected zoonotic assemblages in the NHP from the Maitine Zoo from southern Italy, NHP are considered as important hosts of *G. duodenalis*; it is therefore strongly recommended to conduct the screening of this infection within the routine examinations in zoological gardens all over the world.

## 4.6 References

Altman, D.G. (Ed). Practical statistics for medical research. Chapman & Hall, London (1991) 277-321 pp.

Beck R, Sprong H, Bata I, Lucinger S, Pozio E, Cacciò S. (2011) Prevalence and molecular typing of Giardia spp. in captive mammals at the zoo of Zagreb, Croatia. Vet Parasitol (2011) 175(1–2): 40–46.

Berrilli F, Prisco C, Friedrich KG, Di Cerbo P, Di Cave D, De Liberato C. *Giardia duodenalis* assemblages and *Entamoeba* species infecting non-human primates in an Italian zoological garden: zoonotic potential and management traits. Parasit Vectors (2011) 4(1): 199.

Bowman DD, Liotta JL, Ulrich M, Charles SD, Heine J, Schaper R. Treatment of naturally occurring, asymptomatic *Giardia* sp. in dogs with Drontal Plus flavour tablets. Parasitol Res. (2009)105 Suppl 1:S125-34. doi: 10.1007/s00436-009-1503-0.

Cacciò SM, Beck R, Lalle M, Marinculic A, Pozio E: Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int J Parasitol (2008) 38:1523-1531.

Cacciò SM, Ryan U. Molecular epidemiology of giardiasis. Mol Biochem Parasitol. (2008) 160:75-80. doi: 10.1016/j.molbiopara.2008.04.006.

Capasso M, Maurelli MP, Ianniello D, Alves LC, Amadesi A, Laricchiuta P, Silvestre P, Campolo M, Cringoli G, Rinaldi L. Use of Mini-FLOTAC and Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals. Rev Bras Parasitol Vet. (2019) 28:168-171. doi: 10.1590/S1984-296120180087.

Chen L, Zhao J, Li N, Guo Y, Feng Y, Feng Y, Xiao L. Genotypes and public health potential of *Enterocytozoon bieneusi* and *Giardia duodenalis* in crab-eating macaques. Parasit Vectors. (2019) 1:254. doi: 10.1186/s13071-019-3511-y.

Chon SK, Kim NS. Evaluation of silymarin in the treatment on asymptomatic *Giardia* infections in dogs. Parasitol Res. (2005) 97:445-51. doi: 10.1007/s00436-005-1462-z.

Ciuca L, Pepe P, Bosco A, Caccio SM, Maurelli MP, Sannella AR, Vismarra A, Cringoli G, Kramer L, Rinaldi L, Genchi M. Effectiveness of Fenbendazole and Metronidazole Against *Giardia* Infection in Dogs Monitored for 50-Days in Home-Conditions. Front Vet Sci. (2021) 8:626424. doi: 10.3389/fvets.2021.626424.

## References


Cringoli G, Rinaldi L, Maurelli MP, Utzinger J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc (2010) 5:503-15.

David EB, Patti M, Coradi ST, Oliveira-Sequeira, TCG, Ribolla PEM, & Guimaraes S. Molecular typing of *Giardia duodenalis* isolates from non-human primates housed in a brazilian zoo. Revista Do Instituto De Medicina Tropical De São Paulo (2014) 56 (1), 49–54 doi.org/10.1590/S0036-46652014000100007.

Einarsson E, Ma'ayeh S, Svard SG. An up-date on Giardia and giardiasis. Curr. Opin. Microbiol. 2016; 34:47–52. 10.1016/j.mib.2016.07.019.

Fomsgaard AS, Bornbusch SL, Bueno GL, Noromalala E, Poulsen M, Rasmussen M, Rosenstierne MW, Stensvold CR, Wright P, & Hvilsom, C. Prevalence, infection intensity and genotyping of *Giardia duodenalis* in ring-tailed lemurs *Lemur catta* from European zoos and wild populations. Journal of Zoo and Aquarium research, (2020) 8(4), 253-258. https://doi.org/10.19227/jzar.v8i4.509.

Fung HB, Doan TL. Tinidazole: a nitroimidazole antiprotozoal agent. Clin Ther (2005) 27:1859–1884.

Geurden T, Olson ME, O'Handley RM, Schetters T, Bowman D, Vercruysse J. World Association for the Advancement of Veterinary Parasitology (WAAVP): Guideline for the evaluation of drug efficacy againstnon-coccidial gastrointestinal protozoa in livestock and companion animals. Vet. Parasitol. (2014) 204, 81-86.

Graczyk TK, Bosco-Nizeyi J, Ssebide B, Thompson RC, Read C, Cranfield MR. Anthropozoonotic *Giardia duodenalis* genotype (assemblage) a infections in habitats of free-ranging human-habituated gorillas, Uganda. J Parasitol. (2002) 5:905-9. doi: 10.1645/0022-3395(2002)088.

Hamlen HJ, Lawrence JM. Giardiasis in laboratory-housed squirrel monkeys: a retrospective study. Lab Anim Sci (1994) 44:235–239.

Hashan MR, Elhusseiny KM, Huu-Hoai L, Tieu TM, Low SK, Minh LHN, Nghia TLB, Loc LQ, Y MN, Eid PS, Abed M, Elkolaly SS, Tawfik GM, Huy NT. Effect of nitazoxanide on diarrhea: A systematic review and network meta-analysis of randomized controlled trials. Acta Trop. (2020) 210:105603. doi: 10.1016/j.actatropica.2020.105603.

Kalishman J, Paul-Murphy J, ScheffIerJ, Thomson JA. Survey of *Cryptosporidium* and *Giardia* spp. in a captive population of common marmosets. Lab Anim Sci (1996) 46:116–119.

## References


Karim MR, Wang R, Yu F, Li T, Dong H, Li D, Zhang L, Li J, Jian F, Zhang S. Multi-locus analysis of *Giardia duodenalis* from nonhuman primates kept in zoos in China: geographical segregation and host adaptation of assemblage B isolates. Infect Genet Evol (2015) 30: 82–88.

Köster PC, Martínez-Nevado E, González A, Abelló-Poveda MT, Fernández-Bellon H, de la Riva-Fraga M, Marquet B, Guéry JP, Knauf-Witzens T, Weigold A, Dashti A, Bailo B, Imaña E, Muadica AS, González-Barrio D, Ponce-Gordo F, Calero-Bernal R, Carmena D. Intestinal Protists in Captive Non-human Primates and Their Handlers in Six European Zoological Gardens. Molecular Evidence of Zoonotic Transmission. Front Vet Sci. (2022 ) 8:819887. doi: 10.3389/fvets.2021.819887.

Kowalewski MM, Salzer JS, Deutsch JC, Raño M, Kuhlenschmidt MS, Gillespie TR. Black and gold howler monkeys (*Alouatta caraya*) as sentinels of ecosystem health: patterns of zoonotic protozoa infection relative to degree of humanprimate contact. Am. J. Primatol. (2011) 73(1):75-83.

Kramer JA, Hachey AM, Wachtman LM, Mansfield KG. Treatment of giardiasis in common marmosets (*Callithrix jacchus*) with tinidazole. Comp Med (2009) 59:174-9. Erratum in: Comp Med. (2009) 59:220. PMID: 19389310.

Labberton L, Bakker J, Klomp R, Langermans JA, van Geijlswijk IM. Challenges in oral administration of metronidazole dissolved in drinking water to rhesus monkeys (*Macaca mulatta*). Lab Anim (NY) (2013) 42:213-6. doi: 10.1038/laban.264.

Levecke B, Dorny P, Geurden T, Vercammen F, Vercruysse J. Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. Vet Parasitol (2007) 148(3): 236–246.

Levecke B, Geldhof P, Claerebout E, Dorny P, Vercammen F, Cacciò SM, et al. Molecular characterisation of *Giardia duodenalis* in captive non-human primates reveals mixed assemblage Aand B infections and novel polymorphisms. Int. J. Parasit. (2009) 39:1595–601.

Maesano G, Capasso M, Ianniello D, Cringoli G, Rinaldi L. Parasitic infections detected by FLOTAC in zoo mammals from Warsaw, Poland. Acta Parasitol (2014) :343-53. doi: 10.2478/s11686-014-0249-8.

Manual Veterinary (MSD) by Terri Parrott, DVM, St. Charles Veterinary Hospital Last full review/revision Jan 2020 | Content last modified Feb 2020MSD https://www.msdvetmanual.com/exotic-and-laboratory-animals/nonhuman-primates/parasitic-diseases-of-nonhuman-primates#

v3304837.

Maraha B, Buiting AG. Evaluation of four enzyme immunoassays for the detection of *Giardia lamblia* antigen in stool specimens. Eur J Clin Microbiol Infect Dis. (2000) 19:485-7. doi: 10.1007/s100960000286.

Martínez-Díaz RA, Sansano-Maestre J, del Carmen Martínez-Herrero M, Ponce-Gordo F, Gómez-Muñoz MT. Occurrence and genetic characterization of *Giardia duodenalis* from captive nonhuman primates by multilocus sequence analysis. Parasitol Res (2011) 109: 539–544.

Modry D, Pafco B, Petrzelkova KJ, Hasegawa H. Parasites of Apes, An atlas of Coproscopic Diagnostics Edition Chimaira (2018). p. 98-102.

Montoya A, Dado D, Mateo M, Espinosa C, Miró G. Efficacy of Drontal® flavour Plus (50 mg praziquantel, 144 mg pyrantel embonate, 150 mg febantel per tablet) against Giardia sp. in naturally infected dogs. Parasitol Res. (2008). 103:1141–1144.

Mravcová K, Štrkolcová G, Mucha R, Goldová M. Zoonotic assemblages of *Giardia duodenalis* in captive non-human primates from the largest zoo in Slovakia. J Parasit Dis. (2021) 45:302-305. doi: 10.1007/s12639-020-01324-3.

Nizeyi JB, Cranfield MR, Graczyk TK. Cattle near the Bwindi Impenetrable National Park, Uganda, as a reservoir of *Cryptosporidium parvum* and *Giardia duodenalis* for local community and free-ranging gorillas. Parasitol Res. (2002) 88:380-5. doi: 10.1007/s00436-001-0543-x.

Ortega-Pierres G. Advances in Parsitology *Giardia* and Giardiasis, part B, vol 107. Academic Press Elsevier (2020). p. 176-182.

Oster N, Gehrig-Feistel H, Jung H, Kammer J, McLean JE, Lanzer M. Evaluation of the immunochromatographic CORIS Giardia-Strip test for rapid diagnosis of *Giardia lamblia*. Eur J Clin Microbiol Infect Dis. (2006) 25(2):112-5. doi: 10.1007/s10096-006-0088-0.

Pepe P, Ianniello D, Alves LC, Morgoglione ME, Maurelli MP, Bosco A, Cringoli G, Rinaldi L. Comparative cost-effectiveness of immunoassays and FLOTAC for diagnosing *Giardia* spp. infection in dogs. Parasit Vectors. (2019) 12:158. doi: 10.1186/s13071-019-3425.

Petri WA. Treatment of giardiasis. Curr Treat Options Gastroenterol (2005) 8:13– 17.

Plumb DC. Veterinary drug handbook. Ames (IA): Iowa State Press (2002).

Potkay S. Diseases of the Callitrichidae: a review. J Med Primatol (1992) 21:189– 236.

## References


Ryan U, Caccio SM. 2013. Zoonotic potential of Giardia. Int. J. Parasitol; 43:943– 56.

Ryan UM, Hijjawi N, Feng Y, Xiao L. *Giardia*: an under-reported foodborne parasite. Int J Parasitol (2019). 49:1–11.

Scicluna SM, Tawari B, Clark CG. DNA Barcoding of *Blastocystis*, Protist, vol 157, Issue 1 (2006) p. 77-85, ISSN 1434-4610, doi.org/10.1016/j.protis.2005.12.001.

Sestak K, Merritt CK, Borda J, Saylor E, Schwamberger SR, Cogswell F, Didier ES, Didier PJ, Plauche G, Bohm RP, Aye PP, Alexa P, Ward RL, Lackner AA. Infectious agent and immune response characteristics of chronic enterocolitis in captive rhesus macaques. Infect Immun (2003) 71:4079-86. doi: 10.1128/IAI.71.7.4079-4086.2003.

Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, Das P, Lal AA, Xiao L. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis. (2003) 11:1444-52. doi: 10.3201/eid0911.030084.

Thompson RC. Giardiasis as a re-emerging infectious disease and its zoonotic potential. Int J Parasitol. (2000) (12-13):1259-67. doi: 10.1016/s0020-7519(00)00127-2.

Tysnes KR, Skancke E, Robertson LJ. Subclinical *Giardia* in dogs: a veterinary conundrum relevant to human infection. Trends Parasitol. (2014) 11:520-7. doi: 10.1016/j.pt.2014.08.007.

Vitazkova SK, Wade SE. Parasites of free-ranging black howler monkeys (*Alouatta pigra*) from Belize and Mexico. Am J Primatol. (2006) 68(11):1089-97. doi: 10.1002/ajp.20309.

Weitzel T, Dittrich S, Möhl I, Adusu E, Jelinek T. Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. Clin Microbiol Infect. (2006) 7:656-9. doi: 10.1111/j.1469-0691.2006.01457.x.

Zanzani SA, Gazzonis AL, Epis S, Manfredi MT. (2015). Study of the gastrointestinal parasitic fauna of captive non-human primates (*Macaca fascicularis*). Parasitol Res (2015) 115: 307–312. doi.org/10.1007/S00436-015-4748-9.

# Chapter 5

Overall Discussion

## 5.1 Overall Discussion

Captive non-human primates (NPH) are susceptible to gastrointestinal (GI) parasitic infections, which are often zoonotic and can contribute to morbidity and mortality of these animals (Li et al., 2015).
Despite their impact on the health and welfare of NPH, diagnosis of GI parasites - e.g. soil transmitted helminths (roundworms, hookworms and whipworms) and intestinal protozoa (e.g. *Giardia duodenalis*, *Blastocystis* spp. and *Entamoeba* spp.) is still neglected in NPH by the scientific community and by veterinary practitioners responsible of the sanitary aspects in zoological gardens.
The present thesis provided important insights into the coprological diagnosis of GI parasitic infections in NHP with particular emphasis on the practical use of the FLOTAC (Cringoli et al., 2010) and Mini-FLOTAC (Cringoli et al., 2017) techniques and evidenced the need of using molecular tools to characterize zoonotic parasites like *Giardia duodenalis* in NHP (Berrilli et al., 2011).
The findings of this study added data regarding the diagnosis (Capasso et al., 2019), epidemiology and control (Capasso et al., 2022) of parasitic infections of NHP kept in zoos in Italy and Brazil. Furthermore, the parasitic fauna of wild geladas (*Theropithecus gelada*) was assessed within a study aimed at studying the social behavior and health of wild geladas frequenting the unprotected area of Kundi, Ethiopia (Caselli et al., 2021).
The overall results of this thesis demonstrated that gastrointestinal protozoa and helminths are highly prevalent in captive NHP of zoos in Italy and Brasil as well as in the wild geladas in Ethiopia. There was a large variation in proportion and multiple infections between groups of NHP, which might be explained by differences in host species susceptibility. This study also emphasizes the need for molecular diagnostic tools in NHP to evaluate the clinical importance and zoonotic risk of these infections as already evidenced in previous studies performed at zoos throughout the world (e.g, Levecke et al., 2014; Maesano et al., 2014).
Successful implementation of control programs of GI parasites in zoo environment depends upon precise and rapid diagnosing of infections and treatment based on drugs characterized by high efficacy and safety. Of particular concern is the diagnosis and control of the zoonotic *Giardia duodenalis*. Overall, the findings of the studies performed during the PhD

emphasize the need for traditional diagnostic tools combined with molecular characterization of the parasite and for the elimination of *Giardia* infection by using an association of metronidazole and fenbendazole, in particular for the NHPs that are asymptomatic with marked persistence of cysts, in order to better assess and reduce the zoonotic risk of *Giardia* infection (Capasso et al., 2022). The management of the NHP have to consider the ethological hierarchy of the social group that can make difficult the drug administration. Only by using best practice of management can ensure efficacy of the therapy protocols. The medical training based of positive reinforce increase the success of drug administration. The medical training method based on positive reinforce with food guarantee that every single subject receives the appropriate food and drug.

In conclusion, the need to prevent, diagnose, control, and treat intestinal parasitism trough specific control programs is mandatory for the health and welfare of NHP in order to limit the spread of parasitic infections in animals and humans.

## 5.2 References

Berrilli F, Prisco C, Friedrich KG, Di Cerbo 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(1), 199.

Capasso M, Ciuca L, Guadano Procesi I, Zinno F, Berrilli F, Cringoli G, Rinaldi L, 2022. Synergistic effects of fenbendazole and metronidazole against *Giardia duodenalis* in non-human primates in a zoological garden in southern Italy. Frontiers in Veterinary Science, section Parasitology, in press.

Capasso M, Maurelli MP, Ianniello D, Alves LC, Amadesi A, Laricchiuta P, Silvestre P, Campolo M, Cringoli G, Rinaldi L, 2019. Use of Mini-FLOTAC and Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals. Rev Bras Parasitol Vet 28(1), 168-171.

Caselli M, Zanoli A, Dagradi C, Gallo A, Yazezew D, Tadesse A, Capasso M, Ianniello D, Rinaldi L, Palagi E, Norscia I, 2021. Wild geladas (*Theropithecus gelada*) in crops-more than in pasture areas-reduce aggression and affiliation. Primates 62(4), 571-584.

Cringoli G, Maurelli MP, Levecke B, Bosco A, Vercruysse J, Utzinger J et al., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Protoc. 12, 1723–32.

Cringoli G, Rinaldi L, Maurelli MP, Utzinger J, 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 5, 503-515.

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(3), 236–246.

Li M, Zhao B, Li B, Wang Q, Niu L, Deng J, Gu X, Peng X, Wang T, Yang G, 2015. Prevalence of gastrointestinal parasites in captive non-human primates of twenty-four zoological gardens in China. J Med Primatol 24, 168-173.

Maesano G, Capasso M, Ianniello D, Cringoli G, Rinaldi L, 2014. Parasitic infections detected by FLOTAC in zoo mammals from Warsaw, Poland. Acta Parasitol 59, 343-353.