UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

MANAGEMENT OF GASTROINTESTINAL PARASITES IN WILDLIFE REHABILITATION CENTERS IN BRAZIL.

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Erro de Português	**Portuguese Mistake**
Quando o português chegou	*When the Portuguese arrived*
Debaixo de uma bruta chuva	*Under pouring rain*
Vestiu o índio	*He clothed the Indian*
Que pena!	*What a pity!*
Fosse uma manhã de sol	*Had it been a sunny morning*
O índio tinha despido	*The Indian would have stripped*
O português	*The Portuguese*

Oswald de Andrade

In 2014-2015, the southeast of Brazil faced the worst drought in 80 years.

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Um parágrafo para os amigos.

À Joana, porque tem que
haver sempre
alguém
com cinco parágrafos.
O quinto é para a amizade. And lameness. Not the limping kind.

Ao Kiko, for the loves.

Aan Herwig en Lieve, voor de onvoorwaardelijke steun.

Aan Oma, voor de ontvangst, steun en bezorgdheid. En om mijn grote voorbeeld te zijn.

MANAGEMENT OF GASTROINTESTINAL PARASITES IN WILDLIFE REHABILITATION CENTERS IN BRAZIL.

Parasites are essential and inevitable part of ecosystems, but simultaneously harm their individual host. This duality leads to dilemmas regarding the best approach to these symbionts when conservation medicine is concerned, such as in wildlife rehabilitation centers. In Brazil, tens of thousands of wildlife specimens are admitted in these centers every year.

An online survey was sent to wildlife rehabilitation centers throughout the country, addressing topics like diagnostic testing, deworming and biosecurity. Additionally, a detailed case study of the gastrointestinal parasite profile at one rehabilitation center was performed.

This resulted in an exhaustive characterization of gastrointestinal (GI) parasite management in these centers, with some notorious and returning findings:

a) Overcrowding and/or understaffing, making time one of the most precious resources in these centers. Veterinarians receive 4 to 18 cases a day, having a total time of 26 to 104 minutes to dedicate to a case from beginning to end, which is virtually impossible.
b) Lack of resources (equipment and funding);
c) A worryingly high percentage of inefective treatments. 15 to 47% of the organizations perform treatments without considering diagnostic results. From the organizations that check treatment efficacy, 74% already encountered inefective results, 40% frequently. Only 60% of the treatments performed by the author were efective.

Based on the collected information, guidelines were written for diagnosis and treatment of GI parasites in wildlife rehabilitation centers, in order to optimize time and resources. A diagnostic protocol was proposed with prioritization of certain patients: a) those with clinical signs suggestive of GI parasitism, such as diarrhea and anemia; b) older animals; c) animals under permanent human care; and d) animals under a high amount of stress (e.g. polytraumatized patients). One should test the efficacy of all treatments and keep extensive records. The use of alternative methods for parasite control, such as fungi and plants, is suggested as a measure with a lot of potential and advantages in wildlife medicine.

Key-words: gastro-intestinal parasite management, wildlife rehabilitation, deworming efficacy, coproparasitology, CETAS, CRAS, Brazil.

MANEIO DE PARASITAS GASTROINTESTINAIS EM CENTROS DE REABILITAÇÃO DE ANIMAIS SILVESTRES NO BRASIL.

Os parasitas são componentes essenciais e inevitáveis dos ecossistemas, mas simultaneamente prejudicam o hospedeiro. Esta dualidade leva a dilemas sobre a melhor abordagem a estes simbiontes em contexto de medicina de conservação, como é o caso dos centros de recuperação de animais silvestres. No Brasil, dezenas de milhares de animais silvestres são admitidos nestes centros por ano.

Um questionário *online* foi enviado a centros de reabilitação em todo o país, com perguntas relativas a hábitos de diagnóstico, desparasitação e biossegurança. Adicionalmente, foi efetuado um estudo de caso detalhado do perfil de parasitas gastrointestinais (GI) em um centro específico.

Do inquérito resultou uma caraterização exaustiva do maneio de parasitas gastrointestinais nestes centros. Alguns resultados notórios e transversais são:

- Sobrelotação e/ou falta de pessoal, tornando o tempo num dos mais valiosos recursos destes centros. Os veterinários recebem entre 4 a 18 casos por dia, dispondo de 26 a 104 minutos para dedicar a cada caso do início ao fim, o que é virtualmente impossível;
- Falta de recursos (equipamento e financiamento);
- Uma percentagem preocupante de tratamentos ineficazes. 15 a 47% das organizações desparasitam sem ter em conta os resultados de diagnóstico. De entre as organizações que avaliam a eficácia, 74% já se depararam com desparasitações ineficazes, 40% com frequência. Apenas 60% das desparasitações realizadas pela autora foram eficazes.

Com base na informação recolhida redigiram-se *guidelines* para maneio de parasitas GI em centros de reabilitação de forma a otimizar tempo e recursos. Foi proposto um protocolo diagnóstico com priorização de determinados pacientes: a) aqueles com sinais clínicos sugestivos de parasitismo GI, como diarreia e anemia; b) animais com mais idade; c) animais sob cuidados humanos permanentes; e d) animais com elevados níveis de *stress* (ex. animais politraumatizados). É recomendado que a eficácia de todas as desparasitações seja testada e que sejam mantidos registos detalhados. O uso de métodos alternativos de controlo parasitário (ex. fungos e plantas) é aconselhado como uma medida a explorar com muito potencial e vantagens em medicina de conservação.

Palavras-chave: maneio parasitário de parasitas gastrointestinais, reabilitação de animais silvestres, eficiência de desparasitação, coproparasitologia, CETAS, CRAS, Brasil.

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List of abbreviations, initials and acronyms

BCS	Body Condition Score
CCFS	Wildlife Conservation Center of Ilha Solteira (*Centro de Conservação de Fauna Silvestre de Ilha Solteira*)
CRAS	Wildlife Rehabilitation Center (*Centro de Reabilitação de Animais Silvestres*)
CESP	São Paulo State Energy Company (*Companhia Energética de São Paulo*)
CETAS	Wildlife Triage Center (*Centro de Triagem de Animais Silvestres*)
CEVAP	Research Center on Poison and Venomous Animals of UNESP (*Centro de Estudos de Venenos e Animais Peçonhentos da UNESP*)
CRT	Wildlife Reception and Triage Center of CCFS (*Centro de recuperação e triagem*)
EPG	Eggs per gram
GI	Gastrointestinal
HBWA	High-biodiversity Wilderness Area
HPP	Hydroelectric Power Plant
IBAMA	Brazilian Institute of Environment and Renewable Natural Resources (*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais*)
IAESTE	International Association for the Exchange of Students for Technical Experience
IN	Normative instruction - legal document (*Instrução normativa*)
IUCN	International Union for Conservation of Nature and Natural Resources
IWRC	International Wildlife Rehabilitation Council
LECOP	Laboratory of Parasite Ecology of the Biology and Animal Sciences Department, UNESP - Ilha Solteira (*Laboratório de Ecologia do Parasitismo, Departamento de Biologia e Zootecnica, UNESP - Ilha Solteira*)
NGO	Non Governmental Organization
OPG	Oocysts per gram
RENCTAS	National Network to Combat Wildlife Trafficking (*Rede Nacional de Combate ao Tráfico de Animais Silvestres*)
SZB	Brazilian Society of Zoos and Aquaria (*Sociedade de Zoológicos e Aquários do Brasil*)
UNESP	São Paulo State University (*Universidade Estadual Paulista*)

I) Description of the training period

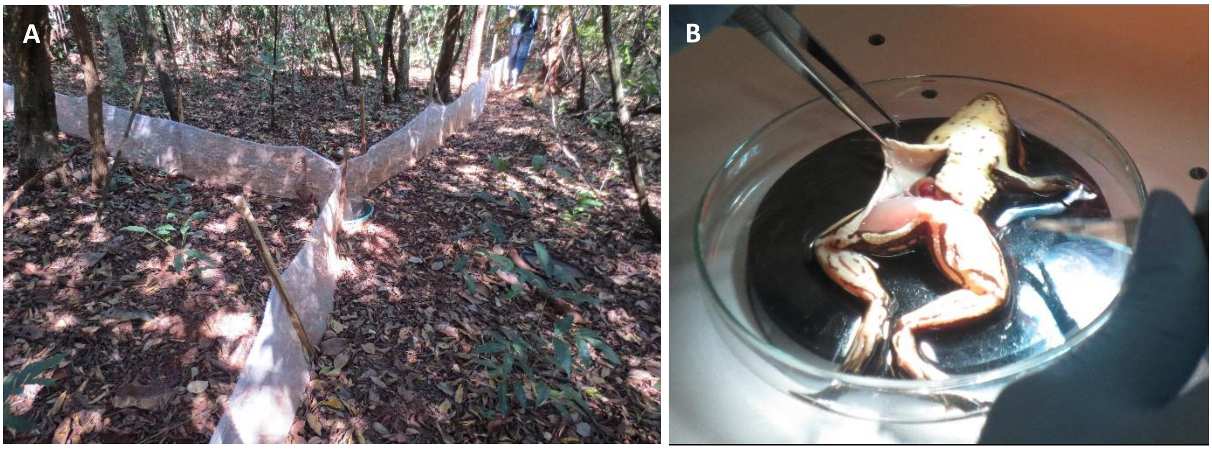
The author performed an 8 month curricular training period for the Integrated Master in Veterinary Medicine in the São Paulo State University - Ilha Solteira Campus, Brazil, initially through the International Association for the Exchange of Students for Technical Experience (IAESTE) exchange program and later on by mutual agreement between UNESP and FMV-ULisboa. The training period involved both laboratory and clinical experience, during which data were collected for this master's project.

Large part of the training period was spent at the Wildlife Conservation Center of Ilha Solteira (CCFS), where the author assisted the attending veterinarian in all of his activities, such as admitting and evaluating new patients, diagnostic work, treatments, routine rounds and check-ups, necropsies, feeding, behavioral conditioning, surgeries and releases (Figure 2).

Samples and relevant data for the project were collected at CCFS and all laboratory work was performed at the Laboratory of Parasite Ecology (LECOP) of the Biology and Animal Sciences Department from the São Paulo State University (UNESP) - Campus of Ilha Solteira. The total of circa 1125 hours were around 40% clinical (450h) and 60% laboratory work (675h). The laboratory work is described with more detail in Chapter III.1.2, but consisted mainly of coproparasitological examinations. The author collaborated in some other projects in course in the laboratory, including the following procedures:

- Active amphibian and reptile capture in the field by visual area search limited by time;
- Amphibian and reptile capture by Y-array drift fence and pitfall traps (Figure 1);
- Parasitological necropsy of amphibians and identification of its parasites (Figure 1);
- Stingray capture and parasitological necropsies in the field (Annex IV).

Figure 1 - Some ongoing projects in LECOP (Laboratory of Parasite Ecology of the Biology and Animal Sciences) in which the author collaborated.



A) Amphibian and reptile capture by Y-array drift fence and pitfall traps; and B) Parasitological necropsy of a frog.

The author was actively involved or performed the following procedures:

- Admission and triage of casualties (n~50);
- Treatment of polytraumatized animals (~30) ;
- Wound management (~20);
- Care of infant animals (birds and mammals) (~40);
- Anesthesia of birds and mammals (~50);
- Surgical procedures:
    - Neutering of domestic cats and dogs (~5);
    - Internal and external fixation of fractures in birds (~30);
    - Tail amputation in primate (*Allouata caraya*) (1).
- Post-chirurgic monitoring and follow-up (~30);
- Physiotherapy in mammals (~10);
- Environmental enrichment for different species (birds, primates, carnivores, reptiles) (~10);
- Behavioral conditioning of animals (primates, psittacids, felids) (~5);
- Release of rehabilitated animals (~20);
- Euthanasia in birds, mammals and reptiles (~20);

Figure 2 - Some clinical cases from the training period (Originals).

A) Jaguar (*Panthera onca*) with pyometra; B) Wing fracture in a toco toucan (*Ramphastos toco*); C) Internal and external fracture fixation in a giant anteater (*Myrmecophaga tridactyla*); D) *Tunga penetrans* in foot of a tapir (*Tapirus terrestris*); E) Orphan giant anteater; F) Blue-and-yellow macaw (*Ara ararauna*) used for environmental education.

- Necropsies of birds, mammals and reptiles (~50);
- Nutritional study in psittacids (collaboration in Master's thesis project);
- Diagnosis and treatment of infectious diseases such as psitacosis, anaplasmosis, enterotoxemia, mange and *Tunga penetrans* and *Serpentoplasma* spp. infections.

The author assisted in the following procedures:

- Orthopedic surgery in large mammals (n=2);
- Vasectomy in large felid - puma (*Puma concolor* - 1);
- Diagnosis and treatment of pyometra in a jaguar (*Panthera onca* - 1);
- Acupuncture (birds and mammals - 5);
- Rooting canal in primate (1).

So far, this work led to the publication of five scientific posters and one article (see Annex IV). Finally, the author had the chance to participate in courses on survey, capture, rescue, identification and management of wildlife delivered at the rehabilitation center. She also had the amazing opportunity to join the biology classes in their weeklong study field trips to the Pantanal and Atlantic Rainforest biomes and to a study center of venomous and poisonous animals - *Centro de Estudos de Venenos e Animais Peçonhentos* – CEVAP (Figure 3).

Figure 3 – Field trips to (A) CEVAP, a study center of venomous and poisonous animals; (B) the Pantanal; and (C) the Atlantic Rainforest.

II) Literature review

1. Introduction

Parasites are an important component of all ecosystems but, compared to domestic animals, the study of wild animal disease is considerably behind. Throughout the years, a general lack of quantitative data has been noticed in this field (Grenfell & Dobson, 1995). The available knowledge usually comes from the study of unrepresentative samples and is often incomplete. The randomness and lack of all-round information is understandable, as the study subjects are difficult to access and restrain and it is hard to obtain a representative sample of a population. Sampling is mostly opportunistic, with parasitic diseases of wildlife being typically investigated by identifying and listing the parasites found in small samples of host species or by examinating feces or carcasses that are found incidentally. The samples are rarely diverse or suitably age-stratified enough to detect heterogeneity in host-parasite associations. Examination of only a small sample may miss heavily infected hosts, as most macroparasites tend to be over-dispersed within the host population, i.e., most individuals are lightly or uninfected and only a few individuals are heavily infected. The detection of heavy infections or highly pathogenic parasites in wildlife is even tougher as these animals typically manifest few recognizable signs of disease, tend to separate and hide when affected, and are hardly findable when already deceased (Gulland, 1995). While individual and punctual parasitological characterization is fairly manageable and has been consistently performed, the relationship between infection and disease (Ballweber, 2001), the impact of a disease on the host population or the distribution of the disease agent in a degree sufficient to understand its epidemiology is much harder to achieve (Gulland, 1995), adding to the low accessibility that the complexity of parasite-host interactions is often very high (Grenfell & Dobson, 1995). Most available information on distribution of infectious diseases in wild animals results from large scale investigations on diseases that also affect men or his domestic livestock (Gulland, 1995).

The study of wildlife diseases is more expensive than the study of domestic animals or even men, starting by the means necessary to access to study subjects and ending with the apparent lack of economical return after achieving the results, when compared to the study of parasitic diseases affecting domestic animals and men, which result in direct improvement of the general health status and eventual increase in productivity. Nonetheless, the importance of studying wildlife infections is globally recognized, not only for a general broadening of scientific knowledge or to get a better understanding of the world we cohabitate (Windsor, 1995; Strona, 2015), but also to apply the gathered information in the management of matters as important as zoonoses or endangered species (Grenfell & Dobson, 1995). With wild animal populations decreasing and the importance of conservation becoming more evident every day, the necessity of thoroughly investigating wildlife diseases on individual and population level is also growing (Gulland, 1995).

2. Parasitism: the bigger picture

The ecological associations between organisms of different species can be classified according to their level of interaction and the consequences of these interactions for each participant, whether negative, neutral or positive (Townsend, Begon, & Harper, 2006).

More than half of all known species live in or on another organism, and almost all organisms serve as host for at least one other (Townsend et al., 2006). This kind of intimate relationship, where organisms of different species live in close association with each other, either permanent or temporarily, is called symbiosis (Ballweber, 2001; Townsend et al., 2006; Bowman, 2013). The varying degree of unilateral or mutual benefit, indifference or harm of particular symbiotic associations gave rise to terms such as mutualism (both organisms benefit from the association), commensalism (one organism benefits from and the other is indifferent to the association), phoresis (one organism is a mere transporter of the other) and parasitism.

Parasitism is a type of symbiotic ecological interaction where one of the parties, the parasite, benefits from the association by living in or on and drawing subsistence from the other, the host, for whom the association is therefore assumed harmful (Ballweber, 2001; Bowman, 2013).

By definition, parasites include any virus, bacteria, protozoan, helminth or arthropod that parasitizes a host (Beldomenico & Begon, 2010; Bowman, 2013). In the field of ecology and biology, if not stated differently, the term is used in this sense, but in the veterinary field the term is most commonly reserved for parasitic helminths, protozoa and arthropods (Bowman, 2013), while virus and bacteria are referred to as infectious diseases agents. From chapter 2 onwards, the veterinary consensus will be used, as it lies closest to the studied groups of parasites in this project.

The term 'parasites' refers to an immense number of different species belonging to a vast variety of taxa and with a daunting diversity of parasitic strategies (Kevin D. Lafferty & Kuris, 2002), so there are many different parameters according which parasites are classified in smaller groups. Frequent classifications include taxonomic division (see

Table 1) and division by location within or on the host, giving rise to terms such as endoparasites (live within the host), ectoparasites (live on the external surface or in the skin of the host or outside of the hosts body), endectoparasites, hemoparasites (live within the bloodstream of the host), parasites of the gastrointestinal tract, parasites of the respiratory system, parasites of the urogenital system, and so on (Hendrix & Sirois, 2007; Bowman, 2013).

Table 1 - Classification of parasites on taxonomic level, with higher detail on macroparasites*.

Microparasites*	Macroparasites*	
	Helminths	**Arthropods**
Virus	Trematoda	Insecta
Bacteria	Cestoda	Arachnida
Protista	Nematoda	Crustacea
	Acanthocephala	

* These terms are frequently found in scientific literature, and were first introduced by Anderson & May (1979) when they created separate models for intensity-dependent and intensity-independent parasites. The intensity-independent models were inspired and are most adequate for bacteria, virus and protozoa, which tend to multiply within the host, have short generation times, a short duration of infection and a tendency to induce immunity in surviving hosts, and were named 'microparasite' models. The intensity-dependent models accommodated parasites that usually don't multiply within the host, have longer generation times, accumulate relatively slowly and tend to generate chronic infections with host immunity being inexistent, short-lived and/or only happening in case of high parasite burdens. These parasites, like many adult parasitic worms, tend to be relatively much bigger in size, and therefore the models were coined 'macroparasite' models (Wilson et al., 2002).
Over time, the terms have been adopted outside the modeling literature based only on size, using the term microparasites to refer to parasitic bacteria, protozoa and virus and macroparasites for parasitic arthropods and helminths. Although this use coincides in most situations with the original definition, one should not forget that there exist large parasites that act in an intensity-independent way (e.g. some larval forms of digenean trematodes) and small parasites that act in an intensity-dependent way (e.g. some coccidians). (Kevin D. Lafferty & Kuris, 2002; Wilson et al., 2002)

2.1. Consequences for the host

Historically, parasites have been defined as organisms which a) use a host as a habitat at least once during its life cycle; b) have nutritional dependence on its host; and c) cause harm to its host (Anderson & May, 1978a). During the parasitic phase(s) of its life cycle, the parasite lives in fact at the expense of the host but, while the parasite is unconditionally benefited, the harm or damage caused to the host may vary from very severe to nearly absent (Bowman, 2013), sometimes making the line between parasitism and commensalism very thin (Townsend et al., 2006).

Although purely theoretically, if the parasite doesn't cause any harm to its host, we would be facing a commensal interaction (Townsend et al., 2006), smaller organisms found in association with humans or with animals or plants that humans value have been called parasites independently of their effect on the host, be it detrimental, indifferent or beneficial (Bowman, 2013). This practice has been adopted in reference manuals as an acceptable convention (Bowman, 2013), justified by the fact that the parasites' pathogenicity can vary greatly, depending on both host and parasite factors (Gómez & Nichols, 2013).

Despite of having the potential to kill their host, parasites do not need it as a prerequisite for successful development (which is the case in parasitoid insects for instance (Kevin D. Lafferty & Kuris, 2002)). In fact, it would harm themselves, as they would most certainly die if they´d cause the death of their host. Therefore it's not surprising that most known parasites such as lice, fleas, ticks, mites, protozoa and helminths appear to do little harm to their host, despite exhibiting the habitat and nutritional requirements of parasites (Anderson & May, 1978a).

It has been stated that the degree of harm caused to the host by a parasite is intimately connected with their co-evolution (Gulland, 1995; Mackinnon & Read, 1999), with parasites becoming less virulent and hosts more resistant (Jaenike & Perlman, 2002). This way, "well-adapted" parasites would inflict little harm to their host, preventing their own eradication (Gulland, 1995; Mackinnon & Read, 1999). Keeping this in mind, the recorded morbidity and mortality cases in individual wild animals have been assigned to "imbalances" in the natural host-parasite interaction (e.g. the introduction of an exotic parasite in a naïve population or the reduction of the host's immune status) (Gulland, 1995; Mackinnon & Read, 1999). This theory is encountered in many literature (Jaenike & Perlman, 2002), but theoretical studies indicate that different co-evolutionary pathways might have been followed depending upon the relationships between parasite pathogenicity and transmission efficiency (Gulland, 1995; Mackinnon & Read, 1999). Indeed, while there are situations where the evolutionary adaptation of the parasite consist in lowering its virulence[1], there are also known examples where the evolutionary selection favored increased transmission rate rather than decreased virulence (Poulin, 1995; Mackinnon & Read, 1999; Townsend et al., 2006). Hosts invariably tend to evolve by increasing their resistance, this way decreasing the degree of harm caused by the parasite, but parasites do not necessarily evolve to protect their hosts (Townsend et al., 2006).

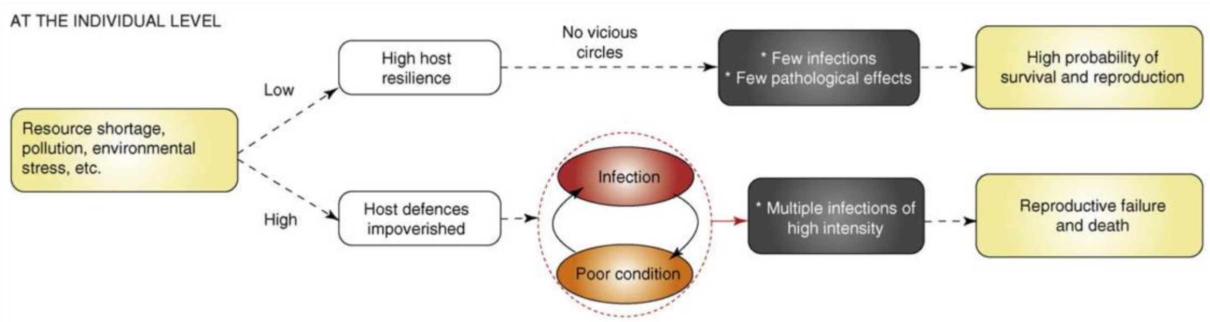
The harm caused to the host and, consequently, the clinical picture, depends on aspects inherent to the parasite, such as the niche and mode of life it adopts within or on the host (Anderson & May, 1978a), the kind and degree of injury it inflicts (Bowman, 2013) and the size of the parasite in relation to the host (Anderson & May, 1978a).

Besides the characteristics of the parasite, the degree of harm also depends on the host itself, namely on its general health status and immunocompetence and indirectly on all factors contributing to it, such as vigor, nourishment, presence of concomitant diseases or other stress factors (Townsend et al., 2006; Beldomenico & Begon, 2010; Bowman, 2013). The host's immune system (immunity and surface barriers) requires energy and nutrients to function, and there is always a trade-off with competing physiological demands, such as age and co-infection. A host in good condition will be better prepared to oppose and/or limit infection than a host in poor condition (Beldomenico & Begon, 2010), and a kind of equilibrium between host

[1] Virulence is defined as the effect of parasite infection on host fitness (Casadevall & Pirofski, 2001).

and parasite where they coexist during longer periods of time without apparent deterioration of the hosts condition may be achieved. As a matter of fact, many parasites cause no apparent harm to their hosts as long as the latter stays healthy and stress-free (Townsend et al., 2006; Beldomenico & Begon, 2010). Both prevalence and intensity of infection[2] are more probable and more severe in host individuals with an underlying poor condition (Bush, Lafferty, Lotz, & Shostak, 1997; Beldomenico & Begon, 2010). Once having infected the host, the parasite will alter the physiological economy of the host by extracting its resources and inducing a nutritionally demanding immune response. It has been suggested that the infection or intensification of the infection results in further deterioration of the hosts condition, which will get even more susceptible, this way creating a vicious circle where host susceptibility and infection work synergistically (Beldomenico & Begon, 2010) (see Figure 4).

Figure 4 - Relationship between host condition and infection intensity at the individual level. Source: Beldomenico & Begon, 2010.



When addressing the degree of harm caused to the host, the intensity of infection is frequently referred, often under the phrasing "light" or "heavy" infection (Anderson & May, 1978a; Gulland, 1995). The limit above which an infection is considered heavy is very variable, as some parasites are extremely harmful to certain hosts even in small numbers, eventually getting the host and themselves killed, while others almost achieve a commensal type of association where even large numbers of parasites cause negligible, if any, harm. Nonetheless, for a given parasite, heavier infections are indeed more likely to cause severe harm or even death of the host (Anderson & May, 1978a; Mackinnon & Read, 1999; Beldomenico & Begon, 2010), and, as referred above, the hosts condition is a big factor influencing the intensity of an infection, specially once inside the vicious circle (Beldomenico & Begon, 2010).

It is unanimous that, when parasitology is concerned, infection does not equal disease (Scott, 1988; Ballweber, 2001). Infection is present whenever the parasite is present in or on the host, while disease is present only when there is a clinical condition that can be observed or

[2] Intensity of infection, also known as worm burden or parasite load, is defined by the number of individuals of a particular parasite species in a single infected host (Bush, Lafferty, Lotz, & Shostak, 1997).

measured (Scott, 1988). Disease may present itself through a huge variety of clinical signs, depending on the niche and mode of life the parasite adopts within or on the host. For gastrointestinal parasites, the most common clinical signs are related to the GI tract, such as diarrhea/low-fecal consistency, abdominal pain, intestinal obstruction and, indirectly, loss of body condition, but there may be also some extra-GI signs. A common one is anemia (e.g. in *Haemonchus contortus* or *Ancylostoma caninum* infections (Lobetti & Schoeman, 2001)). Submandibular edema is also frequently associated to GI and liver parasitism (e.g. Haemonchosis and Fasciolosis, respectively), caused by the hypoalbuminemia and anemia (Pantelouris & Kerkut, 1965).

Absence of clinical signs does not mean that the parasite has no effects whatsoever on the host. Prejudicial effects don't only include the presence of clinical disease but also factors such as increased mortality rate and decreased birth and growth rate (Townsend et al., 2006). As stated before, a parasitic infection extracts host resources and induces a nutritionally demanding immune response (Beldomenico & Begon, 2010), which will obviously trade-off with other physiological functions, such as reproduction or simply growth rate. A simple decrease in growth rate may delay the age at first reproduction (Gulland, 1995). Being infected may increase the susceptibility of detrimental effects or death from other causes such as other infectious agents, but also by causes as simple as predation (Gulland, 1995; Beldomenico & Begon, 2010; Cézilly, Thomas, Médoc, & Perrot-Minnot, 2010). The need to forage more to compensate the extra energy requirement may increase the exposition to predators (Poulin, 1995). A negative association between gastrointestinal nematode prevalence and body condition in African buffalo (*Syncerus caffer*) was observed only if there was a concurrent *Mycobacterium bovis* infection (Jolles, Ezenwa, Etienne, Turner, & Olff, 2008). Similarly, although cestodes in birds are mostly considered not pathogenic and do not usually cause clinical signs, they are frequently found in dead birds together with other infective agents. As in most mixed infections, it is hard to determine each one's specific role. More than once, cestodes have been associated with emaciation and starvation of large numbers of birds during sudden cold snaps, but their role in these deaths remains uncertain (Atkinson, Thomas, & Hunter, 2008).

Besides the nutritional trade-off, parasites are known to induce behavioral and physical changes in their hosts, which may also alter reproduction and survival rates. Parasites have been shown to influence behavioral factors such as movement, social behavior or parental care. Behavioral mechanisms such as territoriality, dominance hierarchies and mate choice have an important role in wild animals' reproduction. Parasite-induced changes in behavior may be just a generic effect of infection (e.g. increase in foraging due to nutritional trade-off), a result of parasite adaptation to enhance transmission or a result of host adaptation to counter infection. Some parasites modify their host behavior,specifically to increase the probability of transmission to a predatory host (Kevin D. Lafferty & Kuris, 2002), such as the case of *Riberoia* spp., which induces the growth of extra legs on its amphibian hosts (Johnson & Mckenzie, 2008) or the *Heterorhabditis* genus which can, with the help of bacteria, color the hosts' skin differently (Poinar, 1975). Some described adaptations of the host are physical removal of ectoparasites (e.g. grooming in primates), self-medication (also in primates) or the search for hotter (behavioral fever) or colder habitats by ectothermic animals (Poulin, 1995).

Given the above, it is difficult to objectively determine the damage done by a parasite to its host, as it is part of a complex network of trade-offs between multitudes of agents and factors. Nonetheless, it is agreed that, overall, parasites have a detrimental effect on their hosts, even though beneficial effects have also been reported. An example are certain immunomodulatory and immunoregulatory characteristics that reduce for instance allergic reactions and other inflammatory related diseases (Correale & Farez, 2007; Hewitson, Grainger, & Maizels, 2009; Maizels, 2009). Due to these characteristics, certain parasitic worms have even been proposed for therapeutic application (Pritchard, 2011).

2.2. Role of parasites in a host population

As stated in the previous chapter, parasites may cause clinical disease, decrease the general condition and immunocompetence of the host, mediate food intake and activity patterns and negatively affect growth rate, reproductive output and survival rate (Anderson & May, 1978a; Gulland, 1995; Nichols & Gómez, 2011). Being so, it is not surprising that they may play a role in regulating[3] or controlling the growth of their host population (Anderson & May, 1978a; Ebert, Lipsitch, & Mangin, 2000).

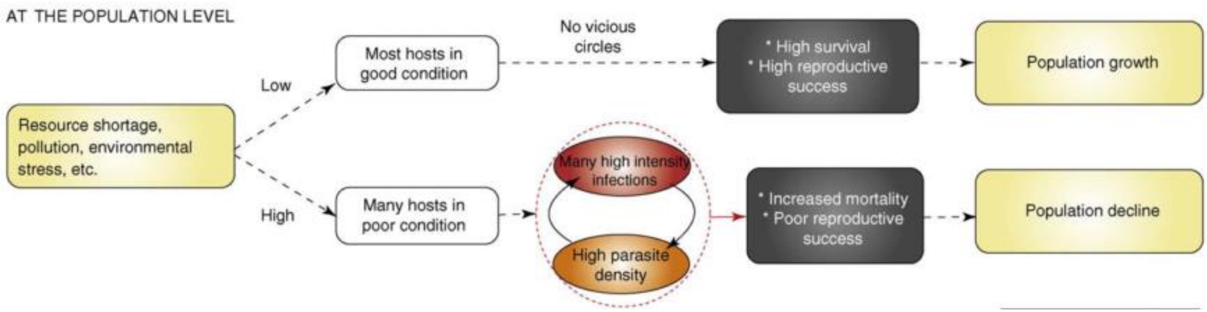
Exotic parasites are known to be able of having devastating effects on their host population, especially when causing epidemics that reduce populations sufficiently to allow stochastic events to lead to their extinction, but also endemic parasites can exert significant effects on host population (Gulland, 1995; Ebert et al., 2000). All parasite-host interactions exhibit stabilizing and destabilizing effects on the population dynamics, although these will vary greatly in extent and proportion (Anderson & May, 1978a, 1978b; Ebert et al., 2000). The impact of a parasite on its host population depends on the trophic connections involving the hosts of a given parasite, the host range, the parasite's virulence and the statistical distribution of the number of parasites per host (Jaenike & Perlman, 2002). The higher the pathogenicity of the parasite, the bigger the impact and the closer we get to a predator-prey type of interaction, with the predator suppressing the growth of its prey population (Anderson & May, 1978a; Ebert et al., 2000).

Also the host's condition plays an important role in the distribution and dynamics of infections and their impact on host population dynamics. As referred in the previous chapter, individuals in poor condition are more likely to become infected and more prone to high infection

[3] A parasite is considered to have a regulatory effect when infected host populations are maintained at a lower density than parasite-free host populations (Ebert et al., 2000; Gulland, 1995).

intensities. These individuals might be the most important source of infection to others. Populations with a large numbers of individuals in poor condition tend to exhibit higher prevalences of infection and a large number of high intensity infections, which may downregulate the population growth (see Figure 5) (Pedersen & Greives, 2008; Beldomenico & Begon, 2010).

Figure 5 - Relationship between host condition and infection intensity at population level. Source: Beldomenico & Begon, 2010.



Most information about the influence of GI parasites on their hosts' populations is generated by theoretical models and laboratory experiments. Evidence from free-living populations is scarce but not absent. One of the best documented examples concerns the effect of *Trichostrongylus tenuis* in red grouse (*Lagopus lagopus*) (Gulland, 1995; Peterson, 2004). Intestinal coccidiosis by *Eimeria* spp. was reported to reduce egg production and fertility and delay maturation of males in wild birds such as the Northern Bobwhite (*Colinus virginianus*) and Japanese Quail (*Coturnix japonica*) (Yabsley, 2008b). Several trematodes have been appointed as the causal agents of severe epizootics in wild waterfowl (Huffman, 2008). Parasitism by cestodes in wild birds was reported to increase mortality, affect plumage quality and sexual ornamentation and, consequently, mate selection (McLaughlin, 2008). Occasional epizootics caused by acanthocephalans in waterfowl have been reported after droughts (Richardson & Nickol, 2008). Most authors agree that research is needed towards the role of parasites in wild host populations, as the effects of these agents, especially the subclinical ones and those in combination with other parasites, are largely unknown (Peterson, 2004; Huffman, 2008; Yabsley, 2008b).

Theoretical models suggest that, in natural populations which live more or less in equilibrium, the extent of the parasite's influence on its host population may be estimated by the mean parasite load per host. This factor should be analyzed with a degree of caution, but, generally, in a balanced population, the lower the mean parasite load per host, the higher the influence of the parasite in the host population (Anderson & May, 1978b; Ebert et al., 2000).

Parasites are deleterious to their individual hosts, but are essential to maintain healthy populations (Nichols & Gómez, 2011). Parasites help to eliminate weak or susceptible host individuals and exert selective pressure on the host population, being at least partially responsible for maintaining a healthier host population and higher levels of genetic diversity, compared to non-parasitized animals (Durden & Keirans, 1996). The diet of predators often shows a bias towards infected prey relative to the prevalence in the prey population (Poulin, 1995). A comparative study in primate species demonstrated that helminth species richness was lower among threatened primates (Altizer, Nunn, & Lindenfors, 2007).

2.3. Role of parasites in ecosystems

Parasites tend to be referred to with a negative connotation, especially in the field of veterinary medicine (Durden & Keirans, 1996; Nichols & Gómez, 2011; Gómez & Nichols, 2013), but they do have a major role in current ecosystems, weren't it only for the fact that parasitism is the most common life strategy on the planet, with parasites outnumbering free-living biodiversity by as much as 50% and having representatives in a wide variety of taxa (Poulin & Morand, 2000; Dobson, Lafferty, Kuris, Hechinger, & Jetz, 2008). Just based on their ubiquity, species diversity, numerical abundance and biomass, one can already suspect that parasites are fundamental components of the ecosystems they belong to, being vital drivers of ecological structure and function (Gómez & Nichols, 2013).

Parasites have been described as crucial components of food webs, contributing to their stability, cohesion and robustness. Food webs are usually considered to be driven mainly by interactions between free-living species (K. D. Lafferty, Dobson, & Kuris, 2006), but parasites exercise a unique role as functional predators (Hudson, Rizzoli, Grenfell, Heesterbeek, & Dobson, 2002), occupying a dominant position in food webs (Smith, Sax, & Lafferty, 2006). Despite this information, published food webs that include parasites are uncommon (Jaenike & Perlman, 2002).

The impact that parasites have on their individual hosts and their population dynamics by mediating matters such as food intake, growth rate, reproductive output and activity patterns (Nichols & Gómez, 2011), most certainly influences the host species' role within the ecosystem. Already in 1948, Park suggested that the presence or absence of a parasite in the host population could shift the competitive advantage of the host species towards another species. The introduction of an exotic parasite or the removal of an endemic parasite can have major impacts on their hosts populations and consequently on community structure, with shifts in species composition and alteration of the ecosystem's balance (Jaenike & Perlman, 2002).

It is unanimous that parasites play a major role in ecosystem dynamics through complex ecological processes, with host-parasite interactions tying together land use and climate change, wildlife and ecosystem ecology, nutrition, stress, pollution and demography (Hudson, 2001). It is therefore important to fight the tendency of disregarding parasites in field studies of natural populations (Jaenike & Perlman, 2002), even though their detection and identification is rather difficult.

2.4. Significance of parasites in conservation biology

The loss of vertebrate life is paid much more attention than the loss of invertebrate life, even though the latter is numerically far superior (Nichols & Gómez, 2011). Also parasites, despite representing the most common consumer strategy on the planet and despite their importance in ecosystems, are often neglected when conservation is addressed (Gómez & Nichols, 2013). When they are considered, it is mostly as a negative feature (Nichols & Gómez, 2011).

The loss of parasite species by itself is a threat, since parasites represent such a significant proportion of total biodiversity on Earth (Durden & Keirans, 1996; Townsend et al., 2006). However, more than a mere number of species, parasites are responsible for fundamental biological relationships. Infection is fundamental to the ecological and evolutionary drivers of biological diversity and ecosystem organization (Marcogliese, 2004). It has been noted that, in some cases, parasites should be a conservation target of their own (Hudson, 1998; Brewer, 2006;) and even the preservation of parasite biodiversity of rare, endangered or even extinct hosts has been highlighted (Windsor, 1995; Durden & Keirans, 1996; Strona, 2015). The loss of ecological interactions often precedes and may affect species functionality and ecosystems at a faster rate than species extinctions (Valiente-Banuet et al., 2015). The incorporation of ecosystem processes into conservation planning should be given more and more importance, since more threatened species are in need of urgent broad scale conservation action because they are dependent on broad scale ecological processes (72%) than because they are in need of the actual physical area (43%) (Boyd et al., 2008). Only this way it will be possible to achieve the goal of conservation biology: to maintain biodiversity, including the evolutionary processes that drive and sustain it (Meffe, Carroll, & Groom, 2006).

Due to their well-known negative effects, parasites are one of the few groups for whom eradication is still a predominant goal within public health strategies, captive breeding and wildlife management programs (Wobeser, 2002). It is common practice to purposely remove visible ectoparasites from rare or endangered animals in order to increase the latter's fitness and chances for survival (Durden & Keirans, 1996; Wobeser, 2002). As stated in the previous chapter, such practices may have great impacts on the ecosystem dynamics, and the decline of parasitic populations may put other species at risk. Host-parasite co-extinction has been described (Durden & Keirans, 1996; Strona, 2015), but also other indirectly involved species may be affected. A documented example involves the African rhinoceros, whose population has been declining, and the ticks that feed on them, whom have also lowered in numbers, an effect that is exacerbated by the purposeful removal of ectoparasites from captured rhinoceros before they are released. Simultaneously, a decline has been documented in the populations of oxpeckers (*Buphagus* spp.), whom feed on ticks from large mammals in southern Africa, including rhinoceroses (Durden & Keirans, 1996). There has not been established a cause-efect relation between these observations, but it is highly suggestive to be careful when addressing blanket eradication of parasites. The common practice of intentional parasite removal, either directly or by the administration of parasiticides, often seems justified if the survival of a host species is at stake (Durden & Keirans, 1996), but considering the complexity of ecosystem balance, it may affect the ecosystems in ways hard to foresee. Several studies suggest that blanket eradication strategies may not go without unintended costs for other species or even for human populations (Correale & Farez, 2007; Rook, 2009).

Fewer parasites would be welcomed by most individual animals, but would at the same time translate into less biodiversity, genetically and immune compromised host populations and damaged ecosystems (Combes, 1996; Durden & Keirans, 1996; Hudson, Dobson, & Lafferty, 2006; Strona, 2015).

2.5. Wildlife parasites and public health

Plenty parasites have zoonotic potential and most of them have a wildlife reservoir (Gómez & Nichols, 2013). Among the main zoonotic diseases that can be acquired through wildlife, namely in Brazil, one can find several parasitic diseases. Most are carried by primates: bertelliosis, capillariosis, echinostomosis, esophagostomosis, malaria, sparganosis and toxoplasmosis (RENCTAS - Rede Nacional de Combate ao Tráfico de Animais Silvestres, 2001). But there are many others, such as visceral larva migrans and cutaneous larva migrans (canids), echinocococosis (foxes, cervids), dirofilariosis (canids, procyonids), trypanosomosis (more than 200 mammal species), fasciolosis (herbivores), giardiosis (carnivores) and leishmaniosis (canids, rodents, equids, anteaters and sloths) (Silva, 2004).Transmission occurs by vectors such as mosquitoes and ticks, ingestion of contaminated water, plants or meat.

Illegal wildlife pets are also an important source of infection, as they do not pass any sanitary control. The stress these animals go through during the illegal wildlife chain may exacerbate shedding of infective forms (RENCTAS, 2001; Lima, 2007).

3. Wildlife rehabilitation and parasitism

3.1. Wildlife rehabilitation - what and why?

Wildlife rehabilitation is defined as "the treatment and temporary care of injured, diseased, and displaced indigenous animals, and the subsequent release of healthy animals to appropriate habitats in the wild" (Miller, 2012: ix).

This practice has forever existed in a home-based form, but, as environmental awareness and the concern for preserving wildlife rose, it has over the past decades evolved into an expanded field with increased knowledge and resources and support from collective experience (Miller, 2012). Nowadays, besides the ongoing home-based wildlife rehabilitators with little training and veterinary clinics providing this kind of care, there are centers exclusively dedicated to this activity, so called wildlife rehabilitation centers or facilities, with qualified professionals in veterinary hospital standard premises (Miller, 2012; Mullineaux, 2014).

The relevance of the rehabilitation of individual wildlife casualties is frequently questioned, with the main arguments against being the high cost of the rehabilitation process versus the low impact of the loss of an individual animal. These programs compete for funds with other more useful conservation programs (Pérez, Meneguz, Dematteis, Rossi, & Serrano, 2005; Branco, 2008). One may also question the ethics of extending the life of a suffering animal instead of relieving it with euthanasia. The main arguments used to justify the treatment and rehabilitation of wildlife are listed below:

- It is our moral and ethical responsibility to counter the negative actions of man on species demographics and individual animal welfare. Besides the purely moral aspect of taking care of the world we live in, this argument is supported by the fact that the majority of the cases admitted at wildlife rehabilitation facilities are direct or indirect victims of human activity: road traffic collisions, poisoning, pet inflicted injuries, disturbance of local environments, electrocutions, illegal poaching and illegal trade (Branco, 2008; Mullineaux, 2014).
- When endangered and/or unique indigenous species are concerned, the investment in individual animals does play an important role in conservation (Tribe, Hanger, Nottidge, & Kawakami, 2005; Saran, Parker, Parker, & Dickman, 2011).
- The animals received in a rehabilitation center are samples from the surrounding ecosystems and the collected data may provide all kinds of information:
    - Identification of environmental problem areas and activities, such as busy roads or polluted water courses, which would otherwise go unnoticed and may affect humans, allowing preventive measures to be taken (Clark Jr, 1999; Ramsden, 2003);
    - The detection of wildlife diseases may be of crucial importance for public health, being an important link in the national disease surveillance affecting conservation, livestock diseases and zoonoses. This is particularly important in emerging/re-

emerging infectious diseases. The animals received at rehabilitation centres serve as sentinel-hosts for pathological and environmental changes. Surveillance, monitoring, precocious diagnosis and control, are the key concepts for these emerging parasitic diseases (Randall, Blitvich, & Blanchong, 2012; Madeira de Carvalho & Alho, 2017);

  - Generate scientific knowledge. During the treatment and rehabilitation of these animals, an incalculable amount of information can be produced in many different fields, including vital parameters and biometric data, epidemiological research, behavioral analysis, toxicological research, physiological research, nutrition, reproduction, and so on. These animals are representative of wild ecosystems and are under normal circumstances hardly accessible, requiring a lot of resources and ethical considerations for these data to be collected (Branco, 2008; Lopes, 2015).

- Last, but not least, environmental public information and education is probably the most important role of wildlife rehabilitation. It is one of the vital, if not the main pillar for successful conservation (Gomes & Oliveira, 2012). Seeing animals victimized by road collisions, poison, firearms, illegal trafficking or loss of habitats is a way of visualizing an usually theoretical concept (Clark Jr, 1999). In order to raise and strengthen environmental and conservation awareness, it is important for the population to have a place to turn to when faced with wildlife casualties and to see that efforts are made to take care of them (Mullineaux, 2014). For these reasons, community support and involvement through volunteer training and public education should be encouraged in wildlife rehabilitation facilities (Miller, 2012).

Clark (1999) resumed that a wildlife rescue program should always rest on three main pillars that include the above-mentioned topics:

1. Care and rehabilitation of individual animals;
2. Environmental education of the public;
3. Participation in management and political activities that require knowledge acquired from captive animals towards the benefit of fauna in general.

All arguments considered, wildlife rehabilitation is accepted as an important activity, fulfilling a welfare, conservation and educational role, as long as it is conducted in a responsible and science based way, following the existing guidelines and not taking the risk of promoting potentially negative effects, such as causing unnecessary suffering to the animals or risking negative ecological repercussions that rehabilitated animals may have on existing populations when translocated (Mullineaux, 2014).

3.2. General outline of the rehabilitation process

3.2.1. Admission and triage

The nature of the admitted casualties varies according to the location of the rehabilitation facility, the groups of animals it targets and the existing infrastructure. There is a worldwide tendency for the bulk of the admissions to be composed by three major groups: immature animals, traumatic injuries (Mullineaux, 2014) and, depending on the legal arrangement of the country, wildlife that was kept in illegal captivity (Branco, 2008; Brandão, 2014).

Immature animals may or may not be injured or orphaned, but are by definition in need of supportive care until they fully develop and are ready to survive on their own in the wild. They are reported to make up 30 to 50% of all admissions in rehabilitation centers around the world (Mullineaux, 2014).

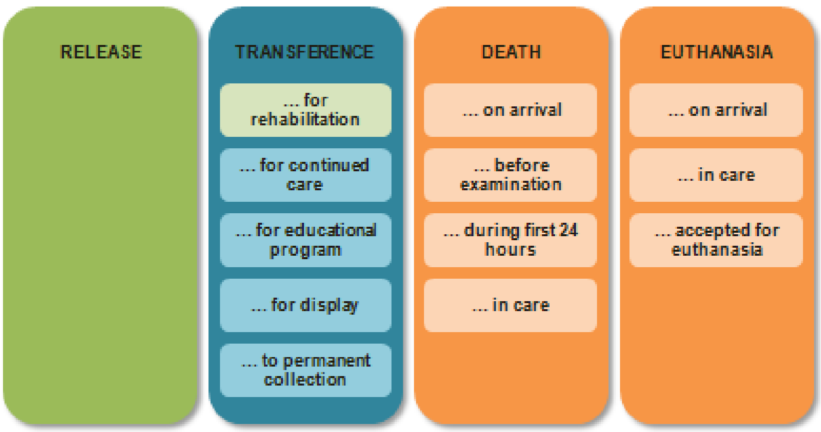
Traumatic injuries are reported to comprise 30 to 43% of all admissions and can have several causes, frequent ones being traffic road collisions (TRC), collisions of birds with cables or windows and predation by domestic animals (Brandão, 2014; Mullineaux, 2014).

The amount of animals coming from the illegal trade chain is much higher in countries where this market is bigger, but even in countries where the market is relatively small it can represent a large part of the admissions (ex. 24% in rehabilitation center in Portugal (Brandão, 2014)).

The low percentage (lower than 10%) of animals admitted due to so called natural causes, such as disease and debilitation doesn't mean there are no clinical cases in nature, but is easily explained by the natural hiding behavior from sick animals and the clear bias towards trauma in human-wildlife interaction areas, such as urban areas and cultivated land and roads, where the casualties are found (Weary, Huzzey, & von Keyserlingk, 2009; Mullineaux, 2014).

Once admitted to a rehabilitation facility, the animals face four possible fates: successful rehabilitation and release, permanent confinement due to factors preventing release, death from its injuries or euthanasia (Miller, 2012) (see Figure 6).

Figure 6 - Possible fates of animals admitted to a rehabilitation facility, divided according to Miller (2012).



The ultimate goal is always to rehabilitate and release these animals in a state of physical and psychological fitness that allows them to survive in their natural habitats, but it is frequent that the physical or behavioral limitations are so significant that releasing prospects are low or inexistent. In those cases, if the physical and psychological wellbeing of the animal can be assured in permanent captivity, one can treat and rehabilitate them in order to be transferred to a facility where they will remain in permanent human care, such as a zoological collection, environmental education or ex-situ breeding program. The decisions about what is best for the animal are not always straightforward and the inclination towards a certain decision may vary between different people or points of view (e.g. veterinary point of view versus conservationist point of view) (Miller, 2012; Mullineaux, 2014).

Everything revolves around the clinical condition of the animal and the suitability for eventual release, but the success of treatment and rehabilitation depends on many aspects, like facilities, training of personnel, veterinary services, funding and availability of release sites (Miller, 2012; Mullineaux, 2014). At all times the welfare of the individual casualty should be the overriding consideration. Unnecessary suffering should be prevented by making the triage decisions as quickly as possible and always consider euthanasia as a valid way of relieving the animal of pain (Cooper & Cooper, 2006).

High mortality rates (including euthanasia) are not abnormal considering the big percentage of trauma cases, which are frequently days old, adding to the fact that ideal treatment protocols of domestic animals are difficult to carry out in human shy and highly stressed, wild animals (Miller, 2012; Mullineaux, 2014). Figures for casualty mortality until 48 hours following admission, either as a result of death or euthanasia, are around 40% (Kirkwood, 2003; Molony, Baker, Garland, & Harris, 2007).

Release rates in the United Kingdom and Australia are reported to round 40%. Release rates are overall higher for birds than for mammals (across all ages), the latter tending towards 30%. Among the juvenile animals, the percentage of traumatic casualties is much lower, which is reflected in higher survival and release rates for this group (Mullineaux, 2014).

The minimum standards for wildlife rehabilitation of the International Wildlife Rehabilitation Council (Miller, 2012) divide the stay of the animal in stabilization (i.e. emergency care), initial treatment (intensive veterinary care), intensive rehabilitation (veterinary medical attention is less intensive and interaction is minimized), intermediate rehabilitation (medical problems are minimal or inexistent, human contact remains minimized and mental stimulation is provided as well as manual physical therapy if necessary) and, finally, pre-release conditioning (larger housing with unlimited activity, where daily exercise is stimulated and the diet should be as natural as possible).

3.2.2. Veterinary clinical care

First aid provision and emergency treatment follow the same basic principles as for domestic species, but some specific knowledge of the ecology, biology and specific problems encountered in the various species is necessary to guarantee a correct veterinary approach and adequate general handling (Mullineaux, 2014).

Wildlife animals tend to be highly stressed by human presence and interaction, and exhibit natural aggressive defensive behavior, which conditions the veterinary approach. Proper handling methods are essential to protect both handlers and patients, and sedation and anesthesia are used with a much higher frequency than in domestic species. The high impact of stress on health and recovery should not be neglected and should shape the veterinary approach. For instance, the advantage of a higher medication administration frequency may be irrelevant due to the stress it causes to the animal, and certain procedures are simply not possible due to the nature of these patients (Miller, 2012) .

3.2.3. Rehabilitation for release

Rehabilitation starts as soon as possible, in most of the cases when the animal is still receiving veterinary care. During the veterinary treatment, manipulation and handling is unavoidable and restriction of activity might be a condition for recovery. But once it is possible, conditions should be provided for the animals to express their natural behaviors and to improve their strength, develop stamina and coordination and restore muscle tonus, as a form of physical therapy and acclimatization to ambient weather conditions. This is accomplished by housing the animals in large and complex enclosures with enough space and enrichment to meet and encourage the species specific patterns of foraging or hunting, playing, resting, sleeping, hiding, predator avoidance (including humans and domestic animals such as dogs) and, if necessary, social responses to conspecifics or cage mates. Physical therapy should be primarily voluntary, but might be stimulated or in some cases forced by caregivers. Depending on the individual casualty, the rehabilitation process might comprise more or less steps and take more or less time for the animal to develop the physical and behavioral traits to be ready for release (Miller, 2012).

On physical level, rehabilitation shows overall good results and animals tend to compensate physical deficits fairly easy, with records existing of wildlife being successfully released even when missing a limb (Brandão, 2014; Geraldes, 2012).

Behavioral rehabilitation is a much harder nut to crack. When the animals have lived in the wild as adults, their natural behavioral traits may be easily restored. Rehabilitation is especially difficult when the animals never learned or developed the required behavioral traits, which happens frequently when they are reared in captivity, and translates into lower survival rates in reintroduction projects. Human imprinting reduces survival rate after release, as it reduces

the animal's ability to respond appropriately to predators and other hazards, or even induces abnormal denning and foraging behavior (Ben-David, Blundell, & Blake, 2002; Tribe et al., 2005; Jule, Leaver, & Lea, 2008).
Some species require larger and more complex enclosures for conditioning than others, such as animals that need deep pools or large predators that need big and secure enclosures where they have to be conditioned for hunting live prey and avoiding humans. Another limiting factor may be the absence of other animals from the same species for whom the inclusion in a group is crucial for a successful rehabilitation. In many instances, cooperation with other rehabilitation centers, which have the adequate conditions, might be the best or only strategy to follow (Miller, 2012).

3.2.4. Release

The primary goal of every rehabilitation facility is to retrieve to nature as many animals as possible, but release of a casualty is a decision that should not be made lightly. Release is an often underestimated component of the rehabilitation process, with potential for high losses (Vogelnest, 2008). Successful release should involve the integration of the animal in its natural habitat, including normal behavior and reproduction (Grogan & Kelly, 2013). This depends on a variety of factors, including clinical, physical and behavioral readiness of the animal, its life stage, the release strategy and the release site (Miller, 2012).
The animal should be at an adequate life stage, have no limiting diseases or physical disabilities, exhibit the behavioral traits and a sufficient level of physical fitness and stamina for essential activities such as foraging or hunting, breeding, migration and territory defense. It should exhibit normal behavior towards its conspecifics, prey, predators and other species it might interact with, including humans. Also the time of the year may be important, especially in migrating species. (Llewellyn, 2003; Miller, 2012; Tribe et al., 2005)
It is not enough for the animal to be an appropriate candidate for release. There has to be a suitable release site available as well. Selection of adequate release areas is critical to the release process, in order to minimize mortality (Miller, 2012; Tribe et al., 2005). The release site should consist of an appropriate habitat, with adequate geography, vegetation and climate to provide shelter, protection, mates for reproduction and an adequate and long-term food and water supply for the species in question. The size of the releasing site, the presence of roadways, human developments, natural or introduced predators and already existing populations of that species should be considered. Detailed knowledge of the species' and the individual's history and behavioral patterns is essential in choosing the correct habitat (Miller, 2012).
The selection of an appropriate release strategy is also a significant factor for the outcome of the process, wherefore the presence of adequate infrastructure is a significant factor (Miller, 2012). The release strategy can be broadly divided into hard release, which consists of simply

releasing the animal back into the wild, and soft release, which involves an initial provision of shelter and food within the release site. The latter is preferred in the majority of cases, while the first is mostly only considered adequate for adult short-term casualties (Llewellyn, 2003). Last but not least, releases should always be done in accordance to the local legislation.

Post-release monitoring of activity and behavior is always desirable. It is essential to evaluate the real success of release process and it allows the identification of factors within the rehabilitation and release processes that influence release success and contribute to decision making about the best approaches to release casualties (Guy, Curnoe, & Banks, 2013; Llewellyn, 2003; Mullineaux, 2014).

3.2.5. Records

Keeping records is of the utmost importance in a rehabilitation program. The statistical analysis of the recorded data can provide extremely helpful results. Knowing what are the most common species and the most common causes of admission allows planning of investments and future endeavors. Parameters such as mortality and release rates allow evaluation of performance and identification of problem points. The use of padronized parameters in different centers allows comparing results and mutual improvement by collaboration. Or the simple consult of old case files may help in new, similar cases (Miller, 2012).

It is highly recommended to use forms for each individual animal with information such as case identification number, species, date of admission, place of origin, cause of admission, anamnesis, initial clinical assessment, weight, contact of finder, weight, final disposition (with date and location), daily forms with information about food, medication and care and data regarding surgery, clinical pathology or necropsy.

Many governmental agencies, such as the US Fish and Wildlife Services or IBAMA (Brazil), require that wildlife rehabilitators report pre-determined information on their activity.

3.3. GI parasite management in wildlife rehabilitation

3.3.1. Importance of parasites in wildlife rehabilitation centers

Parasites play an important role in wildlife rehabilitation facilities for several reasons. Firstly, for their potential effect on the host, the target of the rehabilitation process. As described in chapter II)2. (page 5) onwards, parasites may have a detrimental effect on their host, and when their general condition is compromised, these effects may be exacerbated. The sole fact of the animal being kept in captivity is a stress factor, in addition to the cause of admission itself. Secondly, the introduction of a new animals has a potential effect on ecological, genetic or disease level, especially when animals are translocated (Griffith, Scott, Carpenter, & Reed, 1993; Robison, 2002; Wobeser, 2002; Vogelnest, 2008). Finally, many diseases are transmitted from animals to humans and also from animal to animal, including parasitic agents.

Public health is always a concern in order to protect humans, domestic animals and other wildlife patients (Miller, 2012).

An equilibrium has to be found between these aspects. On the one hand, one wants to guarantee the wellbeing and recovery of the individual animals, while keeping in mind that it will return to an ecosystem where parasites are ubiquitous, meaning that it will be reinfected. Furthermore, parasites are essential parts of healthy ecosystems, and their removal should not be handled lightly (Correale & Farez, 2007; Rook, 2009). On the other hand, one might be introducing new parasites in areas where they did not exist before, causing disturbance on a higher level (Griffith, Scott, Carpenter, & Reed, 1993; Robison, 2002; Wobeser, 2002; Vogelnest, 2008).

Branco (2008), analyzed several definitions of wildlife rescue centers and found that generally the focused unit is the individual animal. The goals are to improve its wellbeing and return it to nature, but the impacts of its return are often not considered. This reflects the conflict between the clinical side with the ecological and conservationist side. There is no wrong or right, but many times, there is lack of communication between different areas to decide on the most adequate path to follow.

3.3.2. Diagnosis of parasitic diseases

Miller (2012) recommends to perform a routine examination for parasites on all new arrivals, with re-examinations during prolonged stays. Diagnostic procedures are the same as described for domestic animals. For GI parasites, one mainly recurs to coproparasitological exams (Bowman, 2013). The wild nature of these animals brings some limitations, as close manipulation is often impossible. For example the Graham technique, where clear-cellulose tape is applied on the perianal area, is not possible to perform unless the animals are properly contained or anesthetized. Identification of egg type is in most cases fairly easy and enough to make clinical decisions (Bowman, 2013). Further parasite identification can be very challenging, since there isn’t much literature and topics like L3 larval stages have almost never been described. Nonetheless, morphologic characteristics can give an idea of a higher taxonomic division of the parasite in question (Ballweber, 2001).

Even though it’s very important to execute diagnostic tests, one should always be careful when interpreting results of positive diagnostic test, remembering that the presence of the agent doesn't imply that it is causing clinical signs (Ballweber, 2001). Coproparasitological and necropsy findings should always be crossed with the case history and clinical signs to arrive at a solid diagnosis (Bowman, 2013).

To simplify the decision making process, egg count thresholds to classify infections as "light" or "heavy" have been established for domestic species (mainly ruminants and horses), and are even used to establish a cut-off point above which an animal is dewormed, independently of the presence of clinical signs. The thresholds are different for different host species and different parasites. For instance, while >500 strongylid eggs/g is considered a high egg count in cattle, for sheep and goats it is only above >5000 eggs/g. At the same time, negative egg counts do not exclude infection, as nonreproductive worms such as arrested larvae or infertile adults may still be present. Results should always be interpreted keeping in mind the biology of both parasite and host (Bowman, 2013). When working with wildlife, a critical mindset is of high importance, since there exists little to none published data concerning what are considered normal or unacceptably high infection intensities.

3.3.3. Treatment of parasitic diseases

Published information, especially peer-reviewed literature, relating specifically to veterinary care and treatment of wildlife casualties is limited (Mullineaux, 2014). Most available publications concern valuable species, either because of their vulnerability statute (endangered species), their iconic status (e.g. unique species of a specific region, such as Koalas) or their commercial value (e.g. African wild game) (Zieger & Cauldwell, 1998; Mullineaux, 2014). Many wildlife veterinary manuals, such as *Fowler's Zoo and Wild Animal Medicine* (Miller & Fowler, 2014), *Parasitic Diseases of Wild Birds* (Atkinson et al., 2008) and *Exotic Animal Formulary* (Carpenter, 2012), indicate dosages and treatment plans for antiparasitic drugs in wildlife. These are often little developed and based on stochastic observations rather than controlled studies, but nonetheless are very helpful to choose and determine treatment plans.

3.3.4. Hygiene and cleaning

Facility cleanliness is an integral part of disease prevention and containment. Cleaning protocols vary considerably based on the species and condition of the animals in care, facility type, and enclosure construction. With these variables in mind, one has to select the cleaning method and the timing of cleaning effort (Miller, 2012).

Cleaning methods include physical cleaning, chemical cleaning and other methods such as flaming or steam cleaning. A basic cleaning technique consist of physical removal of organic matter (feces, food, dirt, etc.) followed by the use of a disinfectant. Physical removal can be dry (scraping, sweeping, picking-up) or with the aid of water and/or a detergent solution to facilitate the removal of certain debris. The removal of organic matter before disinfection is essential for its efficacy, since many disinfectants are inactivated by organic matter or even the presence of soaps or detergents (Miller, 2012).

Different products have different efficacies against different agents, as can be seen in Table 2, but none of the most commonly used products are specifically effective against nematode eggs or larvae. The best way to eliminate these is by simple mechanical means, albeit this method doesn't guarantee complete removal (Miller, 2012). Some highly resistant parasitic forms, such as ascarid eggs, are only eliminated through high heat, such as flaming or steaming (Roussere et al., 2003).

Due to the high prevalence of the roundworm *Baylisascaris procyonis* in raccoons, Miller (2012) recommends that their enclosures should be made of material that can withstand flaming or steam cleaning and even then used only for this species, since this parasite sheds eggs that are highly resistant and its transmission to other species can be fatal.

Table 2 - Properties of disinfectants. Adapted from Miller (2012), page 24.

Property or Spectrum of Action	Gram + bacteria	Gram - bacteria	Bacterial spore	Chlamydia	Fungi and yeasts	Viruses	Protozoa	Effectiveness organic matter
Phenol	high	high	none	none	low	moderate	low	moderate
Quaternary Ammonium Compounds	high	high	none	high	moderate	variable with formulation	moderate	low
Cresol	high	high	none	none	moderate	moderate	unknown	moderate
Alcohol	high	high	none	none	moderate	moderate	moderate	none
Iodophore	high	high	moderate	unknown	high	moderate	high	moderate
Chlorine	high	high	none	low	high	high	none	none
Aldehyde	high	high	moderate	unknown	high	high	unknown	variable with formulation
Chlorhexidine	high	moderate	none	none	moderate	moderate	low	moderate
Chlorine-dioxide	high	high	moderate	unknown	high	high	high	low

none low moderate high variable with formulation unknown

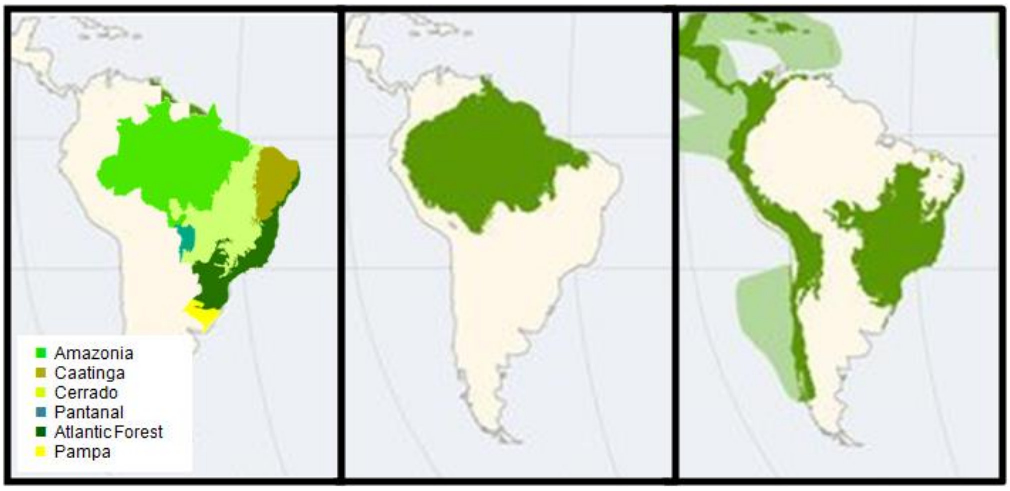
4. Wildlife rehabilitation in Brazil

4.1. The country's role in conservation

Biodiversity is one of the fundamental aspects of nature, responsible for the equilibrium and stability of the ecosystems (Tilman, 1999; Lehman & Tilman, 2000; Loreau, 2000) and Brazil pops up on every list addressing biodiversity richness, as it shelters an estimated 20% of Earth's biodiversity. The country harbors over 100 000 animal species and over 43 000 plants species, with new species being discovered every day. On world level, it ranks #1 in mammal, #2 in amphibian, #3 in bird and #5 in reptile biodiversity (RENCTAS - Rede Nacional de Combate ao Tráfico de Animais Silvestres, 2001; Groombridge & Jenkins, 2002; Branco, 2008; Secretariat for Social Communication of the Presidency of the Federative Republic of Brazil, 2012). These species are distributed among the marine and coastal areas and the country's six biomes: Amazonia, Atlantic Forest, Caatinga, Cerrado, Pampa and Pantanal (Figure 7).

The Amazonia and Atlantic Forest biomes, which account for more than half of the national territory (62.33% (IBGE - Instituto Brasileiro de Geografia e Estatística, 2004)), have the highest values of overall species richness (Jenkins, Alves, Uezu, & Vale, 2015), but that doesn't mean it receives equal conservation priority (Orme et al., 2005; Jenkins et al., 2015). The main factors for prioritization are irreplaceability, most commonly measured by species endemism[4], and vulnerability (Brooks et al., 2006; Brooks, 2010). There are nine major templates of global terrestrial conservation priorities[5] (Brooks et al., 2006; Brooks, 2010), and Brazilian territory is marked, more or less extensively, on all of them. The most highlighted areas are Amazonia and parts of the Atlantic Forest and Cerrado biomes (Figure 7).

Figure 7 - Map of South America showing (A) the Brazilian biomes (IBGE - Instituto Brasileiro de Geografia e Estatística, 2004); (B) Amazonia high-biodiversity wilderness area (HBWA) (Brooks et al., 2006); and (C) Biodiversity hotspots (Mittermeier, Turner, Larsen, Brooks, & Gascon, 2011).



[4] Endemic species is a species restricted to a particular geographic region.
[5] Crisis ecoregions; Biodiversity hotspots; Endemic bird areas; Centers of plant diversity; Megadiversity countries; Global 200 ecoregions; High-biodiversity wilderness areas; Frontier forests; and Last of the wilds.

As shown in Figure 7, parts of the Atlantic forest and Cerrado biomes are classified as biodiversity hotspots, i.e. areas with high numbers of endemic species combined with serious habitat loss (70% or more of the primary native vegetation) (Myers, 1988; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Mittermeier et al., 2011). Currently there are 35 regions worldwide which meet the hotspot criteria, the latest having been recognized in 2011 (Mittermeier et al., 2011; Williams et al., 2011). Combined, they comprise 2.3% of the Earth's land surface (only 15% of the original area once covered with this natural vegetation), but hold at least 50% of all plant species and 43% of terrestrial vertebrates[6] as endemics (Mittermeier et al., 2011). Highly irreplaceable and highly threatened areas such as biodiversity hotspots are the most urgent priority in conservation planning, in need of a reactive approach to prevent substantial biodiversity loss in the immediate future (Brooks et al., 2006; Mittermeier et al., 2011). The future of biodiversity hotspots is a so called reconciliation ecology, combining both conservation goals (sustainability of biodiversity) and human land uses, normally of populations who are socially and economically challenged (Brooks, 2010; Stork & Habel, 2014).

Amazonia, on the other hand, is one of the world's five high-biodiversity wilderness areas (Mittermeier et al., 2003). Mittermeier et al. (2003) identified a total of 24 wilderness areas around the world, defined as areas larger than 10 000km^2, with at least 70% of their historical habitat extent (500 years) and low human population density (<5 people/km^2). These wilderness areas, which include Pantanal and Amazonia, cover 44% of the Earth's land area (historically 52%), but represent a relatively low percentage of biodiversity (Mittermeier et al., 2003). The vast majority of the species of wilderness areas are harbored by only five of them, named high-biodiversity wilderness areas (HBWA) (Mittermeier et al., 2003), where endemic species richness meets the criteria of biodiversity hotspots (Mittermeier et al., 2011). These cover about 7.9% of the Earth's land surface and house 28% of the world's mammals and 20% of the world's amphibians (7% and 11% as endemics, respectively) (Mittermeier et al., 2011). While biodiversity hotspots are the Earth's biologically richest and most threatened terrestrial ecosystems, HBWAs are the least threatened highly diverse regions of the planet (Mittermeier et al., 2011). Highly irreplaceable areas with low threat such as HBWAs are also priorities on a conservation level. They offer considerable opportunities for proactive, large-scale and relatively low cost conservation actions, such as the creation of enormous protected areas like the 3 800 000 ha Tumucumaque National Park in Amapá, Brazil (Brooks et al., 2006; Mittermeier et al., 2011). In addition, these areas play a big role in the planet's climate balance (SECOM, 2012; Viana et al., 2013) and their low vulnerability status may be changing with the expanding search for lands with high cultivation potential, such as the fringes of the Amazon basin for crop culture (Phalan, 2013).

[6] Amphibians, mammals, birds and reptiles.

In conclusion, around 80% of the Brazil's terrestrial surface is considered global conservation priority area, 35% as biodiversity hotspots and the rest as part of the Amazonia HBWA (see Table 3).

Table 3 - Comparison of the Atlantic Forest and Cerrado biodiversity hotspots, Amazonia (high-biodiversity wilderness area) and Pantanal (non high-biodiversity wilderness area) (Myers et al., 2000; Mittermeier et al., 2003, 2011).

	Atlantic Forest	**Cerrado**	**Amazonia**	**Pantanal**
Original extent of primary vegetation	1 227 600 km^2	1 783 200 km^2	6 683 926 km^2	210 000 km^2
Remaining primary vegetation	7.5%	20%	80%	80%
Area protected	35.9%	6.2%	8.3%	2.7%
Occurring plants species	20 000	10 000	40 000	3 500
Endemic plant species	8 000	4 400	30 000	0
Occurring vertebrate species	2 070	1 135	2 523	765
Endemic vertebrate species	613	93	1 061	0

While invertebrates represent the bulk of eukaryotic diversity on Earth and have major roles in ecosystem functioning, they haven't been incorporated in global conservation priority analysis, not due to lack of interest, but because of lack of data (Brooks et al., 2006; Stork & Habel, 2014). However, high congruence has been found with conservation priorities for terrestrial vertebrate species (Brooks, 2010), and the role of biodiversity hotspots in the protection of fungi and invertebrates, including parasitic insects, has been suggested (Stork & Habel, 2014).

The anthropic pressure on the ecosystems and the consequent threats to wildlife are omnipresent and ever growing, ranging from growing urbanization, deforestation, wildfires, introduction of exotic species and livestock breeding to illegal wildlife trade (RENCTAS, 2001; Branco, 2008). Fortunately, Brazil's environmental legislation is considered one of the most advanced of the world, especially when fauna is concerned (Gomes & Oliveira, 2012). A holistic environmental approach was started in 1981, seeking to protect the environment as a whole and including ambitions for a data collection system, adherence monitoring and participation encouragement (Patriota, 2009). The Federal Constitution states that an ecologically balanced environment is a universal right and an essential element for a healthy life quality, wherefore it is a collective duty to protect and preserve it for the present and future generations (Federal Constitution/1988 art225). All native wildlife, also when in captivity (Law 7173/1983) is officially considered state property (Law 5197/1967) and the government is responsible for its protection (Federal Constitution/1988 art23 e 225). The importance of investing in the future generation's education was early recognized and the inclusion of the wildlife protection topic in school books and in radio and television programs is mandatory since 1967 (Law 5197/1967).

The number one threat to biodiversity is the destruction of habitats, so the cornerstone of conservation action should always be habitat conservation, which is best attempted by the creation of protected areas (Brooks, 2010). Brazil has recognized this importance and in fact has been leading the creation of protected areas worldwide (SECOM, 2012). In 2015, 17.6% of the country's continental area was protected (6.1% under full protection and 11.4% in sustainable use) (CNUC/MMA, 2015). While not created with the goal of biodiversity conservation, another 12% of the territory is under formal protection as indigenous land (Jenkins et al., 2015).

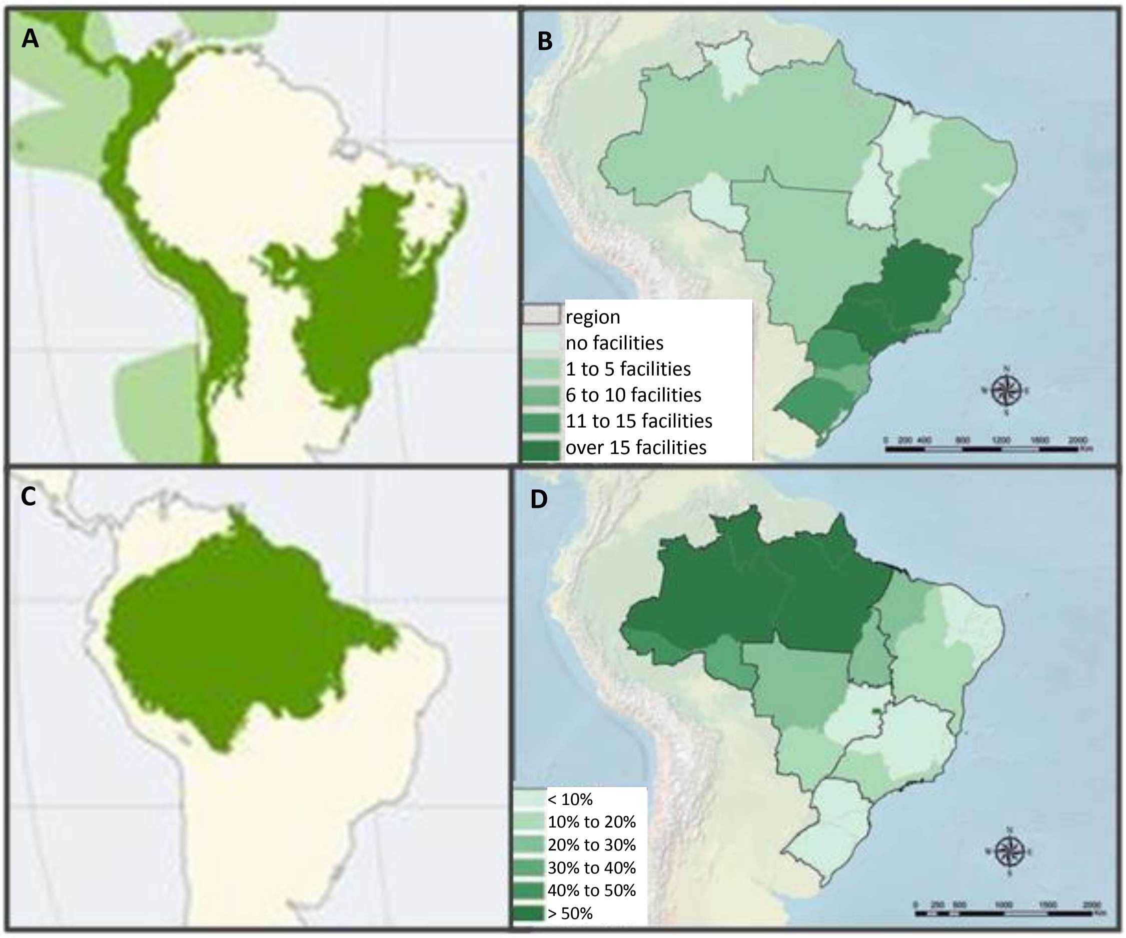
After habitat loss, the second biggest threat to Brazilian native species is their illegal capture for subsistence hunting or, with a much bigger impact, to supply the illegal wildlife trade (RENCTAS, 2001). Illicit wildlife trafficking by definition includes fauna, flora and all their products and byproducts (WWF/Dalberg, 2012). In this document, if not differently specified, the term will be used to address only fauna, its products and byproducts. When including timber and fisheries, illicit wildlife trafficking comprises the fourth biggest illegal trade in the world, after narcotics, people and counterfeit products and has despite all efforts been a growing market, since the economic returns are very big and the risks involved are relatively low (WWF/Dalberg, 2012). Animal wildlife illegal trade alone (excluding fisheries and timber) is estimated to move 8 to 10 billion US dollar each year (WWF/Dalberg, 2012), of which 900 million US dollar in Brazil (RENCTAS, 2001). The most complete report on illegal wildlife trade in Brazil was published in 2001 by a non-profit organization (Rede Nacional de Combate ao Tráfico de Animais Silvestres - RENCTAS) and reports an estimate of 38 million specimens that are removed from the country's habitats every year (RENCTAS, 2001). Sixty percent of the animals involved in illegal trafficking are believed to supply the national demand while the remaining forty percent enter the international market (Lima, 2007). Illegal wildlife trafficking is considered by some to be the most cruel factor that contributes to species extinction (Lima, 2007). For every wild animal product on the market three specimens are estimated to have died and when living animal trade is concerned, only one of every ten captured animals survives. With the exception of some rare, extremely valuable specimens, all animals suffer from abuse during the trafficking scheme, ranging from being drugged, having their teeth and nails cut or pulled out, feathers cut and corneas burnt or perforated or being killed (RENCTAS, 2001).

In 2001, only 0.45% of the animals involved in wildlife trafficking in Brazil were estimated to be apprehended by authorities. Although it is very small percentage, the absolute number of apprehended animals is big. In the 90's decade, between 16 500 and 60 000 animals were apprehended per year (RENCTAS, 2001). In 2005, 25 111 animals were apprehended in the State of São Paulo alone (Pinto, 2006).

Both during habitat destruction or interference and illegal wildlife trade, a considerable amount of wildlife specimens are found in need of support following injury and/or removal from their habitats. And one of the aspects that has to be covered is the ex-situ wildlife care for these animals, which has been done by the establishment of an ex-situ conservation and support network (see chapters below).

It is encouraging to notice that both reactive and proactive conservation initiatives are growing year after year. It is also reassuring that the concentration of proactive conservation measures correlate with the HBWAs, while the reactive measures are way more abundant in the biodiversity hotspots (Viana et al., 2013), exactly as recommended (Figure 8). Jenkins et al., (2015) described a correlation of between rates of protection and higher biodiversity, suggesting that the protection areas have been well defined. Nonetheless, it is essential to continue efforts to reduce habitat loss and fragmentation, expand formal protection areas, stimulate habitat regeneration and produce more scientific data in order to invest in the best measures and the right places (Jenkins et al., 2015).

Figure 8 - The concentration of proactive conservation measures correlate with the HBWAs, while the proactive measures are way more abundant in the biodiversity hotspots.



A) Biodiversity hotspots (Brooks et al., 2006); B) Concentration of ex-situ biodiversity facilities: zoological and botanical gardens (Viana et al., 2013); C) Amazonia high-biodiversity wilderness area (HBWA) (Mittermeier et al., 2003, 2011) and D) Proportion of protected area (fully protected, sustainable and indigenous land) (Viana et al., 2013).

4.2. Legal considerations of ex-situ wildlife management in Brazil

The use and handling of wildlife in captivity in Brazil is strictly regulated and can only be done in properly licensed and registered establishments. The authorized categories of wildlife handling facilities are stated in the Normative Instruction nº7, April 30, 2015 (IN nº 7/2015), which recently substituted IN nº 169/2008. While all considered under the same article, the categories can be split in two main types:

a) Those where wildlife is exploited in a commercial way: these establishments form a legal alternative to meet the existing demands and are an important livelihood, especially in rural communities. This group includes the following categories:

- Commercial breeder: undertaking with the purpose of keeping and breeding wildlife specimens in captivity in order to alienate life specimens or their parts, products and byproducts;
- Merchant of live wildlife animals: commercial establishment with the purpose of alienating live wildlife animals, but not to reproduce them;
- Slaughterhouse authorized to slaughter, avail and alienate parts, products and byproducts of wildlife specimens;
- Merchant of parts, products and byproducts of wildlife fauna.

b) Those who function as a support network for wildlife with conservation, research and environmental education purposes, including:

- Wildlife triage center (CETAS - *Centro de Triagem de Animais Silvestres*): a private or public undertaking with the purpose of receiving, identifying, triaging, evaluating, recovering, rehabilitating and destining wildlife specimens apprehended during legal inspections, rescued or voluntarily turned in. Commercialization of the specimens is forbidden;
- Wildlife rehabilitation center (CRAS - *Centro de Reabilitação de Animais Silvestres*): a private or public undertaking with the purpose of receiving, identifying, triaging, evaluating, recovering, rehabilitating and destining native wildlife specimens with the goal of reintroduction in their natural habitat. Commercialization of the specimens is forbidden;
- Scientific breeding center for conservation purposes: non-profit establishment tied to an authorized conservation program where native wildlife animals are kept and reproduced in captivity, in order to conduct or support conservation and environmental education programs. Exhibition and commercialization of the specimens is not allowed;
- Scientific breeding center for research purposes: non-profit establishment belonging to or connected to a research or education institute, where wildlife specimens are kept and reproduced in captivity in order to conduct or support scientific research and education. Exhibition and commercialization of the specimens are strictly forbidden;

– Wildlife keeper: non-profit undertaking with the purpose of keeping wildlife specimens in captivity and where reproduction, exhibition and alienation are not allowed;
– Zoological garden: undertaking where a collection of wildlife specimens are kept in captivity or semi-liberty, available for public visitation, with scientific, conservationist, educative and sociocultural purposes.

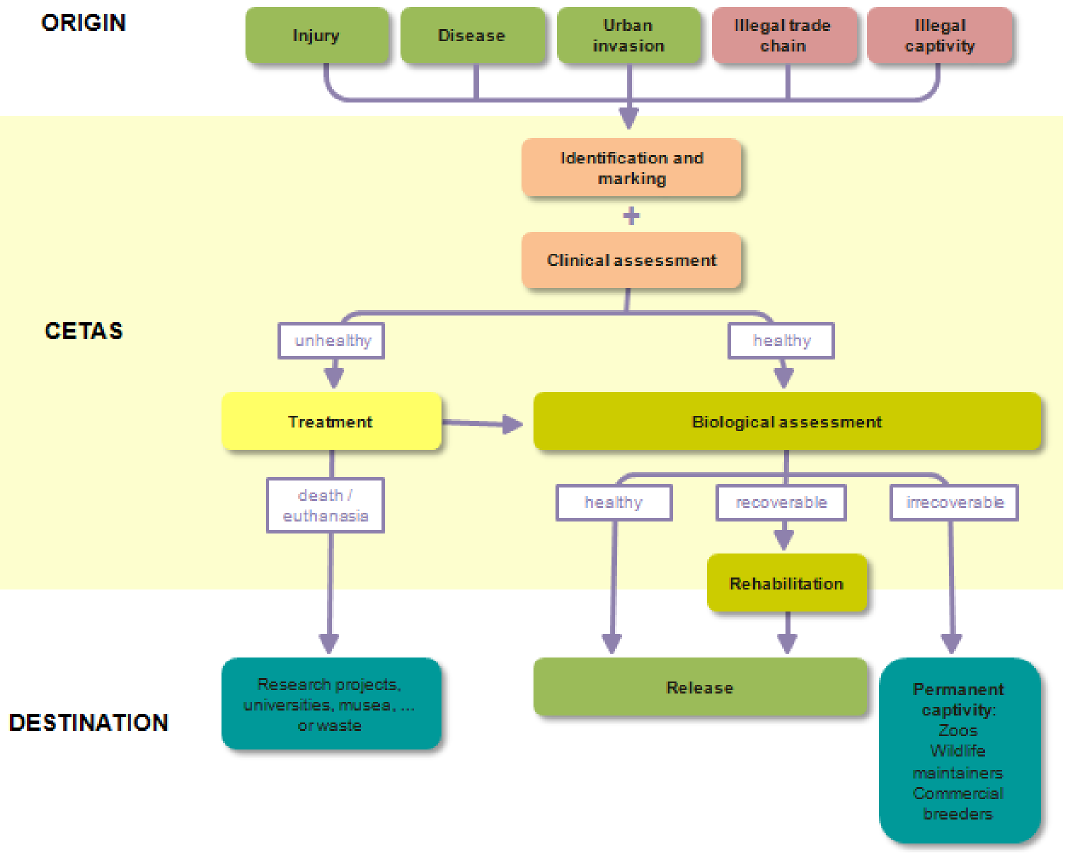
In order to get a license, both architectonical and working plans have to meet all legal requirements. These have to be detailed, containing information such as keeping and/or receiving capacity, hygienic and sanitary measures, individual animal identification system, emergency plan, diet that will be provided, handling and restraining measures, reproductive control and neonatal care (IN nº 7/2015).

Both CETAS and CRAS are very similar in nature, having overlapping functions. They are therefore frequently addressed all under the most commonly used term CETAS (Lo, 2012a), and this connotation will also be applied in this document.

4.2.1. Rescued wildlife management

The idealized flowchart of the journey of rescued wildlife in Brazil is pictured in Figure 9 and will be addressed step-by-step in this chapter.

Figure 9 - Ideal flowchart of the journey of rescued wildlife (Original).



4.2.1.1. Origin

The rescued wildlife received in CETAS are essentially animals which were directly or indirectly victimized by anthropic actions (Romano et al., 2012) and come from two major sources: the illegal wildlife trafficking network or directly from their habitats.

Animals in the illegal wildlife trade may be intercepted at various points of the trade chain:

a) Confiscation during the actual illegal trade chain, between capture and reaching their final owner (Figure 10);
b) Confiscation of illegal wild pets at the owner's property;
c) Voluntary turn-in by owners of illegal wildlife pets, which can be done anonymously and without consequence for the detainer (DF nº 6514/2008). It is frequent for owners of illegal pets to lose the interest in keeping the animal once it grows, develops aggressive behavior or for some other reason doesn't meet their expectations (RENCTAS, 2001). Not having another channel to dispose of the animals, they often release them into nature, without a professional evaluation of the survival ability of the specimen and risking to unbalance the ecosystem by the introduction of non-endemic species. With this measure, the uncontrolled release of these animals can be reduced.

Figure 10 - Twenty-seven blue-fronted amazons (*Amazona aestiva*) confiscated during a routine car search, in the state of São Paulo, Brazil, in the condition they were found (Original).

Wildlife rescued from their habitats are mostly sick or injured animals, predominantly due to anthropic causes such as motorway accidents, electrocution, wildfires, deforestation and loss of parental care (frequently after illegal hunting or motorway accidents involving the mother). Animals that invade urban spaces, creating a potentially hazardous situation for the human population and/or themselves also frequently land in the ex-situ network, but are generally rapidly released.

In order to attend these rescued animals, there have to be a) places to receive, house and treat these animals and b) a way for these animals to reach these centers.

4.2.1.2. Rescue and transportation

In Brazil, the rescue and transportation operations are supported by law enforcement (such as the environmental military police (*Polícia Militar Ambiental*) and highway patrol) and civil protection (fire brigades), who fully invest in rescuing and transporting these animals, sometimes hundreds of kilometers. It also happens that rescue and reintroduction actions are supported by military intervention (Reis, 2011; Portal Brasil, 2015a) or even by protocols with public airlines (Portal Brasil, 2015b; Martins, 2017). Other public and private entities also often bring rescued animals directly to the center and voluntary turn-ins are very frequent (Milanelo & Fitorra, 2012).

4.2.1.3. CETAS

The Brazilian legislation states that apprehended wildlife who aren't eligible for immediate release in their natural habitat (DF nº 6514/2008; DF nº 6686/2008) such as domesticated animals or animals who have no survival chances in their natural habitat (IN nº 28/2009 - IBAMA) for another reason (like injury or disease), should be destined to CETAS.

A CETAS should be located on a totally fenced terrain and include adequate enclosures and equipment to keep, handle, restrain, treat and transport wildlife; an area for food preparation; a veterinary clinic; and a bioterium to provide live prey if necessary. There have to be animal keepers, security service and a supervised externship program. All animals have to be taxonomically identified and there has to be a full quarantine program. Records of the center's release programs should be kept and there has to be literature available to consult. (IN nº 3/2015)

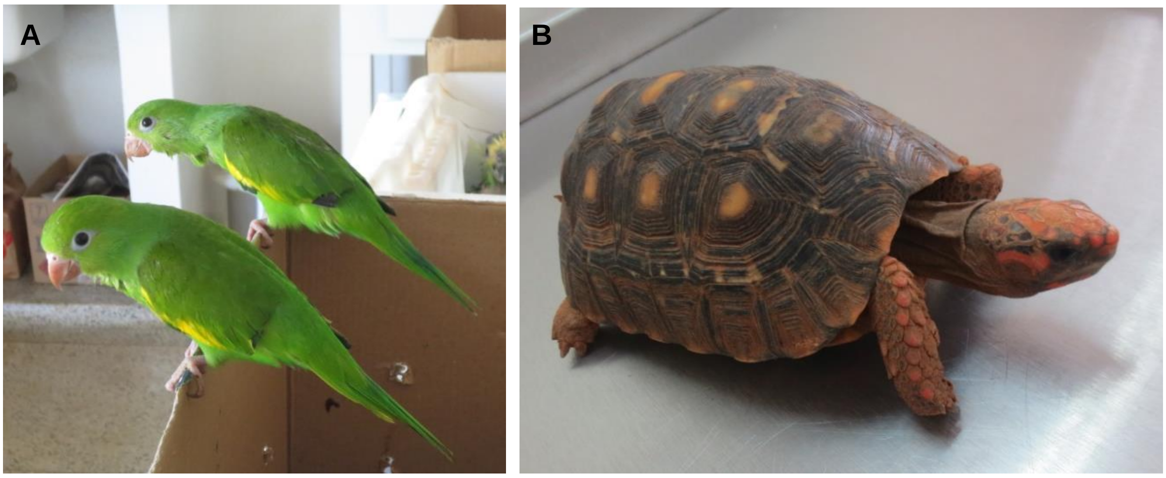
IN nº 23/2014 defines the guidelines and procedures for the functioning of CETAS of IBAMA. These guidelines are also used by many other CETAS, as they describe a thought through course of action and make sure all the data required for the annual reports are generated.

4.2.1.3.1. Reception and triage

All animals entering a CETAS should be registered, given an entry number and be individually identified (IN nº 23/2014) by microchip (all species), leg ring (birds), ear tag or tattoo (mammals) or other individual identification systems (IN nº 20/2013; IN nº 2/2001 - IBAMA). The entrance ID, species and individual identification should be recorded on a registration form together with information about the origin and background of the animal and personal information of the deliverer (IN nº 23/2014). When delivered by law enforcement agencies a copy of the police report should be annexed to the registration form (IN nº 23/2014). The individual identification of the animals is a very important measure, not only for the practical and obvious reason to know which animal it is, but also to avoid the entrance of the animals into the black market. Each animal is accompanied by a paper trail from its capture until its release.

A physical examination is performed by a veterinarian and when necessary further diagnostic procedures and treatments are carried out (IN nº 23/2014). While obviously necessary in sick or injured animals, animals from illegal captivity are often also in need of veterinary attention. Animals are known to suffer terribly during the trafficking progress (between capture and commercialization), but also at their final homes illegal pets are frequently kept in inadequate conditions (Figure 10; Figure 11). Historically this was frequent due to the lack of experience and knowledge about the keeping of these animals, but also today, in spite of the studies and knowledge that have been generated about wildlife keeping, many buyers are unaware of or ignore even the minimal necessities of these animals (RENCTAS, 2001).

Figure 11 - A) Yellow-chevroned parakeets (*Brotogeris chiriri*), confiscated as illegal pets, with the feathers of the wings cut in an inappropriate way; B) The striations and pyramid formations on the shell of this red-footed tortoise (*Chelonoidis carbonaria*) specimen are indicative of inadequate nutrition and are often seen in captive tortoises (Originals).



Besides the clinical assessment, a behavioral assessment is also carried out (IN nº 23/2014), since being clinically healthy, does not equal having the ability to survive in their natural habitat. Based on their background and the clinical and behavioral assessments, the animals are admitted for treatment and/or rehabilitation, or routed for immediate final disposition (IN nº 23/2014).

IN nº 23/2014 includes the following list of suggested standard laboratory exams to be performed during the quarantine period: hematology, blood biochemistry, hemoparasite check, urinalysis, ectoparasite check, stool Gram stain and coproparasitological analysis, including direct observation, flotation and sedimentation techniques. These are followed by a list of recommended diseases to be tested in the different groups of animals for epidemiological research. The referred agents are mainly viral and bacterial, but also include a few protozoan agents and one nematode: dirofilariasis in carnivores in endemic areas. In other words, a high focus on the diagnosis of both ecto- and endoparasitism is recommended, but specific parasitic nematode diseases are not considered to be of epidemiological importance.

The entire stay and evolution of each animal should be documented on clinical forms, including the animal's identification data, anamnesis, biometry data, results of physical exams and laboratory tests, all administered treatments, behavioral assessments and other pertinent observations. In case of death, necropsy should be carried out and registered on a necropsy form (IN nº 23/2014).

4.2.1.4. Disposition

By definition, a CETAS is a place to receive and give the necessary care to rescued wildlife and, once their needs have been attended, destine these animals (IN nº 7/2015; IN nº 23/2014). There are two major options: release into their natural habitat or permanent captivity (IN nº 19/2014). The decision is made based on criteria published by IBAMA, by the International Union for Conservation of Nature and Natural Resources (IUCN) (Romano et al., 2012), and under the terms of IN nº 23/2014, IN nº 19/2014, DF nº 6514/2008 and Law nº 9605/1998.

<u>Release</u>

Detailed legal norms put release in their habitats as the priority procedure (IN nº 19/2014), possible for animals who present behavioral traits of being recently captured and no signs that could compromise their survival chances in the wild, into an area where the species is endemic (IN nº 23/2014). Even specimens who are circumstantially inapt to be released should be integrated in programs aimed for release, population reinforcement or research for reintroduction protocols (Yamashita & Seino, 2012; IN nº 23/2014). I.e.: all efforts should be directed at the reintroduction of apprehended fauna into nature.

The creation of release and monitoring areas and release programs are important measures to make it possible to follow the legislation (Yamashita & Seino, 2012). Licensed releasing areas should be officially registered and provide information concerning their area, conservation status, vegetation, soil use and occupation, springs and water courses, existing native vegetation corridors, presence of mild release infrastructure and an indication of suitable species to be released (IN nº 23/2014).

Immediate release may be carried out by the confiscating agents at the time of inspection if the criteria for immediate release are met (IN nº 19/2014).

Permanent captivity

If release is infeasible or not recommended for sanitary reasons, native wildlife animals are handed to zoos, foundations, entities with scientific or educational character, triage centers, breeders or similar entities, provided that they are entrusted to qualified technicians (IN nº 23/2014; IN nº 19/2014; CONAMA resolution nº 457/2013; DF nº 6686/2008; DF nº 6514/2008). Exceptionally, apprehended animals may be entrusted to a legal guardian, preferentially under the responsibility of public organs or entities. Legal guardians may be environmental, scientific, educational or other types of entities, individual third parties or the accused himself. When entrusted, the animal has to be identified with a primary and secondary identification system. A deadline is defined for the trustee to file the necessary documents to gain permanent custody of the animal, case this is an option (IN nº 19/2014).

Death

The carcasses of animals that died may be destined to research or teaching facilities that formally manifested interest for this material. Carcasses that were not solicited should be treated according to biological waste legislation. In addition, the whereabouts of the carcasses have to be formalized and registered. (IN nº 23/2014)

4.3. Reality and numbers - current situation

CETAS and CRAS were legally defined for the first time in 2008 (IN nº 169/2008), but obviously these type of facilities were already essential before their official recognition, and many developed naturally, without formal action (Lo, 2012a). They usually functioned in existing establishments that were already involved with wildlife, such as zoos and some NGOs (Lo, 2012a) or were founded with the exact purpose of wildlife rehabilitation, such as the CRAS of Campo Grande (Mato Grosso do Sul), in operation since 1988 (Branco, 2008).

There are both governmental and non-governmental CETAS. The first are managed on federal, state or municipal level and the latter are generally maintained by nongovernmental organizations (NGOs), universities or private companies (Lo, 2012a).

In 2008, Lima reported forty-two existing CETAS in Brazil. In 2005, the CETAS Brazil Project was launched, with the main goal of building, renovating and/or expanding 117 CETAS throughout the country. The places for these initiatives would be strategically chosen based on studies of the main wildlife traffic routes and proximity to airports and universities to create the possibility of establishing cooperation (Lima, 2008). Currently, IBAMA owns 24 CETAS distributed in 21 of the 27 federative units (IBAMA, 2017a). In the last decade, several new CETAS were opened in different states and there are still new CETAS under development (Porto, 2008; Conceição, 2010; Mendes, 2010; Lo, 2012b; Mineração Rio do Norte, 2012). Others are being improved and augmented, such as the CETAS/IBAMA in São Luís, Maranhão

(Fundação Josué Montello, 2017). But, at the same time, CETAS are closing due to various reasons, mostly lack of resources (Azevedo, 2017).

Over the last years the responsibility for CETAS/CRAS, reintroduction areas and projects has been transferred from federal to state level (Complementary law 140/2011; Instituto Estadual de Florestas, 2013; Rocha, 2014; Folha Web, 2014).

Some states and municipalities have their own CETAS, such as the CRAS in Mato Grosso do Sul, CRAS-PET-DAEE (*Centro de Recuperação de Animais Silvestres "Orlando Vilas Boas" - Parque Ecológico do Tietê*) and DEPAVE-3 (*Divisão Técnica de Medicina Veterinária e Manejo da Fauna Silvestre*) in São Paulo.

Brazilian legislation obliges companies that use environmental resources that may have any environmental impact to take preventive and compensatory measures, including fauna and flora conservation. These are included in the project from the start and are a requisite for obtaining a license. They vary in depth depending on the magnitude of the environmental impact. Examples of measures are animal rescue before and during the undertaking, ex-situ reproduction programs (fauna and flora) or construction, financing and/or management (for a pre-determined amount of time) of a CETAS. This way, many non-governmental CETAS have been established in the country. (CONAMA resolution nº237/1997; Sousa, 2015)

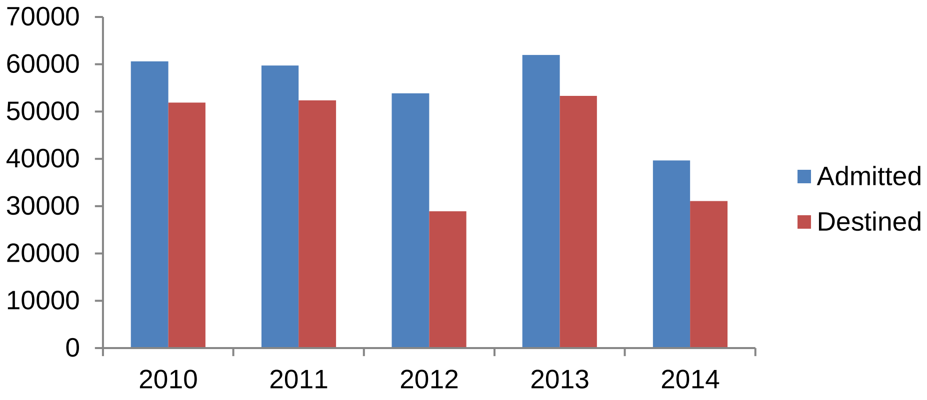
A report concerning the State of São Paulo showed that even though non-governmental CETAS outnumber the governmental ones, the latter receive the vast majority of the rescued animals (80-90%) (Lo, 2012a). This is in accordance to the fact that these establishments tend to be more stable, allowing a more consolidated and long-lasting line of work. Non-governmental CETAS have shown to be more frequently temporary. A clear example of these temporary CETAS are some of the ones created and maintained as a compensatory measure, running out of funding when the period of obligation comes to term, or even before opening (Mariano, 2017; Martins, 2017).

Most zoos, many NGOs and some wildlife breeding centers and university veterinary hospitals, even though not licensed and registered as CETAS, are known to receive rescued wildlife and are resorted to by inspection agencies (Lo, 2012a). Functionally, this is a very positive aspect, as it enlarges the supporting network for rescued wildlife, but it is also a sign that the existing facilities specifically designed for this purpose are not enough and/or are not located in the right places. Ideally, the establishments that provide this service should also register under this category, in order to be legally in order and to be included in the statistics, providing a more realistic picture of wildlife management.

4.3.1. Admissions

The size, conditions, infrastructure and localization of each CETAS can vary greatly, and consequently so can their capacity. There are centers who receive as little as 200 animals or less per year, to others who receive more than 5000 animals per year. During the last years, the amount of animals admitted to CETAS of IBAMA nationwide oscillated between 40 000 and 60 000 animals per year (see Figure 12). During 2003-2011 the CETAS of IBAMA in the state of São Paulo accounted for only 13.75% of the animals received in the registered CRAS/CETAS of the state (Lo, 2012a), not even considering the non-registered establishments who also receive rescued wildlife. If the same tendency exists in other states, the real number of animals who find their way to a CETAS may lay far higher.

Figure 12 - Number of animals received at CETAS of IBAMA from 2010 to 2014 (IBAMA, 2017b)



The majority of the admitted animals are related to the illegal wildlife trade. Animals rescued directly from their habitats represent only a small proportion. The revoked IN nº169/2008 included an estimate of the proportion of animals entering a CETAS as 80% birds, 15% reptiles and 5% mammals. Most reports do not deviate much from these numbers (Destro, Pimentel, Sabaini, & Barreto, 2012; Milanelo & Fitorra, 2012), commonly reporting around 70-80% of avian admissions, but there are centers with significant shifts. In the *CRAS - Batalhão de Polícia Militar Ambiental* (BPA), in Rondônia, in 2010, 2011 and 2013, the admissions consisted of 53% birds, 29% mammals and 18% reptiles (Lima & Silva, 2014).

4.3.2. Final disposition

In the state of São Paulo, between 2003 and 2011, 30.5% of the admitted animals died or were euthanized, 36% were released and 25% still remained at the CETAS. There is a big variation between centers. For instance, mortality rate varied between 17% and 44% and release rate between 17% and 56%. This may be due to differences in management, resources or protocols with releasing sites and programs. One can also see variations in different years for the same center. This may be consequence of different internal and external factors, such as the implementation of IN179/2008, which introduced stricter requirements for release and reintroduction projects and areas, leading to a decrease in release and reintroduction rates in 2009 (Lo, 2012a).

Some centers report release rates higher than 70% (Universidade do Vale do Paraíba, 2014 ; "Criadouro de São José reintroduz animais na natureza," 2015). When considering only animals from the illegal trafficking network, release rates of 38 to 65% were reported (Destro et al., 2012).

In 1999 and 2000, 78% of the apprehended animals in Brazil were released, most of them directly by the confiscating agents, without passing through a CETAS (RENCTAS, 2001). More recent reports, considering only confiscated animals from illegal wildlife trade in the state of São Paulo state that less than 20% of the animals are returned to their natural habitats. From those that were kept in permanent captivity, 20% remained with their owners (Branco, 2008). Disposition to commercial breeders has been a solution for a large numbers of avian species and contributes to the creation of a legal alternative for the wildlife pet demand, but would never be the preferred choice if reintroduction would be an option. These animals are only allowed as breeding animals and may in no situation be commercialized. Descendants may be commercialized from the first or, in case of endangered species, only from the second generation onwards (Portaria nº 118-N / 97). The creation of a legal alternative to supply the existing demand in the pet market through commercial breeders of native wildlife is an important step in the combat of illegal wildlife trade (Gomes & Oliveira, 2012).

Post-release monitoring is performed by some CETAS, as for instance DEPAVE-3, in São Paulo, who performs passive monitoring by reencountering identified animals and active monitoring with mist nets (Romano et al., 2012).

4.3.3. Social role of CETAS

4.3.3.1. Scientific contribution

Much scientific output on wildlife is generated through CETAS, frequently in collaboration with universities. IBAMA itself states that the participation in scientific studies is one of the main goals of CETAS (IBAMA, 2017b).

A simple search in Google Academic ® yields dozens of results with publications done in CETAS all around the country, in the most diverse areas (e.g. parasitology, epidemiology, physiology). A report from only one CETAS concerning the 1990's decade showed yearly outputs as high as 400 pathologic, 750 hematologic and 1100 parasitological tests (Branco, 2008). These studies are not only relevant on conservation, biology and ecology level, but can be also important for public health concerns. For instance, DEPAVE-3 had a role in studies concerning hantavirus and leptospirosis epidemiology (Branco, 2008).

4.3.3.2. Environmental education

Besides the obvious role of being a place to turn to for citizens faced with a wildlife casualty, many CETAS have an active role within the community and encourage public participation and education with programs such as volunteering, externships, involvement of the populations during releases, school visits, guided visits, presence in events, talks and publications (Pellegrini, 2008; Behling et al., 2014; Ascom/Ibama/PI, 2015).

4.3.4. Final considerations

While official environmental organs for inspection against illicit activity were early established and organized, the network to receive the rescued animals was not (Lo, 2012b). CETAS naturally developed and were later actively created, but are still clearly not meeting the existing demands (Nogueira & Sena, 2012).

CETAS are overall reported to be working at or over their maximum capacity, receiving more animals than they can ideally tend to (Associação Mineira de Defesa do Ambiente, 2009; Develey, 2012; Coissi, 2015). In 2000, CETAS were reported to face financial and technical difficulties, being overcrowded and not being able to receive newly apprehended animals (RENCTAS, 2001), and this phenomenon continues until nowadays (Branco, 2008; Gomes & Oliveira, 2012; Azevedo, 2017). The amount of animals entering CETAS is far below the amount of registered apprehensions. In the state of São Paulo, only about half of the apprehended animals are admitted to a CETAS (Lo, 2012b). There are also reports from CETAS with considerable infrastructure problems due to lack of maintenance (Souza, 2014) or even refusing further admissions because they don't have enough resources to feed the animals (Satriano, 2015).

The main problems associated to the difficulties CETAS face are the high maintenance costs, overcrowding, the small number of CETAS and zoos and the lack of scientific knowledge to perform the releases (RENCTAS, 2001; Develey, 2012).

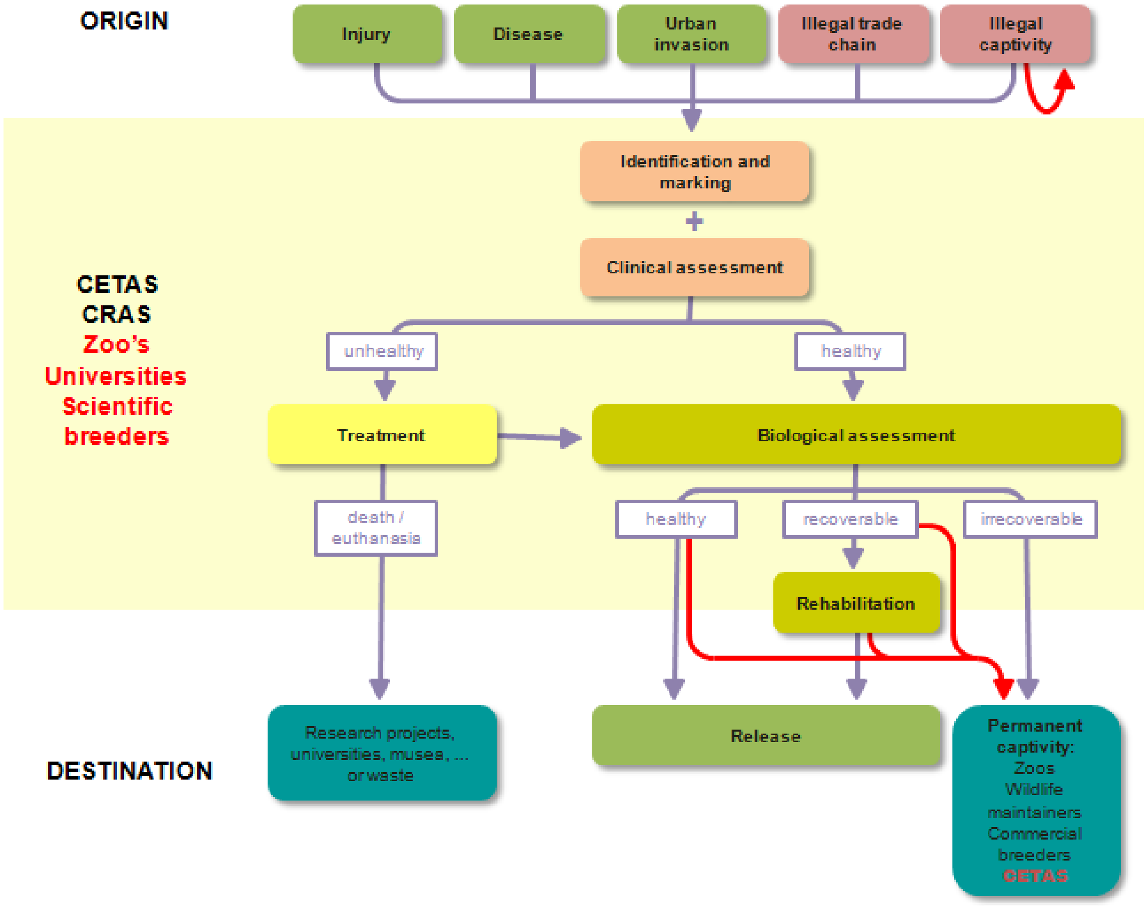
As the maximum capacity is overridden, the reception of a higher number of animals has been suggested to be related with a higher mortality rate (Lo, 2012a). Overcrowding of the CETAS also causes these centers to start refusing animals (Develey, 2012; Coissi, 2015), starting by those who don't need immediate veterinary care, which are mostly illegal pets kept under good housing conditions. Not having a place of destination for these animals, it is frequent for illegal wildlife pet owners to be fined but given permission to keep the animals as legal guardians (Branco, 2008). The lack of centers to receive the apprehended animals directly contributes to an inefficient working of the inspection and control activities (RENCTAS, 2001). Destro, Pimentel, Sabaini, & Barreto (2012) stated that CETAS are essential support structures for the environmental enforcement actions related to fauna in Brazil, and that the creation, implementation and maintenance of more CETAS is one of the structural measures required to improve actions against illicit wildlife trade.

An issue that greatly limits the receiving capacity of the CRAS/CETAS is the difficulty in destining the animals, causing unnecessary overcrowding of the centers (Nogueira & Sena, 2012; Globo Paraíba, 2015). They sometimes keep many animals that have already recovered and are awaiting final disposition, consuming space and resources that could and should be dedicated to attend newly incoming animals. When the species are rare and/or popular, disposition to zoos is quite easy, but in the majority of the cases, it's hard to find a definitive home for the non-releaseable animals. Adding to those, there are two groups of animals with potential to go back to the wild competing for these permanent captive places. First, those clinically and functionally ready for introduction, but without legal reintroduction areas to be reintroduced at and lack of reintroduction programs to accompany the animals during and after the release to evaluate the result. Second, there is a high number of animals who are not ready to be reintroduced but could be rehabilitated, but for whom there are no conditions to complete the rehabilitation process, as the rehabilitation process requires people and infrastructures that are not always in place. The missing elements may be as simple as the lack of a big flight cage to exercise birds, or as hard as installations to teach a large feline to hunt and avoid humans (Ascom/Ibama/PI, 2015; Globo Goiás, 2015). The absence of conditions to rehabilitate animals in the CETAS and the lack of specialized behavioral rehabilitation centers/programs are an important missing link in the animal rescue chain.

The creation of more release areas is essential to solve this problem. Registered release and monitoring areas could be the most important partners of CETAS, and would ideally work as an extension of its work, as the final step of the rehabilitation process. The more and better releasing areas are available, the easier it will be to quickly and efficiently releasing the animals, avoiding clogging of the CETAS with animals awaiting release, the bulk of which are healthy birds originated from the illegal wildlife trade chain. This way animal welfare would be maximized, more attention could be given to animals in need, more animals could be admitted and there would be a retrograde flux of information about the success of release, making space for constant improvements along the whole process. These areas are also ideal places to implement conservation programs, environmental education programs and scientific research. (Nogueira & Sena, 2012)

The legislation to regulate the ex-situ wildlife support system is in place and describes a good and complete system which is pictured in Figure 9. In reality, the ex-situ network is not big enough to respond to all cases in need of care, and some other pathways are followed, as shown in Figure 13.

Figure 13 - The red details show the less ideal but frequent steps that happen in the pathway of rescued wildlife (Original).



III) Experimental work

1. Goals, material and methods

With the main goal of characterization and understanding of GI parasite management in wildlife in Brazil, the experimental work was divided into two parts:

A. A survey directed at organizations all around the country in order to:
   a) learn about their routine diagnostic and treatment approaches to GI parasites;
   b) evaluate differences in approach between animals in temporary and permanent human care;
   c) identify the limiting factors and possibilities for a more thorough GI parasite management.

B. A detailed case study of one of these organizations, in order to:
   a) Get a better understanding and a practical insight of the dynamics and possibilities of GI parasite management within these undertakings.
   b) Characterization of the GI parasitological profile of the wildlife managed by the organization:
      - prevalence and its relation to age, clinical signs, body condition and time in captivity;
      - influence of frequency of sampling;
      - evaluation of antiparasitic drug efficacy.

1.1. Characterization of gastrointestinal parasite management in wildlife in Brazil

1.1.1. Material and methods

1.1.1.1. Eligibility criteria

From all organizations involved in wildlife management in Brazil, the questionnaire was directed at wildlife triage centers (CETAS), wildlife rehabilitation centers (CRAS), scientific breeders for conservation or research purposes, wildlife maintainers and zoological parks. The questionnaire was also sent to all universities that teach veterinary sciences in Brazil, as these may also receive injured wildlife. Wildlife commercial breeders were not included because of the difference in management implied by the goal of maximizing production.

1.1.1.2. Questionnaire

The questionnaire was designed keeping in mind the reality of wildlife rehabilitation in Brazil (described in chapter II)4.3. - page 36) and following questionnaire design guidelines (Crawford, 1997; Dohoo, Martin, & Stryhn, 2003; Pfeiffer, 2013). The language of choice was Portuguese and questions were designed to be easy to answer, namely using closed-ended questions. Option to provide an open answer was always given, as an attempt to meet all possible realities in the diverse group of targeted organizations.

Test and validation

As widely recommended for a satisfactory study design, the questionnaire was trial tested (Hassan, Schattner, & Mazza, 2006; Thabane et al., 2010; Charlesworth, Burnell, Hoe, Orrell, & Russell, 2013). The first version consisted of 23 questions on paper and was tested by a panel of two veterinarians employed at an eligible organization, two veterinarians employed as university professors, one biologist in charge of an eligible organization and one biologist specialized in wildlife parasitology. The overall evaluation was positive and the average time for completion was 10.4 minutes (minimum 8 and maximum 14 minutes and 11 seconds). Some changes were made in the question formulation to turn the questionnaire less repetitive, more versatile and more straightforward, aiming for a completion time under 10 minutes. The improved version (see Annex 1 and 2) was set up as an online questionnaire and tested by a panel of three veterinarians and one biologist. Apart from a few flaws, no further corrections in content were made.

Application

The questionnaire was formulated online with Google Forms® and distributed in January 2016 through several channels in order to reach the different eligible organizations:

- The environmental departments of all state governments and the state departments of IBAMA (Brazilian Institute of Environment and Renewable Natural Resources) were contacted via e-mail to request help in divulgation to all eligible organizations under their jurisdiction;
- The specific terms "CETAS", "CRAS", "*animais silvestres*" (wildlife) combined with the names of each of the 27 states were used in the search engine Google® (www.google.com) and checked until page 5 of the search results. The upcoming names were registered and contacted;
- Zoological parks were individually contacted by e-mail and the questionnaire was also spread by the Brazilian Association of Zoos and Aquaria - *SZB: Sociedade de Zoológicos e Aquários do Brasil*;
- A list of all veterinary education establishments of the country was obtained at the site of *Conselho Federal de Medicina Veterinária* (CFMV, n.d.) and these institutions were individually contacted by e-mail, whenever possible directly to the clinic/hospital or to a responsible for the wildlife department.

1.1.1.3. Data Analysis

All submissions were organized and analyzed in Microsoft® Office Excel® 2007.

1.2. Case study of the Wildlife Conservation Center of Ilha Solteira (CCFS)

1.2.1. The Wildlife Conservation Center of Ilha Solteira (CCFS)

The Wildlife Conservation Center of Ilha Solteira (CCFS - *Centro de Conservação de Fauna Silvestre*) is maintained by the São Paulo State Energy Company (*Companhia Energética de São Paulo* - CESP) since 1979. The center is located at the edge of Ilha Solteira, a small city in the west of the state of São Paulo, separated from the state of Mato Grosso do Sul by the Paraná river and connected to it with the dam and hydroelectric power plant of Ilha Solteira, also managed by CESP (Figure 14). CCFS covers an 18ha fenced area with Cerradão vegetation (Figure 15), the native dry forest associated with the Cerrado savanna biome, and is subdivided in two physically and functionally distinct sections: the zoological park and the wildlife reception and triage center (CRT).

Figure 14 - Location of CCFS in Ilha Solteira, São Paulo, Brazil. (Map data ©2017 Google)

1.2.1.1. Zoological park

The zoological park occupies the larger area of the conservation center and is open for the public during weekends and used for guided environmental education visits on weekdays. Only regional wildlife species are exhibited, as the goal of CCFS is to raise awareness of the local Brazilian biodiversity and its importance. Around 200 animals of nearly forty different species are exhibited in enclosures dispersed across the native vegetation (Figure 15), stating the interdependence between fauna and flora, which is only confirmed by the large variety of free living animals who chose the conservation center as their home and can occasionally be observed by quiet and patient visitors. While some of these free living species have been released in the park, such as brown brockets (*Mazama gouazoubira*) (Figure 16), many more have occupied this area voluntarily, ranging from easy colonizers such as macaws and parakeets to the giant anteaters who climbed the fence and never left (Figure 16). Two open-air bungalows are located at one end of the park and are used as lecture rooms during guided tours and picnic and rest spot for visitors.

Figure 15 - The enclosures within the zoological park are distributed across native vegetation. (Originals)

Figure 16 - Some of the free-living animals within CCFS (Originals).

A) Black howler monkey - *Alouatta caraya*; B) Yellow-chevroned parakeet - *Brotogeris chiriri*; C) Blue-an-yellow macaw - *Ara ararauna*; D) Brown brocket - *Mazama gouazoubira;* E) Black-tufted marmoset - *Callithrix penicillata*; F) Argentine giant tegu - *Salvator merianae;* G) Nine-banded armadillo - *Dasypus novemcinctus*; and H) Giant ant-eater - *Myrmecophaga tridactyla*.

1.2.1.2. Wildlife reception and triage center (CRT)

CCFS receives wildlife specimens that are sick, injured or have invaded urban areas, causing disturbance or even being a threat to humans. It also receives animals that were kept as illegal pets, both after apprehension by authorities or by spontaneous drop-off by the owners. The most frequent causes of admission are illegal captivity, trauma and orphans. These animals are assessed by a veterinarian, treated for any diseases or injuries, rehabilitated whenever possible and then disposed. Reintroduction is the primary goal for any specimen entering the center. When reintroduction is not possible, the animals are kept at the center (often transferred to the exhibition area), or transferred to other organizations. In 2014, CCFS received 197 animals from nature or illegal captivity: 41% were released back in nature or escaped, 36.5% died or were euthanized, 1.5% were transferred to another organization and 21% still remained at the center by September 2015 (Figure 17). The vast majority of the retained animals originated from illegal captivity and lacked behavior traits to be eligible for release. Mortality rate is quite high, but stands within the common rates in wildlife rehabilitation centers, consequence of high rates of frequently days old traumatic injuries and the difficulty in managing human-shy, highly stressed wild animals. The wildlife reception and triage area consists of 22 to 30 enclosures for small, medium and large animals plus portable cages for intensive care patients kept inside the veterinary clinic (Figure 18).

Figure 17 - Disposition of the 197 animals from nature or illegal captivity that were received in CCFS (births and transferred animals were excluded). Data analyzed in November 2015.

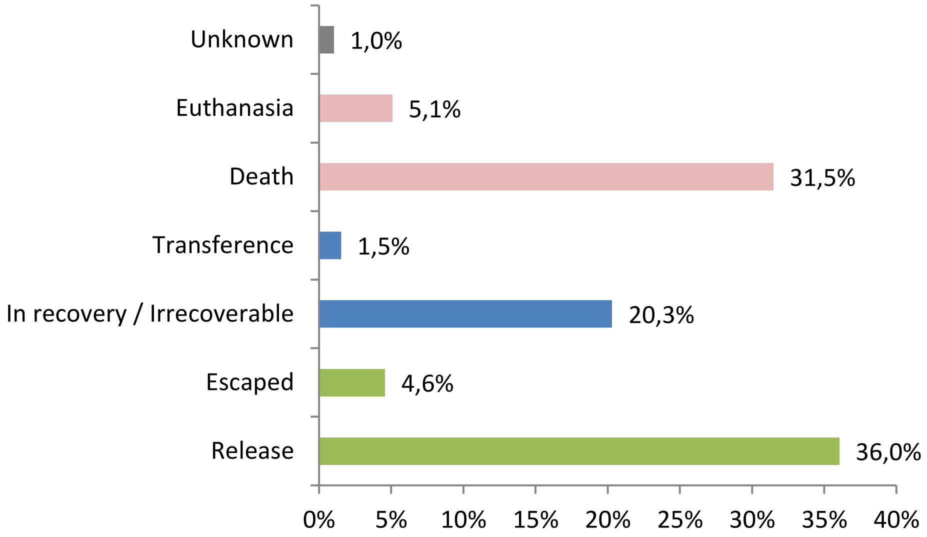


Figure 18 - Examples of enclosures for large (A), medium (B) and small (C) animals (Originals).

1.2.1.3. Remaining infrastructure

Located in the non public part of the center, next to the CRT, we can find the veterinary clinic (with a consultation room, a surgery room and a necropsy room), the administration offices, a kitchen for food preparation for all animals (

Figure **19**), a bioterium with mice, rats and rabbits used for feeding (Figure 20), a lecture room, a museum room, where biological specimens of interest (like skeletons, eggs or feathers) are preserved and a storage barn. At one extremity of the center, there is a big composter, where all biological waste generated in the CCFS is processed. The entrance to the conservation center is guarded twenty-four seven.

Figure 19 - Kitchen for food preparation for the animals in CCFS (Original).

Figure 20 - The bioterium consisted of one room with mice and rats (A) and one room with rabbits (B) (Original).

1.2.1.4. Common hygiene and biosecurity practices

All enclosures are cleaned on a daily basis, with exception of big water reservoirs, like the ones for alligators (*Caiman latirostris*), capybara (*Hydrochoerus hydrochaeris*) or tapirs (*Tapirus terrestris*). Cement and wooden areas are scrubbed with water and a hard broom and dung is removed with a shovel. In the zoological park the cleaning equipment is individual for each enclosure or used in a close range group of maximum three enclosures. In CRT the same equipment is used for all cages. When considered necessary enclosures are cleaned with a flamethrower, especially when parasites were detected.

Traps are set up around the park to catch feral cats (*Felis catus*) that invade the park and open enclosures. When caught they are spayed and then transferred to the competent authorities.

1.2.1.5. Importance

The main goals of the conservation center are stated below, in line with the three pillars considered necessary to a wildlife rescue program by Clark Jr (1999):

a) Supporting the **wildlife management projects** stated in the environmental licenses from the hydroelectric power plants (HPP) managed by CESP. Included are the *ex situ* conservation programs of the marshal deer (*Blastocerus dichotomus*) and jaguar (*Panthera onca*), endangered species whose habitats were affected by the formation of the reservoirs of the Três Irmãos HPP (Tietê river, 30 km before flowing into the Paraná river) and Engenheiro Sergio Motta (Rio Paraná, 300 km downstream of Ilha Solteira);

b) Conducting and collaborating in **research** for *ex situ* wildlife conservation;

Playing an active role in **environmental education**: besides the open visitation days/weekends to the zoological park, CCFS provides guided tours during weekdays, mainly designed for and aimed at children, all free of charge. In 2010, 31 343 people visited the zoo during the weekend and 73 schools benefitted the guided tours, totaling 2672 students and 300 teachers. CCFS also allows students to perform short two week externships to learn about wildlife care and management, gives courses on wildlife identification, rescue and manipulation for police and fire brigades and collaborates with workshops on wildlife survey and capture with the local university (Companhia Energética de São Paulo (CESP), n.d.).

1.2.2. Material and methods

1.2.2.1. Inclusion criteria

Sampling occurred on site from August to October 2014 and May to July 2015. All animals present in the center, either in permanent human care or being rehabilitated were sampled. Exceptions were, for instance, animals released on the day of arrival or animals who live in water and/or whose enclosure was not safe to enter (e.g. *Caiman latirostris* - alligator). Some deceased animals' cadavers from in between the mentioned sample periods were frozen to be included in the study. Since management of wildlife in permanent human care is substantially different than those destined to be released back into the wild, the animals were divided into two main groups depending on the time they spent in human care, with a cut-off point of one year (see Table 4). This is of course an artificial threshold, and some animals may have a recovery longer than one year while others may be declared non-releasable after a short time, but for the majority of cases it is accurate and it is also the same value used in the questionnaires, creating the possibility to compare results.

Free living animals in the center were also eligible for sampling to evaluate the possibility of outside contamination.

Non endemic species were not included in the study.

Table 4 - Division of animals for analysis.

Group	Description
In recovery	In human care for less than one year
Non-releasable	In human care for over one year

1.2.2.2. Sampling

Fecal samples were collected from the enclosure together with the responsible zookeeper during the daily cleaning of the enclosure, to ensure safety and not to disturb the usual routine of the animals. This way, feces were never older than 24h. Sampling was performed during three consecutive days (or three consecutive defecations, in case of animals that did not defecate daily, such as reptiles) whenever possible. Exceptions were animals who were released sooner. Whenever possible, individual sampling was performed. In animals that shared an enclosure and were not easily separated, group sampling was performed, as the animals' wellbeing was always a priority. All deceased animals were necropsied and feces collected.

Free ranging animals in the park were sampled on an opportunistic basis, collecting fecal samples when fresh and identifiable and performing necropsies on deceased animals.

1.2.2.3. Coproparasitology

The fecal samples were stored at 5 ºC and analyzed within 48 hours from sampling. Samples were mixed for homogenization and then analyzed with the techniques described on the following pages. Note that the described quantities represent the ideal procedure, having been reduced proportionally in cases where the size of the sample was very small, such as in some birds.

<u>Simple flotation</u>

This qualitative technique is based on the lower density of many parasite eggs and protozoan cysts relatively to the majority of the other fecal components (Bowman, 2013). Approximately 2 g of feces were mixed with approximately 15 ml of saturated sugar solution (500 g of sugar dissolved in 365 ml of distilled water and 10 ml of 10% formalin, specific gravity 1.27) and then filtered through a tea strainer to remove the larger debris. The solution was transferred to a test tube up to the edge where it formed a meniscus, and the tube was covered with a coverslip, making sure the liquid contacted with it and avoiding the formation of bubbles. After at least 15 minutes, the coverslip was lifted straight up, placed on a microscope slide and scanned thoroughly and methodically under x100 magnification, confirming any doubt at higher magnification (Monteiro, 2011). The focal plane should be set at the same level as the air bubbles, as these will adhere to the coverslip, just as the parasite eggs and oocysts.

This technique is effective to detect the presence of most nematode and cestode eggs and protozoan cysts, but trematode and acanthocephalan eggs tend to be dense and do not appear on these slides. Nematode eggs were classified according to Bowman (2013) as oxyurid, ascaridoid, spirurid, rhabditoid, strongylid or trichinelloid (trichuris or capillarid). Very small protozoan cysts, such as *Giardia* sp. cysts or *Cryptosporidium* sp. oocysts are not detected under x100 magnification, as they require at least x400 magnification (Bowman, 2013).

Simple sedimentation

Sedimentation techniques reveal objects that are too heavy to be evidenced in the flotation techniques, such as trematode and acanthocephalan eggs and protozoans such as amoeba and ciliates (Bowman, 2013). Two to five grams of feces were thoroughly mixed with around 200 ml of water and filtered through a tea strainer to remove the bigger debris. The solution was then transferred to a test tube and left to rest for at least 15 minutes. The supernatant was decanted and a portion of the sediment transferred to a microscope slide with a Pasteur pipette and covered with a coverslip. The slide was than scanned thoroughly and methodically under x100 magnification. If the first slide had a negative result, a second slide was examined as well (Monteiro, 2011).

Frequently objects that are evidenced with the flotation technique also appear on the sedimentation slides, especially if the egg count is high, but sedimentation remains far less sensitive for the detection of these objects (Bowman, 2013).

Direct smear

In cases where the amount of feces available was too small to perform any of the above mentioned techniques, direct smears were performed. For this technique, a very small amount of feces was diluted in a drop of water directly on a microscope slide, covered with a coverslip and scanned under x100 magnification. The suspension should be very thin: if placed on top of a newspaper, the letters underneath should still be legible (Bowman, 2013; Monteiro, 2011).

Cornell-McMaster dilution egg counting technique

While above mentioned techniques are qualitative, the McMaster technique allows the quantification off the objects that appear during flotation methods. Precisely four grams of feces were mixed with 60 ml of saturated sugar solution and filtered through a tea strainer in order to remove the bigger debris. If there wasn't as much fecal material, the amount of feces was altered but the proportion remained 1 g:15 ml. Both chambers of a McMaster counting slide were filled with the solution through a Pasteur pipette. The chamber was left to rest for at least 15 minutes, allowing the parasite eggs and protozoan cysts to settle on the undersurface of the chamber cover. All eggs within the grid in both chambers were counted under x100 magnification. The volume under the grid of each chamber is 0.15 ml, so in total the eggs of 0.3 ml of solution, corresponding to 0.02 g of feces, are counted. By multiplying the number of eggs counted by 50 the estimated number of eggs or oocysts per gram of feces (EPG or OPG, respectively) is inferred (Monteiro, 2011; Bowman, 2013).

Simple sedimentation technique - modified McMaster

The simple sedimentation method (McMaster) as described by Conceição, Durão, Costa, & Correia (2002) was used to obtain a quantitative measure of sedimenting eggs. Ten grams of feces were mixed with tap water and filtered through a tea strainer to remove gross debris, into a one liter sedimentation flask. The sample was left to sediment and then decanted, four consecutive times. The sediment was then resuspended in 50 ml of tap water and this solution was used to fill both chambers of a McMaster counting slide. The chamber was left to rest for a few minutes for the eggs to settle on the bottom of the chambers. All eggs within the grid in both chambers were counted under x100 magnification. Multiplying the amount off eggs counted in both chambers by 5, the estimated number of eggs per gram of feces (EPG) is inferred. If a lot of debris was present, the solution was strained through a sieve before filling another McMaster chamber.

Culture of coccidian oocysts for sporulation

A small amount of feces was mixed with 2.5% potassium dichromate solution in a Petri dish and placed in an incubator at 28ºC for seven days. The cultures were checked daily and more dichromate solution was added to avoid dehydration. The sporulated oocysts were then observed under x400 and x1000 magnification (Monteiro, 2011).

Culture of nematode larvae

Culturing third-stage larvae is an important tool to generically identify nematodes with this kind of infective stage (Bowman, 2013). A certain amount of feces was weighted and mixed with dehydrated equine feces to establish the right degree of humidity in a glass previously rinsed with 0.1% sodium carbonate solution to inhibit molding (Bowman, 2013). The glass was covered with a double layer of gauze and stored in an incubator at 28ºC for 10 days. The cultures were checked daily and a few drops of sodium carbonate solution were added in case they got too dry. At the end of the incubation period, the larvae were recovered and observed at x100 magnification.

Baermann technique

Approximately 5-15 g of feces, wrapped in gauze, hung in lukewarm water in a sedimentation flask and left overnight. The next day, the sediment was aspirated with a Pasteur pipette,

placed onto a slide and covered with a coverslip to be thoroughly and methodically scanned under x100 magnification (Monteiro, 2011) (see Figure 21).
The temperature stimulates the larvae to move from the fecal mass towards its surface and then takes advantage of their inability to swim against gravity, becoming concentrated at the bottom. This technique is recommended for the detection of first stage larvae of lung nematodes, which are shed in the feces after the eggs hatch in the lungs and being coughed up and swallowed (Bowman, 2013).

Figure 21 - Baermann technique for the recovery of first stage larvae of lungworms (Original).

1.2.2.4. Antiparasitic treatment and efficacy testing

Animals with more than 500 EPG and debilitated animals with a positive results were eligible for antiparasitic treatment. The drugs and dosages were selected for each case individually based on the available literature and drugs (Atkinson et al., 2008; Maddison, Page & Church, 2008; Carpenter, 2012; Milagro, Rodríguez, Vega, Diego, & Ponce, 2013; Miller & Fowler, 2014).
The efficacy of treatment was evaluated through a fecal egg count reduction test (FECRT): the fecal egg count was repeated 21 days after deworming and, whenever possible, at day 7 and 14 as well. The egg count reduction was obtained through the formula 1-[(EGP day 21)/(EGP day 0)]x100, or in case of coccidia, OPG. Fecal egg count reductions lower than 90% were considered inefficacious (Madeira de Carvalho, 2001; Cabaço, 2014).

1.2.2.5. Necropsies

All animals that die at CCFS are routinely necropsied. Focus on finding parasites was increased during the sampling period, making sure to lay open and inspect the thoracic and abdominal cavity and all its organs for macroscopic parasites. The contents of the stomach, small intestine and large intestine from large animals were checked against a dark background plastic tray. The mucosa of these organs and all organs from smaller animals were checked with a stereomicroscope Leica S8APO ®. Feces were analyzed with all methods mentioned above with exception of the Baermann technique, which was performed directly with lung tissue (Monteiro, 2011; Bowman, 2013).

1.2.2.6. Helminth fixation

Whenever helminths were found during a necropsy or in a fecal samples, they were fixed for later identification. Nematodes were kept in saline solution (0.9%) while still alive and then fixated with 70% ethanol at 65ºC to promote extension of the parasite. Cestodes and trematodes were compressed between a slide and coverslip, or between two slides, depending on their size and resistance, and fixed with 70% ethanol while compressed. Acanthocephala were left in saline solution (0.9%) at 5ºC overnight to promote proboscis evagination, and then fixed with 70% ethanol at 65ºC. For microscope observation, nematodes were clarified with lactophenol, while cestodes and acanthocephalan were stained with carmine (Amato et al., 1991; Andrade, 2000).

1.2.2.7. Microscopy

All microscopic and stereomicroscopic observations were performed with a microscope Leica DM2500 ® with digital camera Leica DMC2900 ® and stereomicroscope Leica S8APO ® with digital camera Leica DMC2900 ®. All images were recorded and analyzed with computerized image analysis system LAS V4 (Leica Application Suite ®).

1.2.2.8. Body condition evaluation

The body condition of the animals was quantified by giving a score ranging from 1 to 5 (Table 5), similarly to the existing body condition score (BCS) models for cattle (Ferguson, Galligan, & Thomsen, 1994), dogs and cats (Figure 22), rabbits, birds (Figure 23) and guinea pigs (Laflamme, 1997a; Laflamme, 1997b; PFMA - Pet Food Manufacturers Association, 2015). This classification is easily applied to any animal, but should always be done with care for there may be anatomical differences between different species.

Table 5 - Body condition score system.

Body Condition Score	Description
1	Cachectic
2	Thin
3	Ideal body condition
4	Overweight
5	Obese

Figure 22 - Body condition score system for cats and dogs published by the Pet Food Manufacturers Association (2015), based on Laflamme, 1997a; Laflamme, 1997b.

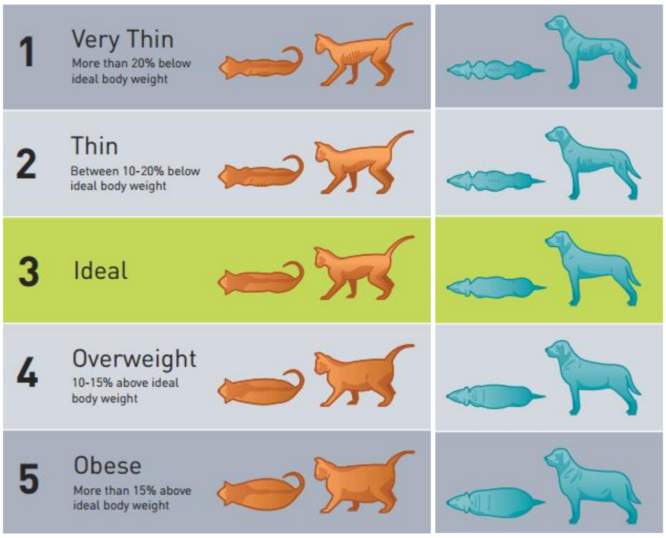


Figure 23 - Body condition score from 1 to 5 (left to right) in necropsied birds, exposing the *pectoralis* major muscles and keel (Originals, based on PFMA (2015)).

1 2 3 4 5

1.2.2.9. Data analysis

Data were organized in Microsoft® Office Excel® 2007 and analyzed with Microsoft® Office Excel® 2007 and GraphPad InStat Version 3.10, 32 bit for Windows® Sep 9, 2009.

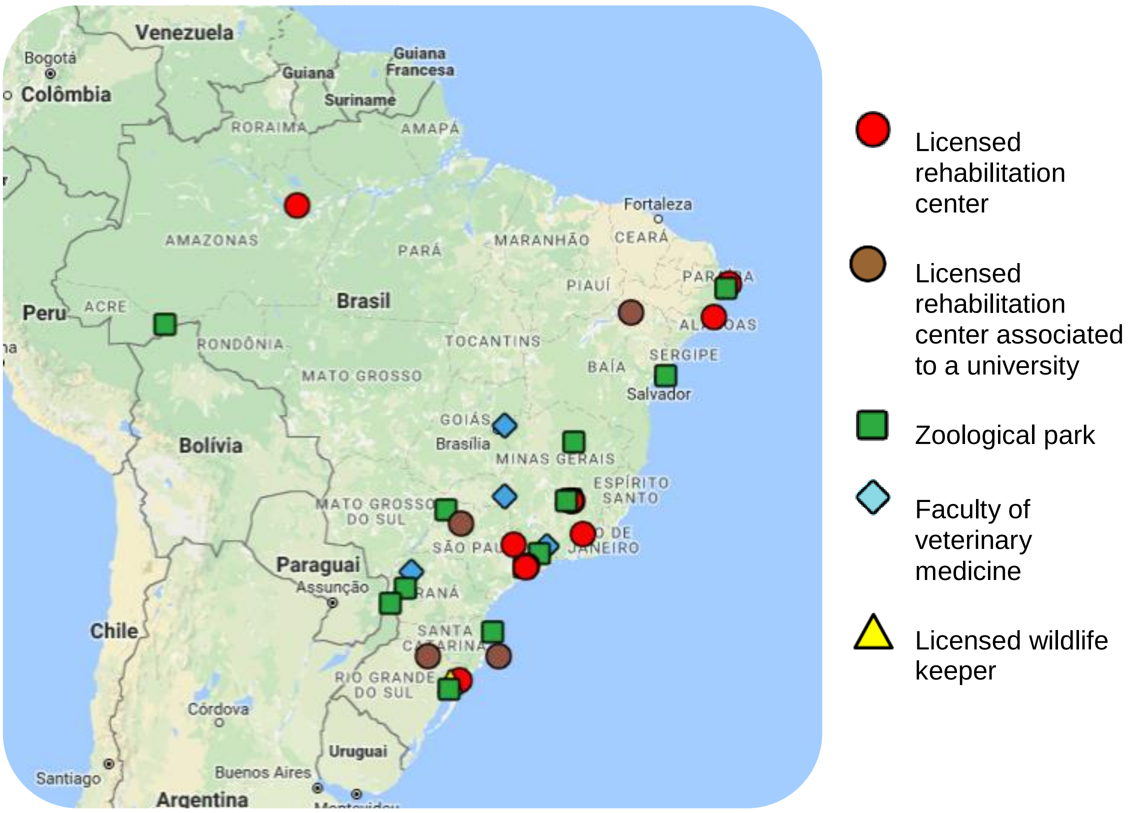
A chi-squared test for independence was used to evaluate the difference of prevalence between groups with different times in captivity, BCS or health status. Considering the distribution of observations in this category, for groups with a different health status, a Fisher's exact test was found to be more adequate. For parameters with more than one category (BCS and age), a chi-square test for trend was applied as well. Differences were considered statistically significant with p values under 0.05.

2. Results

2.1. Characterization of gastrointestinal parasite management in wildlife in Brazil

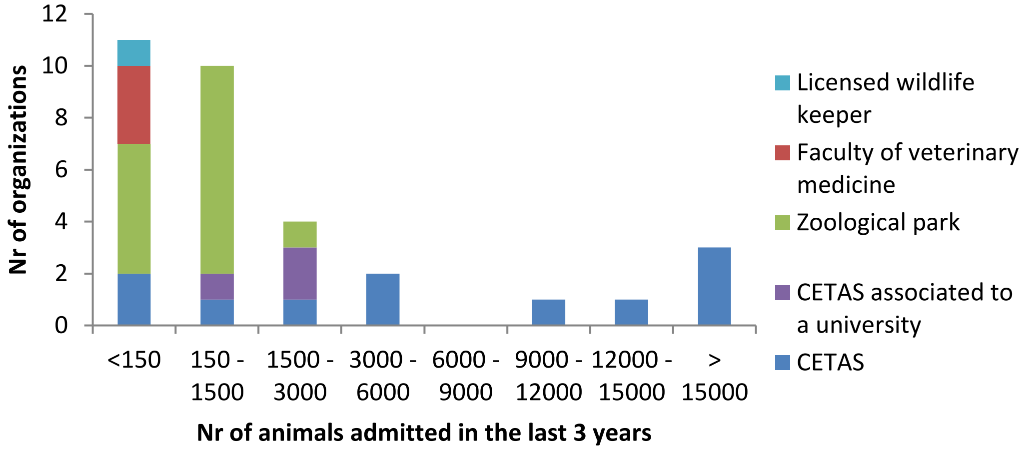
Thirty-two institutions, located in eleven different federative units, answered the questionnaire and are mapped in Figure 24. Fourteen (44%) were licensed rehabilitation centers (CETAS or CRAS), thirteen (41%) were zoological parks, four (13%) faculties of veterinary medicine and one licensed wildlife keeper (2%). When an organization had more than one operating activity, the closest activity to wildlife rehabilitation (or, for instance, questions about animals in permanent human care, the closest activity to the particular topic) was considered. For instance, a zoological park associated with commercial wildlife breeding was classificated as a zoological park. Six CETAS were associated to other operating activities; four of them were associated to universities, one to a commercial breeder of birds of prey and one to a licensed wildlife keeper. For statistical purposes, the first were considered as a separate group, as they are expected to have an easy access to the University's parasitology lab and input from students, and therefore are expected to have a different behavior in parasite management.

Figure 24 - Distribution of the thirty-two participating organizations (Map data ©2017 Google).



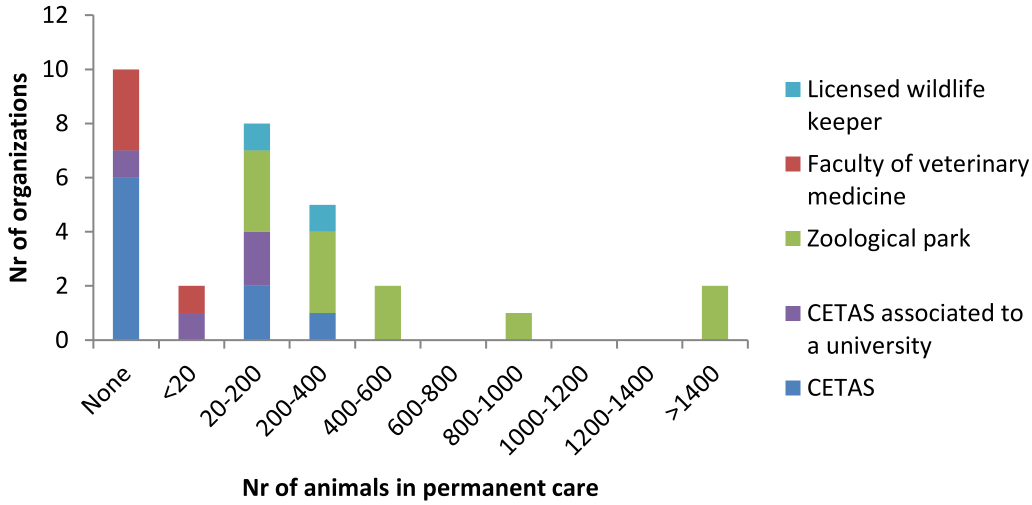
Caseloads (see Figure 25) varied from less than fifty animals per year (34%) up to 500 (31%), 1000 (12%) or more. All faculties of veterinary medicine that responded received a small wildlife caseload (less than fifty animals per year) and the CETAS with similar caseloads were associated to other activities such as commercial breeding or merely keeping nonreleasable wildlife. Admissions from 1000 animal a year up to 5000 or more were only reported by government subsidized CETAS.

Figure 25 - Number of admissions in the last three years (2013-2015).



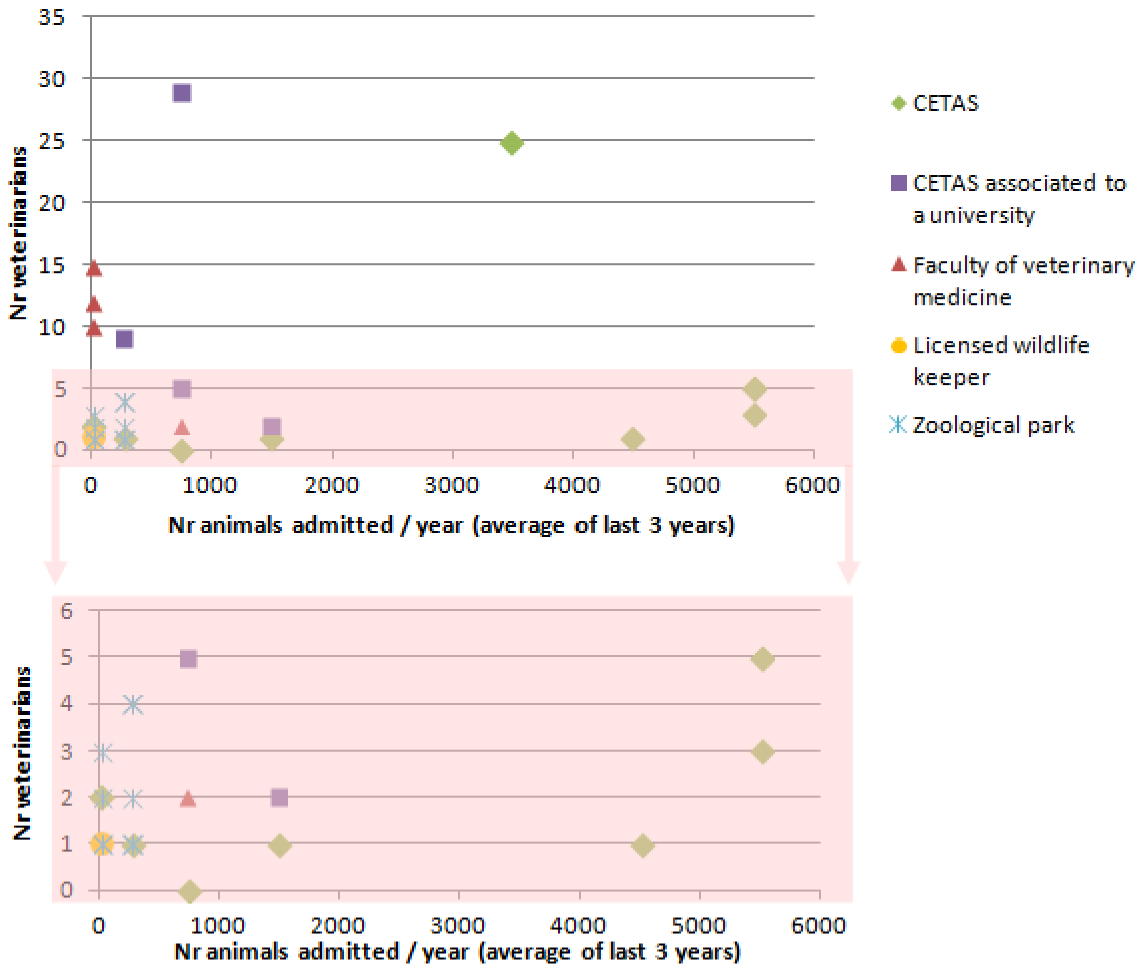
Concerning animals in permanent care (Figure 26), the tendency for CETAS is not to have any, since nonreleasable casualties are disposed to wildlife maintainers, zoological parks or other licensed keepers. Two CETAS noted that although they theoretically do not maintain animals in permanent care, disposition is not always easy. Two entries were excluded for this analysis due to inconsistent answers (e.g. zoological park that reported not maintaining animals in permanent care).

Figure 26 - Number of animals in permanent care.



The number of veterinarians working in each participating organization varied considerably, with noticeable higher numbers in universities, which raises some questions about the scope of the question not having been specific enough, causing the respondents to fill in, for instance, the total number of veterinarians employed in the university clinic, which would not be representative of those responsible for wildlife casualties. Therefore, the average of 4.8 veterinarians is likely to be largely overestimated. The median of 2 and mode of 1 veterinarian per organization should represent the reality in a more accurate way, as can be seen in Figure 27. Except one CETAS that employs 25 veterinarians, all other employ five or less veterinarians. One organization reported not having a permanent veterinarian on site.

Figure 27 - Number of veterinarians employed in the participating organizations.

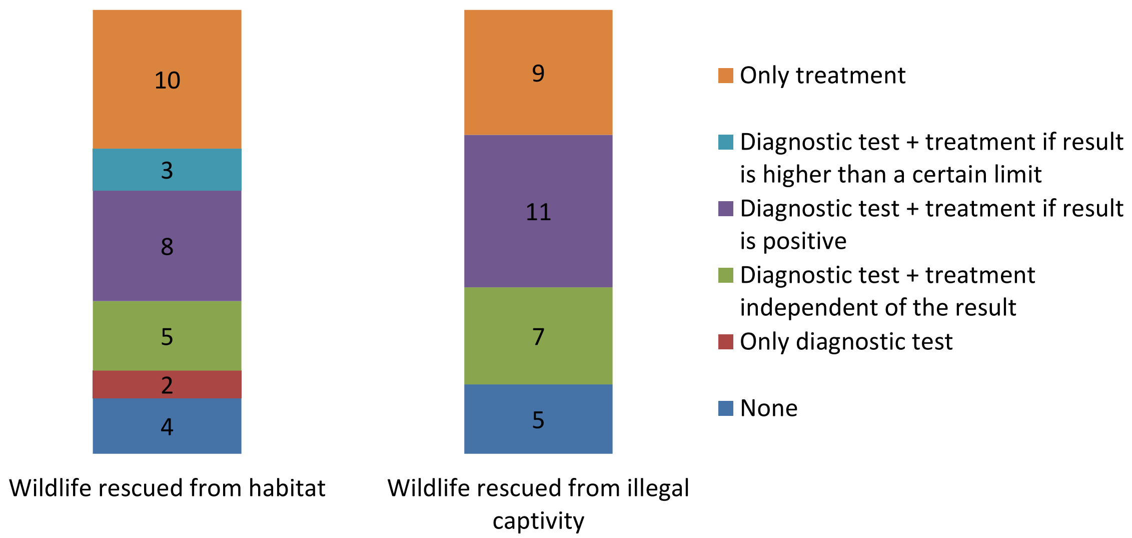


2.1.1. Routine diagnostic and treatment protocols

<u>Admission</u>

Upon admission of the animals (Figure 28), only four organizations (12.5%) didn't diagnose nor treat for GI parasites, unless they show clinical signs that could indicate that the animal is infected. One of these four proceeds this way since they work almost exclusively with one species and previous experience showed a GI parasite prevalence near zero. From the remaining 28 (87.5%) organizations, fifteen (54%) treat all incoming animals originated from their natural habitats, ten of which without performing any diagnostic test. The other thirteen (47%) perform diagnostic tests and eleven of those apply treatment depending upon the results of the tests. When the animals come from illegal captivity, most organizations maintain the same protocols, but five of them are stricter and deworm them more easily than animals originated from their habitats.

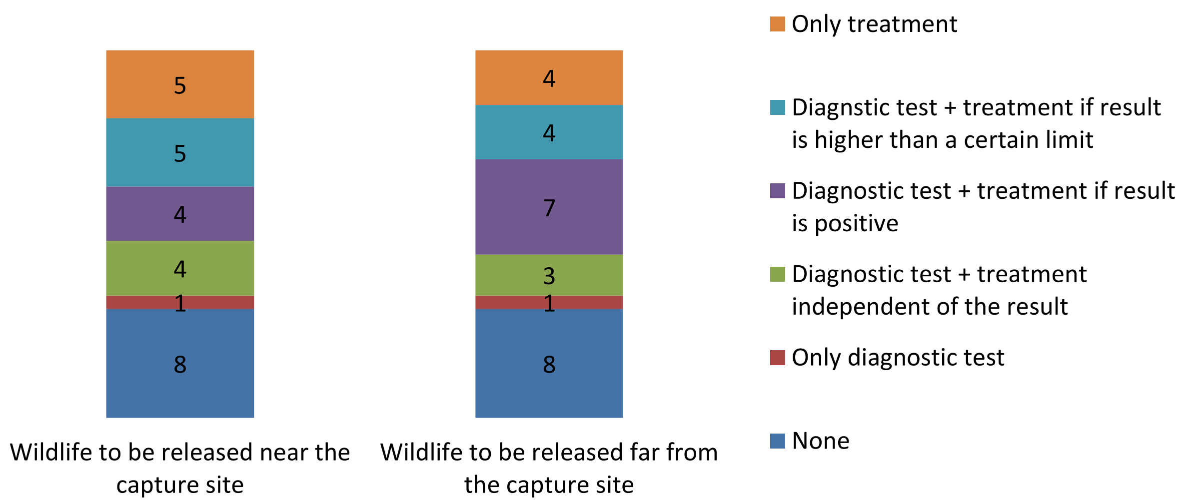
Figure 28 - Routine procedures upon admission of rescued wildlife.



Discharge

Twenty-seven (84%) of the organizations directly release rehabilitated animals back into their natural habitats. Eight (30%) do so without any kind of diagnostic or treatment measures, some of which also do not perform these actions upon admission. Nine organizations (33%) deworm all animals before release, without performing or basing the decision on diagnostic testing. Nine others perform treatment depending on the results of the diagnostic exams and one organization performs only diagnostic tests. When the release is to be done in a geographical area far from the capture location, protocols are generally the same, except for three organizations, two of which are curiously less strict in these cases (Figure 29).

Figure 29 - Routine procedures before release.

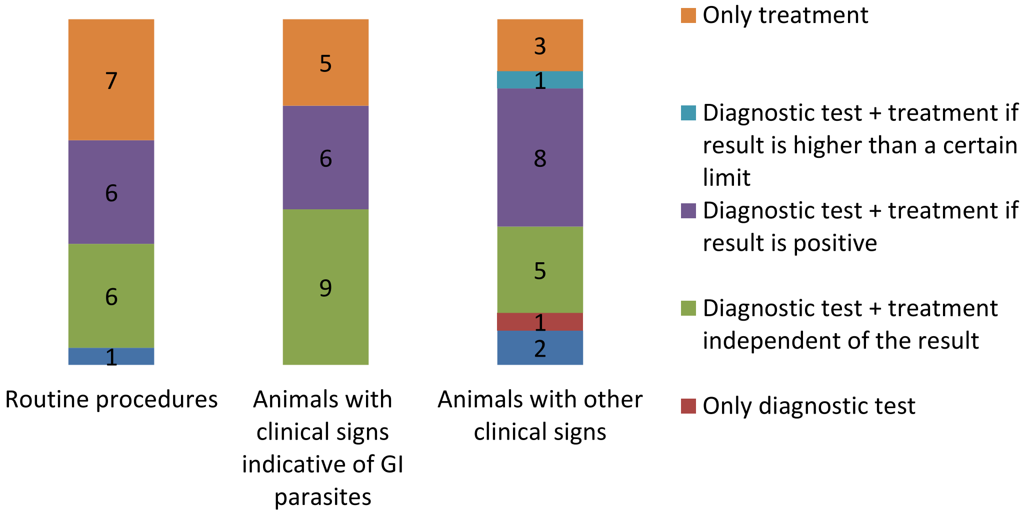


Seven (26%) of the organizations consider the presence of GI parasites to be impeditive to release the animal, fourteen (52%) only if parasite burdens are high and five (19%) do not consider it an issue. The remaining organization has not registered GI parasitism in the species they receive; therefore it doesn't apply to it. Four organizations (15%), consider GI parasitism to be impeditive when the animals come from illegal captivity and three (11%) when they are to be released far from their capture site.

<u>Animals in permanent care</u>

The majority of the organizations that keep animals in permanent care routinely deworm their animals (95%), thirty percent of which after performing diagnostic tests. Another thirty percent only deworm in case of positive results. If these animals present clinical signs indicative of GI parasitism, there is a higher tendency to perform diagnostic procedures. When they show other clinical signs, the tendency is to perform diagnostic tests and deworm them depending on the results. One should note that these results, presented in Figure 30, are a generalization for a large number of very different species, and of course the approach is impossibly the same for all animals. For instance, one institution specified that although they usually perform diagnostic tests and deworm depending on the results, in case of chelonians and crocodilians they perform prophylactic deworming every second year in consequence of the limitation of collecting feces out of the water.

Figure 30 - Approach to GI parasitism in animals in permanent care.



2.1.2. Limiting factors

The main reported limiting factors for not performing diagnostic tests were lack of time, funds and equipment or infrastructure (see Table 6), while the ones for not performing treatment were lack of funds and difficult access to certain drugs (see Table 7). Three of the participating organizations expressed concerns about the difficulty of using efficient administration methods, such as difficult access to animals and administrating routes and rejection or uncertainty of ingestion of oral drugs.

Table 6 - Reported limiting factors for performing diagnostic tests.

Factor	n	%
Economical factors (e.g. costs of reagents or external laboratories fees)	15	47
Lack of time	13	41
Lack of equipment and/or infrastructure	11	34
Lack of information / literature	3	9
Lack of specialized staff	3	9
Other: Lack of institutional support	1	3
None	5	16

Table 7 - Reported limiting factors for deworming.

Factor	n	%
Economical factors (e.g. cost of drugs)	15	47
Difficulty in obtaining the drugs	9	28
Lack of equipment and/or infrastructure	7	22
Lack of training / information / literature on treatment of gastrointestinal parasitoses in wildlife	4	13
Lack of time	4	13
Lack of specialized staff	2	6
Other: drug administration	3	9
None	8	25

2.1.3. Diagnosis

2.1.3.1. Clinical signs

The clinical signs that were considered by the responding organizations as potentially indicative of GI parasitism are presented in Table 8, with diarrhea, weight loss and anemia leading the list (>80%), followed by anorexia, regurgitation and abdominal distension (>60%).

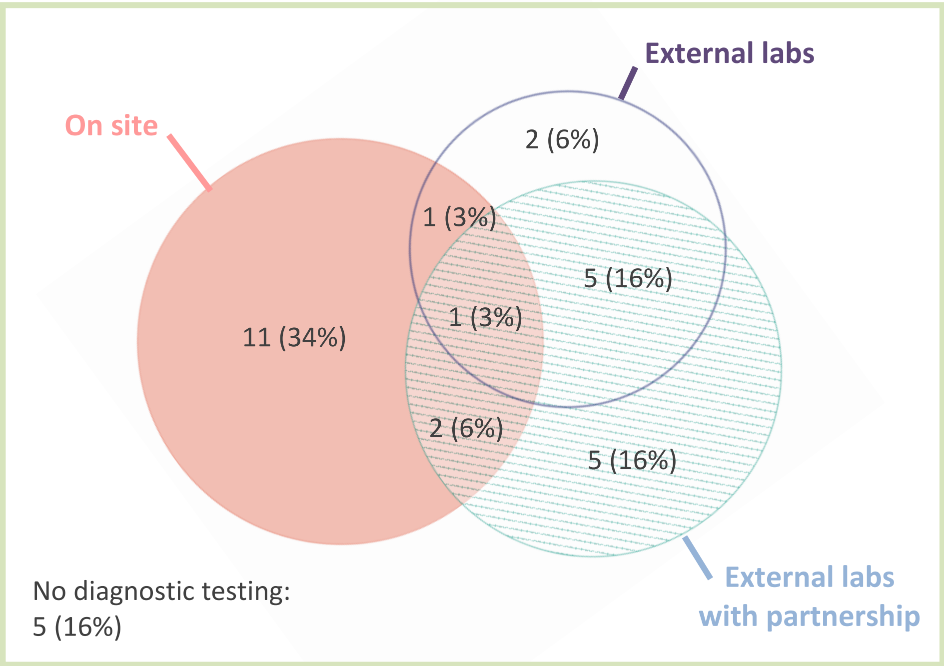
Table 8 - Clinical signs that raise suspicion of GI parasitism.

Clinical sign	n	%
Diarrhea	31	97
Weight loss	28	88
Anemia	26	81
Anorexia	22	69
Regurgitation	21	66
Abdominal distension	20	63
Emesis	19	59
Constipation	18	56
Abdominal pain	18	56
Polyfagia	16	50
Tenesmus	15	47
Nervous signs (e.g.: ataxia)	12	38
Pica and/or parorexia	6	19
Other:		
- hematochezia	1	3
- anal pruritis	1	3

2.1.3.2. Diagnostic tests

Most organizations (n=27, 84%) do perform diagnostic tests for GI parasites, and more than half of those (n=15, 56%) carry them out in their own facilities. Four of these regularly resort to external laboratories, with or without partnership, for certain tests. Forty-one percent (n=13) of the organizations have partnerships in place with external laboratories (such as labs in universities) and merely two (6%) only call upon external labs without partnerships (paid).

Figure 31 - Places where diagnostic tests are carried out, when performed (Venn diagram with proportional areas).



The question about other qualified people within the organization to perform diagnostic exams for GI parasites was answered in quite different ways, with some of the respondents including the number of veterinarians or not specifying the profession. One mistake of the author was to assume that all veterinarians felt prepared or were available to perform these tests, which became clear because some organizations filled in a lower number of veterinarians in this field as the number of veterinarians employed within the facility (obtained in the previous question). Considering this information, one can assert that at least 11 of the organizations contain people with these skills, such as biologists (n=10), zootechnists (n=1), lab technicians (n=2) or students (n=1).

The most used diagnostic tests are simple fecal flotation (n=26, 81%), direct fecal smear (n=19, 59%) and simple fecal sedimentation (n=17, 53%). Only five (16%) of the organizations perform a quantitative test. While CETAS carry out an average of 2.6 different tests, this number is 4.6 in universities. One organization added they carry out a specific test to detect Giardia (see Table 9).

Table 9 - Diagnostic tests usually carried out to detect GI parasites.

Diagnostic test	n	%
Fecal flotation (Willis-Molley or Sheather's solution)	26	81
Direct fecal smear	19	59
Fecal sedimentation	17	53
Centrifugal fecal sedimentation	10	31
Faust (centrifugal flotation with zinc sulfate solution)	7	22
Fecal culture of nematode larvae	6	19
Quantitative egg count - flotation (McMaster)	4	13
Quantitative egg count - sedimentation (e.g. modified McMaster)	3	9
Culture of Coccidian Oocysts for Sporulation	2	6
Graham technique (clear-cellulose tape)	2	6
Other:		
- *Giardia*	1	3
None	2	6
Don't know	2	6

2.1.4. Treatment / deworming

As can be seen in Table 10, as a way to choose the drug and dosage to treat parasitized animals, most organizations evaluate existing literature for each case (n=26, 81%) or base their decision on personal experience (n=19, 59%). The use of fixed deworming protocols by species or groups of animals is less common (n=12, 38%). One organization added that palatability is an important decision factor, reinforcing the difficulty of administrating oral drugs to wildlife species. Another one stated that their drug supply is limited to what city hall is able to provide (Table 10).

Table 10 - Decision factors to select the drugs and dosages to treat parasitized animals.

Decision factor	n	%
Evaluation of the existing literature for each case	26	81
Personal experience	19	59
Fixed deworming protocols by species / groups of animals	12	38
Extrapolation of drug and dosages from domestic animals	11	34
Others:		
- Only drugs that are provided by city hall	1	3
- Palatability	1	3

2.1.4.1. Treatment efficacy

Nearly half (n=15, 47%) of all organizations repeat diagnostic tests after all antiparasitic treatments, and 25% (n=8) repeat them depending on the case or species in question. Eight (25%) organizations do not test treatment efficacy, and one, as stated before, never had to, since the species they work with were never found to be parasitized.

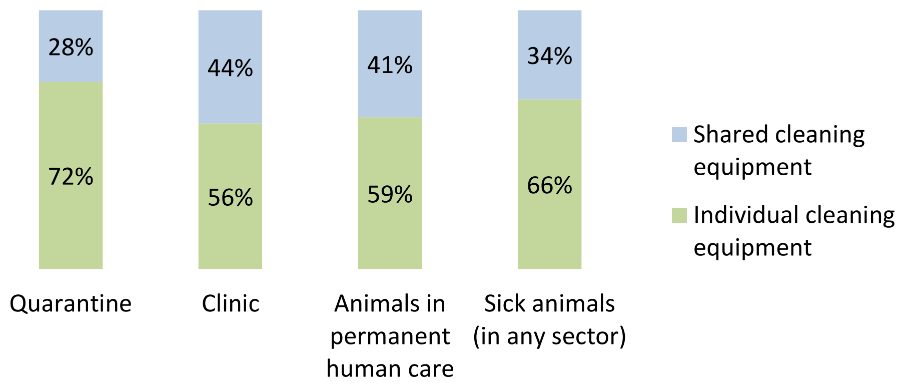
Seventy-four percent (n=17) of the organizations that do test the efficacy of their treatments have already encountered resistances, 9 (39%) reporting them to be frequent. More than one organization reported resistances of coccidians in birds (n=4, 17%), capillarids in birds such as parrots and toucans (n=4, 17%) and trematodes (n=2, 9%). One organization reported resistance of trichinelloids (trichurids and capillarids) in birds and mammals to ivermectin, mebendazol and albendazol. *Giardia* was also reported as a challenging parasite, presenting resistance against treatment with metronidazole especially in primates, although the organization itself suggested that they might be underdosing the drug, since it has a strong flavor, which makes prolonged oral administration in primates very hard. Also for primates, another organization reported to have issues with febendazol, praziquantel, febantel and albendazol, amongst others.

2.1.5. Hygiene and biosecurity

Eighty-one percent (n=26) of the participating organizations clean the enclosures every day, and the remaining 19% do so two to three times a week. Of course, this is a mode parameter, since hygiene procedures obviously vary according to species. For certain species there is no need for such a high frequency (e.g. certain reptiles), for others it can be influenced by technical reasons (e.g. aquatic species), or other variables such as juvenile animals that require a higher frequency (e.g. twice a day).

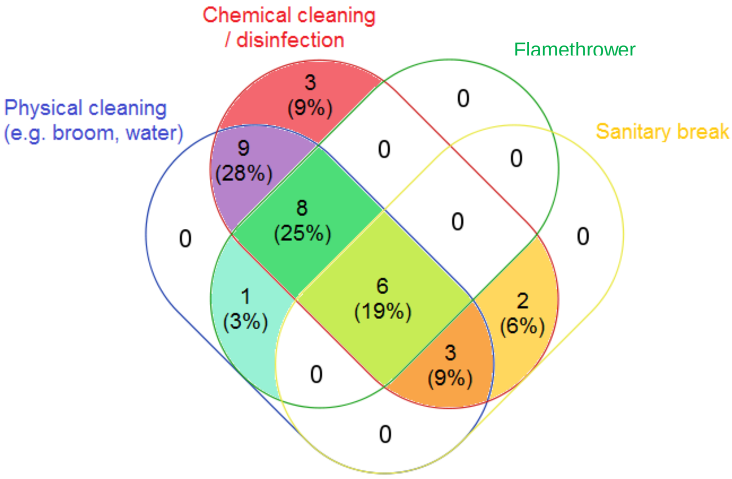
In most cases the cleaning equipment of an enclosure (55-60%) is not used in other enclosures (see Figure 32). For animals in permanent human care, such as in zoos, it is frequent to have separate equipment per sector (e.g. carnivores, primates, ungulates). As desired, in the quarantine sector, there is a higher tendency to use individual equipment (72%) and when an animal shows clinical signs, independently of the sector it is in, 66% of the organizations use individual equipment until the case is resolved. The clinic, however, is the area where the lowest percentage of organizations uses individual equipment (56%).

Figure 32 - Distribution of enclosure cleaning equipment (e.g. broom, waste shovel).



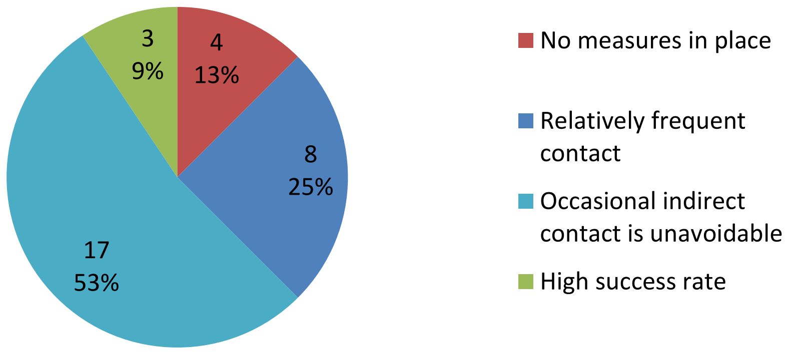
In between different occupants, there are different combinations of cleaning and sanitary methods used by the organizations, schematized in Figure 33. Physical cleaning methods are used in 84% (n=27) of the organizations and chemical cleaning methods in all but one (97%, n=31), with 81% (n=26) using both. Fourteen of these organizations (44%) also use a flamethrower and six of those (19%) implement a sanitary break between residents.

Figure 33 - Sanitary operations in the enclosures in between different occupants.



Most organizations (n=27, 87%) have measures in place to prevent contact of their animals with pests, synanthropic, wild and feral animals, such as nets to keep birds away or traps for cats. Twenty (62%) consider having a good success rate, with seventeen (53%) reporting that occasional indirect contact with these animals is inevitable. Eight organizations (25%) reported that the there is still a relatively high contact frequency despite of the measures in place (Figure 34).

Figure 34 - Efficacy of measures to prevent contact with pests, synanthropic, wild and feral animals.



Almost half (n=15, 47%) of the organizations include products of uncontrolled origin in the feeding scheme of some animals. Many give living or freshly slaughtered prey such as fish (n=6), crustaceans (n=1), birds and/or small mammals (n=8), termites and other insects (n=6).

2.2. Case study of the Wildlife Conservation Center of Ilha Solteira (CCFS)

A total of 287 or more animals of 62 different species were analyzed, sampled in 149 groups (listed in annex III). These animals were distributed as shown in Table 11. In total, 116 groups were submitted to fecal tests and 48 animals were necropsied (Table 12).

Table 11 - Number of sampled animals and group distribution.

Group	# animals	# samples	# animals included in one sample			
			1	2	3	>4
In recovery	91	78	69 (88%)	7 (9%)	1 (1%)	1 (1%)
In permanent human care	183	62	29 (47%)	16 (26%)	6 (10%)	11 (18%)
Free living	13	9	7 (78%)	1 (11%)	0	1 (11%)
Total	287	149	105 (70%)	24 (16%)	7 (5%)	13 (9%)

Table 12 - Distribution of tests performed.

	Coproparasitology	Necropsy
In recovery	40	38
In permanent human care	58	4
Free living	3	6
Total	101	48

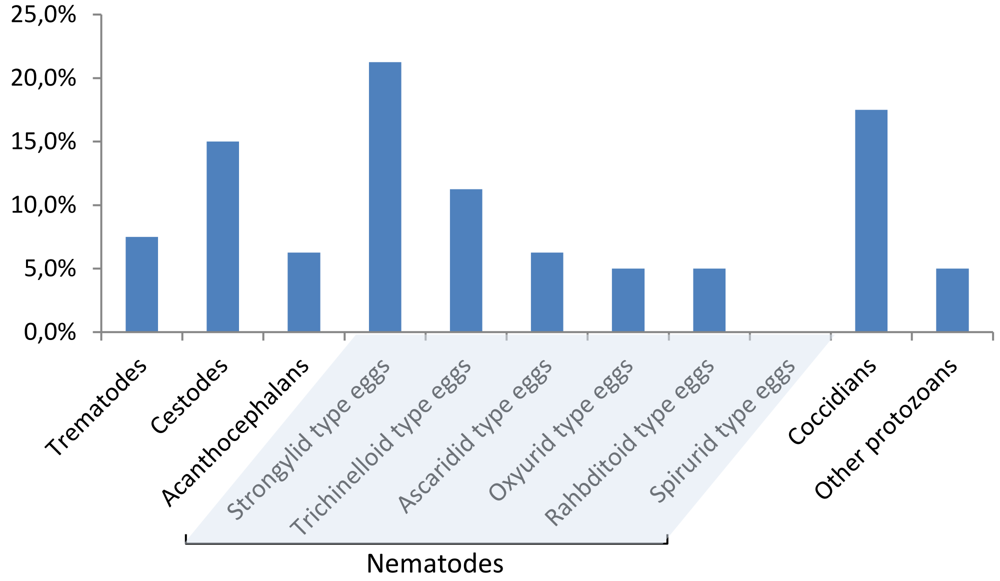
2.2.1. Prevalence

A wide range of parasites were detected, as can be seen in Figure 35. Parasites were classified according to egg type as acanthocephalan, cestode, trematode or nematode, the latter divided into six different egg types as described by Bowman (2013). Protozoa were divided into coccidians (Apicomplexa) and other protozoa (including the only other detected agent: *Balantidium* spp.). No lungworm infections were detected by the Baermann technique.

Two thirds of the positive animals showed only one type of eggs, while the other third presented mixed infections with two (20%) or three (13%) different parasites. Some of the detected parasites were zoonotic agents, such as *Bertiella* spp. in black howler monkeys (*Allouata caraya*) and *Balantidium* spp. in the suids *Tayassu pecari* and *Pecari tayacu*.

Nearly thirty percent (29.5%) of the animals in recovery tested positive, against 46.8% of the animals in permanent captivity. This difference is statistically significant (chi-square test, $p<0.05$).

Figure 35 - Distribution of detected parasite. Division of nematode type eggs according to Bowman (2013).



2.2.1.1. Risk of infection from the environment

To evaluate the parasite pressure from free-living animals within CCFS, free ranging animals in the park were sampled on an opportunistic basis. Three out of nine sampled groups tested positive for GI parasites (Table 13). Special reference goes to the group of black howler monkeys (*Alouatta caraya*), that presented *Bertiella* spp. (Figure 40), which was also diagnosed in all resident black howler monkeys (five animals, three samples) of CCFS. This cestode has an oribatid mite as intermediate host and has zoonotic potential.

Table 13 - Results of the fecal tests and/or necropsies on free ranging animals in CCFS.

		Species	sample size	Result	Diagnosed in in-house animals?
Bird	Passeriformes	*Cacicus haemorrhous*	1	Negative	-
	Psittaciformes	*Ara ararauna*	1	Negative	-
	Psittaciformes	*Ara ararauna*	4	Negative	-
Mammal	**Primata**	***Alouatta caraya***	**1**	***Bertiella* spp.**	**Yes**
	Primata	***Alouatta caraya***	**group**	***Bertiella* spp.**	**Yes**
	Rodentia	***Dasyprocta azarae***	**2**	**Oxyurid, rhabditoid and capillarid eggs**	**Possibly**
	Rodentia	*Dasyprocta azarae*	1	Negative	-
	Xenarthra	*Euphractus sexcinctus*	1	Negative	-
Reptile	Sauria	*Salvator merianae*	1	Negative	-

There are other hosts and intermediate hosts that live within the territory of CCFS, such as giant anteaters and termites (intermediate host) that were not analyzed.

2.2.1.2. Risk of infection through feeding

As mentioned before, living or freshly killed prey is included in the feeding schemes of several animals: mainly carnivores and reptiles but also anteaters or toucans are fed with live or freshly killed prey. In some of these, parasites were detected whose life cycle includes an intermediate host that was part of the feeding scheme. One ocelot (*Leopardus pardalis*) presented diphyllobothriidean eggs, which might have been transmitted through the consumption of freshly caught fish. Several giant anteaters presented parasites that are transmitted by termites, such as the acanthocephalan *Gigantorhynchus echinodiscus*.

The rats and mice of the bioterium were infected with *Hymenolepis* spp. (Figure 36) and pinworms. Once again, it is important to understand the feeding scheme to identify pseudoparasites. The latter showed up in the fecal exam of a puma (*Puma concolor,* <50 EPG) and a snake (*Hydrodinastes gigas*, 100 EPG) as a pseudoparasite (Figure 37).

Figure 36 - *Hymenolepis* spp. egg detected in the rats and mice in CCFS' bioterium (Originals).

Figure 37 - Pseudoparasitism: oxyurid egg found in the fecal sample of a South American water cobra (*Hydrodinastes gigas* – A), compared to the same eggs from the rats in the bioterium (B) used to feed the snakes (Originals).

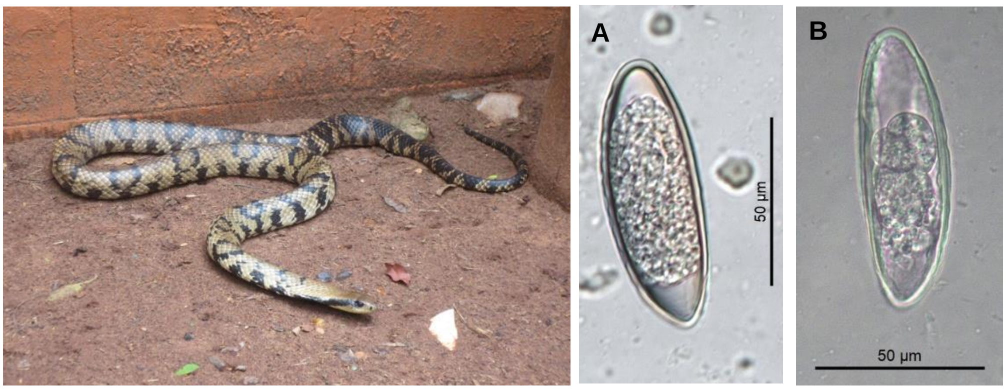


Table 14 - Food of uncontrolled origin included in some species' feeding schemes.

	Species	**Rabbits from bioterium**	**Rats or mice from bioterium**	**Quail** (origin: farm)	**Beef** (slaughtered in CCFS and frozen)	**Fish** (freshly caught)	**Termites**	Results
Mammal	*Chrysocyon brachyurus*		✓	✓				Hookworm eggs
	Leopardus pardalis		✓	✓		✓		Diphyllobothriidean eggs
	Leopardus tigrinus		✓	✓		✓		Negative
	Myrmecophaga tridactyla						✓	*Gigantorhynchus echinodiscus* (acanthocephalan) Strongylid eggs Coccidia
	Panthera onca	✓	✓	✓	✓	✓		Negative
	Puma concolor	✓	✓	✓	✓	✓		Oxyurid egg: pseudoparasite
	Puma yagouaroundi		✓	✓		✓		*Toxocara cati*
	Tamandua tetradactyla						✓	Strongylid eggs Coccidia
Bird	*Ramphastos toco*		✓					Negative
Reptile	*Boa constrictor*		✓					Negative
	Eunectes murinus	✓						Cestode (*Crepidobothrium* spp.) Rhabditoid eggs
	Hidrogynastes gigas		✓					Trematode

2.2.1.3. Evolution of intensity of infection during captivity

Many of the animals admitted in CCFS are polytraumatized and go through a more or less intense recovery period. Additionally, they tend to be much stressed. This may also reduce their immune response and affect the host-parasite balance.

Polytraumatized animals represent a special group of patients, as they are generally healthy until the moment of the accident. In the days following the traumatic event, the strain on their body is very high and their overall resistance decreases. These patients allow evaluating the effect of decreased fitness and stress associated to sudden captivity on the host-parasite balance. Considering the high prevalence of GI parasites in giant anteaters (*Myrmecophaga tridactyla*) and the frequent admission of polytraumatized anteaters (victims of motorway accidents), the author performed follow-up analysis on giant anteaters during their recovery in CCFS, comparing the evolution of egg counts from clinically healthy animals and polytraumatized animals over time. While the parasite load upon admission did not statistically differ from the parasite load of healthy animals, it did rapidly increase as the animals got more and more debilitated (T-test, $p<0.001$) (Figure 38). In one of the animals that was necropsied, the high load of the acanthocephalan *Gigantorhynchus echinodiscus* caused at least partial obstructions at several sites (Figure 39), and was considered a main contributor to the animal death.

Figure 38 - Egg count evolution of a polytraumatized giant anteater from its arrival until euthanasia was performed.

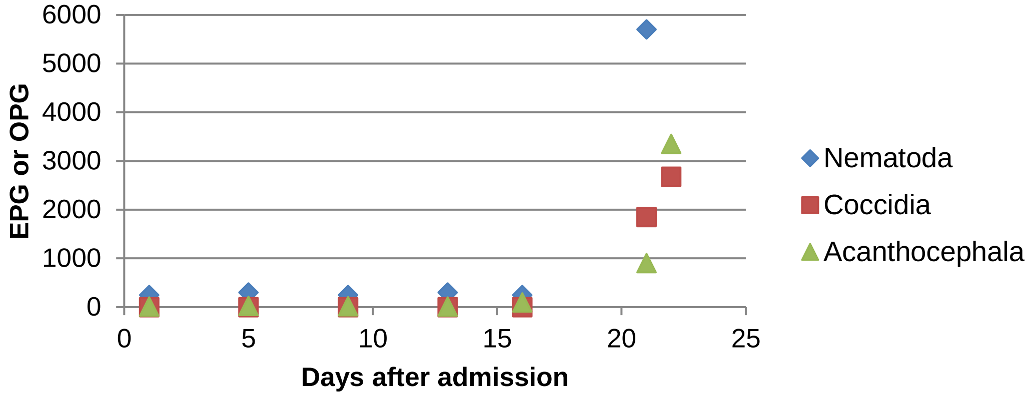
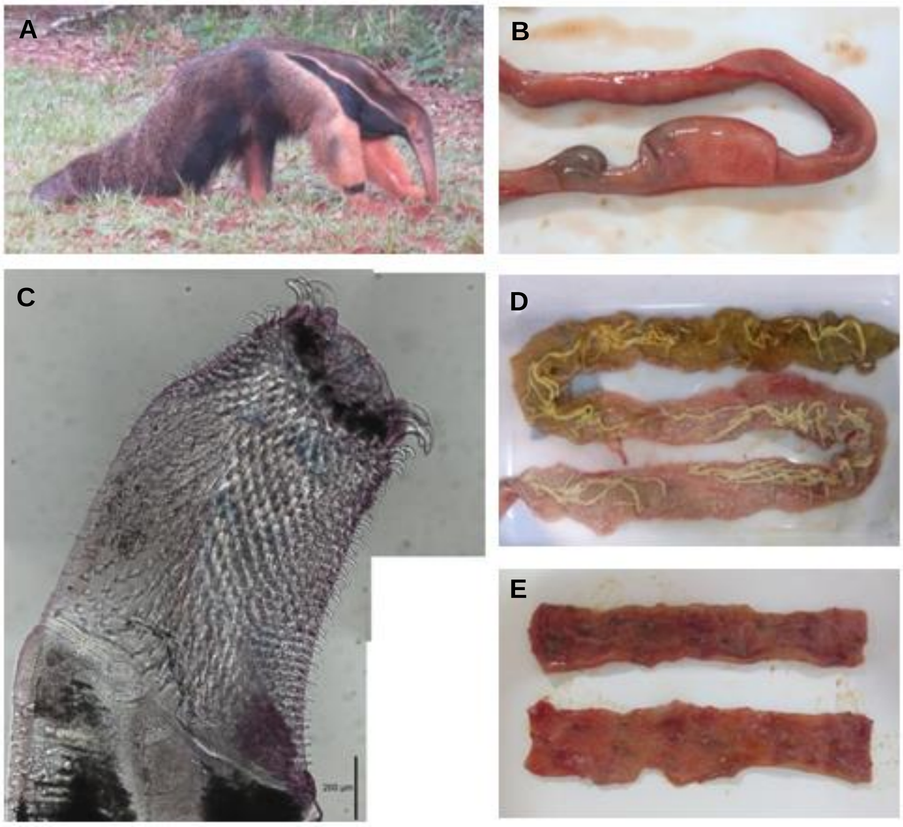


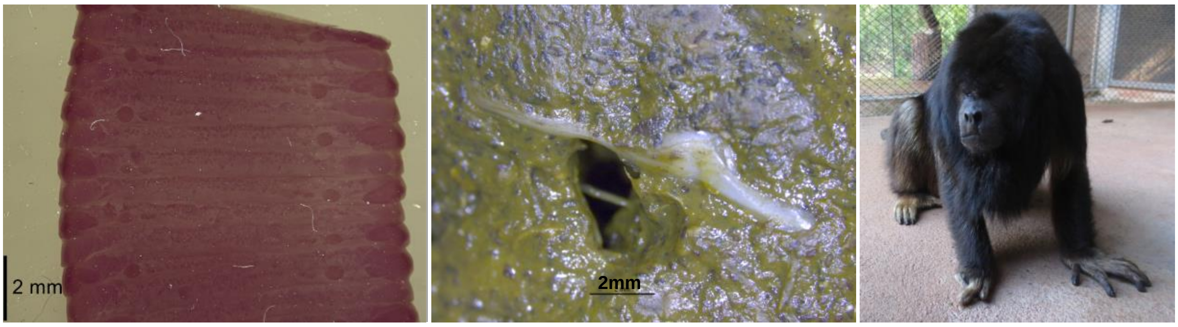
Figure 39 - Presence of the acanthocephalan *Gigantorhynchus echinodiscus* in the small intestine (D) of a giant anteater (*Myrmecophaga tridactyla* - A), causing obstructions (B). The thorny proboscis (C) causes severe lesions of the intestinal wall (E).



2.2.2. Impact of times sampled

In order to evaluate the impact of multiple sampling in this study, all positive cases that were sampled three times (n=33) were evaluated. In total, 50 different parasite eggs were detected in either flotation or sedimentation methods. Of those, 42% were present all three days, 36% only on two days and 22% on only one of the days. The parasites that were detected on only one or two days, mostly showed low egg counts: nineteen (66%) of the parasites that weren't detected in at least one day had egg counts ≤50EPG and another five (17%) ≤500EPG. The remaining 17% had higher egg counts (n=2) or were eggs of *Bertiella* spp. (n=3). This cestode releases proglottids into the feces that are very hard to find (Figure 40), and the eggs tend to appear in the fecal exams only when the samples are a little older, giving time for disintegration of the proglottids.

Figure 40 - Proglottids of *Bertiella* spp. in a fecal sample of a black howler monkey (*Alouatta caraya*) easily go unnoticed as they are small, slender and elastic, resembling mucous (Originals).



When analyzing if an animal was infected or not by any GI parasite detectable with simple flotation and sedimentation methods, 64% of the positive animals tested positively on all three days, 24% on only two and 12% on only one (Table 15).

Table 15 - False negatives in samples that tested positive at least one day out of three.

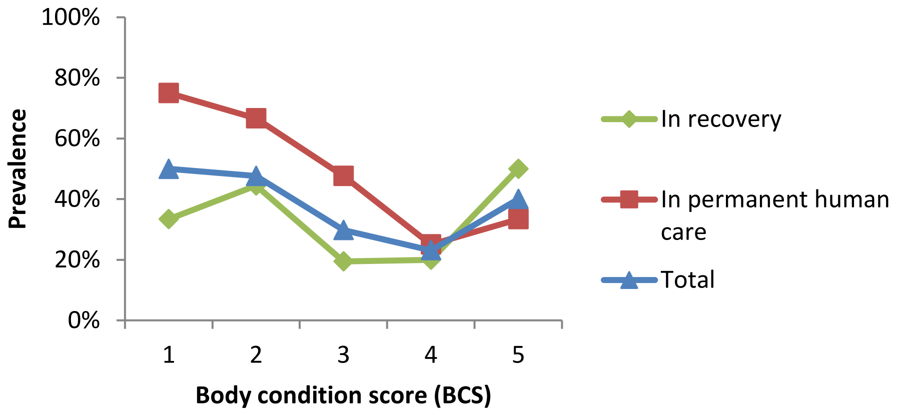
Positive **False negative**	**3 days +** **none -**	**2 days +** **1 day -**	**1 day +** **2 days -**
Parasite type level (n=50)	42% (21)	36% (18)	22% (11)
Host level (infected or not) (n=33)	64% (21)	24% (8)	12% (4)

2.2.3. Correlation between infection and body condition

Body condition score (BCS) evaluation by mere observation showed to be quite difficult, since animals were not always close by in the enclosures and fur and feathers make it extremely hard to correctly evaluate the BCS without palpation. Therefore, only the animals that were directly manipulated or necropsied were considered for this analysis. Also group samples with two or more animals were excluded, as BCS might be different amongst the animals and only one infected animal is enough for the sample to be positive.

No statistical significant difference was found between BCS groups (chi-squared test for independence, $p>0.05$), nor was there a linear tendency (chi-squared test for trend, $p>0.05$). Nonetheless, there seems to be a tendency for higher prevalence in animals with low body condition scores. There were not enough animals with BCS 5 (only five) to allow making a comparison (see Figure 41).

Figure 41 - Prevalence divided by body condition score.



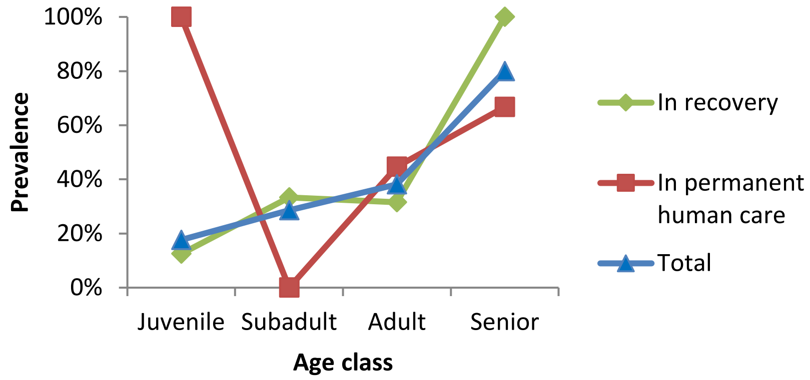
2.2.4. Correlation between infection and clinical signs

No statistically significant difference was found between prevalence and general clinical condition (healthy vs. not healthy, chi-squared test for independence, $p>0.05$). On the other hand, the presence of indicative clinical signs of GI parasitism (such as diarrhea/low fecal consistency or anemia) was highly statistically significant (Fisher's exact test, $p<0.001$), with prevalence of animals that showed these type of signs around 73% versus 31% in animals that didn't.

2.2.5. Correlation between infection and age

For this analysis, groups with mixed ages were excluded, leaving a total of 139 samples, 78 in recovery and 61 in long-term human care. The animals were classificated as juveniles (still in need of maternal care and feeding), young adults (before full development and sexual maturity), adults and seniors. For the total of samples, a significant increasing linear trend of prevalence was found with the advance of age (chi-squared test for trend, $p<0.05$), that can be observed in Figure 42. There were not enough data points in the juvenile and senior categories to test the "in recovery" and "in permanent human care" groups separately. There was only one juvenile and one young adult animal in permanent human care and two senior animals in recovery that are represented by extreme prevalence values (100% or 0%), therefore these results should not be object for analysis. Nonetheless, the curve of "animals in recovery" runs quite closely with the total curve.

Figure 42 - Prevalence among different age groups.



2.2.6. Treatment efficacy

Repetition of egg count at exactly day 21 was not always possible because of particularities from certain species (e.g. snakes may defecate only once every couple of weeks) or due to early release. A total of 30 distinct infections were treated amongst nineteen individuals, as stated in Table 16, and twelve (40%) inefficacious treatments were detected. The most evident ones were acanthocephala in giant anteaters (*Myrmecophaga tridactyla*), capillarids in scarlet macaws (*Ara macao)* and *Eimeria* spp. in toucans (*Ramphastos toco*).

Table 16 - Treatments and results of fecal egg count and oocyst reduction test.

	Species	Parasite	Treatment	EPG/OPG reduction
Mammal	*Felis pardalis**	Cestode (Diphyllobothriidean)	Pyrantel pamoate 128 mg + Praziquantel 75 mg + Febantel 225 mg), PO	100%
	Myrmecophaga tridactyla	Strongylid type egg	Ivermectin 0.3 mg/kg SC	-189%
	Myrmecophaga tridactyla	Strongylid type egg	Ivermectin 0.3 mg/kg SC	97.1%[a]
	*Myrmecophaga tridactyla**	Strongylid type egg	Pyrantel pamoate 725 mg + Praziquantel 250 mg + Febantel 750 mg, PO, repeat after 15 days	100%
	*Myrmecophaga tridactyla**	Strongylid type egg	Pyrantel pamoate 725 mg + Praziquantel 250 mg + Febantel 750 mg PO, repeat after 15 days	100%
	Myrmecophaga tridactyla	Acanthocephala	Ivermectin 0.3 mg/kg SC	-130%
	Myrmecophaga tridactyla	Acanthocephala	Ivermectin 0.3 mg/kg SC	-177.2%
	*Myrmecophaga tridactyla**	Coccidia	Toltrazuril 0.5 g, PO	100%
	*Myrmecophaga tridactyla**	Coccidia	Toltrazuril 0.5 g, PO	100%
	*Tamandua tetradactyla**	Strongylid type egg	Pyrantel pamoate 696 mg + Praziquantel 60 mg	100%
	*Tamandua tetradactyla**	Coccidia	Toltrazuril 0.15 g, PO	100%
	Puma yagouaroundi	Hookworm	Ivermectin 0.2 mg/kg SC	100%

* In human care for > 1 year
[a] Nematodes present at necropsy, ca. 1 month later.

(Continued on next page)

Table 16 - Treatments and results of fecal egg count and oocyst reduction test (cont.)

Bird	*Ara macao**	Capillarid	Ivermectin 0.2 mg/kg IM	63.9%
	*Ara macao**	Capillarid	Ivermectin 0.2 mg/kg IM	14.5%
	*Ara macao**	Capillarid	Ivermectin 0.4 mg/kg IM	0%
	*Ara macao**	Capillarid	Ivermectin 0.8 mg/kg IM	88.3%
	Ara macao *	Capillarid	Mebendazol, 100 mg/kg PO for 5 days, repeat after 10 days	100%
	*Ara macao**	Capillarid	Mebendazol, 100 mg/kg PO for 5 days, repeat after 10 days	100%
	*Asio clamator**	Coccidia	Toltrazuril 25 mg/kg, PO q7d, 3 times	100%
	*Crypturellus parvirostris**	Strongylid type egg	Albendazol, 1 mg, PO, q10d	100%
	*Penelope obscura**	Capillarid	Levamisol 20 mg/kg SC	100%
	*Penelope obscura**	Capillarid	Levamisol 20 mg/kg SC	100%
	Pulsatrix perspicillata	Trematode	Albendazol, 12 mg, PO q10d	100%
	Ramphastos toco	*Eimeria* spp.	Trimetoprim-sulfametoxazol 25 mg/kg PO for 7 days	- 68.7%
	Ramphastos toco	*Eimeria* spp.	Toltrazuril 25 mg/kg PO q7d, 3 times	- 21.9%
Reptile	*Chelonoidis carbonaria*	Strongylid type egg	Pyantel pamoate 72.5 mg, praziquantel 25 mg, Febantel 75 mg PO, repeat after 10 days	75%
	Chelonoidis carbonaria	Trematode	Pyantel pamoate 72.5 mg, praziquantel 25 mg, Febantel 75 mg PO, repeat after 10 days	increase
	Chelonoidis carbonaria	Coccidia	Trimetoprim-sulfametoxazol 30 mg/kg IM, 5 days	100%
	Eunectes murinus *	Cestode (*Crepidobothrium* sp.)	Praziquantel 1.5 mg/kg, Pyantel pamoate 15 mg/kg	100%[b]
	Eunectes murinus *	Rhabditoid type egg	Praziquantel 1.5 mg/kg, Pyantel pamoate 15 mg/kg	99.3-100%[c]

* In human care for > 1 year
[b] Day 10 (1st defecation after deworming): deformed cestode eggs; Day 29 and 39 - 100%
[c] Day 10 (1st defecation after deworming): 100% ; Day 29: 99.3% ; Day 39: 100%.

3. Discussion

The responding wildlife rehabilitation centers (a reactive conservation measure) are more concentrated within biodiversity hotspot areas when compared to HBWAs (Figure 24), meeting the conservation priorities established by Brooks et al. (2006) and Mittermeier et al. (2011).

The analysis of the questionnaires showed that the vast majority of rescued wildlife cases are received by government subsidized CETAS, with admissions reaching over 5000 animals per year. These CETAS with higher caseloads tend not to have any animals in permanent human care, as this is not their function. They instead transfer irrecoverable animals to other organizations such as licensed wildlife keepers and zoological parks, as is intended by law (IN nº 23/2014; IN nº 19/2014; CONAMA resolution nº 457/2013; DF nº 6686/2008; DF nº 6514/2008).

Considering a regular work schedule (40 h/week, 20 days vacation), caseloads vary from about 4 or 5 up to eighteen casualties per veterinarian per day. In general small animal veterinary practice, routine consultations are reported to take from 5 to 30 minute, with clinicians seeing between 5 and 15 patient a day. Larger consultation times are associated with a better service and a lower stress level of the veterinary surgeon (Meehan & Bradley, 2007; Özkul, Genç, Dogan & Özen, 2008; Everitt, Pilnick, Waring & Cobb, 2013). First consultations are reported to take longer than revisits, and the more problems are addressed in a consultation, the longer it takes (Robinson, Dean, Cobb & Brennan, 2014). Although it's hard to compare small animal consultations, where a high percentage of the cases are preventive and about welfare issues (e.g. vaccination), to the admission of wildlife casualties, which are often polytraumatized, orphaned or very debilitated animals, and need a full work-up since they always function like first consultations with no clinical background. The animals frequently need careful physical contention or anesthesia, increasing the complexity of the consult. Furthermore, the wildlife veterinarian is directly or indirectly responsible for the complete follow-up of the case, the consultation being inevitably followed by hospitalization, daily treatments, eventual surgery, feeding scheme, behavioral conditioning, and so on. With the reported caseloads and considering a regular work schedule, the veterinarian can dedicate a rough estimate of about 0h26 to 1h44 to each case from its admission to its full recovery, which is virtually impossible. For domestic animals, communication with the owners are considerable and important portions of the consultations (Meehan & Bradley, 2007; Özkul, Genç, Dogan & Özen, 2008; Everitt, Pilnick, Waring & Cobb, 2013). This aspect is absent in wildlife cases, but is often replaced by communication with the public and environmental awareness actions (Pellegrini, 2008; Behling et al., 2014; Mullineaux, 2014; Ascom/Ibama/PI, 2015).

Considering the above, the CETAS appear to be greatly understaffed, concurring with the recent reports (Associação Mineira de Defesa do Ambiente, 2009; Develey, 2012; Coissi, 2015), and justifying the refusal of further admissions by some centers in order to not compromise the care of the animals under their responsability (Develey, 2012; Coissi, 2015).

The maintainence of an inadequate parasite control is not only an issue of animal care and welfare, but also a public health concern. There are many zoonotic parasites with wildlife reservoirs (RENCTAS, 2001; Silva, 2004; Gómez & Nichols, 2013), and several were found during this study, such as *Balantidium* spp. in suids and *Bertiella* spp. in black howler monkeys. This cestode, transmitted through an oribatid mite, is highly prevalent in black howler monkeys in the wild (75% in this study) and normally causes no clinical signs (Júnior et al., 2008; Oliveira, Prates, Mentz, & Bicca-Marques, 2008), but when it infects humans it can be quite pathogenic (Paçô, Campos, & Araújo, 2003).

<u>Routine diagnostic and treatment protocols</u>

It is important to keep in mind that the results of the questionnaire are probably biased in a positive manner, since overcrowded/understaffed centers will not as easily find the time to answer the questionnaire, which was quite extensive.

The management of GI parasites in rescued wildlife in Brazil seems to be approached with care. More than half of all participating organizations perform diagnostic tests upon admission and around 80% deworm the animals. Nonetheless, around 50% apply antiparasitic drugs without performing any diagnostic testing or independently of the obtained result. The same happens upon release for 33% of the centers. This practice is rather undesirable, as the uncontrolled use of antiparasitic drugs may be unnecessary and potentiate the development of resistances (Kaplan, 2004; Wolstenholme, Fairweather, Prichard, von Samson-Himmelstjerna & Sangster, 2004). Ideally all animals would be submitted to diagnostic tests before performing any treatment (Miller, 2012).

When rescued from illegal captivity, the parasitological profile of the animals may be very different of the “natural” infection pattern, since these animals lived in a different habitat, with a different feeding pattern and in contact with different hosts, including humans. The presence of antropozoonotic parasites is more likely. A higher contamination level of an individual enclosure (e.g. cage) also potentiates infections by fewer species but with a higher intensity of infection, since their environment tends to be more contaminated (Bush, Lafferty, Lotz, & Shostak, 1997; Fromont, Morvilliers, Artois & Pontier, 2001). These factors would justify a tighter parasite control in animals that come from illegal captivity, which is the case for only 5 (~15%) of the participating organizations. It would be interesting to study the difference between the parasitological profile of free-living and captive specimens of the same species, in order to evaluate the magnitude of these differences and create guidelines for parasite control based on more species-specific evidence.

When the animal is to be released, there is some controversy if deworming is recommended or not. From a biological point of view, it is very likely that the animal will be reinfected when back in the wild, and therefore the release with a low parasite load may actually be beneficial, since it may keep certain levels of immunity. On the other hand, the release is rarely done at the place of capture, so translocation of parasites into an uninfected area may be an issue, this being the reason why many authors recommend deworming these animals (McLaughlin, 2008). When the release is to be done far of the capture site, the risk of translocation of parasites is higher (Miller, 2012), which would justify a tighter parasite control (Zieger & Cauldwell, 1998; RENCTAS - Rede Nacional de Combate ao Tráfico de Animais Silvestres, 2001). Another point in favor of deworming is that the release process into an unknown area itself is already stressful, and therefore, the elimination of the pre-existing parasite burdens is welcome (Zieger & Cauldwell, 1998). Miller (2012), recommends that the release of animals is done within approximately fifteen kilometers from the point of capture (and less than one kilometer for reptiles and amphibians) whenever possible and reasonable, in order to maximize their chance of survival and minimize the unnatural spread of parasites, diseases and genetic material among wild populations. From a legal perspective, the Brazilian law indicates that coproparasitological exam (direct fecal smear, flotation and sedimentation) should be done at least three times separated by a fortnight, but it does not state that treatment is mandatory.

Most of the participating organizations did not consider the presence of GI parasites as an impeditive factor for release. Its importance rose a little when parasite loads are high, probably more because it would indicate that the animal is not clinically healthy and therefore not ready for release, rather than problems issued by translocation. This shows that there is a higher focus on the individual animal health and a lesser preoccupation with the ecological risks associated to the rehabilitation actions, an issue that should be paid more attention to (Branco, 2008). Although some reported that they considered GI parasitism as an impeditive factor for release far from the capture site, the routine procedures for close and far releases were nearly the same, if anything a little less strict approach for far releases. This inconsistency may derive from limiting factors such as time (Table 6 and Table 7), or other factors such as the possibility of the animals passing through other organizations during the transport and being dewormed according to their protocols, as commented by one of the organizations.

When addressing animals in permanent care, the focus lays on maximizing the wellbeing of the individual animal, and one aims for a minimal parasite load, similarly to domestic animals (Stull, Carr, Chomel, Berghaus & Hird, 2007). This line of thinking is followed by the large majority of the responding organizations (95%), although the percentage basing their deworming actions on diagnostic result should be higher.

In the case study of CCFS, the animals in permanent captivity showed a statistically significant superior prevalence when compared to the animals in recovery. This result is surprising considering that most of these animals had been dewormed over the past years. Exact numbers are hard to evaluate since there were some breaches in the record keeping of these routine procedures. Even though, from the nine groups whose records specifically mentioned deworming over the previous five years, five tested positive (55.6%).

The higher prevalence amongst animals in permanent human care may be consequence of a variety of factors (Munenea, Otsyulaa, Mbaabub, Mutathic, Muriukid & Muchemie, 1998; Gordón, Prados, Romero, Moreno, Pontes, Osuna & Rosales, 2008):

a) contaminated enclosures - captive animals are often exposed to a larger number of eggs or intermediate hosts than free living animals, especially if the enclosures are crowded. This is one of the reasons appointed for the higher prevalence of capillariosis in captive birds, together with the presence of different closely related host species, since several capillarid species have low host-specificity (Yabsley, 2008a). In this study, 100% (n=7) of the capillarid infections in birds were detected in long-term captive animals.
b) breaches in the deworming protocols;
c) introduction of new, not dewormed animals in the enclosure;
d) stress from prolonged captivity and/or disease;
e) contamination from the environment, especially carried by free-living animals. The examination of free-living animals in CCFS confirmed the possibility of several definitive or intermediate hosts easily transmitting infective forms to the animals from the zoological park by direct or indirect contact and there are hosts and intermediate hosts that live within the territory of CCFS that were not analyzed. In the questionnaire it became evident that, although 87% have active measures in place to minimize contact with free-ranging animals, for the majority (78%) at least indirect contact with these animals is still unavoidable. The presence of an intermediate host (such as mites or termites) in the life cycle of the parasite may turn these infections particularly hard to control, and resident animals will need periodical deworming to be kept parasite free. Animals such as birds frequenting or living in the area and flying over or sitting on the enclosures are also very hard to control. Domestic cats are known to roam around the center and invade some enclosures to steal food (mostly meat) from the carnivores. This could be a possible source of the *Toxocara cati* infection found in eyra cats (*Puma yagouaroundi*). CCFS controls these unwanted visitors by setting up traps to capture the cats, sterilize them and turn them in to local authorities. Free ranging animals are also to be kept in mind when pseudoparasitism is concerned: a toucan (*Ramphastos toco*) presented a very high count of *Bertiella* spp. eggs, a cestode of the black howler monkeys that roamed in the trees above its cage and occasionally defecated in it.

   For animals in recovery that ought to be released, contamination from other wildlife species should not be a problem, as they will also contact with them when free ranging. Knowing that total isolation is difficult to achieve, the focus should be on minimizing the contact as much as possible. Special attention should be given to animals in permanent care whose parasites' reservoir hosts are known to visit the premises. Zoonotic parasites and their hosts should also be given close attention in order to guarantee the health of not only the animals but also the staff. If necessary, deworming of the free-ranging animal may be attempted (Miller, 2012).

f) infection through feeding - the inclusion of uncontrolled products is common (performed in 47% of the organizations) and the analysis of the feedstuff in CCFS confirmed them as a possible source of infection to the animals and also to the handlers, with zoonotic agents such as *Hymenolepis* spp., that can be directly transmitted to humans (Bowman, 2013). This reinforces the importance of controlling (testing and deworming) the bioterium animals, not only to prevent the transmission of certain parasites through feeding, as to create the best conditions for breeding and rearing of these animals and to ensure the safety of the handlers. The easiness in controlling parasitic forms in different classes of feedstuff varies a lot. It is easy to keep a good parasitic control of animals in the bioterium. Freshly caught or slaughtered prey such as fish or meat can be frozen in order to inactivate parasitic forms, but it requires freezing equipment and storage space (Adams, Murrel & Cross, 1997). On the other side of the spectrum there are certain feedstuffs such as termites, which are basically impossible to obtain free off parasitic forms. A balance should be made between the benefits and risks of this type of feeding, and the benefits on nutrition level and environmental enrichment often outweigh the risk of infection. For instance, the feeding of termites to anteaters: in captivity, these animals are usually fed a liquid multi-ingredient mash. Animals that did not grow up in captivity tend to completely reject this food, and even when they consume it, there is still investigation towards optimizing the formula, as it is very hard to match such a specific diet (Nofs, Dierenfeld & Backus, 2017; Stannard, Bekkers, Old, McAllan & Shaw, 2017). In such a case the inclusion of the untested food in their diet is almost essential to meet their nutritional requirement and stimulate their natural behavior, and respect the freedoms of animal welfare (Farm Animal Welfare Council, 1979; Royal Society for the Prevention of Cruelty to Animals, 2009).

The first three discussed points are mainly management related and are easily improved by sharpening deworming, record keeping and hygiene protocols.

Hygiene and biosecurity

Hygienization protocols amongst the inquired organizations were very good, with 81% cleaning the enclosures on a daily basis, as recommended by Miller (2012). The use of separate cleaning equipment for different enclosure, however, is not that widespread. Depending on the sector, only 56% to 72% use individual equipment, the lowest percentage being for the hospitalized patients. This may seem logical from a practical point of view, since cages and enclosures in the hospitalization sector are usually close together in a relatively small area, but this is the place where diseased animals and incoming wildlife casualties are usually lodged when there is no quarantine, so it is important to avoid contact between them.

Also in between residents there is room for improvement in the sanitary methods taken. A minimum of physical and chemical cleaning methods are used by nearly all organizations (>80%), but the recommended use of heat methods (flamethrower or steam) and/or sanitary breaks to break the transmission cycle of more resistant parasites (Roussere et al., 2003; Miller, 2012) is only applied by less than 60%.

Frequency of cleaning depends on several factors, such as type and size of the enclosure, species and age. In mammal species, daily removal of feces and urine is necessary to prevent odor, parasite re-infestation and insect overpopulation, but avian, reptile and amphibian cages usually require less frequent cleaning. Sometimes the ideal frequency from a sanitary point of view is not ideal from a rehabilitation point of view, since many species are very easily stressed. Infant animals tend to be less easily stressed and require much more frequent cleaning because they tend to have limited movement and soil their nest more frequently. When an enclosure changes occupant(s), the bedding material should be changed and it should be properly cleaned and disinfected (Miller, 2012).

Predatory fungi can be a good alternative to chemical and physical desinfectants, namely in very resistant free living exogenous stages, such as ascarid eggs, particularly the ones from *Baylisascaris procyonis*, due to their predatory effect over these parasites in the environment (Cazapal-Monteiro et al., 2015; Madeira de Carvalho et al., 2017).

Diagnosis

The most commonly used techniques for diagnosis worldwide are qualitative flotation and sedimentation techniques and quantitative McMaster technique (Monteiro, 2011; Bowman, 2013). Also among the inquired organizations qualitative flotation and sedimentation techniques were the most common choices. The use of the quantitative McMaster test was surprisingly low (only 13%), although this is a widespread technique to determine if an infection is intense or not and to establish thresholds for deworming (Bowman, 2013). When working with wildlife recovery this concept seems to be even more relevant, since the goal is not to obtain a minimal parasite load, but to maintain the natural parasite-host equilibrium (Jaenike & Perlman, 2002; Meffe, Carroll, & Groom, 2006).

The main reported limiting factors for diagnostic testing in wildlife rehabilitation centers in Brazil were the lack of resources, time and equipment or infrastructure.

The simple fact of performing diagnostic tests to determine whether the animal should be dewormed or not is a way of saving costs, as systemic deworming of all animals is likely to be more expensive than targeted deworming according to the obtained results.

Subcontracting an external laboratory is the most effective way to save time, but it is also the most expensive option, besides delaying the results. Selecting where to perform the diagnostic tests can be a great way of cost saving.

Cooperation with external laboratories such as universities could reduce both the economical and temporal strain on the center, with the added advantage of potentially creating scientific output that may be useful in the future. In this study, CETAS associated to universities carried out an average of 4.6 diagnostic tests, opposed to 2.6 tests in independent CETAS, reflecting the advantage of collaborating with the parasitology laboratory and students. These kind of protocols, maintained by 41% of the participants, have the downside of not always working ideally. Sometimes the analysis are only guaranteed during a certain student's research project or there are periods with no availability to their service (e.g. vacation periods).

More than half of the participants (56%) do perform at least the basic diagnostic parasitological tests on site, as it is the way that provides the fastest answers at the lowest cost. A basic laboratory can be easily set up with a minimal amount of space and investment, as shown in Table 17. For a small lab, consisting of one microscope, two McMaster chamber, ten test tubes, one strainer, one scale, five gobelets, fifty slides, one test tube holders, four stirring rods, three sedimentation cups and five Petri dishes, the grand total is just about under 300€ (1130 Brazilian reais). The variable costs for the most common tests round about 0.07€ (0.25 Brazilian reais) per test. These estimates indicate that resorting to an in-house laboratory can largely reduce costs associated to outdoor fecal testing with the added advante of eliminating the waiting time associated with sending the fecal sample and waiting for the results.

Table 17 - Equipment for a basic laboratory set-up.

		Equipment	Approximate price (€)	Status
Basic equipment for flotation, sedimentation and quantitative egg counts		Microscope*	200	permanent
		Slides	0.03 each	reusable
		Coverslips	0.03 each	consumable
		McMaster chamber	20	reusable
		Sugar	0.80/kg	consumable
		Test tubes	0.15 each	reusable
		Strainer	2.00	permanent
		Pipets	0.03 each	consumable
		Scale **	20	permanent
		Gobelet	2.00 each	reusable
		Test tube holder	3.00 each	reusable
		Stirring rods	0.50 each	reusable
Additional exams	Baermann technique	Sedimentation cup	2.00 each	reusable
		Gauze	0.70/meter	consumable
	Oocysts culture	Potassium dichromate	20/kg	consumable
		Petri dish	0.16 each	permanent
	culture for larvae	Glass	0.10 each	permanent
		Gauze	0.70/meter	consumable
		Charcoal, dehydrated horse feces or similar	0.80/kg	consumable

* A professional microscope that magnifies up to x1000 is always desirable as it can be used for many tests. For simple fecal tests, x400 magnification is enough to detect organisms as small as *Cryptosporidium* spp. and Giardia. ** Some laboratories use a volume measure (e.g. in a syringe) that represent approximately 2 g of feces, but when working with so many different species with different fecal densities and many species that may not excrete enough feces to complete 2 g, the use of a scale is necessary for quantitative coproparasitology.

The problem with indoor testing is that it is quite time consuming and needs some expertise on the subject. The tests are quite easy to carry out and can be taught by the veterinarian to technicians or students in the center. A full coprological examination with the described methods takes around 20-30 minutes (Monteiro, 2011) which is time that can hardly be dispended at these centers that are already working above their capacity (Branco, 2008; Gomes & Oliveira, 2012; Azevedo, 2017).

Considering that the objective is to make decision from a clinical and ecological perspective and not to perform research, one may simplify the diagnostic process in order to save time and resources, such as the technique used in the laboratory of FMV-ULisboa. This method requires weighting, mixing, filtering and then leaving to rest only one fecal aliquot to perform the three main tests, considerably reducing the time and resources spent (Bernardino, 2014; Cabaço, 2014).

Other, more time consuming diagnostic tests can be reserved for selected patients or be overridden by other decision factors. For instance, the modified McMaster method used in this project is a bit more time consuming, but alternatively, one may decide to deworm whenever trematode or acanthocephalan eggs are present, since the first are many times shed in very low numbers despite considerable infections (e.g. with hepatic trematode infections) and acanthocephalans are very aggressive for the mucosa because of their thorny proboscis (Bowman, 2013).

Similarly, the use of fecal culture is not a priority for clinical decisions within the current state-of-the-art of wildlife parasitology, although it can give relevant epidemiological information. The identification will generally go no further than certain parasite groups, since there are nearly no identification keys nor descriptions of L3 larval stages for the vast majority of wildlife parasites. This might be limiting because it hampers the ability to pinpoint the specific parasite in question, with the associated increase in the difficulty of management (medical or otherwise).

Many sources recommend to perform several fecal samplings, in order to detect parasites with intermittent egg shedding (van Gool, Weijts, Lommerse & Mank, 2003). When analyzing the values of the case study of CCFS, if only one random sampling was performed, 73% of the parasites would be detected and 84% of the positive animals would be effectively diagnosed as such. From a clinical point of view, around 90% of the infections that meet the criteria for deworming would be detected. All considered, although it reduces the sensitivity, one single sampling appears to show the best trade-off between time invested and detecting rate.

Ideally, all animals would be analyzed, but such often isn't feasible nor is it a priority from a clinical and biological point of view. Performing fecal tests for all admitted animals is very time consuming, and especially knowing that understaffing is an issue it becomes necessary to select which animals to sample. The selection of patients to be submitted to diagnostic testing can be done in many different ways. The characteristics that were evaluated in this project for the selection of the patients were:

a) BCS - The case study of CCFS didn't show a significant statistical correlation between parasitism and different classes of BCS. This is likely explained by the fact that these parameters are influenced by many different factors, making it hard to find a correlation in a sample this small, where all groups of animals are being handled at the same time, with big variations between species.
b) Clinical signs - Much like what happened with the BCS, the case study of CCFS didn't show a significant correlation between parasitism and general health condition. Again the correlation is hard to make when dealing with such multifactorial parameters. On a more specific note, although they can also be due to other causes, the presence of indicative clinical signs of GI parasitism, such as diarrhea or anemia, were statistically significant correlated with a higher prevalence. This finding is of course to be looked at with care. For instance, all studied giant anteaters in captivity showed low fecal consistency, but it is known that the diet in captivity causes this effect. At the same time, they showed a 100% prevalence for GI parasites, so it's hard to determine which factor is the main cause of the referred sign. The presence of normal feces in equally infected giant anteaters from the wild raises suspicion that it could be just a consequence of nutritional parameters. In the questionnaire, more than 80% of the participants considered diarrhea and anemia indicative of parasitism, but also loss of BCS.
   Although lacking enough data to make any conclusions, a possible link between debilitated animals was discussed when analyzing the evolution of the egg counts in polytraumatized anteaters (Bomon et al, 2015). The presented results only take into account one species and have a low number of observations, but they do concur with published date about the effects of host fitness and stress on the host-parasite balance (Townsend et al., 2006; Beldomenico & Begon, 2010; Bowman, 2013). Further investigation should be performed, but the standard deworming of considerably debilitated animals may be recommended, even when their initial parasite load is low.
c) Age - A significant increasing linear trend of prevalence was found with the advance of age, with senior animals more prone to be infected. An explaining hypothesis may be that the older the animal, the higher the chance of being in contact with the parasite and getting infected, associated with a decrease in immune response (Weksler, 1993).

Adressing the third biggest limiting factor - the reported lack of equipment or infrastructure: this may be perceived as an important hindrance to the process, but as shown above not much equipment is needed, and a small investment will pay itself quickly.

Treatment / deworming

The main reported limiting factors for treatment were lack of funds and difficult access to drugs. Both these factors are hard to reduce, although some alternative management options could diminish the need of chemical parasite control. These measures range from regular cleaning, use of heat, plants or fungi to reduce the presence of infective forms in the environment (Kaplan, 2004; Engström et. al., 2016; Madeira de Carvalho et al., 2017).

Another reported struggle for the treatment of the animals is the difficulty in administrating the drugs. This aspect is not always as easy to handle as it seems. The use of injectable drugs assures that the animals were administered the right dosage, but the animals have to be captured and handled, which may cause a lot of stress, especially if administrations have to be repeated. Administrating the drug through the food or water basically eliminate the stress associated to the administration, but has the disadvantage that often it is not known if the animals consumed the right amount. There are several species (like primates) that are very selective in their food intake, hardly accepting non-palatable or unfamiliar contents in their food. Close monitoring to guarantee a correct intake is important, since the ingestion of a lower dosage than indicated may feed the development of resistances (Kaplan, 2004; Wolstenholme, Fairweather, Prichard, von Samson-Himmelstjerna & Sangster, 2004).

In both the questionnaire and the case study high rates of unsuccessful treatments were reported. 74% of the organizations that retested the animals after treatment had already encountered inefficacious treatments, 39% reporting them to be frequent. Forty percent of the performed treatment in CCFS were unsuccessful. Some of the inefficacious treatments detected in CCFS were also reported in the questionnaires, such as capillarids and coccidians in birds and trematodes.

The term resistance is frequently used when a greater proportion of parasitic organisms within a population are still alive after exposure to an antiparasitic compound, but it's more correct to define it as the selection of resistant phenotypes in parasite populations through regular application of antiparasitic drugs (Bowman, 2013). Heritability is the most important feature of resistance (Lanusse, Alvarez, Sallovitz, Mottier, & Bruni, 2009). In wildlife, antiparasitic drugs are nearly always used off-label, since the drugs are not tested in, nor licensed for wildlife species, and studies are scarce. Therefore, many of the reported so called resistances may just be because of inadequate treatments plans, and the registered inefficacy may be caused by several reasons. For instance, the drug may not be adequate to kill the parasite in question or may not be metabolized in the expected way by the host and therefore not be distributed in the right concentration or distributed in the right tissues (Madeira de Carvalho et. al., 2017).

One of the main reasons for the high percentage of unsuccessful treatments is the lack of literature on these parasites and these host species, leading to the selection of inadequate drugs, dosages and routes of administration (Mullineaux, 2014). The extrapolation of dosages from domestic species may often be inadequate. For instance the use of ivermectin to kill the acanthocephalan that infects giant eaters turned out to be highly ineffective, although the same dosage is used with success to treat *Macracanthorhynchus hirudinaceous* infections in pigs (Milagro et al., 2013). It also proved to be ineffective to reduce the strongylid egg count of these animals, whereas the use of a combined pill with pyrantel pamoate, praziquantel and febantel solved the problem. It is hard to know if we are faced with actual resistances, where resistant phenotypes of parasites were selected through regular application of antiparasitic drugs (Bowman, 2013), or rather the selection of the wrong drugs, dosages or routes of administration, since there are no data about their metabolization in the hosts nor the susceptibility of their parasites (Madeira de Carvalho et al., 2017).

Considering the reality of wildlife rehabilitation centers, with a very diverse and rapidly rotating caseload, it may take a while to find an effective drug against certain parasites. Studies for effective treatment for frequently diagnosed parasites would therefore be useful. Also, the sharing of information through a communication platform or the publication of scientific notes may be a big help to advance the knowledge on antiparasitic treatments efficacy. This is important to avoid the creation of resistances, especially when zoonotic agents are concerned. It may also be the difference between saving an animal or not, as in some severe cases there is no time to experiment with different drugs.

The majority (81%) of the participants does consult the available literature to base their decisions on, and, as a second option, base their decision on personal experience. Since the available literature is so scarce, and personal experience is very valuable but also very prone to bias, retesting the animals is of uttermost importance.

Finally, both for captive and wild animals, the need for new approaches on parasite control is also very important, since control of parasitic infections in captive animals and in the wild is focused almost exclusively on deworming. Repeated anthelmintic use favors the emergence of anthelmintic resistance and increases anthelmintic metabolic waste to be eliminated with the feces or urine of animals generating ecotoxicity (Kaplan, 2004; Madeira de Carvalho et al., 2017). There are some microorganisms which prevent infection of animals by reducing the presence of infective stages in the environment. It is essential to understand this biological control as a regulatory measure whose aim is to control the harmful effects of the parasitic population and not its eradication. Among the various organisms studied as potential biological control agents there are predatory fungi that infect and destroy gastrointestinal nematode larval stages (but also trematode and cestode eggs), acting on their exogenous development stages in the environment (Madeira de Carvalho et al., 2017). Also different plants have been studied for nematode control (Engström, 2016).

4. Recommendations and guidelines

Based on the combination of the findings of the questionnaire and the case study presented in the discussion above, the following guidelines were formulated for GI parasite management in wildlife rehabilitation centers. It was attempted to achieve an equilibrium between what are the needs and what's possible to achieve, in order to optimize time and resources, by prioritizing more susceptible hosts and more prevalent parasites.

Diagnosis:

It is always recommended to perform diagnostic testing before deworming, for the following reasons:

- To know if there is any need for deworming. If negative or if the parasite load is low, treatment is not necessary, unless it's a zoonotic parasite. By avoiding unnecessary treatments the risk of creating resistances is lowered and precious resources are saved;
- To guarantee the usage of the most effective drug. Most deworming protocols do not include all possible agents, from nematodes to trematodes, cestodes and protozoa. Only identifying the group of parasite can make a big difference for treatment efficacy;
- To follow up the evolution of parasite load;
- To know if there is any zoonotic agent present.

Which animals?

- Potentially immunosuppressed animals:
  - Debilitated and polytraumatized animals. Also treat them even if they present low egg counts. Very debilitated animals can be dewormed right away with a large spectrum drug, but should nonetheless be tested, to infer if the right drug was used for the infective agent and to test treatment efficacy later on. These animals should be closely monitored, as deworming may take a dangerous toll on these animals by demanding resources for metabolization and creating a suddenly large amount of dead parasitic forms for the host's body to deal with (Eo, Kwak, & Kwon, 2014);
  - BCS 1/5. BCS did not have a statistically significant relation with prevalence. BCS of 2/5 is common amongst wildlife due to variety of factors, but cachectic animals are likely to be debilitated and possibly have some immunosuppression;
  - Senior animals
- Clinical signs indicative of GI parasitism: diarrhea, low fecal consistency or anemia.
- All animals that are to remain in permanent human care
- Before release - at least three times separated by a fortnight (according to recommendations of Brazillian law)

How many times?

In this study, around 90% of the infections that met the criteria for deworming were detected with only one day of sampling. Considering time restrictions, it would be very good if one sample for every eligible animal could be analyzed.

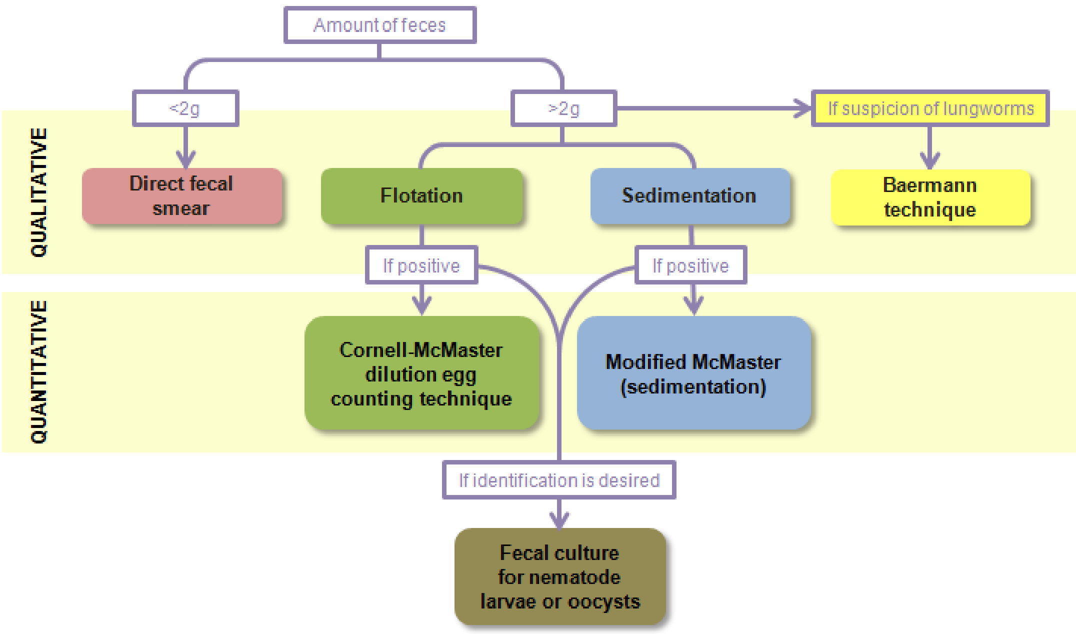
Where to test?

From the various possibilities, the most cost-efficient option should be picked considering that time and resources are the most common limiting factors. In the long run, the option that seems to be the most efficient in terms of time and money saving, is investing in an in-house laboratory and establishing *pro bono* protocols with external laboratories.

Which tests?

A decision tree of the recommended fecal exams in shown in Figure 43. If enough feces are present, the absolute minimum is to perform a simple flotation and sedimentation test. If one of these gives positive results, a quantitative test is desirable to evaluate the intensity of the infection to decide whether to treat.

Figure 43 - Simple decision tree for coproparasitological tests.



To maximize resources and reduce testing time to a minimum, one can use a single test tube to perform three tests:

- mix 2 g of feces with 30 ml of saturated sugar solution (or equivalent proportion) and filter the solution through a tea strainer to remove larger debris;
- Use the solution to
    a) fill a McMaster slide;
    b) fill a test tube up to the edge, forming a meniscus, and immediately covering it with a coverslip;

- Let both rest for 15 minutes;
- Lift the coverslip straight up, place it on a slide and scan thoroughly and methodically under x100 magnification;
- Count the eggs in the McMaster chamber (x100 magnification);
- Discard the supernatant, transfer a drop of sediment to a slide with a Pasteur pipette, cover with a coverslip and scan thoroughly and methodically under x100 magnification.

(Monteiro, 2011; Bernardino, 2014; Cabaço, 2014).

Since the modified McMaster is more time consuming and considering the pathogenicity of trematodes and acanthocephalans, one may opt for deworming whenever these are found. If there are clinical signs that lead to a suspicion of lungworm infections, it is recommended to perform a Baermann test. If there is a need for a better identification of the parasites, fecal culture may be carried out. Collection of parasites at necropsy and identification may be a big help to identify certain eggs in future examinations of the same host species.

<u>Treatment:</u>

Which drug?

The selection of drugs and dosages is ideally based on existing literature, when available, or on personal experience. Personal experience may be more valuable than interpolation from other species, especially when records are kept about previous treatments and their efficacy. The use of alternative parasite control methods such as plant or fungi are highly recommended, as they reduce the concern with resistances and ecotoxicity caused by the repetitive use of antiparasitic drugs (Engström, 2016; Madeira de Carvalho et al., 2017).

Retesting

Repetition of a quantitative fecal exam to perform a fecal egg count reduction test is essential to guarantee efficient treatment.

Records

Keeping records is highly recommended. A simple database as suggested in Table 18 could provide a useful tool to establish parasitic profiles by species and improve treatment efficacy over time. Ideally, there would be an easy way to share this information between organizations like an online platform. Species that consistently present a determined pattern of parasites can be considered for regular deworming.

Table 18 - Suggestion for record keeping of parasite prevalence and efficacy of treatments.

Species	Parasite	Drug	Dosage	Administration route	Initial egg count	Egg count after deworming	Egg count reduction
…	…	…	…	…	…	…	…

Animals in permanent human care:

When the animal is irrecoverable and will permanently stay in human care, the emphasis shifts from maintaining a balance between the "natural" infection level and the clinical recovery of the animal, giving the best possible life quality and health for the remainder of its life. Therefore, diagnostic testing and deworming depending on the results is recommended once or twice a year, similarly to domestic animals (Stull, Carr, Chomel, Berghaus & Hird, 2007). Special attention should be given to those known to carry zoonotic agents, such as primates. Retesting after deworming is still of high priority for the above mentioned reasons.

Hygiene:

Equipment: Use separate cleaning equipment for different groups of animals. Special attention to use separate cleaning equipment for in house animals, animals in recovery and in quarantine (Miller, 2012).

Frequency: Varies according to the species, but for most animals once daily is recommended (Miller, 2012). Contamination of the enclosures may be a key factor in the reinfection of animals within the enclosure.

Methods: Considering the statistically significant increase of prevalence among long-term captive animals highly likely perpetuated by contamination of the enclosure, physical cleaning is a must for daily maintenance. Sterilization with fire torch or steam is recommended for recurrent infections in the same enclosure or when these will change occupants, ideally followed by a sanitary break.

Biosecurity:

Free ranging animals:

- Ideally, contact with free ranging animals should be avoided;
- For animals in recovery that ought to be released, contamination from other wildlife species should not be a problem, as they will also contact with them when free ranging;
- Special attention to:
    - Animals in permanent care whose reservoir hosts are known to visit the premises;
    - Zoonotic parasites and their hosts.

Feeding:

- Bioterium: diagnostic testing and deworming depending on the results is recommended twice a year;
- Freezing of freshly caught or slaughtered feedstuff whenever possible.

5. Suggestions for further research

The performed study consisted of an umbrella approach to the thematic of GI parasitic management in wildlife rehabilitation centers. The questionnaire allowed to get a general picture of this reality in Brazil and to identify certain transversal problems. The combination of the obtained answers with the practical data obtained at CCFS allowed redacting suggestions for GI parasite management in wildlife rehabilitation centers.

Nonetheless, none of the topics was deeply approached, opening questions for many other research lines, like:

- Cost comparison between testing and treating depending on the results vs. treating all animals routinely;
- Importance of deworming hosts before release;
- Risk of translocation of parasites through release;
- Data sharing to fight inefficacious antiparasitic treatment;
- Study the parasitological profile of animals in illegal and permanent captivity, compared to the wild populations.
- Extend research to ectoparasites and hemoparasites;

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Annex I - Survey - Brazilian Portuguese

Caracterização do manejo de parasitas gastrointesinais em fauna silvestre no Brasil

Este questionário está sendo feito no âmbito do projeto de Mestrado Integrado em Medicina Veterinária na Faculdade de Medicina Veterinária da Universidade de Lisboa, sob orientação do Prof. Dr. Luciano Alves dos Anjos (UNESP) e do Prof. Dr. Luís Madeira de Carvalho (FMV-ULisboa).

Tem como objetivo caracterizar o manejo de parasitas gastrointestinais nos centros de conservação, triagem, reabilitação e/ou manutenção de fauna silvestre nativa brasileira, permitindo:

– Conhecer as rotinas de diagnóstico e tratamento de parasitoses gastrointestinais seguidas;
– Compreender as diferenças no manejo de parasitas gastrointestinais de animais mantidos sob cuidados humanos permanentes e animais destinados a reintrodução na natureza;
– Avaliar a possibilidade da translocação de parasitas gastrointestinais entre comunidades de animais separadas geograficamente;
– Compreender quais os fatores limitantes no manejo de parasitas gastrointestinais em fauna silvestre nativa.

O projeto não visa de modo algum avaliar o desempenho das instituições participantes. O anonimato e confidencialidade são garantidos, sendo que os dados recolhidos serão utilizados exclusivamente para fins estatísticos.

O questionário consiste de 25 questões de resposta rápida, requerendo cerca de 15 minutos para completar.
É idealmente respondido por um médico veterinário da instituição.

Desde já, obrigada pela sua participação!

Melody Bomon

*(* = obrigatório)*

Identificação da instituição

1. Nome da instituição: * ____

2. Estado * *(marcar apenas uma opção)***:**

☐ Acre
☐ Alagoas
☐ Amapá
☐ Amazonas
☐ Bahia
☐ Ceará
☐ Distrito Federal
☐ Espírito Santo
☐ Goiás
☐ Maranhão
☐ Mato Grosso
☐ Mato Grosso do Sul
☐ Minas Gerais
☐ Pará
☐ Paraíba
☐ Paraná
☐ Pernambuco
☐ Piauí
☐ Rio de Janeiro
☐ Rio Grande do Norte
☐ Rio Grande do Sul
☐ Rondônia
☐ Roraima
☐ Santa Catarina
☐ São Paulo
☐ Sergipe
☐ Tocantins

3. Cidade: * ____

4. **Tipo de instituição *** *(marcar tudo o que for aplicável)*
   - ☐ Jardim zoológico
   - ☐ Centro de reabilitação de animais silvestres (CRAS)
   - ☐ Centro de triagem de animais silvestres (CETAS)
   - ☐ Mantenedouro de fauna silvestre
   - ☐ Criadouro cientifico para fins de pesquisa
   - ☐ Criadouro cientifico para fins de conservação
   - ☐ Criadouro comercial de fauna silvestre
   - ☐ Comerciante de animais vivos de fauna silvestre
   - ☐ Faculdade de Medicina Veterinária
   - ☐ Outro: ______________________________

5. **Quantos animais de fauna silvestre nativa brasileira de vida livre e/ou apreensão (cativeiro ilegal) recebeu nos últimos três anos? *** *(marcar apenas uma opção)*
   - ☐ Recepção esporádica (<150 animais em três anos)
   - ☐ 150 - 1500
   - ☐ 1500 - 3000
   - ☐ 3000 - 6000
   - ☐ 6000 - 9000
   - ☐ 9000 - 12000
   - ☐ 12000 - 15000
   - ☐ > 15000
   - ☐ A instituição não recebe animais provenientes de vida livre e/ou cativeiro ilegal
   - ☐ Outro: ______________________________

Manejo de parasitas gastrointestinais de animais à entrada na instituição

6. **À entrada na instituição, quais as rotinas de diagnóstico e desparasitação de parasitas gastrointestinais que costumam ser seguidos? *** (Dx = Exame de diagnóstico de parasitas gastrointestinais; Tx = Desparasitação contra parasitas gastrointestinais) *(marcar uma opção por linha)*

	Dx apenas	Dx + Tx em caso de resultado positivo	Dx + Tx em caso de carga parasitária superior a determinado limite	Dx + Tx independente do resultado	Tx apenas	Nenhum	Não aplicável
Todos os animais provenientes de vida livre							
Todos os animais provenientes de cativeiro ilegal							
Todos os animais provenientes de outras instituições							
Animais com sintomatologia clínica que possa indicar parasitismo gastrointestinal							
Animais com outros sinais clínicos / animais debilitados							
Animais que vão ficar sob cuidados humanos permanentes							

Observações; outros critérios de decisão para diagnóstico e desparasitação de parasitas gastrointestinais de animais que entram na instituição:

7. **Quantos animais de fauna silvestre nativa brasileira são mantidos sob cuidados humanos permanentes na instituição? *** (Inclui animais na área de exposição ou sector extra em jardins zoológicos e animais irrecuperáveis.) *(marcar apenas uma opção)*
   - ☐ < 20
   - ☐ 20 - 200
   - ☐ 200 - 400
   - ☐ 400 - 600
   - ☐ 600 - 800
   - ☐ 800 - 1000
   - ☐ 1000 - 1200
   - ☐ 1200 - 1400
   - ☐ >1400
   - ☐ Não são mantidos animais sob cuidados humanos permanentes na instituição *(Passe para a pergunta 9)*

Manejo de parasitas gastrointestinais de animais de fauna silvestre nativa sob cuidados humanos permanentes na instituição

8. **Nos animais de fauna silvestre nativa mantidos sob cuidados humanos permanentes, quais as rotinas de diagnóstico e desparasitação de parasitas gastrointestinais que costumam ser seguidos? *** (Inclui animais na área de exposição ou sector extra em jardins zoológicos e animais irrecuperáveis. Dx = Exame de diagnóstico de parasitas gastrointestinais; Tx = Desparasitação contra parasitas gastrointestinais) *(marcar uma opção por linha)*

	Dx apenas	Dx + Tx em caso de resultado positivo	Dx + Tx em caso de carga parasitária superior a determinado limite	Dx + Tx independente do resultado	Tx apenas	Nenhum
Procedimentos periódicos (pelo menos uma vez por ano)						
Animais com sintomatologia clínica que possa indicar parasitismo gastrointestinal						
Animais com outros sinais clínicos / animais debilitados						

Observações; outros critérios de decisão para diagnóstico e desparasitação de parasitas gastrointestinais em animais sob cuidados humanos permanentes:

Manejo de parasitas gastrointestinais em animais à saída da instituição

9. À saída da instituição, quais as rotinas de diagnóstico e desparasitação de parasitas gastrointestinais que costumam ser seguidos?? * (Dx = Exame de diagnóstico de parasitas gastrointestinais; Tx = Desparasitação contra parasitas gastrointestinais) *(marcar uma opção por linha)*

	Dx apenas	Dx + Tx em caso de resultado positivo	Dx + Tx em caso de carga parasitária superior a determinado limite	Dx + Tx independente do resultado	Tx apenas	Nenhum	Não aplicável
Animais para soltura num local próximo ao seu local de captura							
Animais para soltura num local distante do seu local de captura							
Animais que vão ser transferidos para outra instituição							
Animais com sintomatologia clínica que possa indicar parasitismo gastrointestinal							
Animais com outros sinais clínicos / animais debilitados							

Observações; outros critérios de decisão para diagnóstico e desparasitação de parasitas gastrointestinais em animais que vão sair da instituição:

10. Na soltura de um animal na natureza, a presença de parasitas gastrointestinais é considerado um factor impeditivo? * *(marcar tudo o que for aplicável)*

- ☐ Não, quase nunca.
- ☐ Sim, quando a carga parasitária é elevada.
- ☐ Sim, quando o animal vai ser solto longe do local de captura.
- ☐ Sim, quando o animal é proveniente de cativeiro ilegal.
- ☐ Sim, geralmente.
- ☐ Não é realizada soltura de animais na natureza

Diagnóstico de parasitas gastrointestinais

11. Que sinais clínicos conduziriam à suspeita de parasitose gastrointestinal? * *(marcar tudo o que for aplicável)*

- ☐ Diarreia / redução da consistência das fezes
- ☐ Sintomatologia nervosa (ex. ataxia)
- ☐ Anemia
- ☐ Picacismo e/ou parorexia
- ☐ Perda de peso
- ☐ Vômito
- ☐ Regurgitação
- ☐ Constipação
- ☐ Anorexia
- ☐ Polifagia
- ☐ Distensão abdominal
- ☐ Dor abdominal
- ☐ Tenesmo
- ☐ Não sabe
- ☐ Outro: ______________________________

12. **Onde os exames de diagnóstico de parasitas gastrointestinais são realizados? *** *(marcar tudo o que for aplicável)*
    - ☐ Na instituição
    - ☐ Em laboratório externo com parceria (ex. faculdades)
    - ☐ Em laboratório externo, custeados pela instituição
    - ☐ Não tem costume de fazer exames de diagnóstico de parasitas gastrointestinais
    - ☐ Outro: ____

13. **Quantos médicos veterinários trabalham na instituição? *** *(validação: tem de ser um número entre 0 e 99)* ________

14. **Quantos outros profissionais habilitados para fazer exames de diagnóstico de parasitas gastrointestinais trabalham na instituição? *** (Exemplo: 2 biólogos, 1 zootecnista) ____________________________

15. **Que técnicas são habitualmente usadas para diagnóstico de parasitas gastrointestinais? *** *(marcar tudo o que for aplicável)*
    - ☐ Técnica de flutuação simples (Técnica de Willis-Molley (com solução saturada de sal) ou Técnica de Sheather (com solução saturada de açúcar))
    - ☐ Técnica de Faust (centrífugo-flutuação em sulfato de zinco)
    - ☐ Técnica de sedimentação simples
    - ☐ Técnica de sedimentação por centrifugação
    - ☐ Técnica de McMaster
    - ☐ Técnica de McMaster modificada para contagem de ovos que sedimentam
    - ☐ Técnica de Graham (método da fita adesiva)
    - ☐ Exame direto de fezes
    - ☐ Coprocultura para obtenção de larvas L3
    - ☐ Coprocultura para esporulação de oocistos
    - ☐ Não sabe
    - ☐ Outro: ____

16. **Quais considera ser os principais fatores limitantes para a realização de procedimentos de DIAGNÓSTICO de parasitas gastrointestinais? *** *(marcar tudo o que for aplicável)*
    - ☐ Fatores econômicos (ex: custos associados à compra de reagentes ou à contratação de laboratórios externos)
    - ☐ Falta de tempo
    - ☐ Falta de formação / informação / literatura disponível
    - ☐ Falta de equipamentos e/ou infraestruturas
    - ☐ Nenhum
    - ☐ Outro: ____

Desparasitação contra parasitas gastrointestinais

17. **Quais considera ser os principais fatores limitantes para a realização de procedimentos de DESPARASITAÇÃO? *** *(marcar tudo o que for aplicável)*
    - ☐ Fatores econômicos (ex: custos associados à compra do fármaco)
    - ☐ Falta de tempo
    - ☐ Falta de formação / informação / literatura sobre tratamento de parasitoses gastrointestinais em espécies silvestres
    - ☐ Falta de equipamentos e/ou infraestruturas
    - ☐ Nenhum
    - ☐ Outro: ____

18. **Como é escolhido o princípio ativo e dosagem para desparasitar um animal? *** *(marcar tudo o que for aplicável)*
   - ☐ Existem planos de desparasitação fixos por espécie ou grupo de animais
   - ☐ Extrapolação de princípios ativos e doses de animais domésticos
   - ☐ Avaliação da literatura disponível para cada caso
   - ☐ Experiência pessoal
   - ☐ Outro: ______________________________

19. **Verificam o sucesso da desparasitação? *** *(marcar apenas uma opção)*
   - ☐ Sim, os exames de diagnóstico de parasitas gastrointestinais são repetidos após a desparasitação
   - ☐ Não *(passe para a pergunta 21)*
   - ☐ Outro: ______________________________

20. **Já identificaram resistências aos tratamentos contra parasitas gastrointestinais? *** *(marcar apenas uma opção)*
   - ☐ Sim, com alguma frequência
   - ☐ Sim, mas raramente
   - ☐ Não
   - ☐ Outro: ______________________________

Se sim, alguma que deseja partilhar?

Higiene e biossegurança

21. **Com que frequência os recintos são limpos? *** *(marcar apenas uma opção)*
   - ☐ Diariamente
   - ☐ 2-3 vezes por semana
   - ☐ Uma vez por semana
   - ☐ Menos que uma vez por semana
   - ☐ Outro: ______________________________

22. **O equipamento de limpeza utilizado na higienização dos recintos (vassoura, pá, etc.) é individual nos recintos… *** *(marcar uma opção por linha)*

	Sim	Não	Não aplicável
… da área de quarentena			
… da área de internamento			
… de animais mantidos sob cuidados humanos permanentes			
… de animais que apresentam sinais de doença (independentemente do setor em que se encontram)			

Observações:

23. **Quando um recinto muda de ocupante, que procedimentos de limpeza, higienização e biossegurança são realizados? *** *(marcar tudo o que for aplicável)*
   - ☐ Nenhum
   - ☐ Limpeza física (varrer, água)
   - ☐ Limpeza química / desinfecção
   - ☐ Flamejador
   - ☐ Vazio sanitário
   - ☐ Outro: ______________________________

24. São tomadas medidas para evitar o contato de pragas, espécies sinantrópicas, animais silvestres e assilvestrados com os animais presentes na instituição? * (p. ex. aves silvestres, ratos, gatos assilvestrados, etc.)

- ☐ Sim, com elevada taxa de sucesso
- ☐ Sim, mas o contacto indirecto ocasional com estes animais é inevitável
- ☐ Sim, mas ainda assim há um contacto relativamente frequente
- ☐ Não
- ☐ Outro: ____

25. Há inclusão de produtos de origem não controlada na alimentação de algum animal? * (p. ex. alimento cru ou vivo (peixe, carne, cupim) com status parasitológico desconhecido)

- ☐ Sim
- ☐ Não

Se sim, quais?

MUITO OBRIGADA PELA SUA PARTICIPAÇÃO!

Deseja ser informado sobre os resultados e conclusões do questionário?

- ☐ Sim
- ☐ Não

Deseja partilhar alguma informação?
Sugestões, comentários, protocolos ou peculiaridades não abordados no questionário.

Annex II - Survey - English

Characterization of gastrointestinal parasite management in wildlife in Brazil

This survey is being realized for the Integrated Master's Degree in Veterinary Medicine at the Faculty of Veterinary Medicine of Universidade de Lisboa, with supervision Prof. Dr. Luciano Alves dos Anjos (UNESP) and Prof. Dr. Luís Madeira de Carvalho (FMV-ULisboa).

It's goal is to characterize the management of gastrointestinal parasites in organizations for conservation, triage, rehabilitation or keeping of native Brazilian wildlife, to:

– Know the diagnostic and treatment routines of gastrointestinal parasite infections;
– Understand the differences in the management of gastrointestinal parasites in animals in permanent human care and animals to be released into their natural habitats;
– Evaluate the possibility of translocation of gastrointestinal parasites between geographically separated communities of animals;
– Understand the limiting factors in gastrointestinal parasite management in native wildlife.

The project does not aim to evaluate the performance of the participating organizations. Anonymity and confidentiality are guaranteed, as the data will only be used for statistical purposes.

The survey consists of 25 questions, requiring around 15 minutes to be completed. It is ideally answered by a veterinarian.

Thank you for your collaboration!

Melody Bomon

*(* = mandatory)*

Identification of the organization

26. Name of the organization: * ____

27. State * *(choose only one option)*:

- ☐ Acre
- ☐ Alagoas
- ☐ Amapá
- ☐ Amazonas
- ☐ Bahia
- ☐ Ceará
- ☐ Distrito Federal
- ☐ Espírito Santo
- ☐ Goiás
- ☐ Maranhão
- ☐ Mato Grosso
- ☐ Mato Grosso do Sul
- ☐ Minas Gerais
- ☐ Pará
- ☐ Paraíba
- ☐ Paraná
- ☐ Pernambuco
- ☐ Piauí
- ☐ Rio de Janeiro
- ☐ Rio Grande do Norte
- ☐ Rio Grande do Sul
- ☐ Rondônia
- ☐ Roraima
- ☐ Santa Catarina
- ☐ São Paulo
- ☐ Sergipe
- ☐ Tocantins

28. City: * ____

29. Type of organization * *(choose all options that apply)*

- ☐ Zoological garden
- ☐ Wildlife rehabilitation center (CRAS)
- ☐ Wildlife triage center (CETAS)
- ☐ Wildlife keeper
- ☐ Scientific breeding center for research purposes
- ☐ Scientific breeding center for conservation purposes
- ☐ Commercial wildlife breeder
- ☐ Merchant of live wildlife animals
- ☐ Faculty of Veterinary Medicine
- ☐ Other: ____

30. How many wildlife specimens from their natural habitat or from the illegal wildlife chain were admitted during the last three years? * *(choose only one option)*

- ☐ Sporadic admission (<150 animals in three years)
- ☐ 150 - 1500
- ☐ 1500 - 3000
- ☐ 3000 - 6000
- ☐ 6000 - 9000
- ☐ 9000 - 12000
- ☐ 12000 - 15000
- ☐ > 15000
- ☐ The organization does not rescued wildlife
- ☐ Other: ____

Management of gastrointestinal parasites upon admission

31. Upon admission, which diagnostic routines for gastrointestinal parasites are followed? * (Dx = Diagnostic test for gastrointestinal parasites; Tx = Treatment against gastrointestinal parasites) *(choose one option per line)*

	Only Dx	Dx + Tx in case of positive result	Dx + Tx if the parasitic load is higher than a certain limit	Dx + Tx independent of the result	Only Tx	None	Not applicable
All animals from their natural habitats							
All animals from illegal captivity							
All animals from other organizations							
Animals with symptoms that may indicate gastrointestinal parasitism							
Animals with other clinical signs / debilitated animals							
Animals that will be kept in permanent human care							

Observations; other decision criteria for diagnosis and treatment of gastrointestinal parasites upon admission:

32. **How many Brazilian native wildlife animals are kept in permanent human care in the organization? *** (Includes animals in the exhibition area or surplus sector in zoological gardens and all irrecoverable animals.) *(choose only one option)*
   - ☐ < 20
   - ☐ 20 - 200
   - ☐ 200 - 400
   - ☐ 400 - 600
   - ☐ 600 - 800
   - ☐ 800 - 1000
   - ☐ 1000 - 1200
   - ☐ 1200 - 1400
   - ☐ >1400
   - ☐ There are no animals in permanent human care kept within the organization *(Pass to question 9)*

Management of gastrointestinal parasites in wildlife in permanent human care

33. **In the animals kept in permanent human care, which diagnostic and treatment routines are followed for gastrointestinal parasites? *** (Includes animals in the exhibition area or surplus sector in zoological gardens and all irrecoverable animals. Dx = Diagnostic test for gastrointestinal parasites; Tx = Treatment against gastrointestinal parasites) *(choose one option per line)*

	Only Dx	Dx + Tx in case of positive result	Dx + Tx if the parasitic load is higher than a certain limit	Dx + Tx independent of the result	Only Tx	None
Periodic procedures (at least once a year)						
Animals with symptoms that may be indicative of gastrointestinal parasitism						
Animals with other clinical signs / debilitated animals						

Observations; other decision criteria for diagnosis and treatment of gastrointestinal parasites for animals in permanent human care:

Management of gastrointestinal parasites upon discharge

34. **Upon discharge, which diagnostic routines for gastrointestinal parasites are followed? *** (Dx = Diagnostic test for gastrointestinal parasites; Tx = Treatment against gastrointestinal parasites) *(choose one option per line)*

	Only Dx	Dx + Tx in case of positive result	Dx + Tx if the parasitic load is higher than a certain limit	Dx + Tx independent of the result	Only Tx	None	Not applicable
Animals for release close to the capture site							
Animals for release far from the capture site							
Animals that will be transferred to another organization							
Animals with symptoms that may indicate gastrointestinal parasitism							
Animals with other clinical signs / debilitated animals							

Observations; other decision criteria for diagnosis and treatment of gastrointestinal parasites upon discharge:

35. Is the presence of gastrointestinal parasites considered an impeditive factors for release? *
(choose all options that apply)

- ☐ No, almost never.
- ☐ Yes, when the parasitic load is high.
- ☐ Yes, when the animal will be released far from the capture site.
- ☐ Yes, when the animal is from illegal captivity.
- ☐ Yes, mostly.
- ☐ Release into the natural habitat is not performed by the organization.

Diagnosis of gastrointestinal parasites

36. Which clinical signs would lead you to suspect from gastrointestinal parasites infection? *
(choose all options that apply)

- ☐ Diarrhea / reduced fecal consistency
- ☐ Nervous signs (e.g.: ataxia)
- ☐ Anemia
- ☐ Pica and/or parorexia
- ☐ Weight loss
- ☐ Emesis
- ☐ Regurgitation
- ☐ Constipation
- ☐ Anorexia
- ☐ Polyfagia
- ☐ Abdominal distension
- ☐ Abdominal pain
- ☐ Tenesmus
- ☐ Doesn't know
- ☐ Other: ____

37. Where are the diagnostic test for gastrointestinal parasites carried out? * *(choose all options that apply)*

- ☐ At the organization
- ☐ At external laboratory with partnership (e.g.: universities)
- ☐ At external laboratory (paid by the organization)
- ☐ There is no habit of performing diagnostic tests for gastrointestinal parasites
- ☐ Other: ____

38. How many veterinary surgeons work at the organization? * *(validation: has to be a number between 0 and 99)* _________

39. How many other professional capable of carrying out diagnostic test for gastrointestinal parasites work at the organization? * (E.g.: 2 biologists, 1 zootechnic) _____________________

40. Which tests are usually carried out for gastrointestinal parasite diagnosis? * *(choose all options that apply)*

- ☐ Simple flotation (Willis-Molley technique (with saturated salt solution) or Sheather technique (with saturated sugar solution))
- ☐ Faust technique (centrifugal-flotation with zinc sulphate)
- ☐ Simple sedimentation technique
- ☐ Centrifugal sedimentation technique
- ☐ McMaster technique
- ☐ Modified McMaster to count eggs that sediment
- ☐ Graham technique (adhesive tape method)
- ☐ Direct smear

- ☐ Fecal culture to obtain L3 larvae
- ☐ Fecal culture for oocyst sporulation
- ☐ Doesn't know
- ☐ Other: ____

41. Which factors do you consider as limiting to realize DIAGNOSTIC procedures for gastrointestinal parasites? * *(choose all options that apply)*
- ☐ Economical factors (e.g.: costs of buying reagents or hiring external laboratories)
- ☐ Lack of time
- ☐ Lack of training / information / literature
- ☐ Lack of equipment and/or infrastructure
- ☐ None
- ☐ Other: ____

Treatment of gastrointestinal parasites

42. Which factors do you consider as limiting to realize TREATMENT for gastrointestinal parasites? * *(choose all options that apply)*
- ☐ Economical factors (e.g.: costs of drugs)
- ☐ Lack of time
- ☐ Lack of training / information / literature on treatment of wildlife gastrointestinal parasite infections
- ☐ Lack of equipment and/or infrastructure
- ☐ None
- ☐ Other: ____

43. How it the drug to treat a gastrointestinal parasite infections chosen? * *(choose all options that apply)*
- ☐ There exist fixed treatment protocols per species or group of animals
- ☐ Extrapolation of drugs and dosages of domestic species
- ☐ Evaluation of the available literature for each case
- ☐ Personal experience
- ☐ Other: ____

44. Is the efficacy of the treatment tested? * *(choose only one option)*
- ☐ Yes, the diagnostic tests are repeated after treatment
- ☐ No *(pass to question 21)*
- ☐ Other: ____

45. Have you already identified ineffective treatments / resistances? * *(choose only one option)*
- ☐ Yes, quite frequently
- ☐ Yes, but rarely
- ☐ None
- ☐ Other: ____

If yes, which ones?

Hygiene and biosecurity

46. How frequently are the enclosures cleaned? * *(choose only one option)*
- ☐ Daily
- ☐ 2-3 times per week
- ☐ Once a week
- ☐ Less than once a week
- ☐ Other: ____

47. The cleaning equipment (broom, shovel, etc.) is individual in the enclosures… * *(choose one option per line)*

	Yes	No	Not applicable
… in the quarantine area			
… in the hospitalization area			
… of animals in permanent human care			
… of animals that are sick (independent of the area they live in)			

Observations:

48. When an enclosure changes occupant, which cleaning, sanitation and biosecurity measures are taken? * *(choose all options that apply)*

- ☐ None
- ☐ Physical cleaning (swiping, water)
- ☐ Chemical cleaning / disinfection
- ☐ Heat (flaming, steam)
- ☐ Sanitary break
- ☐ Other: ______________________________

49. Are there measures in place to prevent contact of the animals with pests, synanthropic, wild and feral animals? * (e.g.: birds, mice, feral cats, etc.)

- ☐ Yes, with high success
- ☐ Yes, but indirect contact with these animals is inevitable
- ☐ Yes, but nonetheless the contact is relatively frequent
- ☐ No
- ☐ Other: ______________________________

50. Are products of uncontrolled origin included in the feeding scheme of some animals? * (e.g.: raw or live prey (fish, meat, termites) with unknown parasitological status)

- ☐ Yes
- ☐ No

If yes, which ones?

THANK YOU VERY MUCH FOR YOUR PARTICIPATION !

Would you like to be informed about the results and conclusions of the study?

- ☐ Yes
- ☐ No

Would you like to share anything?
Suggestions, comments, protocols or details that were not covered by the survey.

Annex III - Number of sampled species and animals.

Class	Group	Species	# samples	# animals
Bird	Accipitriformes	*Rupornis magnirostris*	1	1
	Anseriformes	*Dendrocygna autumnalis*	4	4
	Apodiformes	*Eupetomena macroura*	1	1
	Cariamiformes	*Cariama cristata*	1	1
	Cuculiformes	*Crotophaga ani*	1	1
	Falconiformes	*Falco sparverius*	2	2
	Galliformes	*Crax fasciolata*	1	3
		Penelope obscura	4	6
		Penelope superciliaris	1	2
	Passeriformes	*Cacicus haemorrhous*	1	1
		Gnorimopsar chopi	2	2
		Lanio cucullatus	1	2
		Pitangus sulphuratus	1	1
		Tyrannus savana	1	1
	Pelecaniformes	*Ardea cocoi*	1	1
	Piciformes	*Pteroglossus castanotis*	1	1
		Ramphastos toco	9	22
	Psittaciformes	*Amazona aestiva*	8	35
		Ara ararauna	7	14
		Ara macao	3	6
		Brotogeris chiriri	4	5
		Brotogeris tirica	1	1
		Eupsittula aurea	4	8
		Psittacara leucophthalmus	7	15
	Rheiformes	*Rhea americana*	1	2
	Strigiformes	*Asio clamator*	1	2
		Athene cunicularia	1	1
		Megascops choliba	1	1
		Pulsatrix perspicillata	1	1
		Tyto furcata	6	11
	Tinamiformes	*Crypturellus parvirostris*	2	6

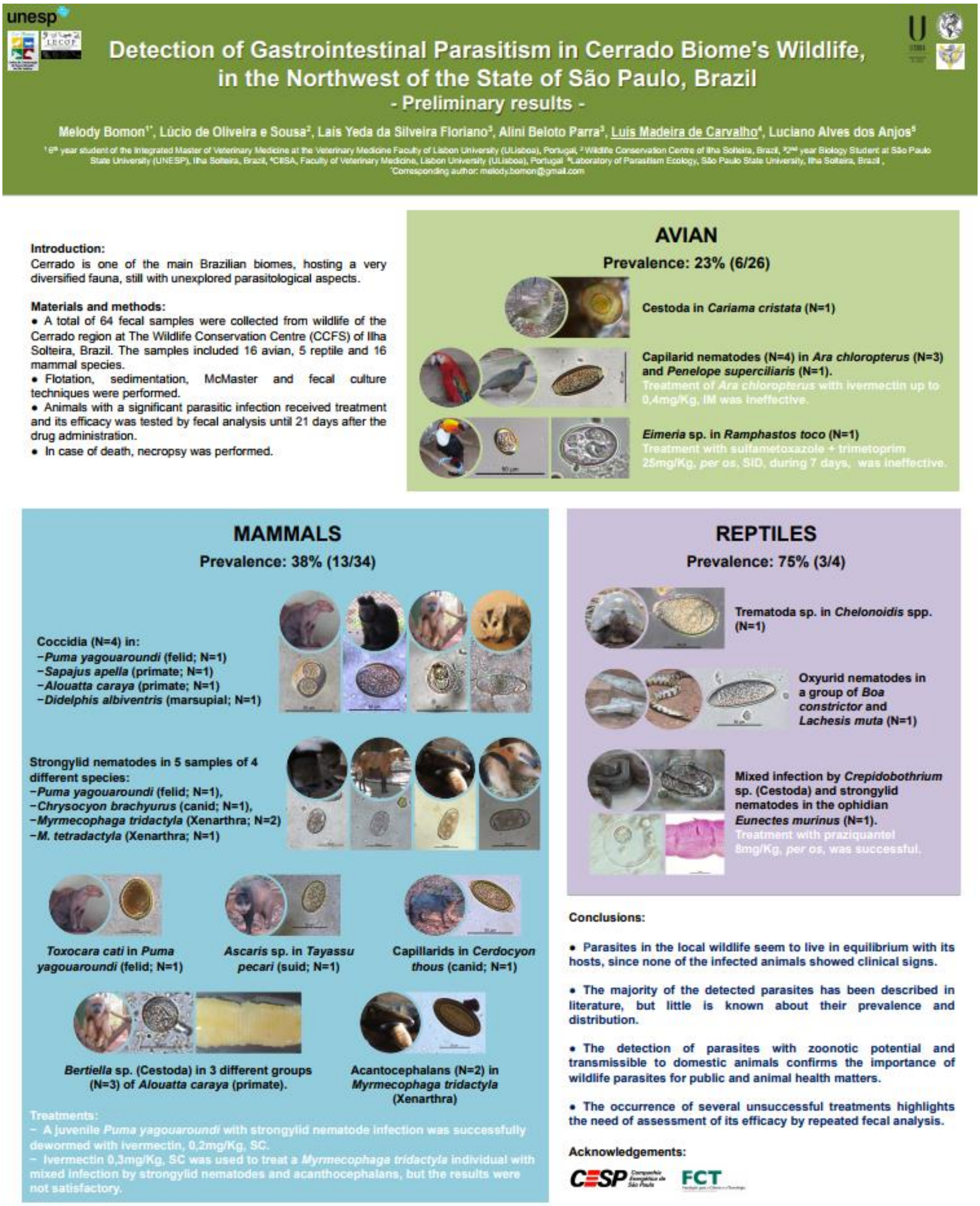
(Continued on next page)

Annex III - Number of sampled species and animals (cont.)

Class	Group	Species	# samples	# animals
Mammal	Artiodactyla - ruminantia	*Mazama gouazoubira*	2	2
	Artiodactyla - suidae	*Pecari tayacu*	1	3
		Tayassu pecari	4	12
	Carnivora - Canidae	*Cerdocyon thous*	4	5
		Chrysocyon brachyurus	1	2
	Carnivora - Felidae	*Leopardus pardalis*	2	2
		Leopardus tigrinus	1	2
		Panthera onca	2	2
		Puma concolor	4	4
		Puma yagouaroundi	3	4
	Carnivora - Procyonidae	*Procyon cancrivorus*	2	2
	Didelphimorphia	*Didelphis albiventris*	1	1
	Lagomorpha	*Sylvilagus brasiliensis*	1	1
	Perissodactyla	*Tapirus terrestris*	1	2
	Primata	*Alouatta caraya*	9	11
		Sapajus apella	1	4
		Sapajus nigritus	3	3
	Rodentia	*Coendou prehensilis*	1	1
		Dasyprocta azarae	2	3
		Hydrochoerus hydrochaeris	1	1
	Xenarthra	*Euphractus sexcinctus*	2	2
		Myrmecophaga tridactyla	8	8
		Tamandua tetradactyla	2	3
Reptile	Chelonia	*Chelonoidis carbonaria*	2	2
		Chelonoidis spp.	1	40
	Ophidia	*Boa constrictor*	3	3
		Caudisona durissu	1	1
		Epicrates crassus	1	1
		Eunectes murinus	1	3
		Hydrodynastes gigas	1	2
	Sauria	*Salvator merianae*	1	1
	TOTAL:	**62 species**	**149 samples**	**293 animals**

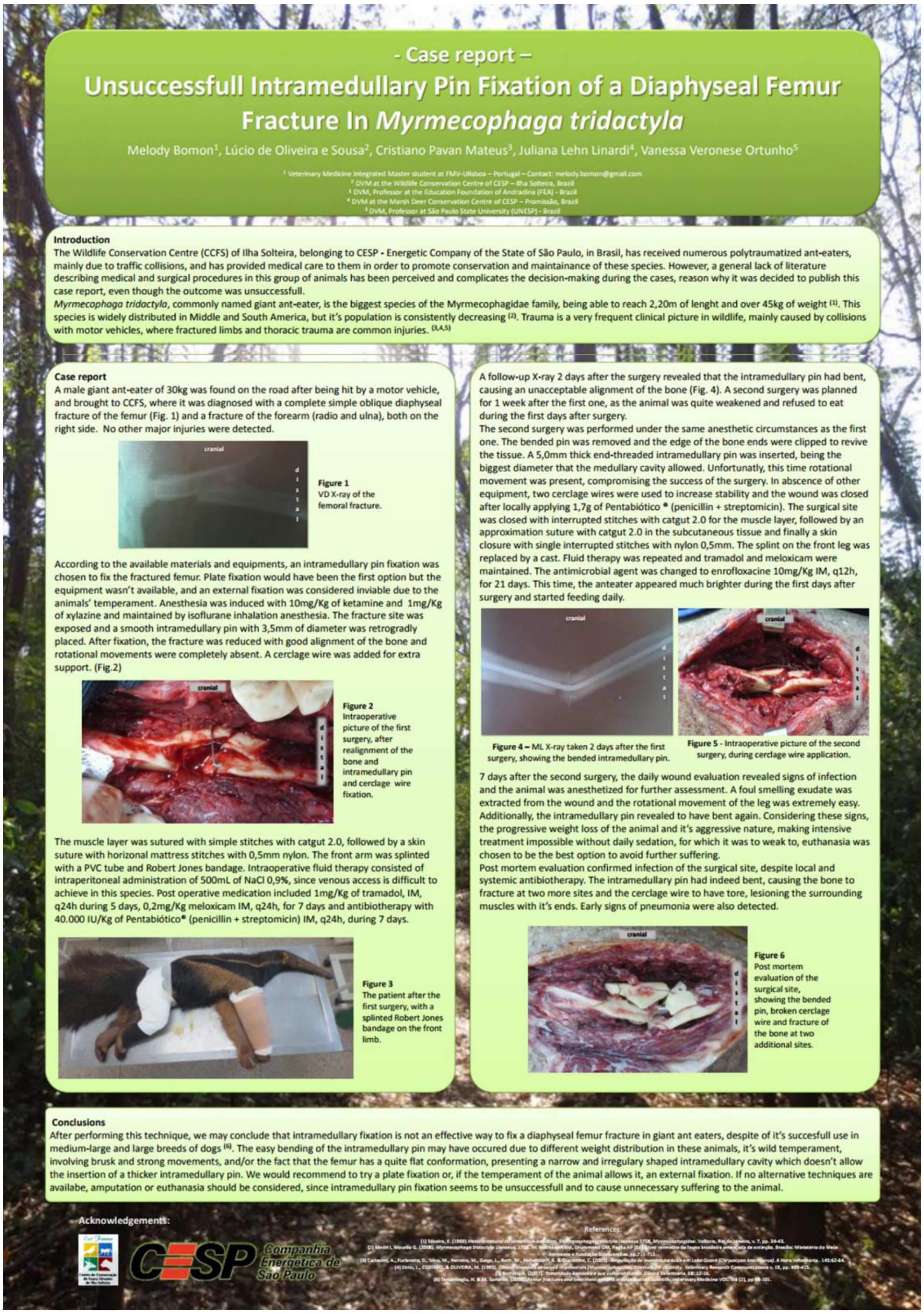
Annex IV – Posters and publications

Bomon, M., Sousa, L. O, Floriano, L. Y. S., Parra, A. B., Carvalho, L. M. and Anjos, L. A. (2014, November). Detection of Gastrointestinal Parasitism in Cerrado Biome's Wildlife, in the Northwest of the State of São Paulo, Brazil - Preliminary results. Poster presented at the XVII Portuguese Parasitology Congress, Coimbra, Portugal, November 2014.

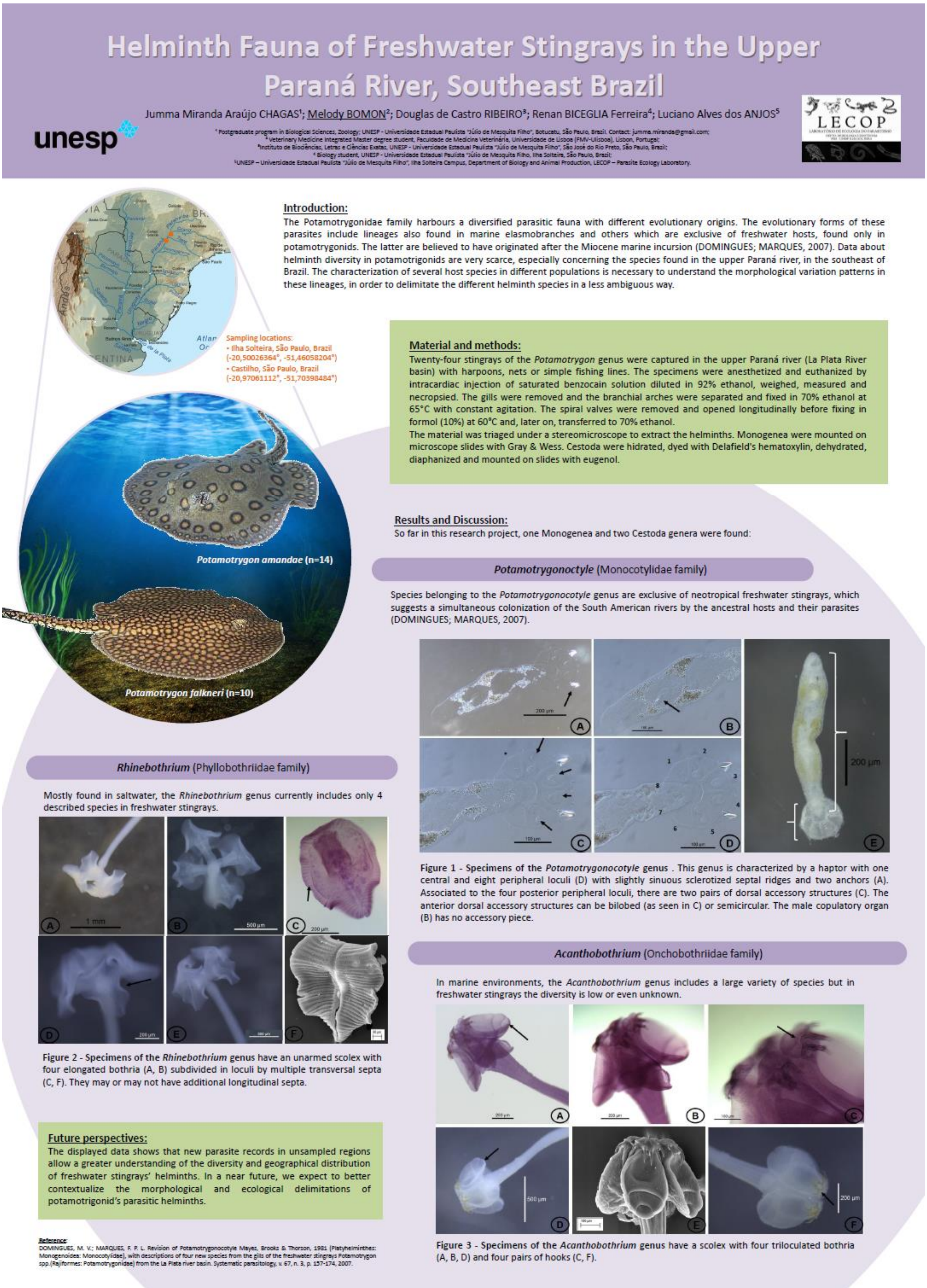


Bomon, M., Sousa, L. O., Mateus, C. P., Linardi, J. L. and Ortunho, V. V. (2014, November). *Case report : Unsuccessfull Intramedullary Pin Fixation of a Diaphyseal Femur Fracture In Myrmecophaga tridactyla*. Poster presented at III International FAUNA Conference, Lisbon, Portugal.

Bomon, M., Souza, L. O. and Ortunho, V. V. (2015). Fixação com pino intramedular em fratura do fêmur em Tamanduá Bandeira, Myrmecophaga Tridactyla 1- Linnaeus, 1758. Relato de caso. *Revista Brasileira de Higiene e Sanidade Animal, 9*(3), 535-542.



Chagas, J., Bomon, M., Ribeiro, D., Biceglia, R. and Anjos, L. A. (2015, November). *Helminth Fauna of Freshwater Stingrays in the Upper Paraná River, Southeast Brazil*. Poster presented at IV International FAUNA Conference, Lisbon, Portugal.



Bomon, M., Capeletti, V., Souza, L., Linardi, J., Carvalho, L. M. and Anjos, L. A. (2015, November). *Case studies - Gastroparasitological Profile of Polytraumatized Giant Anteaters (Myrmecophaga tridactyla).* Poster presented at IV Symposium on Wildlife and Exotic Animals-IAAS-UTAD, Vila Real, Portugal.

- Case studies -

Gastrointestinal Parasite Profile of Polytraumatized Giant Anteaters (*Myrmecophaga tridactyla*)

Melody Bomon[1]; Vanessa Capeletti[2]; Lúcio de Oliveira e Sousa[3]; Juliana Lehn Linardi[3]; Luís Madeira de Carvalho[4]; Luciano Alves dos Anjos[5]

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[4] CIISA, Faculty of Veterinary Medicine, Universidade de Lisboa (FMV-ULisboa), Lisbon, Portugal;
[5] UNESP – Universidade Estadual Paulista "Júlio de Mesquita Filho", Ilha Solteira Campus, Department of Biology and Animal Production, LECOP – Parasite Ecology Laboratory.

Introduction:

The Wildlife Conservation Center (CCFS) of Ilha Solteira (São Paulo, Brazil) receives and provides veterinary care to several polytraumatized giant anteaters every year. (See Table 1)

These animals are generally healthy until the moment of the traumatic event, but in the subsequent period the strain on their body is very high and their overall resistance decreases, making it possible to evaluate the effect of decreased fitness on the parasite-host balance.

Cause of admission		n
Motorway accident	Polytraumatized adult	5
	Healthy infant separated from their polytraumatized mother	2
	Orphaned but healthy infant	1
	Orphaned polytraumatized infant	1
Urban invasion		1
Total		10

Table 1 - Giant anteaters admitted in CCFS between September 2014 and August 2015.

Material and methods:

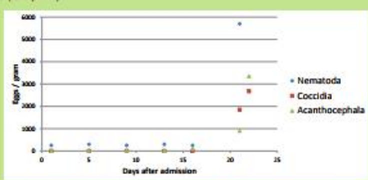
- Four admitted and four healthy captive giant anteaters' gastrointestinal parasite load was evaluated by coproparasitological analysis (flotation, sedimentation, McMaster and modified McMaster).
- The polytraumatized anteaters were classified as healthy pre-trauma (good body condition, traumatic event presumably occurred in the last 48 hours) or debilitated (poor body condition, traumatic event presumably occurred more than 48h before admission) and sampled at arrival and during the course of treatment. In case of death, necropsy was performed (n=3).
- The healthy captive animals were sampled during three consecutive days.
- Five individuals were dewormed and followed-up for 14 to 21 days.
- Statistical analysis was done with GraphPad InStat® version 3.10

Coproparasitology

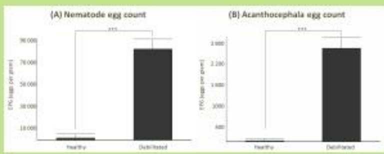
Overall prevalence was high, with seven (87,5%) of the tested animals showing at least one type of gastrointestinal parasite.

- Nematode prevalence: 87,5% (n=7)
- Acanthocephalan prevalence: 50% (n=4)
- Coccidia prevalence: 37,5% (n=3)

Initial egg count of admitted animals classified as healthy pre-trauma did not statistically differ from the healthy long-term captive anteaters, but increased as they became debilitated (Graph 1). Being so, the nematode and acanthocephalan egg counts of debilitated animals were significantly higher than the healthy samples (Graph 2).



Graph 1 - Egg counts of a polytraumatized giant ant-eater from its arrival until euthanasia was performed.



Graph 2 – Mean±SD of nematode (A) and acanthocephalan (B) egg counts from healthy and debilitated giant anteaters. T-test, *** P<0,001

Necropsy

Necropsies revealed gastric nematodes (n=3) and cestodes (n=1) and the acantocephalan *Gigantorhynchus echinodiscus* (n=3) in the small intestine. The latter caused mucosal inflammation (n=3) and partial intestinal obstruction (n=1), being considered a contributor to the animals' death.

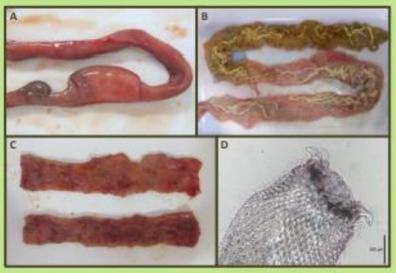


Image 1 - Infection by *Gigantorhynchus echinodiscus* in the small intestine. (A) Bowel obstruction; (B) Parasite load; (C) Macroscopic aspect of the mucosa after parasite removal; (D) Anterior extremity of *G. echinodiscus*

Deworming

Administration of ivermectin (0,2-0,3 mg/Kg, SC) induced a > 95% reduction of nematode egg count in 2 healthy long-term residents without co-infection by Acanthocephala, but, in two individuals with nematode and Acanthocephala co-infection, none of the egg counts was satisfactorily reduced.

The administration of albendazol *per os* to one individual also revealed to be unsuccessful.

Conclusion and future perspectives:

- **Gastrointestinal parasite prevalence seems to be high, with a healthy host-parasite balance.**
- **When the hosts' fitness is decreased by a third factor, such as trauma or prolonged anorexia, this balance is disturbed and the parasite load increases exponentially, increasing its harmful effects on the host and contributing to the hosts further deterioration or even death.**
- **Therefore, it'd be recommended to deworm and follow-up polytraumatized or other debilitated giant anteaters as a standard procedure to ensure a better chance of recovery.**
- **Proper drug tests should be done to establish an effective deworming protocol.**

Acknowledgements:
CESP Companhia Energética de São Paulo

Bomon, M., Carvalho, L. M. and Anjos, L. A. (2016, November). *Management of gastrointestinal parasites in rescued wildlife in Brazil (preliminary results).* Poster presented at V International FAUNA Conference, Lisbon, Portugal.

MANAGEMENT OF GASTROINTESTINAL PARASITES IN RESCUED WILDLIFE IN BRAZIL

(Preliminary results)

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Introduction:

In Brazil, rescued wildlife, such as traumatized wild living animals or animals seized from illegal captivity, are ideally and most frequently admitted in wildlife rehabilitation centers situated all over the country. These centers are often far away and/or overcrowded, thus zoological parks, hospitals of veterinary medicine faculties and other similar facilities also receive and care for these animals, making the ex-situ wildlife network stronger and more complete.

Material and methods:

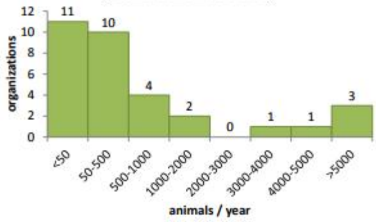
A questionnaire concerning the management of GI parasites in wildlife in Brazil was sent via e-mail to a vast number of wildlife rehabilitation centers, zoological parks and universities with veterinary medicine courses in January and February 2016. The questionnaire contained a total of 26 questions, was formulated online with Google Forms® and the language of choice was Portuguese. Data analysis was performed using Microsoft® Office Excel® 2007.

Results:

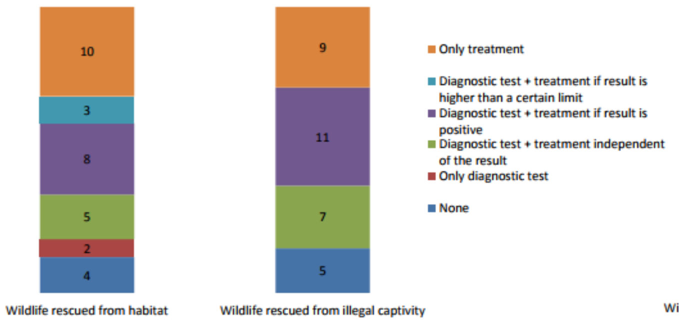
- Total: 32 answers

● 14 (44%) licensed rehabilitation centers, 4 of which associated to a university (●)
■ 13 (41%) zoological parks
◇ 4 (13%) faculties of veterinary medicine
△ 1 licensed wildlife keeper.

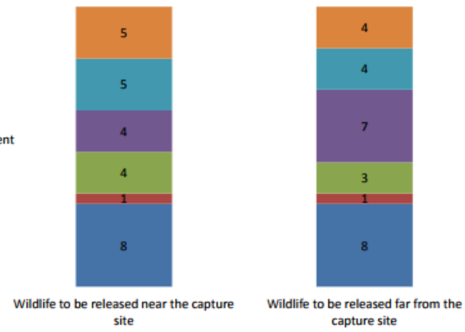
Distribution of case loads



Routine procedures upon admission



Routine procedures before release



Nearly **50%** of all organizations **repeat diagnostic tests** after all antiparasitic treatments.

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25% (n=8) repeat them depending on the case or species in question.

74% (n=17) of these have already encountered **resistances**,
25% (n=9) report them to be **frequent**.

Main reported limiting factors for

DIAGNOSIS	TREATMENT
Time Funds Equipment and/ or infrastructure	Funds Difficult access to certain drugs

Conclusions and future perspectives:

- The management of GI parasites in rescued wildlife in Brazil appears to be approached with care and detail, since around 80% of the institutions perform deworming, whether guided or not by previous testing, and at least 50% check its efficacy.
- The questionnaire was designed to obtain a general picture of this reality but might be biased since overcrowded/understaffed centers will not as easily find the time to answer the questionnaire.
- It is recommended for each of these aspects to be further investigated to evaluate if the used approaches are the most advisable.