



UNIVERSIDADE DE LISBOA
Faculdade de Medicina Veterinária

EASTERN COTTONTAIL RABBIT (*Sylvilagus floridanus*) ADMISSION CAUSES AND
CORRESPONDING OUTCOMES AT THE WILDLIFE REHABILITATION CENTER OF
MINNESOTA: A RETROSPECTIVE STUDY FROM 2011 TO 2017

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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***“I’ve learned that if you want people to join in any kind of conservation effort,
you have to help them to care with their hearts, not just their heads.”***

Jane Goodall

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ABSTRACT

Eastern cottontail rabbit (*Sylvilagus floridanus*) admission causes and corresponding outcomes at the Wildlife Rehabilitation Center of Minnesota: a retrospective study from 2011 to 2017

The eastern cottontail rabbit (*Sylvilagus floridanus*) is one of the most frequently admitted species throughout United States rehabilitation facilities. About one quarter of the annual admissions at the Wildlife Rehabilitation Center of Minnesota (WRCMN) has been comprised by this species and significant increasing intakes have been registered.

Data regarding eastern cottontails' admissions at the WRCMN between 2011 and 2017 was studied and it was observed that the leading admission causes were linked with domestic animals interactions, according to previous results in other wildlife rehabilitation centers (WRCs) reporting the major impact of cats and dogs attacks, especially concerning the studied species. A considerable intake of orphaned rabbit kits, often appearing clinically healthy on arrival, was identified. The majority of the cottontails were humanely euthanized on admission and the overall release proportion in the studied period was approximately 23%.

Age, body weight, body condition, certain admission causes and tested clinical signs categories were significantly associated with the outcomes ($p < 0.01$). The development of clinical decision trees, conducted in this study and based on Fast and Frugal Trees (FFT) algorithms, may be a helpful tool to support future triage in WRCs, or to aid diagnosis or treatment establishment. The period in treatment (PT) or length of stay, an important estimator of daily costs and animal welfare, was also examined.

The identification of factors linked with a better prognosis and subsequent release may support the triage process and resources management, which are commonly scarce in the wildlife rehabilitation field, enabling the improvement on animals' welfare as well.

This study reinforces the importance of public education and urgent establishment of measures to avoid anthropogenic interference in wildlife casualties, preponderant in the eastern cottontail admission causes. Furthermore, it highlights the great value of WRCs database study, not only leading to a better understanding of wildlife threats and subsequent conservation actions implementation, but also to enable future improvement of rescue, rehabilitation and release procedures.

Key-words: eastern cottontail rabbit, wildlife rehabilitation, triage, outcomes, Minnesota, USA.

RESUMO

Causas de admissão do coelho *eastern cottontail* (*Sylvilagus floridanus*) e respetivos desfechos no Centro de Recuperação de Animais Silvestres do Minnesota: um estudo retrospectivo de 2011 a 2017

O coelho *eastern cottontail* (*Sylvilagus floridanus*) representa uma das espécies mais frequentemente admitidas nos centros de recuperação dos EUA. Sensivelmente um quarto das admissões anuais no Centro de Recuperação de Animais Silvestres do Minnesota (CRASMN) tem sido constituído por esta espécie e tem sido verificado um aumento significativo relativamente às suas admissões.

Foram explorados os dados relativos às admissões dos *eastern cottontails* no CRASMN entre 2011 e 2017, sendo verificadas como principais causas de ingresso as interações com animais domésticos, de acordo com os resultados prévios verificados noutros centros de reabilitação de animais selvagens (CRAS) que sublinharam o impacto dos ataques de cães e gatos, especialmente no que toca à espécie em estudo. Foi identificada uma admissão considerável de láparos órfãos, frequentemente saudáveis à chegada. A maioria dos *cottontails* foi humanamente submetida a eutanásia no ingresso e a proporção total de devoluções à natureza atingiu aproximadamente 23%.

A idade, condição e peso corporais, determinadas causas de admissão e categorias de sinais clínicos testados foram significativamente associadas com os desfechos da sua recuperação ($p < 0,01$). O desenvolvimento de árvores de decisão clínica, elaboradas neste estudo e baseadas em algoritmos *Fast and Frugal Trees* (FFT), poderá constituir uma ferramenta útil no apoio de futuras triagens em CRAS ou no estabelecimento de diagnósticos e tratamentos. O período em tratamento, um importante dado para estimativa dos custos diários e do bem-estar animal, foi também analisado.

A identificação dos fatores associados a um melhor prognóstico e subsequente devolução à natureza poderão auxiliar o processo de triagem e a gestão de recursos, muitas vezes escassos na área da reabilitação de fauna, permitindo também um melhoramento do bem-estar animal.

Este estudo sublinha a importância da educação do público e a urgência no estabelecimento de medidas que evitem interferência antropogénica na fauna silvestre, tendo sido identificada como preponderante no conjunto de causas de admissão dos *eastern cottontails*. É destacado o grande valor das bases de dados dos CRAS, que permitem não só um melhor entendimento das ameaças à vida selvagem e subsequente implementação de ações de conservação, mas também por permitirem uma futura melhoria dos procedimentos de resgate, reabilitação e devolução à natureza.

Palavras-chave: coelho *eastern cottontail*, reabilitação de animais silvestres, triagem, consequências, Minnesota, EUA.

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LIST OF ABBREVIATIONS

Acc - Accuracy

CNS - Central Nervous System

C.F.R - Code of Federal Regulations

DH - Definitive Host

DNR - Department of Natural Resources

FFT - Fast and Frugal Tree

GI - Gastrointestinal

ha - Hectare

HPA - Hypothalamic-pituitary-adrenal

IUCN - International Union for the Conservation of Nature

IQR - Interquartile range

kg - Kilogram

LM - Larva migrans

M - Mean

mcu - Mean cues used

Mdn - Median

NLM - Neural larva migrans

OLM - Ocular larva migrans

OR - Odds ratio

PH - Paratenic Host

pci - Percentage of cues ignored

PT - Period in treatment

PCR - Polymerase chain reaction

RSPCA - Royal Society for the Prevention of Cruelty to Animals

SNS - Sympathetic Nervous System

VLM - Visceral larva migrans

wacc - Weighted accuracy

WRC - Wildlife Rehabilitation Center

WRCMN - Wildlife Rehabilitation Center of Minnesota

1. ACTIVITIES DEVELOPED DURING THE CURRICULAR TRAINEESHIP

The author's 6th year curricular traineeship was accomplished at the Wildlife Rehabilitation Center of Minnesota (WRCMN), United States of America. The training period started on September 4th, 2017 and was completed by January 12th, 2018, with a total amount of 720 working hours.

Dr. Leslie Reed, from WRCMN, was the traineeship supervisor and Prof. Doctor Luís Madeira de Carvalho, from FMV-ULisboa, was the co-supervisor.

The WRCMN is one of the oldest wildlife hospitals of the USA, being a 501c3¹ nonprofit organization, supported only by private donations in order to care for the large number of injured, ill and orphaned wildlife admitted – around 13,000 wild animals in 2017, representing more than 185 species. The medical staff comprises nine people and relies on the support of more than 600 volunteers. The hospital has state-of-the-art facilities, fully equipped with digital radiology, ultrasound and surgery suite. Besides the clinical and rehabilitation work, the wildlife center focus on public education through social media that engage the Minnesotan community towards the importance of wildlife care and also, an annual open house day, receiving hundreds of interested members of the public. Furthermore, this wildlife hospital offers teaching experiences, welcoming annually 20-30 veterinary students and veterinarians interested in wildlife fields, from around the world.

At the WRCMN, the author followed the routine activities performed by the medical staff at the beginning of the training period, and then worked with gradually increasing autonomy in several tasks, under supervision. As an intern, the activities included:

- the handling and restraint of songbirds, waterfowl, reptiles (turtles and snakes, mainly), amphibians and a wide variety of mammals, including rabies' vectors such as bats, opossums, raccoons and foxes;
- admission and triage, carrying out the initial physical examination, diagnosis and institution of the appropriate treatment;
- performance of further rechecks and treatments of current patients under rehabilitation;
- assistance as "meds help", carrying out the preparation and administration of fluids and drugs, working alongside veterinary technicians;
- performance of wound management and debriding procedures, management of avian fractures and bandage placement, besides assistance with small surgeries, namely turtle shell repair, toe/tail amputations, feather imping and observation of orthopedic surgery procedures;
- assistance with waterfowl gastric lavage and lead poisoning treatment protocols;

¹ Section 501c3 refers to the section of the U.S. Internal Revenue Code that permits federal tax exemption of nonprofit organizations, namely public charities or private foundations (Foundation Group, 2018).

- physiotherapy and birds' flight training support;
- anesthetic induction and monitoring of mammals, birds and reptiles;
- participation on diagnostic imaging procedures and positioning, besides further image interpretation;
- diagnostic procedures as blood sample collection, skin diagnostic tests and crop swabs;
- undertaking birds, mammals and reptiles euthanasia procedures;
- diet preparation, gavage feeding and the establishment of environmental enrichment.

The author also participated in a study involving eastern cottontail rabbits, considering a possible zoonotic babesiosis case: the author took blood and spleen samples of euthanized individuals that were sent to an external lab for further polymerase chain reaction (PCR) analysis. Additionally, database study and analysis were performed as part of the present master thesis project, based on WRCMN records from 2011 to 2017.

The author developed a 12-hours shadowing shift at the Raptor Center of the University of Minnesota, College of Veterinary Medicine, specialized in the rehabilitation of sick and injured raptors. Moreover, there was the opportunity to participate in the monthly "Zoo Rounds" at the University of Minnesota, College of Veterinary Medicine. Here, case-studies about zoo and wildlife medicine subjects were presented by students, professors and wildlife professionals, giving a broad vision on the current diseases and related challenges concerning the North American wildlife.

After this experience, the author developed a complementary externship, under the program ERASMUS +, that took place at Mallydams Wood Wildlife Center, Hastings, in the UK, from February 5th, 2018 to April 17th, 2018, with a total amount of 350 working hours. This medical center is part of the Royal Society for the Prevention of Cruelty to Animals (RSPCA), the largest animal welfare charity in the UK, specialized in animal rescue and care. This allowed to reinforce practical experience and knowledge in the wildlife rehabilitation field, training and following this hospital routine, besides working closely with British fauna species.

2. INTRODUCTION

Morbidity and mortality in wildlife populations may arise from natural and anthropogenic processes, although it is alarming that recent human-wildlife conflicts have promptly increased in frequency: several authors agree that the greatest majority of injuries and disease observed in wildlife are the direct result of human contact (Pokras & Porter, 1994; Bewig & Mitchell, 2009; Schenk & Souza, 2014). There is a significant number of wild animal species that have successfully adapted to urban and suburban regions, such as in many parts of North America, thanks to their ability to take advantage of immensely fragmented habitats. However, those populations are exposed to different stresses that impose substantial constraints on their biology, forcing them to change natural behaviors and strategies to succeed and, consequently, increasing disease susceptibility (Ditchkoff, Saalfeld, & Gibson, 2006). However, some apparently untouched ecosystems are also under human pressures. For example, the illegal wildlife traffic and its consequences in animals' welfare account for the majority of wildlife casualties in Central and South America (Drews, 2003).

In response to the emerging ecological health problems, there was the necessity to develop a new interdisciplinary field that would ally human and public health, epidemiology, veterinary medicine, toxicology, ecology and conservation biology: the Conservation Medicine subject (Tabor, 2002). Wildlife Rehabilitation Centers (WRCs) are in a unique position to monitor ecological changes and the anthropogenic effects on wildlife health (Sleeman & Clark, 2003; Sleeman, 2008). Therefore, wildlife presented to these facilities for treatment may be a valuable biomonitoring tool to assess environmental problems (Sleeman, 2008).

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, p. ix, free translation). Besides the improvement of many wild animals welfare, one of the most important efforts of WRCs is public education, through the presentation of clinical cases and experiences, building public sensibility towards the value of wildlife and the importance of healthy ecosystems, giving advice and preventing many human-induced threats. Furthermore, the research conducted in WRCs based on the usefulness of respective databases (Pyke & Szabo, 2017) and the developed clinical work may influence public policy decisions regarding conservation (Sleeman, 2008).

Some authors defend the moral and ethical significance of treating wildlife, since most of the injuries are a result of human activities (Bewig & Mitchell, 2009; Tomlinson, 2016), even though it is contested that the rehabilitation of injured individuals of common species has, in general, no significant influence at the overall population level (Wobeser, 2007). Pyke & Szabo (2017) refer scarce confirmation of direct contribution to threatened species conservation as well. The justification of wildlife rehabilitation is frequently questioned, regarding the interference with natural selective processes, increased disease transmission and the unsuitable translocation of animals (Sleeman & Clark, 2003).

There is an increased need to determine not only the admission circumstances in WRCs – in order to monitor the changing health status of the surrounding ecosystem, reduce the anthropogenic impact and study different approaches to decrease the number of wildlife casualties (Schenk & Souza, 2014), but also to understand the factors associated with survival and subsequent release, in such a way that makes it possible to support the triage process with accurate data. This will allow professionals working at WRCs the ability to focus efforts and resources on individuals presenting with a higher likelihood of successful recovery, therefore safeguarding animals' welfare (Molony, Baker, Garland, Cuthill, & Harris, 2007; Grogan & Kelly, 2013; Tomlinson, 2016). The importance of this resource management refers to the availability of facilities and personnel, as well as the treatment cost (Wobeser, 2007), as most of the WRCs have limited funds (Sleeman, 2008) and rely on monetary support through donations or charities.

In the United States of America, one of the animal species that has thrived by relying on human proximity is the eastern cottontail rabbit (*Sylvilagus floridanus*) (Burton & Doblar, 2004). A study based on wildlife casualties' records submitted by eighty-two organizations throughout North America, from 2011 to 2015, revealed that the most frequently admitted species due to all causes of injury or illness was collectively the eastern cottontail rabbit (Loyd, Hernandez & McRuer, 2017).

Successful eastern cottontail rabbit rehabilitation is considered a challenging process by several authors: the rabbit's vigorous sympathetic response is very difficult to manage in captivity (Bewig & Mitchell, 2009; Schott, 2017) and rabbit kits are demanding to hand-raise (Evans, 1987; Bewig & Mitchell, 2009; Cowen, 2016). From 2011 to 2017, the eastern cottontail rabbit made up 26% of all the species admitted at the WRCMN.

This master thesis dissertation was originated on the curricular traineeship developed at the WRCMN, from September 4th, 2017 to January 13th, 2018; data concerning eastern cottontails' admissions from 2011 to 2017 at this WRC was collected and studied.

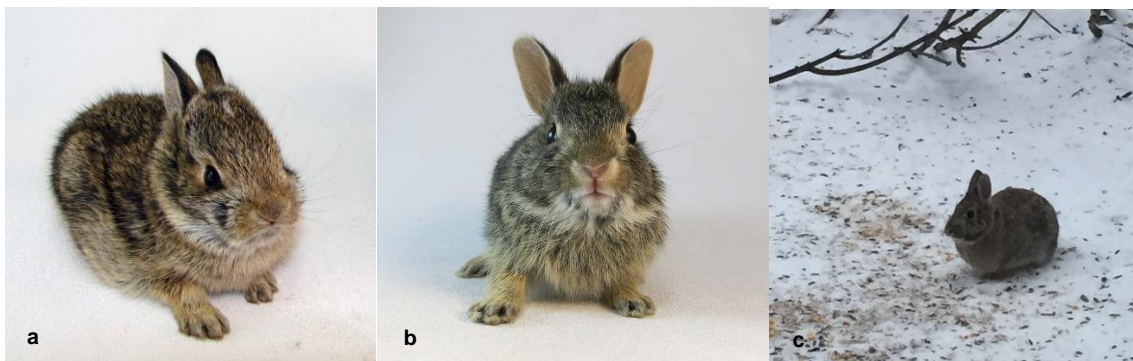
3. LITERATURE REVIEW

3.1. The eastern cottontail rabbit (*Sylvilagus floridanus*)

3.1.1. Taxonomy and morphologic features

The eastern cottontail rabbit (*Sylvilagus floridanus*) (J.A. Allen, 1890), also known as Florida cottontail, belongs to the order Lagomorpha, family Leporidae, and presents the widest geographic distribution of any member of the genus *Sylvilagus*, identified as cottontails. This genus includes 18 recognized species and all of them are New World forms (Chapman, Hockman & Ojeda, 1980; Nielsen & Berkman, 2018). More than 30 subspecies of eastern cottontails have been described (Nielsen & Berkman, 2018).

Figure 1 – (a) Infant, (b) juvenile and (c) adult eastern cottontail rabbits [Source: (a) and (b), courtesy of WRCMN; (c) original].



S. floridanus is considered a medium to large cottontail, weighting from 0.8 to 1.5 kg, although females are slightly bigger than males and, within its range, the body size rises from south to north and from west to east (Nielsen & Berkman, 2018). This species presents long and dense fur, gray to brown on the upper regions of the body and white over the venter and tail (Chapman & Litvaitis, 2003) (Figure 1). The ears are longer in proportion to its head size than found in most cottontails (Nielsen & Berkman, 2018). The distinctive white tail possibly functions as a “flash marking”: the predator is attracted and follows the last white “tail flash”, though the rabbit has evaded on a different direction meanwhile, providing time to hide in a safe cover (Whitaker & Hamilton, 1998).

3.1.2. Geographic distribution and habitat

Concerning its broad distribution, *S. floridanus* occurs from southern Canada into northwestern South America, including Venezuela. Formerly, this species inhabited the Eastern United States from the Rocky Mountains to the East Coast and as far north as New York (Nielsen & Berkman, 2018), although it has spread naturally beyond its original distribution and has been introduced deliberately into the western side of the continent, associated with hunting industries in order to increase their resources. In the 1960s this species has been introduced

into Europe as well, namely into Italy, France and Spain, where it has widespread, being considered invasive (Cooke, Flux & Bonino, 2018).

Within its range, the eastern cottontail rabbit is widely distributed throughout an ample variety of habitats (Chapman et al., 1980; Whitaker & Hamilton, 1998), specifically disturbed, early successional² or shrub-dominated (Chapman & Litvaitis, 2003): abundance of forage and dense understory vegetation cover are essential to their habitat requirements (Chapman & Flux, 2008), in order to avoid predators and to enable insulation against heat loss in winter and heat gain in summer (Althoff, Storm & Dewalle, 1997). Moreover, this species is frequently found in residential areas of large cities (Whitaker & Hamilton, 1998).

3.1.3. Feeding behaviors

Usually, cottontails occupy a form from sunrise to sunset (Althoff et al., 1997) and two feeding periods are selected for active foraging: dusk and dawn. They feed on a broad variety of plants depending on the season and geographic location (Nielsen & Berkman, 2018). Spring and summer diets consist of herbaceous species (clover, timothy and alfalfa), being the fall and winter periods a transition to a diet based on woody perennials, with buds and tender twigs of many small trees types, bushes and vines (Whitaker & Hamilton, 1998; Nielsen & Berkman, 2018).

3.1.4. Reproductive cycle, lifespan and mortality

S. floridanus is the most prolific species of all the members of the genus (Whitaker & Hamilton, 1998; Chapman & Litvaitis, 2003). However, *Sylvilagus*' gestation period and onset of breeding depend on latitude: the onset of reproduction occurs later at higher latitudes (Conaway, Sadler & Hazelwood, 1974) and the gestation period is shorter for these distributions as well (Chapman, 1984). This can be explained by the advantage for populations in northern regions, so that a maximum number of rabbit kits are originated by shorter gestation periods during the period of suitable weather and vegetation growth. Contrariwise, in southern distributions it would be beneficial that the gestation length was longer, in order to allow infants to born more developed and independent, being able to evade predators (Chapman, 1984).

The mean gestation period of the eastern cottontail averages 28 days (Chapman & Litvaitis, 2003) and 3 to 7 litters of 3 to 6 rabbit kits are originated over a breeding season that extends from February through September in northern regions of its range, being a wider period in the southern areas. Breeding in young-of-the-year juveniles has been reported by several studies, varying considerably (from 4.4% to 52% of the young-of-the year being reproductively active)

² A successional habitat develops after a natural disturbance in the original one, implying ecosystem compositional and structural changes and precedes its re-establishment. Successional modification is dominated by annual and perennial herbs, shrubs and trees that colonize these areas originated by river action, glaciation, or abandonment of cleared land. Early successional habitats attract wildlife that favor dense coverage and provide many food sources; however, these habitats are transitory, since they need disturbance to be maintained. (Askins, 2001; Swanson et al., 2011)

between populations from distinct North American states (Chapman, Harman & Samuel, 1977; Chapman et al., 1980).

Although about 80 to 85% of the populations are comprised by juveniles and great reproduction rates occur, the eastern cottontail is confronted with high mortality rates as well, as r-selected species (Whitaker & Hamilton, 1998): the annual survival for an adult is, in general, 20% to 40%, but it can be as small as 5% (Nielsen & Berkman, 2018). Predation is probably the most important factor in this species' mortality and the main direct cause of population regulation (Chapman & Litvaitis, 2003) though weather conditions and human harvest are other examples of primary mortality factors (Nielsen & Berkman, 2018). Therefore, a quarter of the population, or less, survives two years and very few individuals reach three or more years of lifespan (Whitaker & Hamilton, 1998).

3.1.5. Economic and ecological importance

The eastern cottontail is widely considered the most important game animal in the United States (Chapman et al., 1980; Chapman & Litvaitis, 2003; Smith, 2018), being hunted for sport, meat and fur (Nielsen & Berkman, 2018). Despite eastern cottontail rabbits are managed as a game species by state resource agencies in this country, the comprehension of long-term trends is hampered by hunt statistics that have been assembling several leporid species together, which is further complicated by natural fluctuations in population densities. Additively, data regarding cottontails and hares are seldom shared between state agencies (Smith, 2018). *S. floridanus* was classified as a Least Concern (LC) species by the IUCN Red List in 2008. Regardless of being considered very abundant species, several authors agree that its populations declined significantly during the twentieth century (Chapman & Litvaitis, 2003; Bosch, Benson & Mead, 2016; Nielsen & Berkman, 2018). These changes can be explained by alterations in land use and habitat fragmentation (Smith, 2018): in the Eastern region of the United States, urbanization and maturing forests are the main reason for habitat decrease, whereas intensive agriculture is the principal responsible in the Midwest (Chapman & Litvaitis, 2003). Extended drought and increased predation are other possible explanations (Smith, 2018); concerning subpopulations of *S. floridanus*, hunting pressure, human perturbation, predation from invasive species and, in some regions, livestock competition and habitat fragmentation constitute other threats (IUCN Red List, 2008). Contrariwise, Chapman & Litvaitis (2003) argue that the eastern cottontail supports heavy hunting pressures because of its high reproductive rates. Habitat management is considered the key to increase these populations (Chapman & Litvaitis, 2003; Nielsen & Berkman, 2018).

By contrast, these animals are at times interpreted as pests, becoming a nuisance (Whitaker & Hamilton, 1998), damaging flowers, vegetables, trees and shrubs in diverse places such as suburban yards, tree plantations and rural fields, at any time of the year. However, commercial fields or plantations destruction infrequently represents economic significance (Craven, 1994).

Cottontails, as other lagomorphs, play an essential role in many predator-prey food chains, thanks to population great abundance and the intermediate body size of the individuals (Chapman & Flux, 2008). Therefore, a wide variety of small to medium-sized carnivores - many canids, felids, mustelids, raptors and snakes - prey on them (Chapman & Litvaitis, 2003). The intricate reliance of two of the most endangered carnivores, the Iberian lynx (*Lynx pardinus*) and the imperial eagle (*Aquila adalberti*), on the European wild rabbit (*Oryctolagus cuniculus*) survival, is a worthy example of rabbit's importance in food chains, since it represents most of these predators' diet, the risk of extinction of both species is related with the decline of wild rabbit numbers (Delibes-Mateos, Smith, Slobodchikoff & Swenson, 2011).

S. floridanus also represents potential public health concern, since it is a known tularemia reservoir, apart from being host of ectoparasites which may carry zoonotic rickettsial diseases, such as Rocky Mountain spotted fever (Nielsen & Berkman, 2018).

3.2. Wildlife rehabilitation: admission causes and outcomes

3.2.1. The admission process and triage

One of the first steps of the rehabilitation process is to register the information provided by the caller or person presenting the injured wild animal (Meredith, 2016). It is essential to gather as much data as possible, prior to the clinical examination, such as where the animal was found, what clinical signs were observed and if any first aid or treatment were administered before (Richardson, 2016). It is frequent that members of the public try to care for these animals for several days and veterinary support is only sought when the animal's condition begins to deteriorate, which makes the rehabilitation process more challenging (Pokras & Porter, 1994). It is important to bear in mind that the reason for admission as described by the person who found the injured animal may not be related to the definitive diagnosis (Grogan & Kelly, 2013). A common example is an animal that is found by the side of the road. This scenario does not mean the animal was actually hit by a car, it may have been debilitated by a primary disease and was not able to escape from the injuries (Pokras & Porter, 1994). Another example is a fledgling bird found on the ground, apparently with no injuries and admitted as an orphan, but subsequently identified as a cat (*Felis catus*) attack victim after a thorough clinical examination (Grogan & Kelly, 2013). Therefore, a full physical exam is necessary in order to support a precise and comprehensive admission data: in order to achieve an accurate prognosis, all injuries must be assessed, since multiple concurrent illnesses are common (Schott, 2017). After the clinical examination and subsequent diagnostic tests, performed before or after first aid, the decision of further treatment or euthanasia should be reached. Several factors must be taken into account regarding animal's features, such as age and sex (for example, mammal females with pelvic fractures leading to dystocia in the future) and species' natural history and behavior. Treatment effectiveness should be assessed, based on prognostic indicators

(Meredith, 2016), such as illnesses and injuries severity (Molony et al., 2007) and other clinical signs such as emaciation, considerable parasite burdens and certain fracture types (Meredith, 2016). Concerns about the animal suitability for further release should be considered and planned: the animal must recover from the original injury/illness and secondary issues, present the ability to avoid predators, find food in the wild and the normal behavior for the species. Additively, it should not carry potential pathogens or zoonosis, nor represent a risk to the population, humans or the environment (Sleeman et al., 2003). The availability of veterinary skills and equipment, the compliance with legislative requirements and also, release conditions (suitable sites, time of the year) should be considered (Meredith, 2016).

In terms of wildlife welfare, it is pertinent to understand factors associated with survival and consequent release of the rehabilitated animals. Rehabilitation should not be attempted when it is not expected that the individual would survive the treatment process, or if there is a chance that it would remain permanently disabled or unable to survive in the wild, as previously mentioned; euthanasia is required as soon as possible to avoid further suffering and distress (Grogan & Kelly, 2013). When quality of life in a captive or semi-captive environment can be assured, exceptions may be considered, but that is rarely the case, since maintenance of a wild animal in permanent captivity is hardly justified in respect to welfare. Captive breeding programs of rare or endangered species, educational initiatives, or the use as imprint models to allow rearing young animals of the same species are some possible justifications (Meredith, 2016). Rabbits are extremely stressful, therefore they are poor education or exhibit animals, so euthanasia of non-releasable individuals should be considered (Schott, 2017).

Thus, the key is to allocate resources to ones presenting with higher chances of recovery (Molony et al., 2007; Grogan & Kelly, 2013; Tomlinson, 2016). Throughout the rehabilitation period and despite the mentioned efforts, animals may suffer mortality because their injury or illness becomes too severe, or because they do not respond to the treatment given, or further complications may occur (Molony et al., 2007).

3.2.2. The importance of data recording

Records logged at WRCs can also be considered a valuable material by providing information on population biology, behavior and habitat modifications, as well as diverse rehabilitation procedures and treatments, with their relative efficiency and effectiveness. Furthermore, rescued animals may be used as bioindicators of pathogens and chemical contamination (Pyke & Szabo, 2017), and reflect the natural or anthropogenic threats to wildlife (Molina-López, Mañosa, Torres-Riera, Pomarol & Darwich, 2017). It is known that wildlife casualties are mostly associated with human environments and activity (Pokras & Porter, 1994; Burton & Doblár, 2004; Molina-López et al., 2017) and it is likely that human-wild animal conflicts will increase, as the interface between wild and urbanized areas is becoming progressively unclear (Schenk & Souza, 2014).

A study conducted by Molony et al. (2007), based on medical records of eight animal species (the European wild rabbit was not included in the study) admitted between the period of 2000 and 2004 from the four WRCs run by the RSPCA in England, found that for all the studied species, the severity of the injury or illness symptom(s) was the only significant predictor of whether an individual survived to be released or not: the more severe the injury or illness, the less likely the individual was to be released; Molina-López et al. (2017) used a severity of illness/injury scoring system as well, concerning the study of morbidity and outcomes at a WRC. The reason for admission was excluded from the statistical analysis because of its high collinearity with the severity of the injury/illness symptoms. Sex, age, time of admission (given that not all WRCs are open 24h), year and season of admission, body mass on admission, and length of time in care were not significant predictors in any of the studied species. These results support the importance of a good triage, since the attempted treatment of the animals that presented severe injuries on admission could have a negative impact on welfare by prolonging suffering. Lastly, 39% of the wildlife casualties admitted within that period were released and 55% of them survived the first 48 hours after admission (Molony et al., 2007). Other studies reported distinct overall release rates: at the WRC of Torreferrussa, Spain, over 50% of the admissions were released (Molina-López et al., 2017); between 31 and 45% of the animals cared for at Australian WRCs went back to the wild (Tribe & Brown, 2000).

3.3. The eastern cottontail rehabilitation

3.3.1. Main challenges

Several authors agree that successful eastern cottontail rabbit rehabilitation is pointed as a defiant process: rabbits are more susceptible to the effects of stress than most other species, being difficult to manage in captivity (Bewig & Mitchell, 2009; Schott, 2017) and orphaned infants are one of the most demanding animal species to hand-raise (Evans, 1987; Bewig & Mitchell, 2009; Cowen, 2016).

3.3.1.1. Stress

When a stressful stimulus is presented to an animal, behavioral and physiological changes occur in order to adjust homeostasis and improve its chances for survival (Tsigos & Chrousos, 2002). Thus, two responses to acute stress occur: the almost instantaneous fight-or-flight reaction, with the stimulation of the sympathetic nervous system (SNS) and the release of catecholamines, epinephrine and norepinephrine; and the slower response mediated by a hormonal cascade along the hypothalamic-pituitary-adrenal (HPA) axis, that results in the adrenal glands secretion of glucocorticoid hormones (Reeder & Kramer, 2005). The heart rate increases, the blood pressure elevates and the mobilization of energy sources to the central

nervous system (CNS) and somatic muscle occur, to allow the animal to react quickly to the stressful event (Dickens, Delehanty & Romero, 2010).

As prey species, rabbits are highly stress-sensitive animals, being very challenging to manage in captivity, especially in an intensive care setting – patients may decompensate during examination and diagnostic procedures (Varga, 2014; Huynh, Boyeaux & Pignon, 2016; Schott, 2017). Captivity represents significant consequences on the SNS: merely holding wild animals may induce chronic stress because individuals are exposed to persistent stressors and consecutive acute stress responses, leading to immunosuppression, impaired reproduction and dysregulated metabolism (Dickens et al., 2010). Otherwise, the long-term stressful environment associated with captivity of wild rabbits may be a basic adaptive mechanism that allows animals to face life threatening and energy demanding situations. Letty, Aubineau, Marchandeu & Clobert (2003) measured circulating corticosterone and fecal glucocorticoid metabolites in European wild rabbits (*O. cuniculus*), during a quarantine captivity period before translocation and release. It was found that moderately elevated stress levels were negatively associated with body condition, though positively related with survival after release.

The sympathetic stimulation has effects on the gastrointestinal (GI) function: noradrenergic impulses lead to constriction of the abdominal viscera arterioles, decrease the GI motility and tone, inhibit its secretion and induce sphincters contraction. Besides this, glycogenolysis and gluconeogenesis are stimulated, thus elevating blood glucose levels and free fatty acids in order to mobilize resources to respond to the stressful stimulus (Klein, 2013). In rabbits, this event culminates in reduced GI motility, which may imply consequences on cecum microbiota balance – frequently leading to disbiosis – and digestive function. Therefore, enterotoxemia or gut stasis are possible results from any presented stressful event. The carbohydrate metabolism is affected as well, thus diarrhea, hepatic lipidosis, liver failure and death are potential concerns (Bewig & Mitchell, 2009; Varga, 2014).

As capture and hospitalization are stressful events for wild lagomorphs and gut stasis is a possible consequence, Richardson (2016) advises the administration of GI stimulants as ranitidine, metoclopramide and/or cisapride for hospitalized patients.

When the rabbit is confronted with an unfamiliar environment and frequent handling in a clinic context, stress-induced cardiomyopathy is possible: the endogenous release of catecholamines, in a dose-dependent way, is able to induce noteworthy cardiomyopathy in New Zealand white rabbits, with acute or delayed consequences (Downing & Chen, 1985). There is also a substantial decrease of coronary blood flow consequently to vasoconstriction (Simons & Downing, 1985). In extreme situations, heart failure and death are possible, due to catecholamine release (Varga, 2014).

Schadt & Hasser (1998) state that the rabbit presents two distinct defense behaviors when acute stressors are imminent in the wild: active defense and freezing/hiding. Cardiovascular

responses are also different, since the exposure to stressful sensory stimuli can result in increased or decreased somatic activity: the defense action is associated with tachycardia and increased cardiac output; oppositely, a passive response such as “freezing” behavior is related with no increment in these parameters. During hospitalization, both types of responses are expected, so stress should be minimized as much as possible (Huynh et al., 2016).

Creating a quiet, predator-free environment, with a sight barrier to reduce visual stimulation are some housing measures that can be adopted during the rehabilitation process, in order to decrease stress (Bewig & Mitchell, 2009). Additively, handling properly is imperative, since wild lagomorphs can panic to such an extent that spinal fractures can occur during a struggle (Richardson, 2016).

3.3.1.2 “Orphaned” cottontails

Well-intentioned members of the public who bring them to a WRC frequently mistake eastern cottontail rabbit kits as orphans. This occurs because the public observes infrequent visits by the doe to her nest, concluding that there was abandonment or neglect, which is not accurate. Eastern cottontail rabbits have a distinct parental behavior, in which the mother visits the nest not more than twice a day, to quickly feed the kits. This behavior avoids attracting predators’ attention to the offspring. Most of the time, if the nest is not destroyed or threatened by predators, human intervention or disturbance is avoidable. Non-natural hand-rearing of abandoned or orphaned rabbits should only be attempted when all efforts of returning the infant to the wild have failed (Burton & Doblár, 2004), although the mortality rate is high (Varga, 2014).

Rabbits are born altricial, therefore virtually hairless, with sealed eyelids and ear canals, reduced crawling aptitude and helpless (Harkness, Turner, VandeWoude & Wheler, 2010). From the birth to the first week of age, the cottontail weights approximately 20-35 g. Eyes are completely open at 2-3 weeks and weaning should occur at 3-6 weeks of age (King, 2007), becoming independent with about 7 to 8 weeks of age (Pollock, 2013).

Infant cottontails are commonly presented to a WRC in a hypothermic, hypoglycemic and dehydrated state, which can result in lethargy, shock or even coma (Pokras & Porter, 1994; Bewig & Mitchell, 2009; Schott, 2017). After a thorough examination, if no obvious injuries that support euthanasia are found, supportive care should be managed promptly (Schott, 2017): once warmed and rehydrated, *per os* fluids, such as sodium lactate solution mixed with milk replacer formula can be provided – 75% electrolyte solution with 25% formula. At each following feeding, the amount of given formula can be increased until 100% formula concentration is achieved (King, 2007). Hairless animals need to be maintained at 35°C /95°F (Pokras & Porter, 1994).

Orphaned rabbits are one of the most demanding animals to hand-raise in captivity (Evans, 1987; Bewig & Mitchell, 2009; Cowen, 2016). Blind and naked neonates, in particular, present

reduced survival chances, since they require constant care and have poor thermoregulation and immunity (Meredith, 2016).

3.3.1.3. GI disease in infants and weanlings

The main difficulties associated with hand-rearing rabbit kits are related to feeding and digestion: milk aspiration at feeding time, enterotoxemia, bloat and diarrhea (Richardson, 2016), which will be discussed further. Trauma due to improper tubing and ruptured esophagus are also possible (Reed, personal communication, April 30th, 2018).

Aspiration is possible when the fluids or formula given to the infants are forced through the tube or syringe so quickly that they retrograde up the esophagus and are accidentally inhaled into the trachea (Bewig & Mitchell, 2009), or the animal is not suckling appropriately (Richardson, 2016).

In the wild, rabbit kits are fed with large amounts of high-protein and fat concentrated milk, distinctive of this species, during a once-daily nursing (Bewig & Mitchell, 2009; Carabaño, Piquer, Menoyo & Badiola, 2010; Cowen, 2016), being completely dependent on this diet until 10-15 days of age, when they begin to complement their feedings with small volumes of solid food (Richardson, 2016). During the suckling period, the rabbit's GI tract is virtually sterile. This is possible because of the substrate present in the fatty portion of the doe's milk, which reacts enzymatically with the youngsters' stomach wall content and is transformed into an antimicrobial factor. This antimicrobial fatty-acid product, also known as "stomach oil" or "milk oil" is mainly constituted by octanoic and decanoic acids and provides protection against infection (Cañas-Rodríguez & Smith, 1966). Hand-reared rabbit kits fed on milk replacer formula or milk of another species (generally, goat or cat milk is provided) do not develop the "milk oil" and become more susceptible to bacterial infections introduced while they are being artificially fed (Davies & Davies, 2003; Bewig & Mitchell, 2009; Varga, 2014). Sterile syringes and feeding tubes are suggested and each feed should be prepared just previous to being given, in order to avoid these issues (Varga, 2014).

Since milk replacers are a nutritional compromise comparatively to the doe's milk and it is essential to provide adequate calories to the rabbit kits, these animals require regular feeding in captivity, contrarily from the once-daily feed in the wild (Bewig & Mitchell, 2009), but attention should be taken in order to avoid overfeed or force-feed (Varga, 2014). King (2007) supports a designed feeding schedule based on the rabbit's features (eyes closed or opened) and body weight; Bewig & Mitchell (2009) believe that the number of feedings depends on infant's body weight, appetite and general condition; however, Cowen (2016) defends that a minimum of 5 feeds per day should be provided.

Furthermore, different milk replacer formulas are suggested by distinct authors (King, 2007; Oberly, 2015; Cowen, 2016; Paul & Friend, 2017). In the Colorado Wild Rabbit Foundation, Paul & Friend (2017) compared two milk replacer formulas, derived from commercial products,

to understand the association with outcomes in unweaned desert (*Sylvilagus audubonii*) and eastern cottontail rabbits. It was found that one of the tested milk replacers was superior for raising the infant desert cottontails, with higher release rates and reduced pre-weaning GI disease, however, conclusions for eastern cottontails were not significant. GI disease (with signs of diarrhea, bloat, GI stasis, cecal disbiosis, or a combination of all) was the largest cause of mortality in the sampled infants. In both species, GI disease prior to weaning had 100% mortality as a result, regardless of which formula was provided. Curiously, 71% of the eastern cottontails developed either pre- or post- weaning GI disease throughout the study (with 79% of mortality); individuals of this species that did not develop GI disease had a release rate of 100%, despite of which milk replacer they received.

Oberly (2015) tested two different formulas from the habitually provided to infant eastern cottontails at the Ohio Wildlife Center, which did not significantly increase survival, compared to the hospital's regular formulas. The only significant factor linked to release was body weight presented upon admission.

As the young rabbits age, they start to ingest maternal cecotrophs that remain intact in their stomach and are involved in the mucinous coating and maintaining the microbial contents along with the "milk oil". This allows the microbes to pass into the intestine and to colonize the cecum (Davies & Davies, 2003). Cecotrophy is initiated when solid food starts to be consumed (Carabaño et al., 2010) and, during the same period, the production of stomach antimicrobial factors is reduced (Davies & Davies, 2003). The gastric pH decreases from 5-6.5 to the adult level of 1-2, providing a barrier against microbial colonization of the stomach and small intestine (Davies & Davies, 2003; Varga, 2014).

Weaning is considered an extremely critical and risky period for GI disease in the rabbit (Davies & Davies, 2003; Gidenne, García, Lebas, & Licois, 2010; Richardson, 2016), especially for the orphaned individuals (Varga, 2014), and as they approach weaning age, the incapability to handle stress rises (Evans, 1987). The protection of the growing rabbit against enteric infections depends on the coordinated management from one defensive mechanism to another (Davies & Davies, 2003). Additionally, the fiber intake is thought to play a major role in the development of specific and non-specific enteropathies during this period. A fiber deficiency prevents the quantitative and qualitative improvement of the cecum microbial activity in the young rabbit, since the intestinal transit becomes slow, favoring digestive issues and voluntary feed intake decreases (Gidenne, Jehl, Segura & Michalet-Doreau, 2002). Furthermore, a fast weaning should be avoided considering its consequences, namely gastric or cecum tympanism, or intestinal bacterial overgrowth (Cowen, 2016).

Pathogens involved in weanling rabbit specific enteropathies may include *Escherichia coli*, *Clostridium spiroforme*, *Clostridium piliforme*, *Salmonella* Typhimurium and *S. Enteritidis* (Richardson, 2016). *C. spiroforme* and *Eimeria* spp., often accompanying GI disease in young domestic rabbits, were recognized as important agents of morbidity and mortality in three

weanling cottontail species undergoing rehabilitation in the Colorado Wild Rabbit Foundation, including *S. floridanus*. *C. spiroforme* was identified in Gram stains of weanling cottontails' feces, which presented diarrhea; necropsy and histopathology findings were consistent with clostridial enterotoxemia. Weanlings passed soft, malformed cecotrophs which were not consumed; contrarily, it was identified severe watery diarrhea in unweaned cottontails. Furthermore, severe coccidiosis was confirmed via necropsy and histopathology in suspected cases, besides the presence of oocysts in feces of rabbits with clinical signs. In addition to diarrhea, GI stasis and cecal impaction were observed, with signs of abdominal pain. The authors reported the appearance of both diseases on arrival and during the rehabilitation process. It was verified that early specific treatment implementation (metronidazole for clostridiosis and toltrazuril for coccidiosis) reduced considerably the morbidity and mortality within weanlings (Paul & Friend, 2019).

Treatment attempts are often unsatisfactory, however fluid therapy, analgesia, nutritional support, antibiotics and toxin binders are some possibilities (Richardson, 2016); probiotics use is controversial and there is not an agreement about their implementation in weaning GI disease (Bewig & Mitchell, 2009; Cowen, 2016). Euthanasia may become mandatory in severe enterotoxemia cases. Prevention is key: grass hay as a source of fiber and assorted vegetables should be provided and fresh cecotrophs from healthy adult rabbits are recommended, in order to safeguard the intestinal tract transfaunation with healthy bacteria and protozoa (King, 2007; Bewig & Mitchell, 2009; Varga, 2014). Moreover, handling these animals should be reduced as much as possible to decrease possible effects of stress on gut motility and implementation of good hygiene principles should be instituted (Richardson, 2016). Prokinetic drugs are also suggested around the time of weaning to ensure normal GI motility (Cowen, 2016).

3.3.2 Major admission causes and outcomes

The most common admission circumstances of the eastern cottontail rabbit vary according to age, recorded in a table to simplify (Table 1).

Some species' rehabilitation outcomes were more thoroughly studied than others, mainly raptors' morbidity and admission causes (Rodríguez, Rodríguez, Siverio & Silverio, 2010; Molina-López, Casal & Darwich, 2011; Molina-López & Darwich, 2011). As far as the author knows, the information is scarce regarding lagomorphs' rehabilitation results, particularly. Concerning the eastern cottontail, Oberly (2015) reported a release proportion of 34% for the overall eastern cottontails admitted at the Ohio Wildlife Center, during 2014. 37% of the infant and juvenile cases were released that year.

Table 1 - Most common reasons for admission of the eastern cottontail rabbit in WRCs, regarding age class (based on Pokras & Porter, 1994; Bewig & Mitchell, 2009; Richardson, 2016; Schott, 2017).

Neonates and infants	Juveniles	Adults
Abandoned or, more frequently, apparently abandoned – classified as “orphaned”	Found in a debilitated condition, often due to starvation and/or hypothermia	Most frequently, primary disease (e.g. neurologic) that enables succumbing to a secondary injury
Nest disturbance or destruction	Human-induced trauma: road traffic accidents, machinery, hit by lawn equipment, abuse	
Rescued from a predator (domestic cats and dogs, mainly)		

Note: These references did not specify age ranges definitions. Although there are several age estimation techniques applied in cottontails (Chapman & Litvaitis, 2003), they may not be easily accomplished in a WRC context. Here, “neonates and infants” may be considered all animals that have not reached independence (until 7 to 8 weeks); “juveniles” from that stage on, until “adult” age, when body weight averages from 0.8 to 1.5 kg (Pollock, 2013).

Grogan & Kelly (2013) reported the following outcomes for the European wild rabbits (*O. cuniculus*) presented to RSPCA wildlife facilities: approximately 10% of the animals died in cage, 55% were euthanized, 25% were released and 10% presented other resolution, not specified in the respective article. Another retrospective study, conducted by Molina-López et al. (2017), at the WRC of Torreferrussa, Spain, including data from 1995-2013, had the goal to assess the morbidity, outcomes and cost-benefits of the rehabilitation facilities. Rabbits and rodents were studied collectively and it was found that 3.5% of this group was admitted in “Captivity” category (animals captive for more than 6 months and/or confiscated due to poaching or illegal pet trade), 63.1% was “Orphaned”, 19.6% victims of “Trauma”, 10.0% “Infectious disease”, 2.6% “Misplacement”, 0.6% “Metabolic or nutritional”, and 0.6% from other causes. “Mortality” proportion was approximately 30%, “Euthanized” corresponded to 20% and nearly 50% of the animals were “Released” (the small remainder corresponds to “Captivity”, here referring to the animals kept permanently captive after rehabilitation). “Orphaned”, “Captivity” and “Misplacement” categories presented the best release rates, contrarily to “Infectious disease” (with 0% of released animals) and “Trauma”, consisting the worst outcomes in these species. To correctly understand the results, it is important to consider myxomatosis, to which *Sylvilagus* and *Lepus* species have variable susceptibilities, however it represents a highly lethal viral disease that affects the European rabbit population (Lemos de Matos, McFadden & Esteves, 2014): since the treatment is often unrewarding, euthanasia is advisable in these cases (Richardson, 2016). Furthermore, an increasing severity of the clinical condition on admission was shown to be related with decreasing release proportions in rabbits and rodents (Molina-López et al., 2017).

3.3.2.1. Trauma-related admission causes

The main causes leading to traumatic injuries in wild lagomorphs are diverse and include road traffic accidents, gun shots, entrapment, farm/garden machinery and predation. The consequent injuries are mostly fractured limbs and soft tissue wounds. When head trauma occurs (discussed further), neurological signs, jaw fracture and ocular damage are possible (Richardson, 2016).

It is essential to see beyond the traumatized animal: commonly there is already a compromise caused by a primary disease (for example, presentation of neurological symptoms caused by *Baylisascaris procyonis*, discussed further; vision compromise), making it more likely that this animal becomes attacked, hit by a vehicle, or unable to evade capture (Richardson, 2016; Schott, 2017). Therefore, it is necessary to re-evaluate the animal after stabilization, with the possibility to reveal the primary disease (Schott, 2017).

3.3.2.1.1. Automobile collision

Roads induce deep ecological effects in both terrestrial and aquatic ecosystems (Trombulak & Frissell, 2000) and occupy a significant area of total available territories - mainly in urban and densely populated regions (Burton & Doblar, 2004). Roads impact in the mortality of wildlife species (from road construction and from collision with vehicles which may affect the demography of many species), modifies animal behavior, besides the physical and chemical environment, allows the dispersal of exotic species and increases the use of remote areas by humans (Trombulak & Frissell, 2000). Several factors, such as the animal's age, the vegetation cover along the roadside and the season of the year have influence on roadkill: young animals are more frequently affected, different land-covers appeal different animal species and it is known that summer months are the seasonal peak of roadkill for mammals and birds (Burton & Doblar, 2004; Langley, 2018)

Automobile strike ranks as one of the most frequent presentation causes at WRCs, after orphan and cat predation circumstances (Burton & Doblar, 2004). Between 1995 and 1998, 8.12% of the mammals admitted at the Ohio Wildlife Center were assumed to be hit by a vehicle. The eastern cottontail rabbit was one of the three mammalian species that made up the vast majority of the affected cases in this WRC (Burton & Doblar, 2004).

The prognosis of the animals hit by vehicles is generally guarded: in a study conducted in the Wild Clinic in East Tennessee, the cases recorded as result of automobile strikes presented the highest fatality risk (0.715) and also the highest percentage of cases with consequent euthanasia, across all animal groups (Schenk & Souza, 2014).

3.3.2.1.2. Domestic animals interactions

Free-ranging domestic animals may prey on wild animals, causing repercussions on wildlife populations with varying degrees, once predation is additive or compensatory to other causes

of mortality. This is especially influenced by the increasing expansion of urban areas into rural habitats and human settlements creating high densities of pets (Coleman, Temple & Craven, 1997; Baker, Bentley, Ansell & Harris, 2005; Young, Olson, Reading, Amgalanbaatar & Berger, 2011). Wildlife may suffer direct impacts from domestic animals interactions, such as injuries, associated bacterial infections and stress consequent from the capture, besides indirect effects such as dependent juveniles' mortality, when an adult is attacked, besides the competition with native predators (IWRC, 2018). Where habitats have been fragmented (urban and suburban areas), islands and parks, these negative effects are even more important; endangered and ground-dwelling wildlife are the most impacted (Jessup, 2004).

a) Cats (*Felis catus*)

Loss, Will & Marra (2013) estimated that 1.3-4.0 billion birds and 6.3-22.3 billion mammals are killed by free-ranging cats (*F. catus*) across the contiguous United States, annually. The IUCN considered the domestic cat as one of the 100 worst invasive alien species globally, based on its serious impact on biological diversity (Lowe, Browne, Boudjelas & De Poorter, 2000). Feral cats are the most involved in these effects and may surpass all other sources of anthropogenic mortality of U.S. birds and mammals (Loss et al., 2013).

Firstly, it is essential to distinguish cats' varying degrees on human dependence: feral cats are completely independent from humans, although urban and farm colonies, besides stray cats are most of the times partially reliant on people food source (Baker et al., 2005). Most of domestic cats are concentrated in regions where people live, instead of isolated in undeveloped areas. Besides rural free-ranging cats, which have easier contact with wildlife, urban house pets also take live prey when allowed to roam outside (Coleman et al., 1997).

Domestic cats present several advantages when competing with native predators, having a dependable food source (not influenced by changes in populations of prey) and people protect them from disease and predation; cats have a superior reproductive ability; and contrarily to numerous native predators, territoriality does not limit cats' densities (Coleman et al., 1997; Burton & Doblar, 2004). An additional concern is linked with diseases that cat may transmit to wildlife (e.g. FeLV to wild felids) and recognized zoonosis, including toxoplasmosis and rabies (Coleman et al., 1997; Jessup, 2004). Cats have been the domestic animal species with more confirmed cases of rabies, from 2011 to 2016, within the U.S. (Ma et al., 2018).

b) Dogs (*Canis familiaris*)

Dogs (*C. familiaris*) are the world's most common member of the order Carnivora (Vanak & Gompper, 2009; Ritchie, Dickman, Letnic & Vanak, 2014) and are intensely associated with human communities, since food and shelter are directly or indirectly provided: the number of dogs in an area may be predicted on the number of humans. The free-ranging behavior (more or less contained) represents a problem to wildlife (Gompper, 2014).

As it happens with cats, they can be categorized as owned or un-owned; rural or farm dogs; stray or feral; sylvatic or wild; pet or working dogs; although this classification is not always easy and may change concerning the same animal. Animals from all these categories can be free-ranging, however, dog interactions with wildlife are more expected to occur in rural settings (Ritchie et al., 2014). The likelihood to interact with wildlife can be predicted based on where the dog lives and if it is more or less sympatric with wildlife, and its dependence on humans to be fed. Therefore, urban (owned or not) and fully constrained dogs, are highly reliant on human food and do not interact with wildlife very often, unless when accompanying humans into natural areas; oppositely, rural dogs have a better chance to roam freely compared with urban dogs, in which health and nutritional state influence the possibility of interacting with wildlife, that is, if the human-derived food is insufficient, they must range broadly to scavenge or hunt, besides the higher possibility to live near natural areas or wildlife reserves (Ritchie et al., 2014).

Dogs may impact on prey by killing them or inducing fear, which may influence negative changes in prey behavior (ceasing normal activities such as foraging, parental care, resting), physiology and habitat use (prey will alter their spatial distribution in regions where dogs are allowed to roam). They also have influence in native predators, as they can be perceived as competitors for resources or as a potential prey; dogs are possible pathogens carriers (e.g. rabies and canine distemper) and their human reliance is an advantage to reach considerably higher population densities, compared with wild canids (Vanak & Gompper, 2009; Gompper, 2014). Likewise, small and medium-sized carnivores tend to avoid areas with high dog activity (Vanak & Gompper, 2009; Ritchie et al., 2014; Weston & Stankowich, 2014).

c) Importance as one of the main admission causes at WRCs

Domestic animal predation is a consistent cause of admission to rehabilitation centers across the United States (Burton & Doblar, 2004). Loyd et al. (2017) investigated the records from eighty-two wildlife rehabilitation organizations throughout North America, from 2011 to 2015, and it was determined that domestic animal predation, in general, was the second most common cause of injury (14%) to the admitted animals. Juveniles of all taxa exceeded the adult admissions due to this cause, which can explain that most of the cat attacks occurred in the spring and summer. Moreover, fewer cat interactions occur in winter possibly because migratory birds are not present, many mammals are dormant and fewer owners allow cats to roam outside during colder months (McRuer, Gray, Horne & Clark Jr., 2017). In total, 8% of the wildlife presented was affected by domestic cats: 68% of the individuals attacked by cats died during treatment or were euthanized because of the high severity of injuries presented; the proportion of dog attacks for the same disposition was 54%. The treatment period was studied as well, being on average 6 days in care before death or euthanasia and 30 days in care before release. It is important to realize that these numbers are possibly underestimating

the effect of domestic pet predation on wildlife admissions, since the not witnessed cases were not included in the referred study.

Schenk & Souza (2014) carried out a study in the Wildlife Clinic of East Tennessee and concluded that 20% of all cases presented between 2000 and 2011 were due to domestic pets interactions, namely 14% of all cases related with cats and 6% of all cases associated with dogs. Regarding fatality risk, the injuries induced by a cat presented a worse scenario (0.675) than dog-related cases (0.600), across all taxa. Adding to that, cat interactions had the highest percentage of deaths during treatment.

In Lindsay Museum of Walnut Creek, a WRC in California, 24% of birds, 12% of mammals and 15% of reptiles were admitted with cat-related causes, within a period of approximately 9 months (Jessup, 2004).

Between 2000 and 2010, cat interaction was the second leading cause of admission for small mammals (15 %) at the Wildlife Center of Virginia, with a mortality rate of 71% (McRuer et al., 2017).

d) Consequences and injuries

The features and effects of cats' *versus* dogs' bites should be explored, to allow a better understanding on the different outcomes and mortality rates presented by their wildlife prey. Cats present long, slender incisor teeth and fang-like canines capable of inflicting apparently minor puncture wounds (Figure 2) on either side of the prey, at the skin surface— holding the prey until it is still and then using the same teeth to shear the flesh. However, the bites can penetrate deeply, instilling microorganisms into the traumatized subcutaneous tissue, an ideal breeding ground for opportunistic pathogens transferred from the cat's oral microbiota. This represents a challenge because bones, joints and tendons may be potentially punctured and can be easily missed, being also extremely problematic to debride and disinfect (Love, Malik & Norris, 2000; Dendle & Looke, 2008; Freshwater, 2008). Therefore, if cat interaction is suspected, antibiotics on admission are required, even if wounds are not perceptible (McRuer et al., 2017; Schott, 2017). Further cat-induced wounds may include subcutaneous emphysema and hemorrhage, degloving injuries³ or skin lacerations and septicemia, the latter possibly resulting in further clinical signs, such as cardiovascular shock, neurological deficits and respiratory distress (Loyd et al., 2017; McRuer et al., 2017). In contrast, dog's teeth and jaws are aimed to crush bones and tear flesh, producing crush injuries, lacerations and abrasions to their prey (Love et al., 2000; Dendle & Looke, 2008). Other noticeable differences between the two domestic predators that should be taken into account: the greater number of free-roaming cats (owned and stray/feral), compared to the fact that free-roaming dogs are relatively unusual in North America; the possibility that dog attacks are more visible since they take place in the owner's yard due to containment, whereas cat attacks may occur in diverse

³ Avulsion of the skin from the underlying tissue (Loyd et al., 2017).

locations, consequently representing more diverse prey taxa, not often returned to the residence (Lloyd, Hernandez, Carroll, Abernathy & Marshall, 2013). Differences between the hunting behavior presented by dogs (which attack the neck and shoulders) and cats (which play with prey for prolonged periods) imply that dog attack prey are less likely to be presented alive at the WRC, compared to a cat prey (Lloyd et al., 2017).

The most frequently cultured pathogen isolates from both cat and dog bites are *Pasteurella* species (Talan, Citron, Abrahamian, Moran & Goldstein, 1999), being more often identified in cat bites isolates, than from dog bites (Talan et al., 1999). This may be explained by the fact that *P. multocida* is a natural inhabitant of the oral cavity and nasopharynx in most healthy domestic felines (Freshwater, 2008). It is also described that cat bites are a complex mixture of aerobe (*Moraxella* and *Neisseria*), facultative anaerobe (streptococci, staphylococci, *Corynebacterium* and *Pasteurella*) and anaerobe pathogens, the last ones being more frequently isolated from cat bites than from dog bites, associated with the nature of the produced wound (Talan et al., 1999). Mouro, Vilela & Niza (2010) identified *Pasteurella multocida* and *Staphylococcus pseudintermedius* as the most frequent microorganisms in dog bites, however, *Clostridium perfringens* was identified as the most frequent strictly anaerobe and there was an association between clinical infection and the presence of strict anaerobes. In a prospective clinical study developed by Goldstein, Citron and Finegold (1980), of all dog bite wounds analyzed, 74% presented facultative anaerobic pathogens, including several strains of *Streptococcus viridans*, *Staphylococcus aureus* and *P. multocida* and 41% had anaerobic pathogens isolated, such as *Bacteroides* and *Fusobacterium* species (Talan et al., 1999). Regarding infection possibility, 20-80% of cat bites became infected, in contrast to only 3-18% of dog bite wounds (Freshwater, 2008).

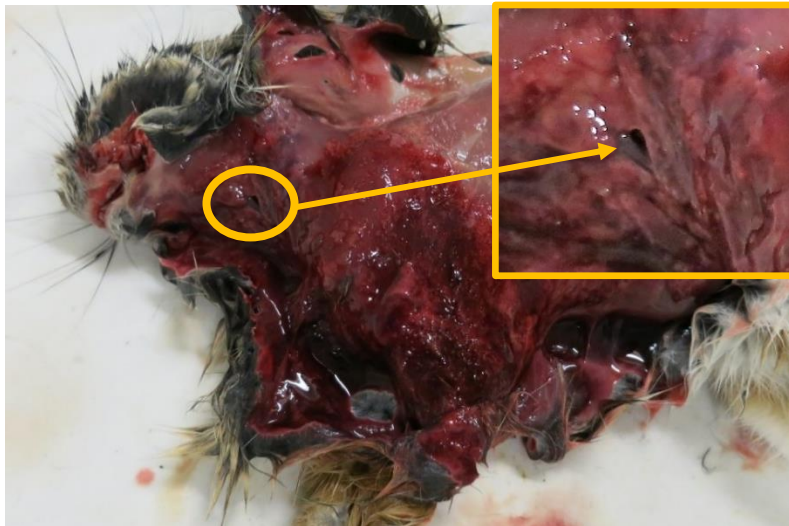
Empirical antibiotherapy for dog and cat bites should be directed against *Pasteurella*, streptococci, staphylococci, and anaerobes; namely antibiotics that are usually prescribed for routine infections of skin and soft tissue (Talan et al., 1999).

The eastern cottontail is one of the most frequently affected species by cat attacks in the majority of North America regions, except in the Southwest and Central Mountains areas (Lloyd et al., 2017); moreover, it was the second small mammal species more frequently admitted with this underlying cause at the Wildlife Center of Virginia, where 26% of all the cottontails had interacted with cats (McRuer et al., 2017). This species is presumably more exposed to cat attacks because of its dawn and dusk habits, ground-foraging behaviors (Lloyd et al., 2017) and its abundance nearby human structures (McRuer et al., 2017). Particularly, young rabbits which interacted with domestic animals, present a guarded prognosis (Bewig & Mitchell, 2009).

In rabbits, predators' bite injuries are one of the possible causes resulting in abscess development, secondary to infection in the damaged tissue. These abscesses are well-encapsulated, slow-growing and rather painless; pyogenic bacteria such as *P. multocida* and

S. aureus are commonly isolated. Culture and susceptibility are recommended to choose an effective antibiotic, although its distribution in infected tissue and the effect on cecal microbiota are decisive (Varga, 2014).

Figure 2 - Adult cottontail rabbit necropsy, with cat interaction history on admission. Extensive subcutaneous hemorrhage on the left side of the body and a puncture wound over the left side of the neck (amplified) were identified. This animal was admitted with associated open mouth breathing, possibly due to upper airways injury (Source: courtesy of WRCMN).



3.3.3. Clinical signs presented on admission

As prey species, rabbits hide signs of illness and/or injury for their own defense, which makes its recognition difficult and medical management becomes more defying (Huynh et al., 2016). The pain assessment is paramount to define the severity of the condition and to create a treatment plan, though it is exceptionally challenging to assess pain in rabbits (Keeble, 2006). A healthy rabbit is vigilant, responsive and alert of its surroundings; in response to pain, it becomes quiet, immobile and is not aware of the environment (Varga, 2014). An efficient clinical examination is always significant, but this is especially true for wild lagomorphs. A quiet environment is essential during the full clinical examination, so sudden movements, unnecessary handling and loud noises should be avoided; the eyes can be covered with a hand or towel; and while lifting and carrying, the spine and hind limbs should be supported (the animal can be wrapped in a towel). Anesthesia may be necessary, when assessing a struggling rabbit (Richardson, 2016).

In the present dissertation, it is intended to approach clinical signs categories reported in cottontail rabbits on admission at rehabilitation settings, focusing on the most frequently found within WRCMN casuistic.

3.3.3.1. Musculoskeletal signs

In wild rabbits, traumatic injuries frequently result in soft tissue wounds and fractures (Richardson, 2016). Lagomorphs' skeleton consist of 8% of the total bodyweight (Harkness et al., 2010), being encased in a comparatively large and powerful muscle mass, which contributes over 50% of the bodyweight (Richardson, 2016). Despite these features allowing the fast evasion from predators (Richardson, 2016), bones are quite brittle and susceptible to fractures (Harkness et al., 2010; Varga, 2014; Richardson, 2016), particularly on long bones and lumbar spine, which support large muscle masses (Harkness et al., 2010). Fractures are habitually complex, presenting multiple fragments, although rabbit bone heals rapidly (Varga, 2014).

The age seems to influence the fracture type in the wild rabbit. Juveniles have incomplete growth plate calcification, which makes them susceptible to fracture (Harkness et al., 2010), usually of the simple long bone form (Schott, 2017). Adding to this, young rabbits are not experienced or fast, which makes them more vulnerable; thus, this situation results in a good to guarded prognosis, with appropriate fixation. Tibial fractures are the most common, followed by femoral, regarding long bone fractures in young rabbits (Schott, 2017). Contrarily, adults are extremely aware of predators and are capable of reaching high speeds, thus it is presumable that fractures are frequently associated with a primary systemic illness (for example, neurological disease) and are rarely able to be fixed, and multiple, comminuted and open fractures are common (Schott, 2017).

When limb fractures occur, the goal is to reestablish bone alignment and immobilize the fracture site, to allow fast healing. The particular shape of rabbits' limbs does not enable the application of satisfactory splints, slings or bandages, thus surgery is required in several cases. External fixation is frequently the key for fracture resolution in rabbits; when internal fixation is preferred, it is accomplished with pinning instead of plating, because of the small bones and thin cortices (Varga, 2014).

A number of factors should be taken into account, in order to make the decision of treatment attempt or euthanasia: higher number of fractures are related with a poorer prognosis; open fractures, intra-articular fractures, luxations and subluxations are associated with a high risk of decreased range of motion, chronic pain and arthritis, presenting a poor prognosis; limb amputations are not acceptable in wild rabbits. When the skull is involved, the presence of neurological signs should be considered (Schott, 2017). In order to deliberate release, these animals rely on speed and agility to survive in nature, so about 100% function is required after fracture healing (Schott, 2017).

Concerning muscle disease, capture myopathy, characterized by metabolic acidosis, muscle necrosis and myoglobinuria and related with pursuit, capture and restraint (Paterson, 2014), is probably present in adult cottontails presented to rehabilitation (Schott, 2017). Muscle damage due to stress of capture was reported in other lagomorph species, namely translocated

European wild rabbits (*O. cuniculus*) (Calvete, Angulo, Estrada, Moreno & Villafuerte, 2005) and European brown hares (*Lepus europaeus*) (Paci, Bagliacca, Lavazza, 2006), which is in accordance with the indication that prey species are more susceptible to this complex and multifactorial disease. Significant morbidity and mortality may occur, however, diagnosis is only possible with the combination of history, clinical signs and pathology, necropsy and histopathology (Paterson, 2014).

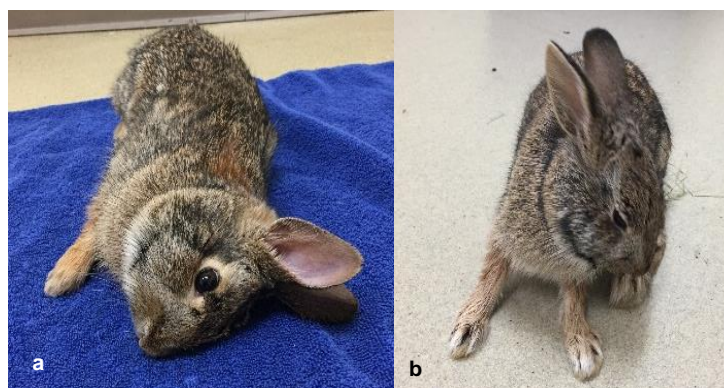
3.3.3.2. Neurological signs

The neurological examination of captive wildlife is demanding. Since the animal is extremely frightened and the sympathetic tone is elevated, several misleading neurological signs may be presented, such as muscular rigidity and decreased response to visual, auditory or tactile stimuli. In wildlife, most neurological signs are caused by trauma, larva migrans (LM) or toxicoses; skull and spinal fractures are frequent (Pokras & Porter, 1994).

Because rabbits are stressed species, frequently freezing during the clinical exam, many individuals do not present menace response (Vernau, Osofsky & LeCouteur, 2007) and the deep pain test may not be reliable, because of their stoical nature (Keeble, 2006). Adding to the history, it is essential to identify the manifestation of the neurological abnormality and localize the lesion, whether it is singular or multifocal/diffuse. However, clinical signs will be similar regardless of the type of lesion that caused the loss of nervous system function (Vernau et al., 2007). The exam should begin with tests or regions improbable to cause pain and requiring minimal handling (Varga, 2014) and it is suggested the following sequence for the neurological examination of the rabbit: general observations (mental status, posture, gait), palpation, examination of postural reactions, spinal reflexes and cranial nerves function and sensation assessment (Vernau et al., 2007).

Signs of neurologic disease in pet rabbits are common (Varga, 2014) and may comprise behavioral changes, head tilt (also known as torticollis) (Figure 3), nystagmus, tremors, paresis, paralysis, seizures, generalized muscular weakness, falling, rolling, ataxia and depressed mental status (Fisher & Carpenter, 2012; Varga, 2014). Secondary skin, digestive or urinary tract issues may be manifested (Varga, 2014).

Figure 3 – (a) Eastern cottontail presenting a severe head tilt to the left (one ear lower than the other) (original). (b) Eastern cottontail presenting a left head turn (ears in normal position and face turning towards the rump) (De Risio, 2005) (original).



Otitis interna, pasteurellosis or other bacterial infections, encephalitozoonosis, cranial or vertebral trauma, spondylosis, heat stress, toxemia and, in the United States, neural larva migrans (NLM) are the main causes of neurological disease in pet rabbits (Fisher & Carpenter, 2012). Further, in this section, the most important causes of neurologic disease reported in wild rabbits will be discussed.

a) Baylisascariasis or neural larva migrans (NLM)

Baylisascaris is a genus of ascaridoid nematodes and most of its species have a similar life cycle, with carnivores as definitive hosts (DH) (except for *Baylisascaris laevis*, occurring in rodents) and presenting an extensive range of paratenic hosts (PH) (Sapp et al., 2017), usually small mammals or birds (Kazacos, 2001). The raccoon (*Procyon lotor*) is the DH of *Baylisascaris procyonis*, the most well-studied parasite of the genus, with increasing public health importance (Saap et al., 2017), affecting free-ranging and captive wildlife, domestic animals, and humans (Kazacos, 2016). *B. procyonis* may cause three LM syndromes: visceral larva migrans (VLM), ocular larva migrans (OLM) and neural larva migrans (NLM), the last one leading to severe neurologic disease and death (Kazacos, 2016). *Baylisascaris columnaris* (with skunks as DH) and *Baylisascaris melis* (with badgers as DH), closely related to *B. procyonis*, are also possible causes of clinical LM in animals and humans (Kazacos, 2001): it is known that *B. columnaris* has the ability to produce clinically significant NLM in susceptible species, as rodents, rabbits, ratites and nonhuman primates (Kazacos, 2016).

B. procyonis is presently enzootic in raccoons in North America, Europe, and parts of Asia, leading to outbreaks of NLM and OLM in animals and humans in these regions. In the United States, *B. procyonis* is more common in the Northeast and Midwest, where prevalence among raccoons may reach 68-100% (Kazacos, 2016) and it is known that millions of eggs per day are shed in their feces, being extremely resistant in the environment, which enables potential transmission to susceptible hosts (Kazacos, 2001).

The raccoon, except in very heavy infections with intestinal obstruction, will appear to be clinically healthy, since extensive migration does not occur in the DH; however, in the PH, eggs hatch in the small intestine and larvae migrate aggressively through the host's tissues and invade the brain. Progressive CNS disease is usually evident by 9-10 days post-infection (Kazacos, 2001) and is a result of the larvae migration in the brain, where it promotes the most important lesions, namely mechanical damage, tissue necrosis and inflammation. Then, larvae become encapsulated in eosinophilic granulomas, which may occur in a variety of organs, until ingested by raccoons, who will predate or scavenge the debilitated or dead PH, perpetuating the cycle (Figure 4) (Kazacos, 2016).

Early clinical signs in small mammals include lethargy or nervousness, tremors in the front paws, mild head and/or body tilts, circling or jumping and alterations over the hair coat; then progressing to numerous combinations of severe head and/or body tilts, arching of the head and neck with "stargazing" or arching the body, ataxia, continuous circling, lateral recumbency, rolling, blindness, extension and rigidity of the forelimbs, motor weakness, coma and death (Kazacos, 2016).

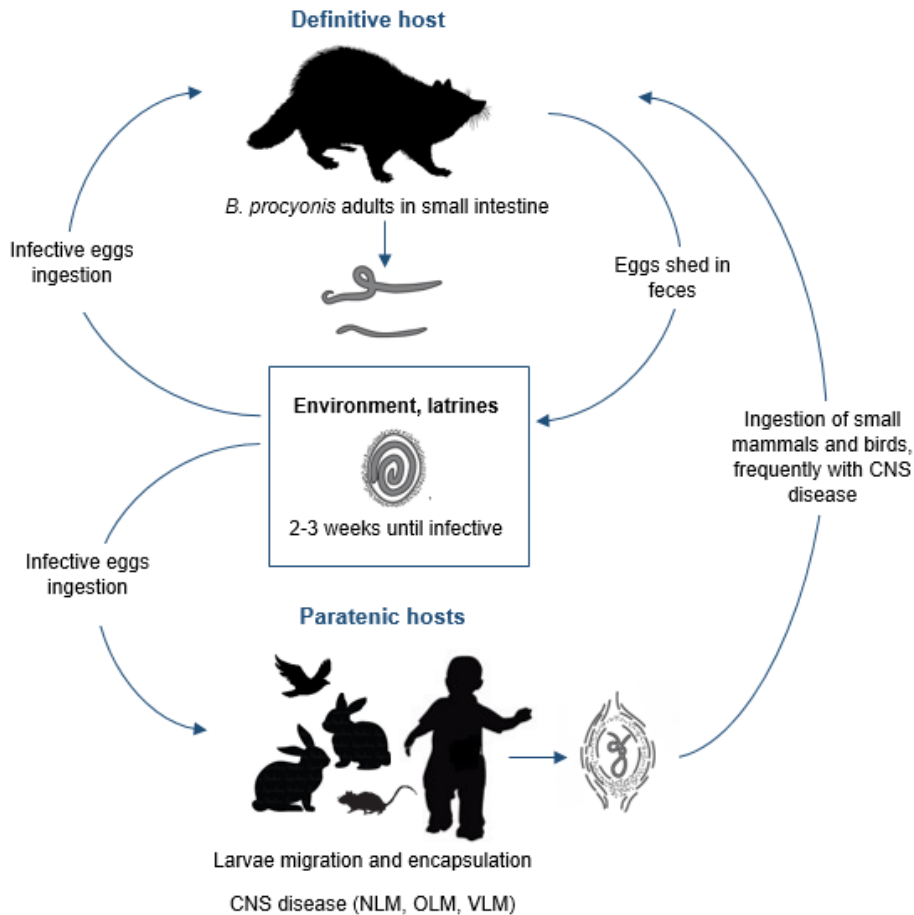
The diagnosis of *B. procyonis* in non-DH is based on clinical signs, history of exposure, laboratory findings (serology, cytology, and cerebrospinal fluids), necropsy and histopathology. However, the confirmatory diagnosis is restricted to the identification of larvae in/from tissues, besides the positive serology that is only indicative of infection. In the DH, the diagnosis is based on the identification of *Baylisascaris* eggs in the feces using fecal flotation methods, identification of the worms passed in feces, or in the necropsy (Kazacos, 2001).

NLM due to *Baylisascaris* carries a guarded to poor prognosis, with or without treatment. Frequently, this diagnosis is not considered until CNS signs are pronounced and consequently, irreversible; anthelmintic treatment at this point is usually ineffective (Kazacos, 2001).

Preferred sites of raccoon defecation, where their feces and *B. procyonis* eggs accumulate are called latrines and the cottontail rabbit is a common forager in these places, thus being exposed. There are several reports about its *B. procyonis* natural susceptibility and experimental infection (Kazacos, 2016). In a study conducted by Sapp, Murray, Hoover, Green & Yabsley (2018), 11% of the inquired wildlife rehabilitators that work with raccoons throughout the U.S and Canada reported *B. procyonis* infections in PHs: cottontail rabbits were reported several times, though it is unclear whether these cases were acquired in the WRC, or if these animals were admitted already infected.

Figure 4 - *Baylisascaris procyonis* life cycle (original, based on Kazacos, 2016).

Source: Kazacos, 2016; <http://getdrawings.com/raccoon-silhouette#raccoon-silhouette-3.png>;
<http://getdrawings.com/mouse-silhouette#mouse-silhouette-21.png>;
<http://getdrawings.com/child-silhouette-clipart#child-silhouette-clipart-30.jpg>;
<http://getdrawings.com/rabbit-silhouette-clip-art-free#rabbit-silhouette-clip-art-free-1.jpg>;
<http://getdrawings.com/bird-in-flight-silhouette#bird-in-flight-silhouette-32.png>



b) Encephalitozoonosis

Encephalitozoon cuniculi is a microsporidian and obligate intracellular parasite, that still generates discussion considering the clarification of its fungal origin (Künzel & Fisher, 2018) and is the etiologic agent of encephalitozoonosis, a worldwide relevant disease in rabbit populations, though it can affect a number of mammalian species, including humans, but also birds (Hinney, Sak, Joachim & Kváč, 2016). *E. cuniculi* presents a direct life cycle with both horizontal and vertical transmission and the most common source of infection is the ingestion of infected rabbit urine containing spores (Künzel & Fisher, 2018). Cell rupture is associated with inflammatory response and granulomatous lesions occur, affecting primarily the brain, kidney or eyes (Künzel & Fisher, 2018); in domestic rabbits, unapparent to mild renal insufficiency, phacoclastic uveitis and/or neurologic signs are possible, the latter comprising behavioral changes, head tilt, nystagmus, ataxia, rolling, or seizures, frequently following a stressful event. However, a significant percentage of seropositive rabbits are asymptomatic.

Immunocompromised humans may be affected, therefore *E. cuniculi* presents zoonotic potential (Fisher & Carpenter, 2012). Encephalitozoonosis diagnosis *in vivo* is still considered very difficult, since several rabbits present a chronic asymptomatic infection; a combination of physical examination, serology and ruling out relevant differential diagnoses are suggested (Künzel & Fisher, 2018). Currently, there is not a recognized treatment protocol in rabbits, though it can be based in inhibition of spore proliferation, anti-inflammatory therapy, supportive care and physiotherapy (Künzel & Fisher, 2018).

In respect to wild Lagomorphs, *E. cuniculi* has been limitedly reported in the European rabbit (*O. cuniculus*) and in the European brown hare (*L. europaeus*) - generally with low prevalence compared with domestic populations - and in the eastern cottontail rabbit as well (Hinney et al., 2016). In Northwestern Italy, 9.72% of the sampled *S. floridanus* tested positive to PCR for *E. cuniculi* and it was found with higher prevalence in the CNS and skeletal muscle, contrarily to the domestic rabbit (Zanet, Palese, Triscioglio, Alonso & Ferroglio, 2013). As far as the author knows, there are not any current reports in the American cottontail populations or any descriptions about the clinical signs presented by wild rabbits.

c) Toxoplasmosis

It is known that the eastern cottontail is susceptible of *Toxoplasma gondii* infection (Duszynski & Couch, 2013), an obligate intracellular protozoon. In the sexual stage it is specific to felids, the DHs, where it is localized in the intestine and the oocysts are shed in their feces, contaminating water, soil and the environment (Fredebaugh, Mateus-Pinilla, McAllister, Warner, & Weng, 2011), infecting a wide range of homoeothermic intermediate hosts (IH) in its asexual phase, in which it becomes systemic, localized in vital organs, muscle tissue and nervous system (Cenci-Goga, Rossitto, Sechi, McCrindle & Cullor, 2011). This enables the enzootic maintenance of *T. gondii* in the food chain, as it represents a source of parasite transmission to carnivores feeding on infected tissues (Fredebaugh et al., 2011); congenital infection and ingestion of infected contaminated food, water or soil are other possible transmission routes (Cenci-Goga et al., 2011).

In rabbits, the infection is frequently subclinical, although sudden anorexia, pyrexia, CNS signs such as posterior paralysis or seizures, and death are possible (Varga, 2014).

The increased presence of feral cats in natural areas influences wildlife through the spread of *T. gondii*. In a study developed in a natural area in Illinois (Fredebaugh et al., 2011), small home range wild mammals, including the eastern cottontail, presented a significantly higher prevalence of antibody to *T. gondii* at sites with a high frequency of cat occurrence, being feral cats the most likely source of environmental contamination. Thus, rabbits that graze an area visited by cats are more exposed, since the source of infection is cat feces containing oocysts (Varga, 2014). Smith & Frenkel (1995) studied the prevalence of antibodies to *T. gondii* in wildlife of Missouri and Kansas, from 1974 to 1987: the prevalence of *T. gondii* in antibody

titers of *S. floridanus* sera was 17%. However, in cottontails introduced in Northwestern Italy, *T. gondii* DNA was found in a lower prevalence, 2.08% (Zanet et al., 2013). Domestic rabbits (*O. cuniculus*) were found to present inflammatory lesions to toxoplasmosis, whereas the mountain hare (*Lepus timidus*) presented acute visceral toxoplasmosis (Gustafsson, Uggla & Järplid, 1997), but little is known about the cottontail susceptibility.

Despite of the zoonotic character of toxoplasmosis, *T. gondii* is only transmissible from rabbits to humans who handle or eat undercooked rabbit meat (Varga, 2014), which is a concern regarding its importance as game species.

d) Cranial trauma

When cranial trauma occurs, the risk of brain injury is possible: associated clinical findings may comprise skull and/or jaw fractures, head bleeding or wounds, epistaxis, hemorrhage from the oral cavity and/or ears, anisocoria, miosis or mydriasis, hyphema, retinal detachment, depression or altered mental state, seizures, head tilt and cranial nerve deficits (Johnson, 2012). Physical examination, including neurologic assessment, history, radiography and computed tomography (the last one usually not available in rehabilitation settings) may help the diagnosis; if head trauma is evidenced in rabbits, oxygen, fluid therapy and thermal support (Johnson, 2012), besides cage rest, limiting stress and handling to keep intracranial pressures normal (Schott, 2017) are advised.

When moderate to severe neurological signs are associated with skull fractures, or if the skull fracture is open or cerebrospinal fluid is leaking, euthanasia is recommended. In cases in which treatment is attempted and neurological signs do not improve in 5 days or worsen at any point in time, euthanasia should be considered as well (Schott, 2017).

e) Spinal trauma

Vertebral fracture or luxation may follow penetrating or blunt spinal trauma (e.g. forceful handling, such as being picked up and shook by a dog, or incorrect handling by a person), leading to spinal cord compression, which commonly results in posterior paresis or paralysis (Johnson, 2012). Spinal trauma is very frequent in young and adult wild cottontails presented for rehabilitation (Schott, 2017). Rabbits are notably susceptible because of their hind limbs' extremely developed muscles and their fairly fragile vertebral column (Johnson, 2012); additively, the spinal cord extends the whole length of the vertebral column, consequently, injury at any level will affect both upper and lower motor neurons (Keeble, 2006). Typically, injury occurs at the lumbosacral level and vertebral fracture is more common than dislocation. Clinical signs are dependent on the level of compromise to the spinal cord and may include posterior paresis or paralysis, absence of skin sensibility over the lumbar region, besides urinary and fecal incontinence (Fisher & Carpenter, 2012). Deep pain assessment is advised: if it is absent, the prognosis is poor and the animal should be euthanized (Schott, 2017). The

clinical diagnosis of vertebral trauma may be confirmed radiographically (Fisher & Carpenter, 2012); euthanasia is recommended if any bony alterations in the spine are evident, since chronic spinal pain is expected (Schott, 2017). Management of spinal trauma may be attempted, according to the prognosis, which is dependent on the site and severity of the injury and clinical signs (Fisher & Carpenter, 2012).

The eastern cottontail rabbits is concomitantly the most important game species in the U.S. (Chapman et al., 1980; Chapman & Litvaitis, 2003; Smith, 2018) and one of the most frequent patients, regarding intakes at WRCs in the same country (Lloyd, Hernandez & McRuer, 2017), being especially important to study in order to understand the main threats that this species is currently facing and concerning its broad geographical distribution (Chapman et al., 1980; Nielsen & Berkman, 2018).

The main admission causes are linked with domestic animals predation, the admission of infants and juveniles that are frequently mistaken as orphaned animals (Burton & Doblár, 2004), nest and habitat disturbance or destruction, and traumatic lesions with anthropogenic origin, such as automobile strikes (Pokras & Porter, 1994; Bewig & Mitchell, 2009; Richardson, 2016; Schott, 2017). Consequently to these causes, the most relevant clinical signs in cottontails admitted at WRCs are associated with musculoskeletal signs, frequently related with traumatic injuries; neurological signs, with trauma or parasitic diseases as the main causes (Schott, 2017); and hypothermia, hypoglycemia and dehydration presented by rabbit kits, mainly (Pokras & Porter, 1994; Bewig & Mitchell, 2009; Schott, 2017).

4. AIMS OF THE STUDY

The main purpose of the present dissertation was to test possible associations between factors on arrival (intrinsic to the animal, such as age, body weight and body condition, plus admission causes and clinical signs presented) of the eastern cottontails admitted into the WRCMN, with the respective outcomes, in order to guide professionals working with *S. floridanus* at WRC settings throughout the triage process, safeguarding animal welfare and efficiently managing resources in the future.

It is also intended to study *S. floridanus* annual intakes within the studied period and to characterize the admitted animals in terms of age, body weight and body condition. Furthermore, this study is expected to present the proportion of different admission causes, which may represent potential threats (natural or anthropogenic) faced by this species, and the clinical signs presented on arrival. Comparing periods in treatment or lengths of stay for the most common admission causes is also aimed.

5. MATERIAL AND METHODS

5.1. The database and studied parameters

All the cases referring to eastern cottontail rabbit admissions from January 1st, 2011 to December 31st, 2017 were exported from the WRCMN database (FileMaker[®] Pro) to Microsoft Excel[®] spreadsheets.

In total, there were 18,985 cases of this species recorded in the mentioned period, namely 3,926 individuals admitted into the clinic facility and 15,059 into the mammal nursery. Each registered individual had a specific case number identification associated with several parameters; the ones included in this study are listed below.

Most of the cottontails' information included in the studied database did not contain sex, as it was classified as undetermined in most of the cases, because it is considerably challenging to identify in young rabbits (Bewig & Mitchell, 2009); only obvious males and lactating or pregnant females were marked. WRCMN team considers the stress of sexing unworthy for data record, therefore sex analysis was not included in this study.

5.1.1. Age

Cottontail's age is recorded in three possible classes: infant, juvenile or adult. This classification is based on maturity features and body weight assessed on admission: as any other mammal species at WRCMN, infants are generally admitted with eyes closed and juveniles with eyes open; the difference between juveniles and adults is more difficult to objectively describe, though the higher body weight in adults (averaging 0.8 to 1.5 kg) may be considered the main difference (Nielsen & Berkman, 2018).

5.1.2. Care facility: nursery *versus* clinic

Two separate care wards are possible destinations for cottontails cared for at the WRCMN: the mammal nursery, where milk replacer formula is provided to infants and younger juveniles; and clinic, where hay and assorted vegetables are given to juveniles and adults eating independently.

Infants are immature, therefore hand-rearing is essential and they are always forwarded to the nursery facility; juveniles with eyes open and at all times when it is uncertain that they are weaned, they proceed to the mammal nursery; when eating independently, juveniles are directed to the clinic facility. Adults are always sent to the clinic ward.

Whenever considered important, cases were divided into nursery and clinic sections during the statistical analysis, regarding the important differences in care between each one, as referred above.

5.1.3. Body Condition

The general body condition of most wild mammals may be evaluated by measuring the amount of subcutaneous adipose tissue through pinching the skin (Bewig & Mitchell, 2009), inspecting hindlimb muscle mass and assessing bony prominences of the spine, ribs and pelvic bones, as used in domestic species (Meredith, 2016).

In WRCMN, three possible classes of body condition scores on admission are considered and are also adopted in this study: emaciated, thin and good body condition. This classification is based on a 1 - 5 scale, such as the one proposed by Pet Food Manufacturers' Association (2015) for domestic rabbits, adapted from methods used in cats, dogs and large domestic animals. At the WRCMN, the following correspondence is made: 1/5 corresponds to emaciated, 2/5 is classified as thin and 3/5 corresponds to a good body condition. Body condition scores such as 4/5 and 5/5, which are associated with overweight and obese categories respectively, are possible in pet rabbits (Varga, 2014); however, they are rarely seen in rescued cottontails at this WRC.

5.1.4. Body Weight

Until 2016, infants and juveniles that could not be successfully reunited with their nests and presenting less than 50g of body weight on intake were humanely euthanized; from that year on, it was increased to 65g. In the past experience of WRCMN medical staff, low survival and unsuccessful rehabilitation have been evaluated with individuals under these cut-off values, therefore, as a welfare safeguard and prevention of further suffering, this is a principle followed at this WRC. The measure unit used for the body weight was the kilogram (kg).

5.1.5. Period in Treatment (PT)

This parameter was defined as the length of time that the animal remained at the WRCMN, namely the period of days since the date of admission until the date of release or death.

5.1.6. Admission Causes

Circumstances of admission mean any and all reasons an animal was brought into care: some are known by the finder⁴ on admission (e.g. a cat brought the cottontail rabbit to the owner, therefore is classified as animal interaction with a domestic animal), others are discovered after the physical examination or through diagnostic procedures at the WRC (e.g. projectiles identified on radiographs, thus the associated circumstance would be "projectile"). It is possible that the responsible person for the admission data recording classifies one, several or undetermined circumstances for each animal case. Likewise, if the primary circumstance is known but the secondary is unidentified, it is just recorded the first one. When more than one

⁴ Injured animals are most of the times collected by members of the public and, more rarely, by conservation or police officers; the WRCMN does not participate in wildlife collection.

admission causes were attributed to an animal, only the main cause was considered for the present dissertation.

In this study, only the primary admission circumstances were analyzed, with one exception, based on previous studies conducted at other WRCs that reported a great importance of domestic animals attacks (Burton & Doblár, 2004; Jessup, 2004; Schenk & Souza, 2014; Lloyd et al., 2017; McRuer et al., 2017), therefore “cat”, “dog” and “non-domestic animal” were differentiated within the “animal interaction” class.

- 1) Animal interaction – refers to contact with another animal, leading directly or indirectly to the cottontail admission to the WRC.
 - a) Domestic animal – direct or indirect contact with one or more animals that have been domesticated for a human environment, though it also includes feral individuals.
 - i) Dog – injury caused by a domesticated or feral dog.
 - ii) Cat – injury caused by a domesticated or feral cat.
 - b) Non-domestic animal – a form of animal interaction where the admitted animal had either direct or indirect contact with a wild animal. In the WRCMN database, this category was divided into “same species” and “different species”. The author did not consider imperative to study this detail for the cottontail rabbit, because fights between conspecifics rarely occur, once other dominance-submission behaviors take place (Whitaker & Hamilton, 1998).
- 2) Collision – associated with injuries resulting from an impact with either a stationary (this may include powerlines/wires or natural features) or a moving object (this may include car/truck/motorcycle, train, bicycle, motorized farm or yard equipment, impact with natural features and human propelled object). For the studied species and based on the literature (Burton & Doblár, 2004), collision with moving objects may be considered the leading cause within this category.
- 3) Entrapment – refers to confining causes, from which escape is difficult or impossible for the cottontail rabbit. It may occur from devices designed to capture animals (e.g. fishing gear, leg/body hold trap/snare, humane/cage trap, glue trap), or from others whose primary function was different than to capture animals (e.g. sporting/landscaping netting, fence, litter or garbage). Spaces where the animal is free to move without physical restrictions, but not allowed to escape (e.g. buildings, window well/outdoor stairwell, duct work, vehicle, pool, storm drain/sewer).
- 4) External substance contamination – this category includes petrochemicals (oil, grease, paint or other petrochemical products that may affect the animal) and botanicals (referring to the presence of tree sap, burrs, or other botanical products) found on the animal and contributing to its capture.

- 5) Environment – pertaining to environmental conditions that directly or indirectly affect the admitted animal, as fire, smoke, seismic event, or weather related.
- 6) Projectile – this includes any propelled object which affects the animal, namely any projectile pushed from a weapon (gunshot or bow/arrow) or a non-weapon (e.g. falling objects).
- 7) Nest/habitat destruction – refers to the disturbance or damage of a nest, burrow, or habitat leading to the animal's injury or displacement.
- 8) Orphan – any reason associated with displaced healthy or injured young animals, dependent on their progenitors for survival, when there is a considerable probability that parents are known or suspected to be dead, have not returned to care for the young after a significant period of time, or have rejected the youngsters. This category includes failed attempts to unite the young with parents.
- 9) Inappropriate human possession – includes any case when the cottontail rabbit is removed from its natural habitat and is in human possession because of perceived risk by the rescuer, to be kept as a pet, for unauthorized or untrained rehabilitation, or as a nuisance animal.
- 10) Confiscation – describing when the animal has been legally seized from another person due to illegal possession, violation of permit conditions or perceived abuse.
- 11) Referral – perceived as any rabbit transferred from one rehabilitation facility to another for further rehabilitation.
- 12) Born in captivity – as a result of natural or assisted birth, most of the times infants born at the WRC from pregnant females in care.
- 13) Undetermined – englobes all unknown events and indeterminate causes.

5.1.7. Clinical signs presented on admission

Based on the previously referred literature and WRCMN casuistic, only the most frequent and important clinical signs reported in cottontail rabbits on admission were included in this dissertation. The animals were studied as presenting *versus* not presenting each clinical sign category on admission, meaning that the same animal could present several clinical sign groups coexisting. Degrees of severity for each one of the clinical signs are not presented nor studied.

- 1) Clinically healthy – whenever any sign of injury is absent and the animal is apparently healthy on admission.
- 2) Trauma – whenever signs of physical injury are identified in a cottontail.
- 3) Neurological signs – only clinical signs with CNS origin were approached, being divided according to its origin, namely brain or spine. This category was focused on clinic rabbits, since only 4% of nursery rabbits had this clinical sign category registered in the database.

- a) Brain signs – includes all signs with intracranial origin, such as vestibular system (head tilt, vestibular ataxia, circling, rolling), cerebellar (wide-based stance, intention tremors), cerebral cortex lesions (behavioral changes), or visual system, for example (De Risio, 2005).
 - b) Spinal signs – reduced or absent deep pain reflex, paresis, paralysis, muscular weakness are possible presentations.
- 4) Dehydration - this may be due to extended time without access to food and water before the animal is admitted, or due to underlying disease causing water and electrolyte loss. The assessment is the same as in domestic species, examining skin turgor, mucous membranes, capillary refill time and eyes appearance (Mullineaux & Keeble, 2016). This category was focused on nursery cottontails, since it is considered an important sign on admission, especially for rabbit kits and juveniles (Pokras & Porter, 1994; Bewig & Mitchell, 2009; Schott, 2017).
- 5) Hypothermia – on admission, rectal temperature should be assessed in collapsed animals, and routinely when possible; only over 2 weeks of age most mammals are able to maintain their thermoregulation (Cowen, 2016; Mullineaux & Keeble, 2016). This category was focused on nursery cottontails, since it is considered an important sign on admission, especially for rabbit kits and juveniles (Pokras & Porter, 1994; Bewig & Mitchell, 2009; Richardson 2016; Schott, 2017).

Despite GI disease being a preponderant issue in young cottontails in rehabilitation settings (Bewig & Mitchell, 2009; Varga, 2014; Oberly, 2015; Cowen, 2016; Richardson, 2016; Paul & Friend, 2017; Paul & Friend, 2019), this was not found as a frequent clinical sign category identified on arrival, within nursery nor clinic (e.g. only 57 cottontails were admitted into the nursery ward with diarrhea signs; however it was registered that at least 1,325 cottontails developed diarrhea while undergoing treatment in the studied period), therefore it was not studied as such.

5.1.8. Resolution

This field is related with the outcome of each admitted animal. It can be divided into the seven following categories:

- 1) Dead on arrival - when the animal does not present any vital sign when entering the WRCMN facilities;
- 2) Died - meaning that the animal died after admission, somewhere between being admitted and going to the rehabilitation ward, thus before or after the first clinical exam;
- 3) Died in cage - related with the death of the animal while the rehabilitation process is taking place, therefore after the hospitalization at the rehabilitation ward;

- 4) Euthanized on arrival - every time that euthanasia is deliberated and conducted following the first clinical exam on admission, therefore the rehabilitation process does not take place in these cases;
- 5) Euthanized - refers to the decision of euthanasia after the beginning of the rehabilitation process, usually due to deteriorating or not resolving condition;
- 6) Released - applies to all the animals that had successfully recovered and were released into their original habitats. In some cases, after the initial triage, if it is clear that the animal is not injured, is possibly returned to its nest (rabbit kits) and does not need any further rehabilitation, this resolution is applicable as well.
- 7) Transferred - when the animal is forwarded to a different licensed rehabilitator, most of the times infants or juveniles needing further rehabilitation and more individualized care.

5.1.9. Inclusion requirements for the present dissertation

During the general study of the database, conflicting data was identified and discussed with medical staff members, in order to resolve these issues whenever was possible. However, all cases with incompatible data which could not be rectified (unconceivable age/body weight association, inaccurately registered body weight and PT) were excluded from further analysis (n = 276). All the cases where information was not presented, in one or more of the referred parameters, were included in the statistical analysis (n = 1,196), therefore, the available information of these cases was not lost.

5.2. Software for data recording and analysis

5.2.1. Microsoft Excel®

A preliminary assessment of the database was performed with Excel 2013®: it allowed a broad view of the information, giving the opportunity to detect and exclude conflicting data and to create tables and graphs, which gave an insight of what statistical tests would suit better for each group of data.

5.2.2. R software

Data analysis was conducted with The R Project for Statistical Computing, version 3.5.1. Median (*Mdn*) and interquartile interval (*IQR*) were designed to study the PT at the WRCMN. Two-sample Wilcoxon test was chosen to assess the difference between *Mdn* body weights for each outcome, besides *Mdn* days in treatment for each outcome. Chi-square test of independence was used in order to assess the association between age, body condition, admission causes and clinical signs with the respective outcomes. Odds ratio (OR) were used to express the measure of association between each factor presented on arrival, tested with

chi-square, and the outcome “dead before release”, when appropriate: $OR = 1$, meaning that the studied factor does not affect the odds of this outcome; $OR > 1$, represents that the studied factor is associated with higher odds of the referred outcome and $OR < 1$, meaning that the studied factor is associated with lower odds of this outcome. The estimation of the caseload trend during the study period was carried out by linear regression model. An alpha level of 0.01 was used for all statistical tests.

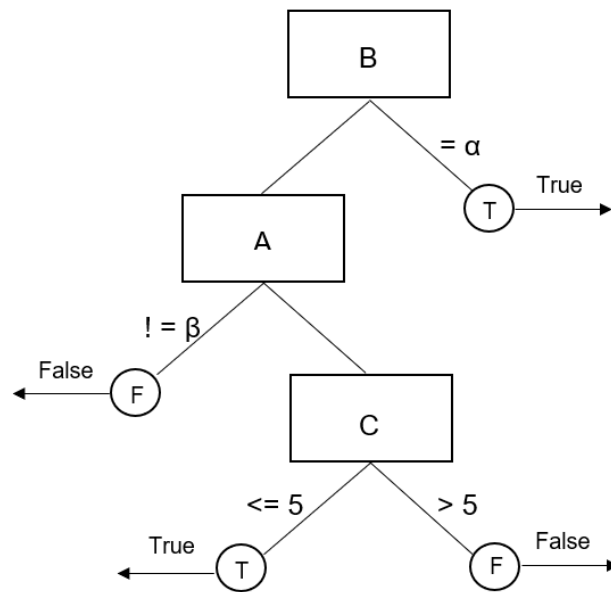
5.2.3. Decision trees: Fast and Frugal Trees (FFTs)

Data mining appeared recently as a fresh approach to data analysis, enabling to expand novel and deep understanding of large datasets which can be employed to support decision-making: the areas of biomedical and health care are an example of its successful application, in which decision-making is of major importance (e.g. diagnosis process, treatment options selection, prognosis prediction) (Yoo et al., 2012). Fast and Frugal Trees (FFTs) (Phillips, Neth, Woike & Gaissmaer, 2017a) are supervised learning algorithms that allow to make fast and accurate binary classification decisions, based on limited cues of information (usually, 1 to 5). A decision tree is non-compensatory, meaning that it uses just a partial subset of all cue information: once a decision is made based on some subset of the existing information, no additional information is considered or can change the decision. Additionally, it uses information in a specific, sequential order (Phillips, Neth, Woike & Gaissmaier, 2017b). FFTs can make good predictions since they are relatively robust against a statistical issue named overfitting, which implies excel in hindsight (fitting) but the lower accuracy in foresight (prediction) (Gigerenzer & Brighton, 2009). There are some examples of the value of FFTs in health care, namely in coronary artery disease diagnosis (Green & Mehr, 1997) or diagnosing depression (Jenny, Pachur, Williams, Becker & Margraf, 2013).

In order to create a decision tree, the model scrolls all the dataset observations; when a cue (binomial or quantitative) is significant, it is selected and inserted in the tree; then, a decision threshold is determined for each quantitative cue. The cue order is given and the respective exit (positive or negative) for each one, as well (Phillips et al., 2017).

For better understanding, a hypothetic FFT was created (Figure 5): each cue is contained in a rectangle (node), decisions are represented within circles (leafs) and branches represent answers to cue-based questions. Branches linking nodes to leaves are called exit branches. This example is composed by five cues (A, B and C), in which A and B are binomial, and C is quantitative. The goal is to know if the answer to the hypothetic problem is True (T) or False (F). The following interpretation can be made and in the following order: if B is equal to α , the answer is True; otherwise, check if A is different from β , the answer is False; if not, check if C is equal or less than 5, the answer is True, but if C is more than 5, the answer is False.

Figure 5 – Example of a FFT (original).



In the present dissertation, FFTs (R package version 1.3.5.) were studied as a possible support to triage when an eastern cottontail arrives at the WRCMN, for each one of the facilities, and based on age, body condition, body weight, all admission causes and selected clinical signs. As data missing values are currently not permitted in the algorithm, all cases with non-available information were promptly excluded ($n = 1,196$).

Then, the original data was randomly divided into two subsets: a 50% training set for model fitting and a 50% testing set for prediction. The chosen algorithm was *dfan*, which does not assume cue independence, and a maximum number of levels was set to 5, to avoid the development of an extensive tree.

The best possible balance between sensitivity and specificity was the goal in the developed tests; however, it is possible to benefit one and consequently decrease the other, as discussed in the next chapter. Here, sensitivity represented the proportion of cases with positive criterion values (released) that were correctly predicted and specificity was linked to the proportion of cases with negative criterion (dead before release) correctly predicted by the FFT (Phillips et al., 2017).

6. RESULTS AND DISCUSSION

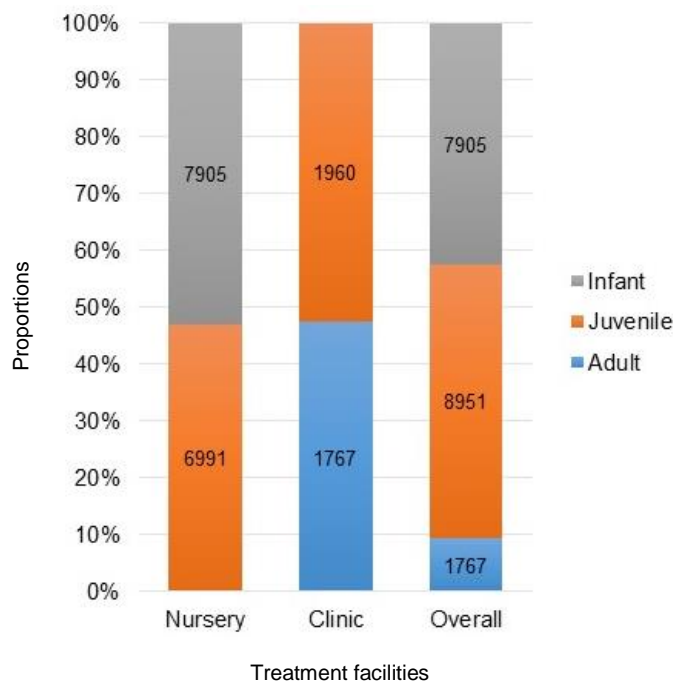
6.1. Sample description

After preliminary data processing, the studied clinical cases (N = 18,709) represent all the eastern cottontails admitted from 2011 to 2017 at the WRCMN, excluding cases with conflicting data: 3,764 were admitted into the clinic ward and 14,945 into the mammal nursery. As referred above, cases with missing information in one or more fields (n = 1,196) were included in the statistical analysis whenever it was possible, although they are not graphically represented. It is essential to notice that multiple staff members and admission volunteers recorded animals' admissions, creating possible reporting biases.

6.1.1. Age

A description of eastern cottontail age proportions in each WRCMN facility and the overall results are presented in Graph 1. Throughout the studied period, infants (42%) and juveniles (48%) admissions overcame adults' (10%), which may be explained by the fact that young animals are more vulnerable to disease and predation, being more easily caught; additively, they are frequently rescued in groups, belonging to the same nest, which means that many of these cases may be associated (Lloyd et al., 2017). Other explanation may be the life cycle of this species, since the great majority of the eastern cottontails' populations consists of juveniles (Whitaker & Hamilton, 1998) and adults present low survival proportions (Nielsen & Berkman, 2018), therefore it is more probable to admit younger cottontails at a WRC.

Graph 1 - Age proportions for each treatment facility and for the overall population of eastern cottontails admitted into the WRCMN between 2011 and 2017.

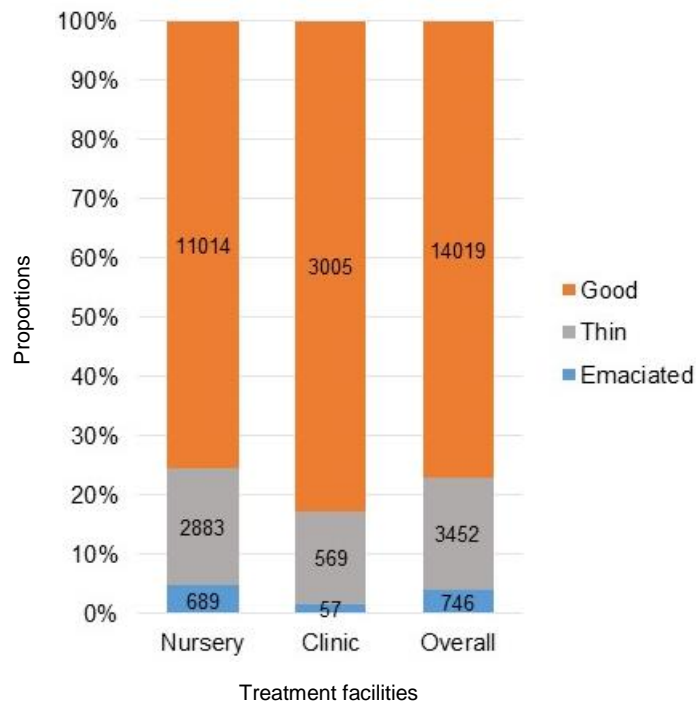


6.1.2. Body condition

Overall, 77% of the overall cottontails were classified as presenting a “good” body condition at the moment of intake and this was true for all age ranges. Furthermore, 19% of the total rabbits were classified as “thin” and approximately 4% as “emaciated” (Graph 2). Nursery rabbits, namely infants and younger juveniles, presented the greatest proportions of animals classified as “thin” (20%) and “emaciated” (5%) categories, compared with more mature rabbits (16% and 2%, respectively).

Possibly, the current body condition classification is rather biased, since it is established to include three possible categories, being equally used to classify every different species admitted at the WRCMN.

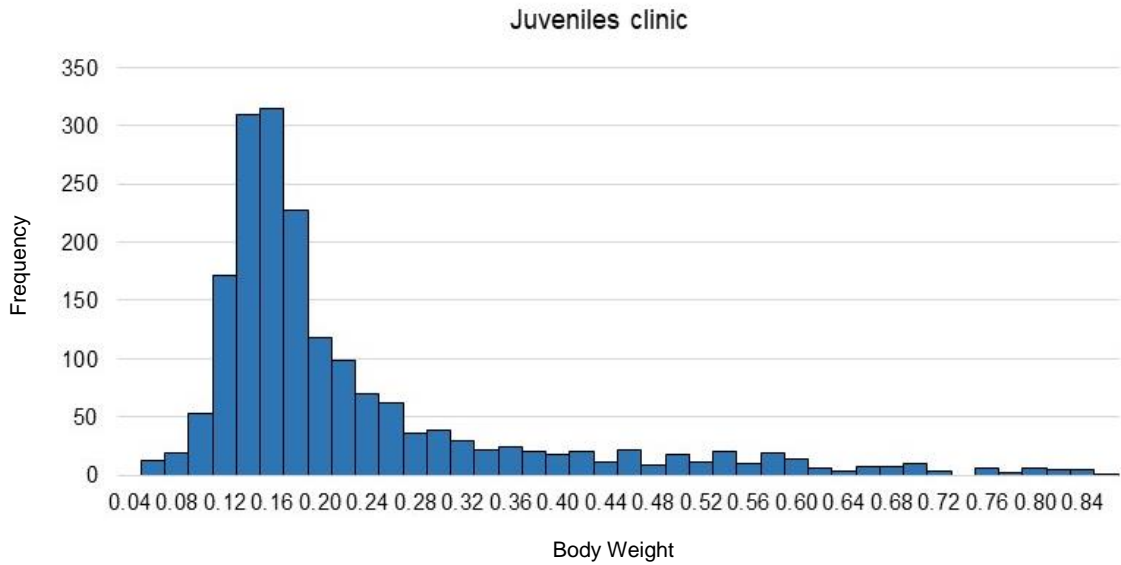
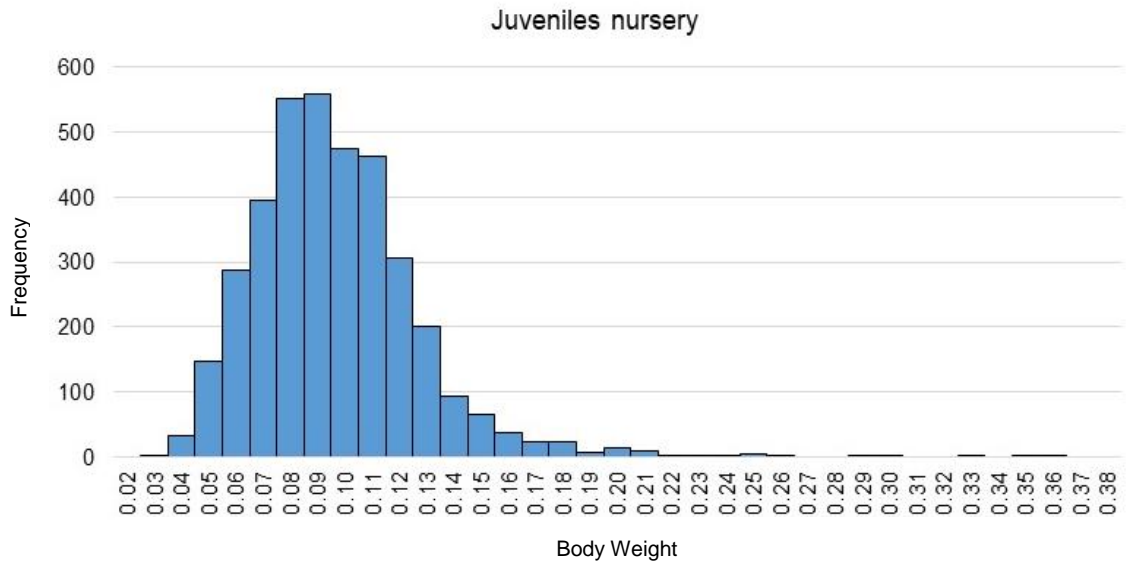
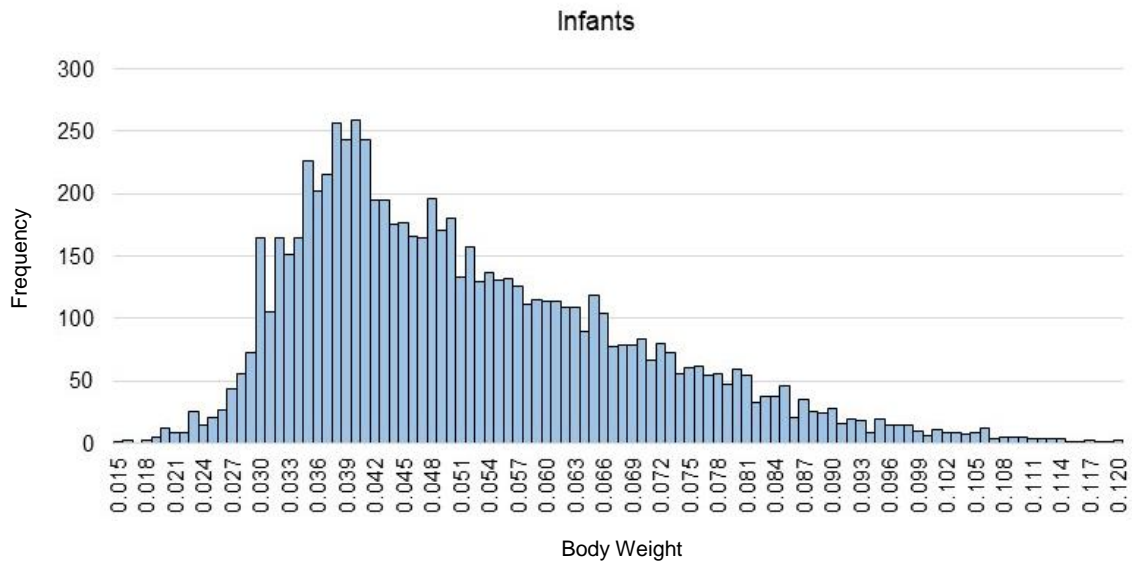
Graph 2 - Body condition proportions regarding each facility ward and the overall eastern cottontail population admitted into the WRCMN from 2011 to 2017.



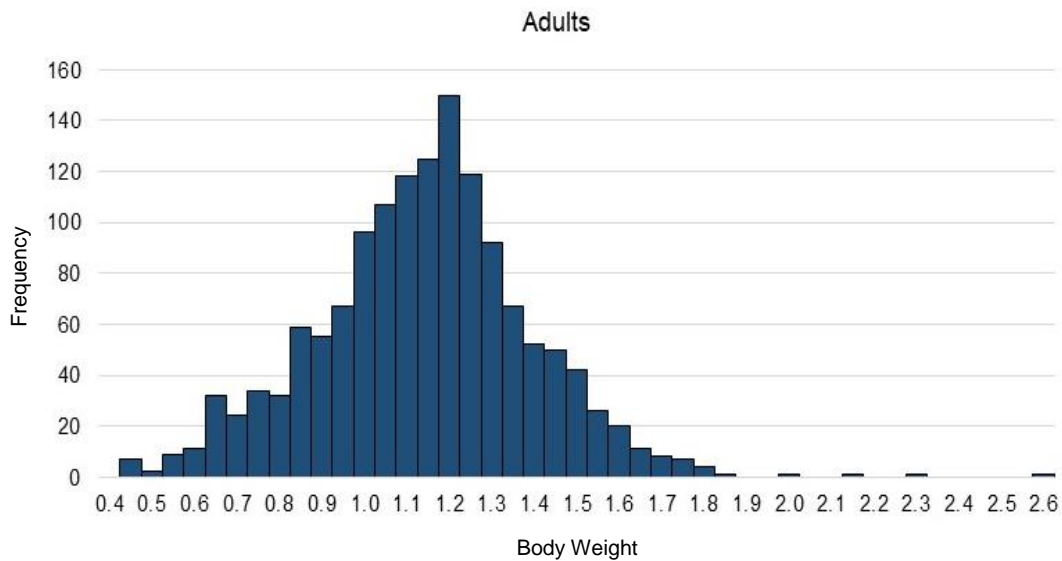
6.1.3. Body weight

Body weights (kg) on admission are presented in Graph 3, including all age classes: infants ($M = 0.05$, $SD = 0.02$; $Mdn = 0.05$, $IQR = 0.04 - 0.06$), nursery juveniles ($M = 0.09$, $SD = 0.03$; $Mdn = 0.09$, $IQR = 0.07 - 0.11$), clinic juveniles ($M = 0.22$, $SD = 0.15$; $Mdn = 0.17$; $IQR = 0.14 - 0.24$) and adults ($M = 1.12$, $SD = 0.26$; $Mdn = 1.13$; $IQR = 0.97 - 1.28$). Infants and all juveniles presented asymmetric body weight distributions, namely positively-skewed; however, adults presented negatively-skewed body weight distribution. These results indicate that younger animals were admitted with higher body weights comparatively to the mode; contrariwise, adults were admitted more frequently with lower body weights relatively to the verified mode.

Graph 3 - Body weight presented on admission, within each age range representing all cottontails with registered body weight admitted at the WRCMN from 2011 to 2017.



Graph 3 – Continuation.



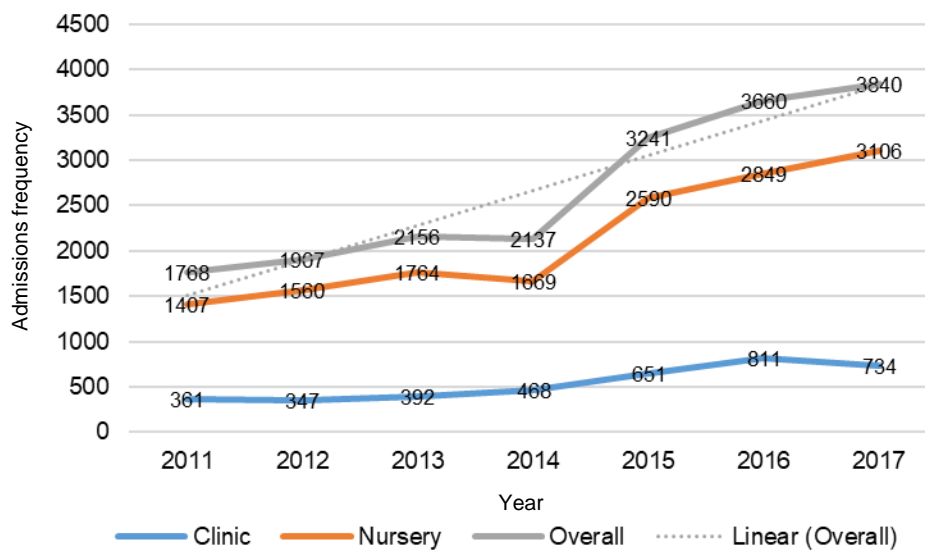
6.1.4. Case load tendency from 2011 to 2017

Linear regression examination showed a significant increase of the total number of eastern cottontails admitted throughout the 7 years of study ($B = 385.96$, $t = 6.82$, $R^2 = 0.90$, $F(1,5) = 46.56$; $p < 0.01$). Nursery cases mostly contributed for this growth, as it is presented in Graph 4.

The increasing number of cottontails keeps track of the rising total of all animals admitted at the WRCMN along the referred period (in 2011, 7,866 animals were admitted; in 2017, 12,928 intakes were registered).

Threats that cottontail rabbits face, as those directed to other wildlife species, might have been intensified over the years, clarifying this significant increase. Other possible justification is the improvement on social awareness about wildlife welfare and protection, besides the progressive recognition of the importance associated with WRCs' work, probably related with a wider divulgence of their mission through media (Molina-López et al., 2017).

Graph 4 - Number of eastern cottontail rabbits intakes at the WRCMN from 2011-2017, for each one of the treatment wards and overall population. Linear regression model was applied for the overall group.



6.2. Primary admission causes

6.2.1. Overall population

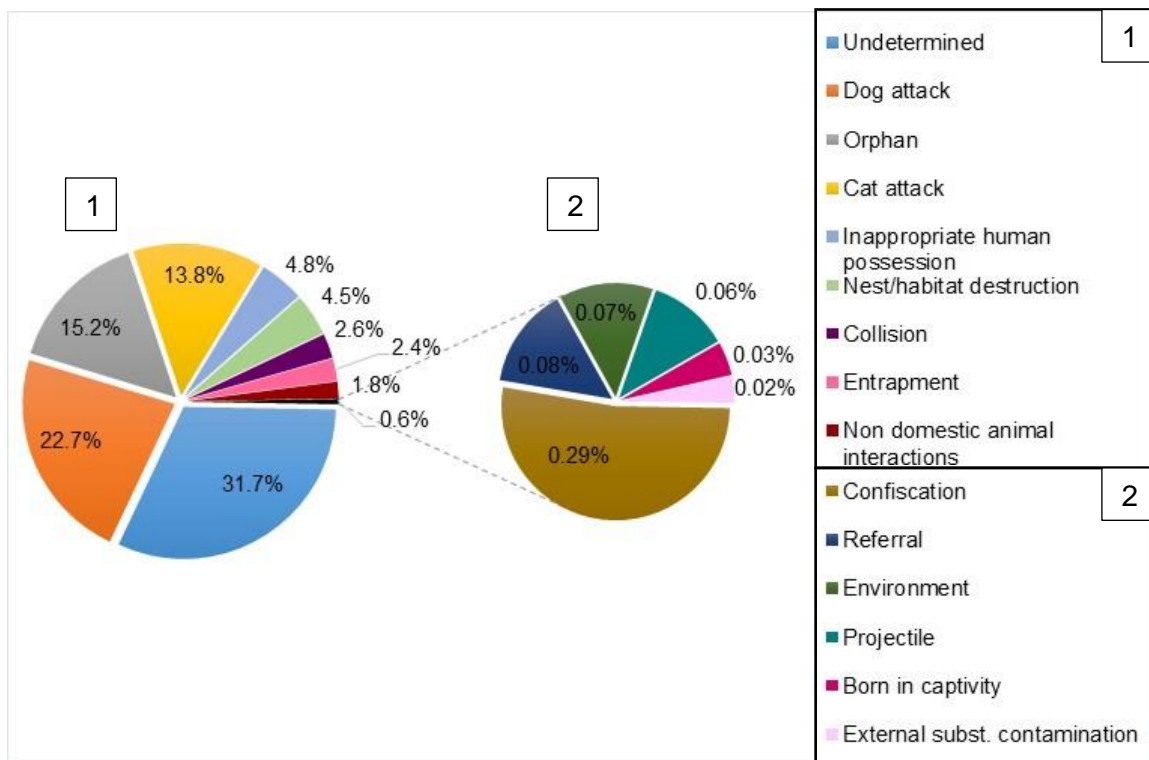
As presented in Graph 5, 32% of all the cottontails had “undetermined” admission circumstances registered. Lloyd et al. (2017) reported that 20% of admissions listed by 82 wildlife organizations across North America had the same classification.

Interaction with domestic animals (37%) was the leading cause of admission: about 23% of the cottontails faced “dog attack” and 14% a “cat attack”. These results are in accordance with past studies reporting domestic animal predation as one of the leading intake causes in WRCs across the United States (Burton & Doblar, 2004), although they exceed previously registered proportions, shown in section 3.4.2. of this dissertation (Jessup, 2004; Schenk & Souza, 2014; Lloyd et al., 2017); only the Wildlife Center of Virginia presented a higher cat interaction proportion and within the admitted eastern cottontails (26%) (McRuer et al., 2017). There is a possibility that these numbers are underestimated, since animal interactions are registered only if witnessed or if there is a clear indication supported by the clinical examination; furthermore, there is a considerable proportion of “undetermined” causes. Moreover, only animals that are still alive after the attack and accessible to rescuers are eligible for rehabilitation (McRuer et al., 2017).

“Orphan” was the following leading cause (15%); in the Wildlife Center of Virginia, for instance, this was the most common cause of admission in small mammals (46.2%) (McRuer et al., 2017).

Other admission circumstances commonly listed as causes of decline for wildlife species, such as “inappropriate human possession” (5%), “nest/habitat destruction” (5%) and “collision” (3%) (Burton & Doblar, 2004) were found less important than domestic animals interactions.

Graph 5 - Admission causes proportions for the overall eastern cottontail population admitted into the WRCMN from 2011 to 2017.



6.2.2. Nursery versus clinic cottontails

Nursery rabbits were admitted mainly due to “domestic animal interactions” (38%), followed by “orphaned” youngsters (19%), “nest/habitat destruction” (5%) and “inappropriate human possession” (5%). More than a half (65%) of the orphaned kits were infant rabbits.

More mature animals, admitted into the clinic facility, had “undetermined” circumstances (52%) more frequently registered, then “domestic animal interactions” (31%), followed by “collision” (6%) and “entrapment” (6%).

One of the main differences between clinic and nursery wards’ admission causes was the proportion of cats and dogs within “domestic animal interaction”. Most of the nursery rabbits’ interactions were linked with dogs (70%), contrarily to clinic rabbits, which were mostly related with cat attacks (76%). A possible explanation for this difference is dog’s behavior, which may affect younger rabbits resting in nests more easily: dogs may damage small mammals’ burrows and even walk over top of them, instigating disturbance (Ritchie et al., 2014).

McRuer et al. (2017) reported that juvenile mammals had the greatest frequency of cat interaction, followed by neonates, then adults, at the Wildlife Center of Virginia. This may be explained by the fact that fewer adults are available compared to rabbit kits; there is an amplified level of difficulty in capturing larger prey; and adult animals are more experienced and capable of escaping or defending themselves. This was also verified in the WRCMN within cat interactions, since 76% of the attacked animals were juveniles, 21% were infants and 3% were adults.

6.2.3. Direct and indirect anthropogenic influence

At least 42% of all the admissions had direct or indirect anthropogenic influence, only counting with the clear classification of domestic animals' interactions, confiscated animals and all the cases where "inappropriate human possession" or "projectile" were assured. This number is certainly larger, since the categories "entrapment", "collision", "orphan", "external substance contamination" and "nest/habitat destruction" can be classified as either natural or anthropogenic. For example, and concerning the studied species, "collision" is mostly reported in literature as vehicle strike; in respect to "nest/habitat destruction", there is still a major public ignorance regarding cottontails' natural history, whom unnecessarily intervene and bring young rabbits into care when they do not need to, thus some rabbits classified as "orphaned" may be actually "kidnapped" from their nests by humans; and "entrapment" is several times associated with human structures (Burton & Doblar, 2004; Oberly, 2015). Moreover, there is a large proportion of "undetermined" admissions, which hampers anthropogenic estimates. A more detailed look to secondary and tertiary classifications of each admission cause (not contemplated in this dissertation, besides that not all cases go that further on detail) would be very useful to have a more precise idea of human impact on WRCMN intakes.

Schenk & Souza (2014), reported that 31% of the animals admitted into a Wildlife Clinic in East Tennessee had anthropogenic influence, with direct human interactions (human induced trauma and hit by automobile) being less frequent than indirect interactions (domestic animals attack) in the studied population; however, in the WRC of Torreferrussa, Spain, this proportion rose up to 64% of all the admissions (Molina-López et al., 2017).

Legislation protecting wildlife and respective law enforcement and, fundamentally, public education towards human negative impacts on nature would be the key to decrease the proportion of injured wild animals requiring care and rehabilitation (Sleeman, 2008). These issues are discussed for "inappropriate human possession" and "domestic animals interactions", as presented below.

6.2.3.1. Inappropriate human possession

According to the Code of Federal Regulations (C.F.R)⁵, certain wildlife species are protected within the U.S. in respect to taking, possessing or transporting, such as migratory birds (Migratory Bird Permits, 2017), endangered and threatened wildlife (Endangered and threatened wildlife and plants, 2017). However, as far as the author knows, C.F.R does not state specifications about generalized native wildlife protection.

State wildlife agencies, such as Department of Natural Resources (DNR), design each state exceptions and particularities concerning wildlife protection: for instance, according to Minnesota DNR regulation (2018), only a person in the possession of a hunting license may

⁵ The codification of the general and permanent rules, published by the departments and agencies of the Federal Government of the United States (Government Publishing Office, 2018).

take, buy, sell, transport or possess protected wild animals in this state. "Taking" englobes chasing, shooting, capturing, trapping or netting these animals. The eastern cottontail is considered a protected mammal within Minnesota, since it may be taken during established hunting seasons, as authorized.

In the U.S., wildlife rehabilitators must hold permits or licenses from the state and federal governments, although there are exceptions and requirements diverge from state to state (National Wildlife Rehabilitators Association, 2015). Nevertheless, the International Wildlife Rehabilitation Council (IWRC) (2018a) recognizes the nonexistence of legal framework for rehabilitation of all wildlife species, which can entail incorrect intervention by the general public.

6.2.3.2. Domestic animal interactions

There are an estimated 50-157 million free-ranging cats in North America only, which involves political, social, public health and conservation issues (McRuer et al., 2017).

Humans' responsibility, towards the welfare of both cats and wildlife they may distress is unequivocal: some support trapping, neutering and releasing (TNR) cats as a solution, which includes sterilizing, feeding, maintaining colonies and eventually reducing free-roaming cat populations; however, wildlife biologists, ecologists and conservation agencies disagree with this type of program (Jessup, 2004), essentially because a TNR cat is not able to reproduce, but it remains a threat to native species and a potential disease reservoir (Barrows, 2004).

With respect to dogs, it is considered important to see beyond their lethal effects, since there are multiple ways that these animals cause disturbance to wildlife. Free-roaming dogs tend to be nocturnal and take advantage of variable home range sizes (from 1 ha to 2,500 ha), which means that they are more likely to affect a good part of native mammals, presenting nocturnal habits as well. Moreover, free-ranging dogs can predate a wide variety of mammals and this may have deep effects at population and community levels. However, leashed or controlled dogs do not seem to present any significant effect on species richness or abundance (Ritchie et al., 2014).

In the U.S., there is presently legislation at a federal and state level, in order to prevent domestic animals' interactions with wildlife. According to the C.F.R (Enforcement, penalty, and procedural requirements for violations of subchapter C rule, 2017), dogs and cats running in a national wildlife refuge and observed by a licensed official in the act of killing, injuring or harassing wildlife, may be disposed. At a state level, Minnesota DNR (2018) enforces that only accompanied dogs, or under the owner's control, are permitted on wildlife management areas; from April 16th through July 14th, dogs must be leashed. Furthermore, it is allowed to hunt rabbits with dogs throughout the hunting season, except from April 16th to July 14th, or by permit.

The IWRC (2018b) encourages and supports domestic animals' population control through the neutering of non-breeding cats and dogs. Education of dog and cat owners is also reinforced,

focusing that cats should be kept indoors and supervised while outside and dogs should be constrained when unsupervised. Dogs should be walked on a leash, unless in an area where impacts on wildlife can be reduced; vaccination and deworming of domestic animals, in order to decrease infectious disease potential, is also recommended. Moreover, the IWRC (2018b) advises humane removal of feral populations, through the rehabilitation and adoption of suitable animals into domestic environments and humane euthanasia when other efforts fail. Jessup (2004) adds the importance of more supportive adoption and fostering programs.

6.3. Clinical signs presented on admission

Overall, 37% of the cottontail rabbits were apparently healthy on arrival; the proportion of “clinically healthy” rabbits that were presented at the WRCMN was very low within clinic (5%) which is in accordance with the idea that more mature rabbits have to be severely injured to be rescued for rehabilitation (Schott, 2017); conversely, almost a half of the nursery cottontails appeared healthy on admission (Table 2), which may have been unreasonably brought into care by members of the public (Burton & Doblár, 2004; Oberly, 2015). Similarly, McRuer et al. (2017) reported a large number of healthy orphans admitted for rehabilitation in the Wildlife Center of Virginia.

Approximately 28% of overall cottontails were presenting trauma signs; being more prevalent within clinic (54%), than in nursery rabbits (21%). Moreover, it was possible to conclude that more than a half (57%) of the cottontails with neurological signs presented trauma signs associated concomitantly. There are two conceivable justifications: on the one hand, it is possible that trauma lesions may have caused neurological signs; on the other hand, neurological signs (instigated by a parasitic disease, for example) may predispose the rabbit to further trauma, since the mental state may be altered.

Table 2 – Proportion of cottontails with selected clinical signs categories presented on admission, within each treatment ward, between 2011 and 2017.

Clinical signs category	% of cottontails with selected clinical sign categories on admission, within each treatment ward	
	Nursery	Clinic
Clinically healthy	45%	5%
Dehydration	28%	15%
Hypothermia	7%	4%
Trauma	21%	54%
Neurological (CNS)	4%	37%
Brain	2%	25%
Spinal	1%	12%
Others	11%	17%

6.4. Resolution and outcomes

The association between the variables age, body condition, body weight, admission causes and selected clinical signs categories presented on intake with the outcomes “dead before release” (which included “died”, “died in cage” and “euthanized” cases) *versus* “released”, were tested. Dispositions on arrival, such as “dead on arrival” and “euthanized on arrival”, besides “transferred” resolutions were not included, therefore only the animals that went through all the rehabilitation process in WRCMN were considered.

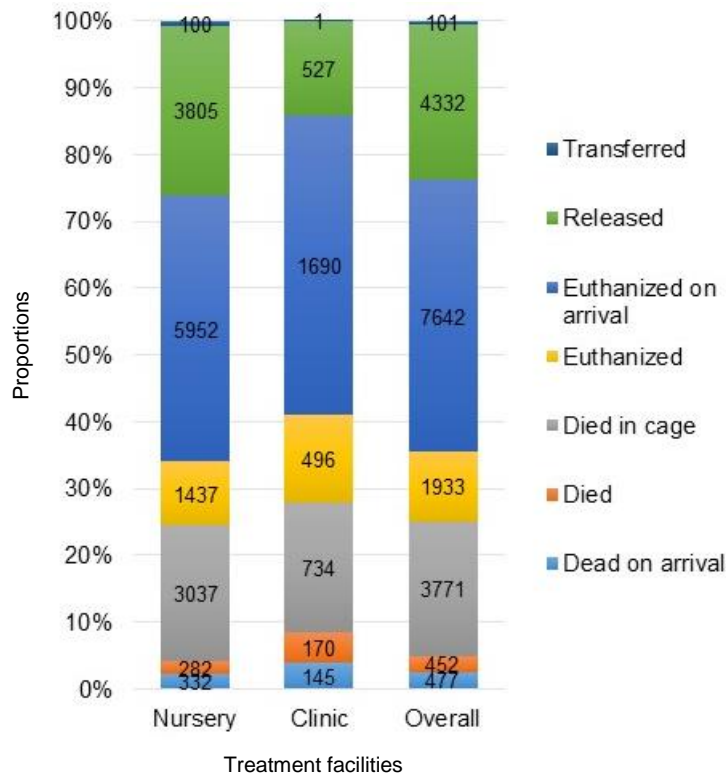
6.4.1. Overall case resolutions

Overall, “euthanized on arrival” (41%) was the leading result for the cottontail rabbits admitted during the studied period (Graph 6). It is important to mention that infants and juveniles under the designed cut-off weights made up 55% of all the rabbits included in this resolution category. The second main disposition was “released” (23%), followed by “died in cage” (20%).

These results emphasized the importance of a good triage, since a considerable proportion of the cottontails were humanely euthanized after the initial clinical assessment. Despite more than a half of these animals were euthanized because of their reduced body weight/maturity and consequent reduced chance of survival, the remaining rabbits were euthanized possibly due to severe injuries, poor prognosis, the non-existent chance of release and/or the animals’ welfare was compromised.

Concerning “released” proportions, about one quarter of the animals admitted into the nursery ward were released in the studied period (26%), greatly contributing for the overall proportion, however clinic had a lower proportion of released rabbits (14%). Oberly (2015), reported that overall eastern cottontails had an annual release of 34%, though infants and juveniles presented a higher release rate (37%) in the Ohio Wildlife Center.

Graph 6 - Outcomes for each treatment facility and for the overall eastern cottontail population admitted at the WRCMN from 2011 to 2017.



6.4.2. Age - outcomes

The major proportion of infants was “euthanized on arrival” (56%) and “died in cage” (18%); the leading resolution of the juveniles rehabilitated in mammal nursery was “released” (39%); for juveniles rehabilitated in clinic, the main resolution was “euthanized on arrival” (31%), followed by “released” (22%) (Graph 7). If the rabbits under the minimum body weight cut-offs and concomitantly euthanized on arrival are omitted, the results are very different for infants, particularly: “euthanized on arrival” would only represent 15% of this age class and “released” would increase to 27% (Graph 8).

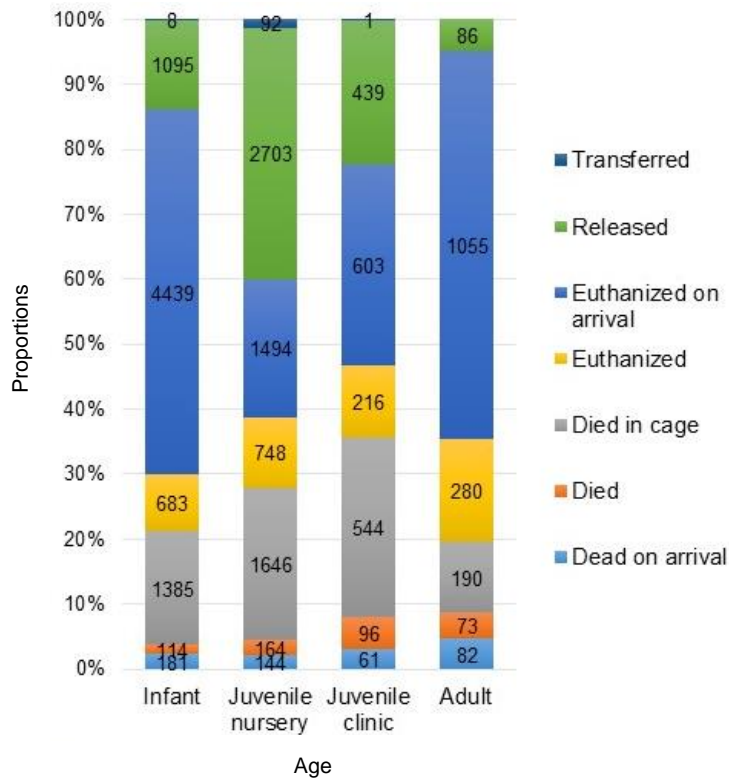
Adults presented effectively “euthanized on arrival” (60%) as the leading outcome, followed by “euthanized” (16%). Only 5% of the adults were released back to their natural habitats (Graphs 7 and 8).

It was possible to conclude that age was significantly associated with the outcomes “dead before release” / “released”, within nursery [$\chi^2 (1, N = 8,538) = 263.84; p < 0.01, OR = 0.47$] and clinic [$\chi^2 (1, N = 1,925) = 87.61; p < 0.01, OR = 0.31$], but also within the overall population [$\chi^2 (3, N = 10,463) = 532.31; p < 0.01$]. Therefore, the proportion of infants, juveniles admitted in clinic and adults that died before release was significantly higher than the proportion of individuals that were released; in contrast, juveniles raised in the mammal nursery presented a significantly higher percentage of released animals, obtaining the best survival outcomes. Compared with infants, this age class thrived more successfully, probably because of increased maturity and less hand-rearing dependence. Juveniles that were forwarded to the

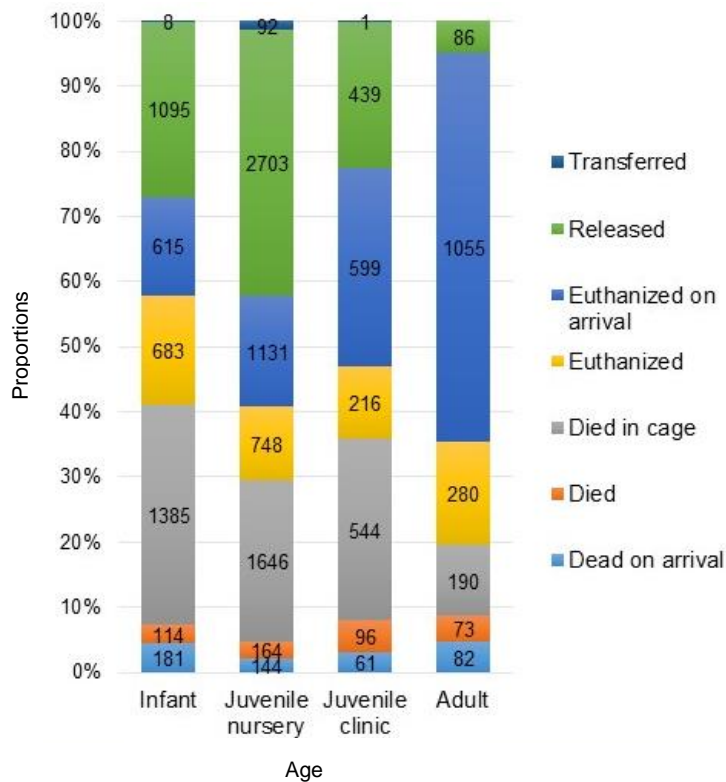
mammal nursery presented best outcomes compared with the ones that were admitted already weaned, rehabilitated in the clinic facility. This may be explained in part by the increasing maturity of these juveniles that present more severe injuries, since they are faster and more agile than the youngest ones, to justify their rescue and further rehabilitation; it is not possible to infer, based on this study, if factors linked with the milk formula/hand-rearing have any influence on these results. Following the same line of thought, adults had the lowest survival proportions in care, which is according with the fact that these animals have to be severely sick to be rescued (Schott, 2017).

“Died in cage” was an important resolution for infants (18%), besides nursery (24%) and clinic (28%) juveniles, which can be related with injury/illness worsening, the absence of response to the given treatment, the emergence of further complications and/or the stress resulting from the captivity environment. In nursery animals, this outcome could be related with their tendency to develop GI disease throughout the rehabilitation process (Bewig & Mitchell, 2009; Varga, 2014; Oberly, 2015; Cowen, 2016; Richardson, 2016; Paul & Friend, 2017; Paul & Friend, 2019), although it is not possible to infer this hypothesis without further study. The author consulted the necropsy reports, concerning 35 juvenile cottontails that developed diarrhea and were sent to the Veterinary Diagnostic Laboratory of the College of Veterinary Medicine, University of Minnesota, throughout the studied period, to have a broad idea of what etiologic agents could be found in juveniles that developed GI disease signs at WRCMN. Bacterial [non-hemolytic (n = 16) and β -hemolytic (n = 5) *E. coli*; *C. difficile* toxins (n = 4)] and rotaviral infections (n = 15) were often identified. Despite these results may not be representative of all cases with morbidity and mortality within nursery animals, and some of them might be related with outbreaks, they lead to the idea that it is important to investigate further on this subject.

Graph 7 - Resolutions for each age class of eastern cottontails admitted at the WRCMN from 2011 to 2017.



Graph 8 - Resolution for each age class of eastern cottontails admitted at the WRCMN from 2011 to 2017, excluding all cases under the minimum body weight cut-offs and concomitantly euthanized on arrival.

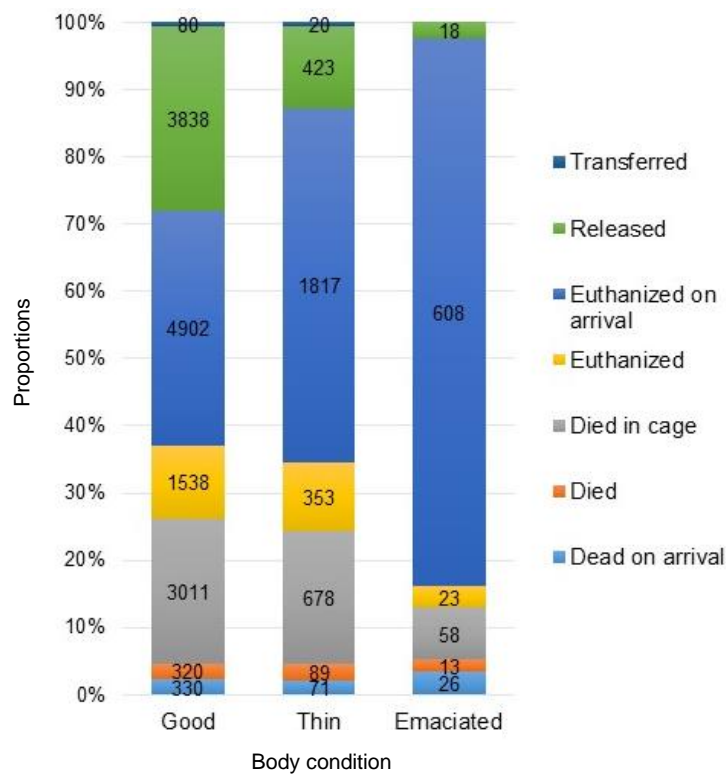


6.4.3. Body condition - outcomes

Although all body condition classes were associated with a greater proportion of “dead before release” compared with “released” animals, a “good” body condition was related with the best proportion of survival, with 27% of the rabbits classified in this category being released. The worst results belonged to “emaciated” individuals, with only 2% of the animals being released (Graph 9).

Body condition was significantly associated with the outcomes within both nursery [$\chi^2 (2, N = 8,455) = 176.02; p < 0.01$] and clinic [$\chi^2 (2, N = 1,908) = 13.93; p < 0.01$], besides the overall population [$\chi^2 (2, N = 10,363) = 180.28; p < 0.01$], as well.

Graph 9 - Body condition scores on admission and respective resolutions' proportions of all the eastern cottontails admitted at the WRCMN from 2011 to 2017.



6.4.4. Body weight - outcomes

Testing nursery and clinic *Mdn* body weights, both are significantly different for each one of the outcomes, as shown in Table 3. However, when age categories were separately tested, since different body weight ranges were observed for each age class (Graph 3), only juveniles, in both nursery and clinic, appear to have their body weight *Mdn* significantly different for each outcome ($p < 0.01$), as it is presented in Table 4.

In nursery, a higher body weight was related with a better chance of being released, possibly because it was associated with improved maturity and minor dependence on hand-rearing and care. Otherwise, in clinic, a higher body weight may be linked with greater mortality, which is

still according with the idea that more mature rabbits are usually rescued with more severe injuries (Schott, 2017).

Molony et al. (2007) did not find the body weight on admission as a significant release predictor in eight animal species (none of them referring to wild leporids) admitted in four WRCs belonging to the RSPCA, in England; however, Oberly (2007) found it significantly associated with the outcomes in eastern cottontail youngsters.

Table 3 - Treatment facilities and respective body weight *Mdn* and *IQR* (kg) for each outcome, with associated Wilcoxon test results.

Treatment facility	Body weight (kg)		Wilcoxon rank sum test
	Dead before release	Released	
Nursery	<i>Mdn</i> = 0.07	<i>Mdn</i> = 0.09	<i>W</i> = 6454600
	<i>IQR</i> = 0.06 – 0.09	<i>IQR</i> = 0.07 – 0.10	<i>p</i> < 0.01
Clinic	<i>Mdn</i> = 0.30	<i>Mdn</i> = 0.16	<i>W</i> = 484200
	<i>IQR</i> = 0.16 – 1.01	<i>IQR</i> = 0.12 – 0.25	<i>p</i> < 0.01

Table 4 - Age classes and respective body weight *Mdn* and *IQR* (kg) for each outcome, with associated Wilcoxon test results.

Age	Body weight (kg)		Wilcoxon rank sum test
	Dead before release	Released	
Infants	<i>Mdn</i> = 0.06	<i>Mdn</i> = 0.06	<i>W</i> = 1240900
	<i>IQR</i> = 0.05 – 0.07	<i>IQR</i> = 0.05 – 0.08	<i>p</i> = 0.01
Juveniles nursery	<i>Mdn</i> = 0.08	<i>Mdn</i> = 0.10	<i>W</i> = 2452400
	<i>IQR</i> = 0.07 – 0.1	<i>IQR</i> = 0.08 – 0.11	<i>p</i> < 0.01
Juveniles clinic	<i>Mdn</i> = 0.17	<i>Mdn</i> = 0.15	<i>W</i> = 234920
	<i>IQR</i> = 0.14 – 0.25	<i>IQR</i> = 0.12 – 0.18	<i>p</i> < 0.01
Adults	<i>Mdn</i> = 1.11	<i>Mdn</i> = 1.16	<i>W</i> = 18706
	<i>IQR</i> = 0.95 – 1.25	<i>IQR</i> = 1.02 – 1.34	<i>p</i> = 0.02

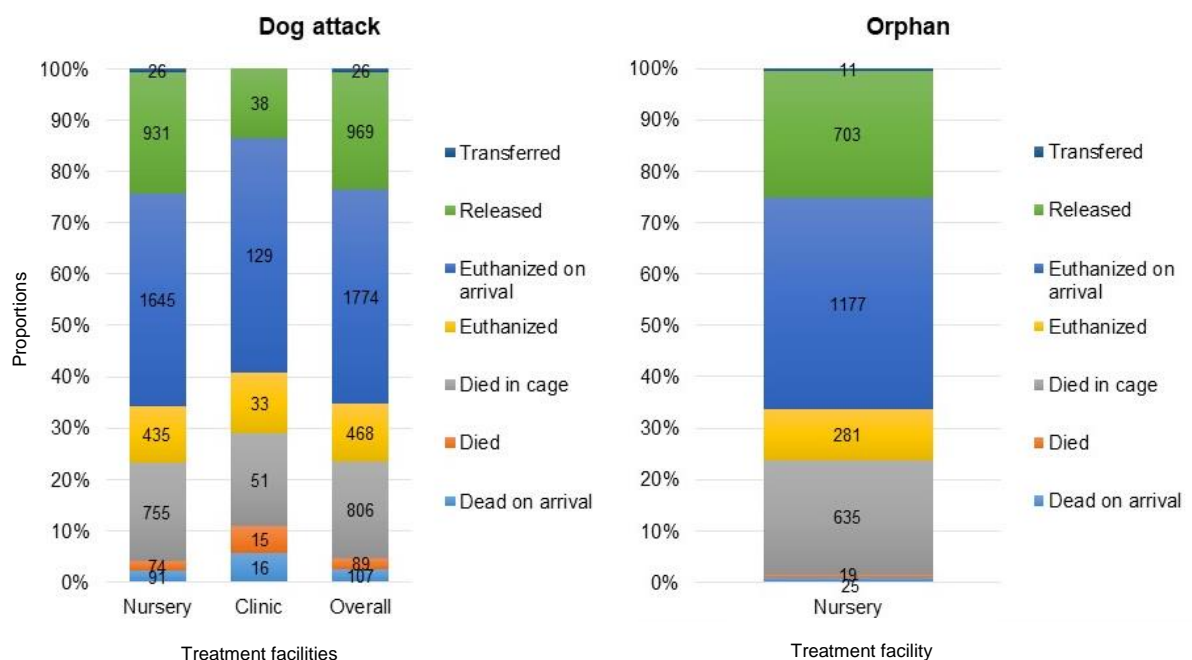
6.4.5. Admission causes - outcomes

The most frequent admission causes and respective outcomes are shown in Graph 10: circumstances with higher release proportions were “entrapment” (44%), “inappropriate human possession” (42%) and “nest/habitat destruction” (34%). This may be explained by the fact that, individually, these admission causes do not present a direct risk of injury compared with the remnant, however, they still may predispose to dehydration, starvation or even trauma. For instance, “entrapment” with dense glue materials, which adhere to hair persistently, can induce stress and compromise the animal’s thermoregulation; other possible scenario is when the cottontail gets trapped in a fence and consequently becomes physically injured. “Inappropriate human possession” may be related with well-intentioned individuals whom ignore wildlife species needs and biology, resulting in improper hand-rearing and imprinting of youngsters for example, although the incorrect perception of a long-term pet or even abuse may be possible

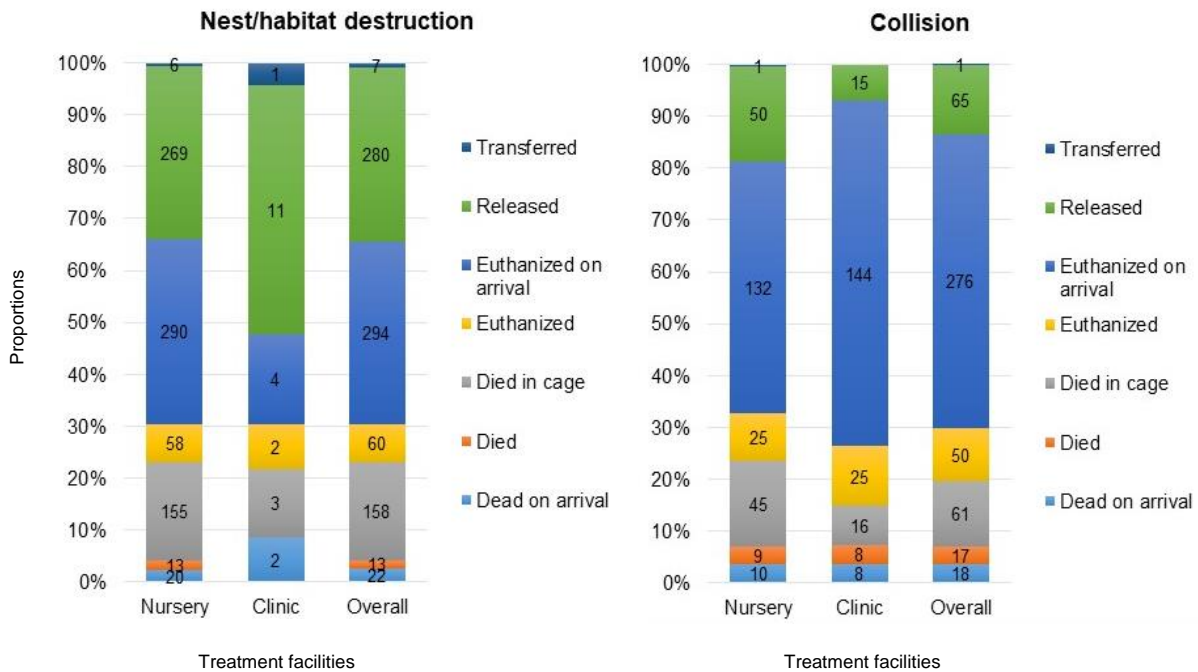
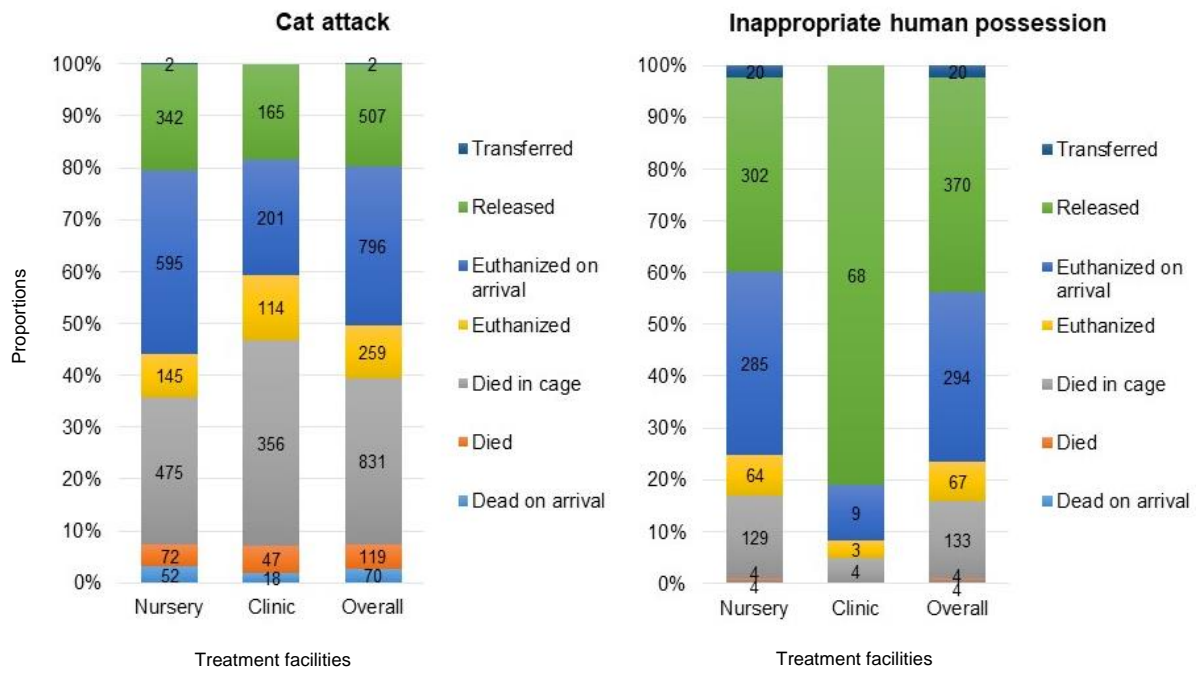
(Burton & Doblár, 2004). In respect to “nest/habitat destruction”, as referred in the section about anthropogenic influence, implies avoidable hand-rearing and stress which jeopardize the possibility that these animals are released back to the wild. “Orphaned” cottontails, which had a release proportion of 25%, might have been incorrectly classified in some cases, meaning that they were effectively a result of nest disturbance instead. Regarding other studies’ results, Lloyd et al. (2017) reported that wild animals cared for in North American WRCs, with nest/habitat destruction as admission cause or classified as orphans, presented 45% of release proportions. In a three-year study conducted in the Ohio Wildlife Center, 37% of all the admitted orphans were intentionally disturbed by members of the public (Burton & Doblár, 2004).

Contrariwise, “collision” (13%), “cat attack” (20%) and “dog attack” (23%) presented the worst release proportions. These results are more or less according with previous admission causes studies in WRCs, where 24% of wild animals affected by cat interactions were released while 54% of the animals that interacted with dogs were dead before release, either euthanized or in captivity (Lloyd et al., 2017). A high fatality risk for animals with automobile collision (0.7), followed by cat-related cases (0.675) were reported; dog interactions cases were associated with a fatality risk of 0.6 (Schenk & Souza, 2014). Survival of cat-related admissions is lower, because of the nature of injuries (deep but small puncture wounds, difficult to identify), multiple and internal injuries, besides wounds that lead to septicemia (Lloyd et al., 2017). It is important to remind that mortality is not only depending on the circumstance of injury, but also related with the species and intrinsic tolerance to stress, injuries’ severity and chronicity, adding to the possible treatment resources (McRuer et al., 2017).

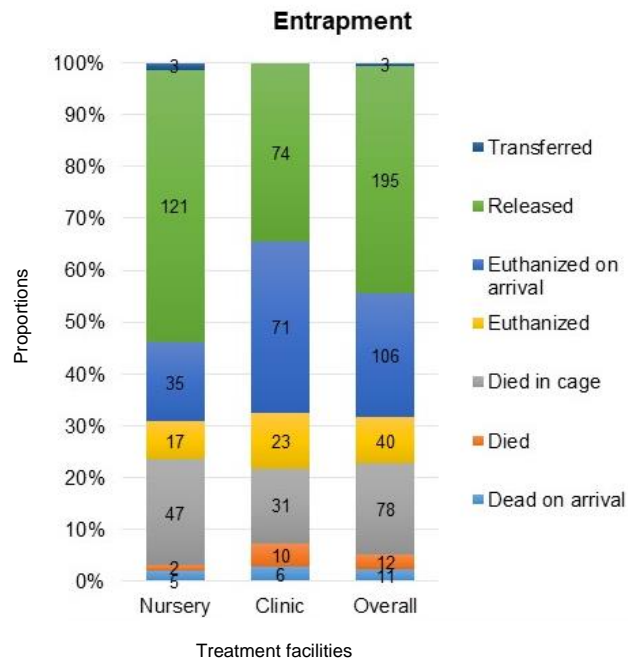
Graph 10 - Most common admission causes and respective outcomes for the eastern cottontails admitted at the WRCMN from 2011 to 2017, within each treatment facility and for the overall population.



Graph 10 – Continuation.



Graph 10 – Continuation.



Nursery and clinic animals were separately tested to assess whether each cause would be significantly associated with the outcomes. Relatively to nursery rabbits, admission causes such as “cat attack”, “entrapment”, “inappropriate human possession” and “nest/habitat destruction” were significantly associated with the outcomes. Rabbits with “cat attack” as admission cause were more frequently “dead before release”, contrarily to the remnant causes, which were more frequently related with “released” animals.

Regarding clinic, “entrapment”, “inappropriate human possession”, “nest/habitat destruction”, “non-domestic animal attack” and “undetermined” circumstances were significantly associated with the outcomes (Table 5). Animals that presented “entrapment”, “inappropriate human possession” and “nest/habitat destruction” were more frequently “released”, in contrast to “non-domestic animal attack” and “undetermined”, which were more frequently linked with the outcome “dead before release”.

Table 5 – Chi-square test results for the statistically significant associations between admission causes and outcomes, within each treatment facility (the remnant non-significant results are presented in Annex 1).

Admission cause	Chi-square test	
	Nursery	Clinic
Cat attack	$\chi^2(1, N = 8,561) = 61.58$ $p < 0.01, OR = 1.72$	See annex 1
Entrapment	$\chi^2(1, N = 8,561) = 31.78$ $p < 0.01, OR = 0.43$	$\chi^2(1, N = 1,928) = 51.72$ $p < 0.01, OR = 0.29$
Inappropriate human possession	$\chi^2(1, N = 8,561) = 55.46$ $p < 0.01, OR = 0.50$	$\chi^2(1, N = 1,928) = 157.58$ $p < 0.01, OR = 0.03$
Nest/habitat destruction	$\chi^2(1, N = 8,561) = 20.85$ $p < 0.01, OR = 0.66$	$\chi^2(1, N = 1,928) = 13.93$ $p < 0.01, OR = 0.44$
Non-domestic animal attack	See annex 1	$\chi^2(1, N = 1,928) = 7.44$ $p < 0.01, OR = 0.44$
Undetermined	See annex 1	$\chi^2(1, N = 1,928) = 59.87$ $p < 0.01, OR = 2.37$

6.4.6. Clinical signs on admission – outcomes

6.4.6.1. Nursery

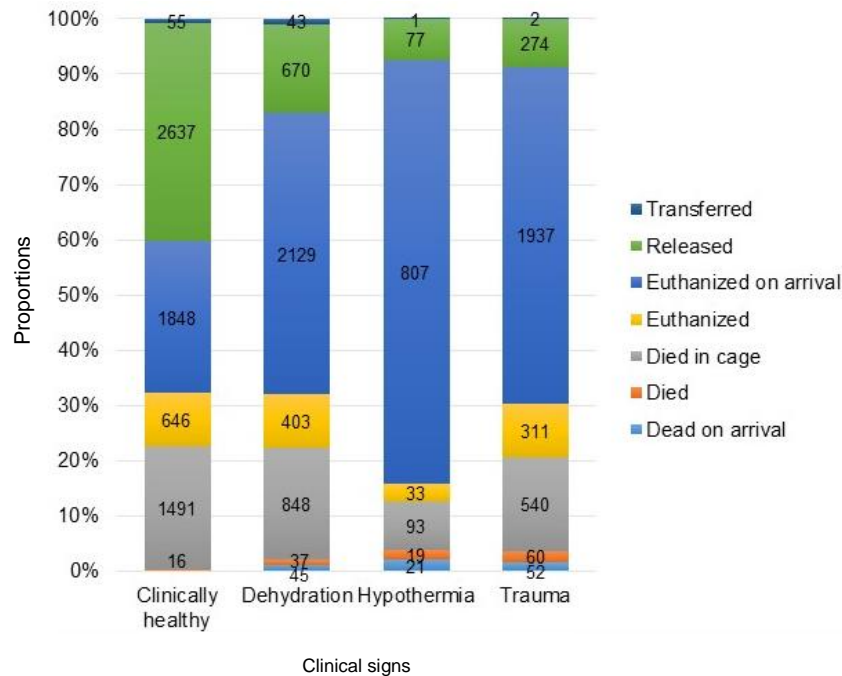
“Clinically healthy” cottontails presented the best release proportions (39%). The high proportion of “euthanized on arrival” (28%) may be justified with the large number of cases of rabbit kits under the body weight cut-offs, which made up 25% of the nursery cottontails that were apparently healthy (this was also valid for the nursery rabbits that did not have this classification) (Graph 11). However, 32% of these died or were euthanized during the rehabilitation process, which is in accordance with studies that highlight this species’ challenging hand-rearing, with GI disease identified as the main problem (Bewig & Mitchell, 2009; Varga, 2014; Oberly, 2015; Cowen, 2016; Richardson, 2016; Paul & Friend, 2017; Paul & Friend, 2019); other possibility is the fact that the animals may be already ill when admission takes place, although there are no identifiable clinical signs associated.

When hypothermia was present, release proportions were low (7%); dehydrated animals had slightly better prognosis (16%). In neonates, hypothermia occurs consequently to multiple routes of heat loss. Poor prognosis in hypothermic pet rabbits on admission was previously reported by Di Girolamo, Toth & Selleri (2016): the risk of death was 3 times that of normothermic rabbits, with probabilities of mortality doubled for each 1°C decrease of rectal body temperature under the reference range (38.0°C-39.9°C or 100.4°F-103.8°F). Hypothermia may cause coagulation, electrolyte and acid-base abnormalities, apart from organ dysfunction (Huynh et al., 2016).

As reported in the literature, infant rabbits frequently present hypothermia, hypoglycemia and dehydration signs, on admission at WRCs (Pokras & Porter, 1994; Bewig & Mitchell, 2009; Schott, 2017); however, as the analysis was developed considering the presence/absence of the clinical sign, and taking into account that trauma, dehydration and hypothermia could be

associated together in the same animal, the results can be biased. However, it is rare to find an animal with just one clinical sign, since multiple illnesses/injuries frequently occur together (Schott, 2017).

Graph 11- Nursery eastern cottontails admitted from 2011 to 2017 at the WRCMN: clinical signs presented on admission and respective resolutions proportions.



Concerning nursery cottontails, clinical signs such as “clinically healthy”, “dehydration”, “hypothermia” and “trauma” were significantly associated with the outcomes, as it is presented on Table 6. “Clinically healthy” animals were more frequently released, oppositely with the individuals presenting the remaining clinical signs.

Table 6 - Nursery eastern cottontails admitted from 2011 to 2017 at the WRCMN: chi-square test results for the association of selected clinical signs categories with the respective outcomes.

Clinical Signs	Chi-square test
Clinically healthy	$\chi^2(1, N = 8,561) = 495.45; p < 0.01, OR = 0.37$
Dehydration	$\chi^2(1, N = 8,561) = 107.54; p < 0.01, OR = 1.74$
Hypothermia	$\chi^2(1, N = 8,561) = 8.79; p < 0.01, OR = 1.52$
Trauma	$\chi^2(1, N = 8,561) = 523.27; p < 0.01, OR = 3.05$

6.4.6.2. Clinic

About 82% of the cottontails that were apparently healthy were successfully released, although there was still a moderate proportion of dead and euthanized individuals, that possibly were already ill and did not demonstrate clinical signs, or it may correspond to animals that did not cope well with captivity (Graph 12). McRuer et al., 2017 reported that most wildlife admitted for cat interactions was categorized as injured (86%). This leads to the idea that even

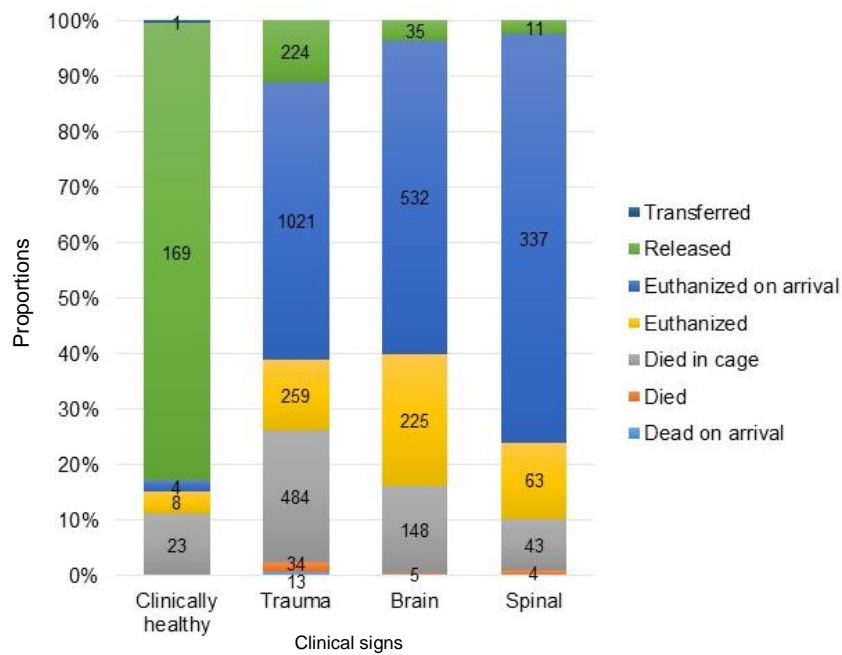
apparently healthy animals may have an underlying injury, for example puncture wounds caused by cat attacks, which are not always evident, being sometimes very challenging to identify.

The release proportion of animals presenting “trauma” (with and without concurrent neurological signs), was 11%; cottontails presenting CNS neurological signs such as “brain” (4%) or “spinal” (2%), had a very poor prognosis; these proportions were sensitively the same when neurological signs occurred with no simultaneous “trauma”, which indicates that no matter the cause that originated neurological signs, the prognosis is very poor. Contrariwise, if cottontails with “trauma” and without concurrent neurological signs are focused, the prognosis is slightly better (15%).

It is possible to observe that traumatized rabbits had a slightly worse prognosis in the mammal nursery (9% release proportion), compared with the clinic (11%). Molina-López et al. (2017) reported a release rate of about 35% for traumatized rodents and rabbits admitted at the WRC of Torreferrussa, Spain, although “trauma” casualties had the higher proportion of euthanasia across all animal species groups, possibly related with severe disabilities involving fractures, neurological deficits or soft tissue injuries.

Based on the uncertainty and vast possible causes of neurologic signs in cottontails with brain origin (as presented in the section 3.5.2. of this dissertation), the author consulted the necropsy reports sent by the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, throughout the studied period, to have a broad idea of what causative agents could have been identified. Most of the animals with clinical history, including this sign category (in total, 7 animals were submitted to necropsy), presented brain lesions consistent with trauma in some cases ($n = 2$), or with lesions highly suggestive of *B. procyonis* larvae migration tracks ($n = 4$). *T. gondii* or *E. cuniculi* were never evidenced as possible causes in these reports. Taking into account that *B. procyonis* is reported in high prevalence ($> 60\%$) in some or most areas of Minnesota (Kazacos, 2016), it is possible to hypothesize that it may be a major cause of neurological disease in cottontails admitted into the WRCMN.

Graph 12- Clinic eastern cottontails admitted from 2011 to 2017 at the WRCMN: clinical signs presented on admission and respective resolutions proportions.



Regarding clinic cottontails, clinical signs categories on admission such as “clinically healthy”, “trauma”, “brain” and “spinal” were significantly associated with the outcomes, as it is presented on Table 7. “Clinically healthy” was associated with a higher number of “released” rabbits, oppositely to the remnant signs, which were related with more “dead before release” cases.

Table 7 - Clinic eastern cottontails admitted from 2011 to 2017 at the WRCMN: chi-square test results for the association of selected clinical signs categories with the respective outcomes.

Clinical Signs	Chi-square test
Clinically healthy	$\chi^2(1, N = 1,929) = 364.06; p < 0.01, OR = 0.05$
Trauma	$\chi^2(1, N = 1,929) = 25.59; p < 0.01, OR = 1.68$
Brain	$\chi^2(1, N = 1,929) = 94.49; p < 0.01, OR = 5.21$
Spinal	$\chi^2(1, N = 1,929) = 21.61; p < 0.01, OR = 3.99$

Although all tested clinical signs were significantly associated with the outcomes, Molony et al. (2017) presented the severity of the injury/illness symptoms as the only significant release predictor, despite its high collinearity with the reason of admission, which was excluded for statistical analysis in this study. Molina-López et al. (2017) categorized injuries severity as well and the cases where lesions were classified as “very severe” (including major injuries, emaciation, paralysis, blindness and respiratory distress), were related with the lowest release rates (about 5% for rodents and rabbits).

The possibility to categorize illness/injury in WRCMN database would be valuable, in order to examine the resulting influence in outcomes in future studies, besides representing further triage support.

Moreover, Molony et al. (2007) suggested a minimum of 10% survival cut-off to attempt treatment based in injury severity and taking into account animal welfare. This could be a possibility to WRCMN as well, even without the results for injuries severity, it is known the example of clinic cottontails with brain or spinal neurological signs having less than 5% chance of survival, therefore, euthanasia on arrival could be considered for these cases.

6.5. Period in Treatment (PT)

Molina-López et al. (2017) presented WRC length of stay or PT as an important estimator of wildlife rehabilitation daily costs, which includes staff, food and medical resources. Moreover, this parameter may lead to a cost-benefit index examination, which is also based in the admission causes and respective prognosis for a designed zoological group.

Meredith (2016) highlights that even if an injury or illness can be treatable (e.g. a repairable fracture), this period has to be considered, since long hospital stays and consequent stress in some wildlife species may not compensate the chance of being recovered and released. Additionally, prolonged human proximity and diverse stimuli while in captivity, possibly resulting in stress, can affect the rehabilitation process itself (Molony et al., 2007). Other important factors are the loss of territory that may be a consequence of long PTs and also the resultant time of the year for release, of special relevance to species that migrate or hibernate, but also for the variance of food resources. In addition, the breeding season must be considered, which requires rapid return to the nest if the animal has dependent offspring, for example (Meredith, 2016). The cottontail is not a territorial species and it does not migrate or hibernate (Whitaker & Hamilton, 1998); however, frigid winters or adverse conditions at certain times of the year may prevent release, meaning that a prolonged captivity is necessary if the time window for effective release is missed. For instance, in WRCMN, there is an established minimum cut-off temperature for release that has to be considered during the coldest winter's months, of approximately $-7^{\circ}\text{C}/20^{\circ}\text{F}$, if the animal is in care for more than 4 weeks, in order to acclimatize.

In the present dissertation, only the PT, expressed as *Mdn* in days and respective *IQR*, will be studied and discussed.

6.5.1. Nursery versus clinic

Overall, the *Mdn* stay at the WRCMN was 1 day for both the nursery (*IQR* 1 - 9) and the clinic (*IQR* 1 - 3) cottontails, which can be justified by the considerable proportion of animals being euthanized on arrival, referred above as the most frequent resolution for this species.

The *Mdn* stay at the WRCMN for individuals that died before release was significantly lower from the released PT, for either nursery or clinic animals, as it is presented in Table 8.

Table 8 - PT - *Mdn* (*IQR*) - for each treatment ward and respective outcomes regarding eastern cottontails admitted at the WRCMN from 2011 to 2017, with Wilcoxon rank sum test results presented.

Treatment ward	Period in treatment	Period in treatment	Wilcoxon rank sum test
	Dead before release <i>Mdn</i> (<i>IQR</i>)	Released <i>Mdn</i> (<i>IQR</i>)	
Nursery	5 (2 – 9)	12 (2 – 17)	$W = 5904900$ $p < 0.01$
Clinic	2 (2 – 3)	12 (5 – 22)	$W = 162250$ $p < 0.01$

6.5.2. Main admission causes

Observing the main admission causes for nursery cottontails and comparing each *Mdn* of hospital stay for released animals (Table 9), it is possible to infer that PT *Mdn* were similar, between 11 and 15 days. “Cat attack” implied the highest *Mdn* for released animals (*Mdn* = 15, *IQR* 11 - 19), which is justifiable since all confirmed cases have to start prophylactic antibiotherapy, which may be time-consuming; conversely, it was the cause with the shortest PT for animals that died / were euthanized during the treatment (*Mdn* = 3, *IQR* 2 - 6). McRuer et al. (2017) reported that small mammals admitted at the Wildlife Center of Virginia following cat interactions spent lower PTs compared to animals with other admission reasons, possibly because of injury severity or compromised quality of life that lead to euthanasia.

The *Mdn* PT for all the presented causes was significantly lower for “dead before release” cottontails, compared with the ones that survived.

Table 9 - Nursery: PT - *Mdn*, (*IQR*) - for each main admission cause and respective outcomes regarding eastern cottontails admitted at the WRCMN from 2011 to 2017, with Wilcoxon rank sum test results presented.

Outcomes	Cat attack	Wilcoxon test	Dog attack	Wilcoxon test	Orphan	Wilcoxon test	Nest/habitat destruction	Wilcoxon test
Dead before release	3 (2 – 6)	$W = 21224$	7 (3 – 10)	$W = 440700$	6 (3 – 10)	$W = 188270$	5 (2 – 9)	$W = 21324$
Released	15 (11 – 19)	$p < 0.01$	12 (1 – 18)	$p < 0.01$	13 (8 – 17)	$p < 0.01$	11 (3 – 15)	$p < 0.01$

Concerning clinic animals and considering the respective most common admission circumstances, the *Mdn* time of stay at the WRCMN for released animals was lower when “entrapment” occurred (*Mdn* = 8, *IQR* 1 - 17), oppositely to “collision”, which had a more time-consuming rehabilitation (*Mdn* = 20, *IQR* 15 - 30). All the studied admission causes had the same *Mdn* for animals that succumbed before being released (*Mdn* = 2), which were lower

than nursery's *Mdn* PTs, possibly because mature juveniles and adults arrived in a more deteriorated condition at the WRC.

The *Mdn* PT for all the presented causes was significantly lower for “dead before release” rabbits, compared with “released” animals, as shown in Table 10.

Table 10- Clinic: PT – *Mdn (IQR)* - for each main admission cause and respective outcomes regarding eastern cottontails admitted at the WRCMN from 2011 to 2017, with Wilcoxon rank sum test results presented.

Outcomes	Cat attack	Wilcoxon test	Dog attack	Wilcoxon test	Entrapment	Wilcoxon test	Collision	Wilcoxon test
Dead before release	2 (2 – 3)	$W = 4059.5$ $p < 0.01$	2 (2 – 4)	$W = 560$ $p < 0.01$	2 (1 – 4)	$W = 1528$ $p < 0.01$	2 (1 – 4)	$W = 24$ $p < 0.01$
Released	14 (9 – 22)		14 (7 – 25)		8 (1 – 17)		20 (15 – 30)	

6.5.3. Clinical signs

“Clinically healthy” animals rehabbed in the nursery had the highest PT for animals that did not survive (*Mdn* = 7, *IQR* 3 – 10), possibly represented by orphans or young animals that were hand-raised but did not thrive. Contrariwise, traumatized youngsters had the highest hospital stay for released individuals (*Mdn* = 17, *IQR* 12 – 22) (Table 11).

Cottontails admitted with “hypothermia” did not present significantly different PT medians for both outcomes. Possibly, animals entering in a hypothermic state and released in the same admission day were successfully reunited with their nests.

Table 11 – Nursery: PT – *Mdn (IQR)* - for selected clinical sign category and respective outcomes regarding eastern cottontails admitted at the WRCMN from 2011 to 2017, with Wilcoxon rank sum test results presented.

Outcomes	Clinically healthy	Wilcoxon test	Hypothermia	Wilcoxon test	Dehydration	Wilcoxon test	Trauma	Wilcoxon test
Dead before release	7 (3 – 10)	$W = 2214000$ $p < 0.01$	2 (1 – 4)	$W = 4832$ $p > 0.01$	4 (2 – 8)	$W = 260770$ $p < 0.01$	3 (2 – 7)	$W = 26946$ $p < 0.01$
Released	11 (1 – 16)		1 (1 – 17)		14 (1 – 19)		17 (12 – 22)	

Concerning clinic cottontails, the ones that were apparently healthy upon admission and subsequently released had the lowest PT (*Mdn* = 2, *IQR* 1 – 9), however, the difference between hospital stays was not significant between both outcomes, contrarily to the other clinical signs (Table 12).

Released animals that presented “trauma”, “brain” and “spinal” signs on admission spent longer periods at the WRCMN, especially the ones with “spinal” neurological signs, staying for more than a month (*Mdn* = 34, *IQR* 25 – 46). Furthermore, traumatized clinic rabbits had longest recovery periods than nursery individuals with the same clinical sign, possibly because a higher proportion of these animals had neurological signs associated with trauma (57%), compared to youngsters (12%).

Table 12 - Clinic: PT – *Mdn* (*IQR*) - for selected clinical sign category and respective outcomes regarding eastern cottontails admitted in the WRCMN from 2011 to 2017.

Outcomes	Clinically healthy	Wilcoxon rank sum test	Trauma	Wilcoxon rank sum test	Brain	Wilcoxon rank sum test	Spinal	Wilcoxon rank sum test
Dead before release	<i>Mdn</i> = 3 (2 – 8)	<i>W</i> = 3268 <i>p</i> > 0.01	<i>Mdn</i> = 2 (2 – 3)	<i>W</i> = 8310.5 <i>p</i> < 0.01	<i>Mdn</i> = 2 (2 – 5)	<i>W</i> = 1208.5 <i>p</i> < 0.01	<i>Mdn</i> = 2 (2 – 4)	<i>W</i> = 9 <i>p</i> < 0.01
Released	<i>Mdn</i> = 2 (1 – 9)		<i>Mdn</i> = 19 (13 – 27)		<i>Mdn</i> = 17 (11 – 29)		<i>Mdn</i> = 34 (25 – 46)	

6.6. FFT method

6.6.1. Nursery

Based on the nursery decision tree plot (Figure 6), it is possible to make the following reading from the top row of the plot, which shows the main dataset information: 56% of the sampled cottontails are predicted to die before release, whilst 44% are predicted to be released. From 14,945 cases, only 8,561 had the outcomes died before release/release (all the cases with dispositions on arrival or transferred were not considered); then, incomplete cases were removed, resulting in 8,385 cases that were split randomly and equally into two subsets, in which training included 4,192 cases.

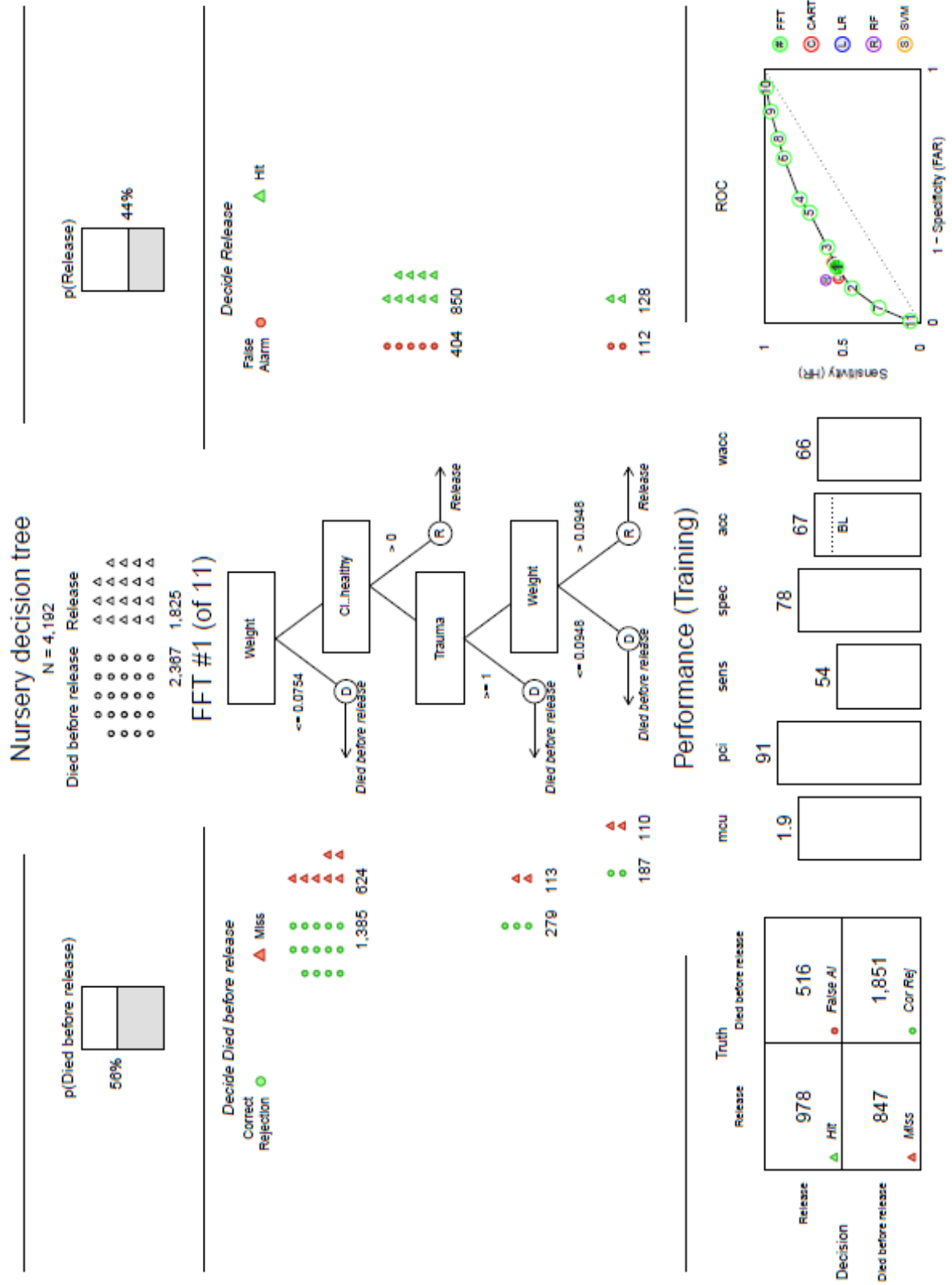
The middle row shows the decision tree and how many examples were classified at each level of the tree; it could be understood that body weight on admission should be assessed firstly – if it is equal or less than 0.075 kg, it’s predicted that the animal may die before release; otherwise, the next cue focus on the clinical signs presented, if the rabbit is clinically healthy, it may have a good chance of being released; if it is not apparently healthy and if it is presenting trauma signs, it is predicted that it will die before release; if not, the body weight should be checked again – if the animal weights more than 0.095 kg, it is probable that it will be released. Over the bottom-left of the plot, a 2 x 2 table represents the number of observations that were hits (true positives), false alarms (false positives), misses (false negatives) and correct rejections (true negatives). The accuracy of an algorithm is based on the capacity of improving

the frequencies of hits and correct rejections, while minimizing false alarms and misses. Moreover, this section presents mean cues ignored in making classifications (*mcu*) and percentage of cues ignored when classifying cases (*pci*), as measures of speed and frugality; sensitivity (*sens* or hit-rate), specificity (*spec* or correct rejection rate), accuracy (*acc*) and weighted accuracy (*wacc*, which quantifies how an algorithm balances *sens* and *spec*) are also presented. A receiver operating characteristic (ROC) graph on the bottom-right of the plot presents the arrangement among sensitivity and specificity in classification algorithms, meaning that when one increases, the other decreases. Numbered circles represent the *acc* of 11 different FFT algorithms with distinct trade-offs between both measures, whilst numbers signify the rank order of algorithm performance regarding their *wacc* values – the present plot is FFT #1 with the highest *wacc* in training; additional points represent competing classification algorithms (Phillips et al., 2017). In this case, FFT#1 has a higher specificity than competing algorithms, at the cost of a lower sensitivity.

This FFT was developed with the best possible *wacc*, although if one desires to be more certain to predict if the animals are effectively released, the sensitivity should be favored, however, specificity is disadvantaged; oppositely, if one wants to be more sure that the animals do not have a good chance to survive, specificity should be improved. This may help to guide the decisions on arrival, in order to understand which animals should proceed with the rehabilitation process and which ones should be humanely euthanized on arrival, to minimize the stress and suffering, besides allowing allocation of resources to the ones that have a better chance.

These results are according with previous statistical tests presented above, referring to the significant association between clinical signs and the outcomes, as well as the significant difference between *Mdn* body weights for both outcomes. Here, most of the factors (91%), except for body weight, “clinically healthy” and “trauma signs”, were ignored to predict if animals would be released or would not survive.

Figure 6 - Nursery FFT: on the first row, information about the dataset is presented; on the second row, the decision tree itself, plus how many examples were classified at each level of the tree; on the last row, acc, speed and frugality and other statistical measures can be consulted.



6.6.2. Clinic

Based on the clinic decision tree plot (Figure 7), it is possible to observe that 72% of the sampled cottontails are predicted to die before release, while 28% are predicted to be released. From 3,764 cases, 1,928 had the considered outcomes; then, incomplete cases were excluded, resulting in 1,870 that were split randomly and equally into two subsets, in which training included 935 cases, as presented in this plot.

From the middle row, it is possible to infer that body weight on admission should be checked – if it is less than 0.188 kg, it is predicted that the animal may be released; otherwise, and if the admission cause is undetermined, it is predicted that the rabbit will die before release; if the admission cause is determined and “cat attack” was assured, the animal may not survive; if not, neurological signs with brain origin should be assessed – when they are present, it is predicted that the rabbit won’t be alive until release.

The most balanced *wacc* was achieved, as shown in the plot over the bottom-right, with a relatively high sensitivity (therefore, a good hit rate), at cost of a lower specificity (weaker correct rejection rate). However, it was better in both parameters, compared to all other competing algorithms.

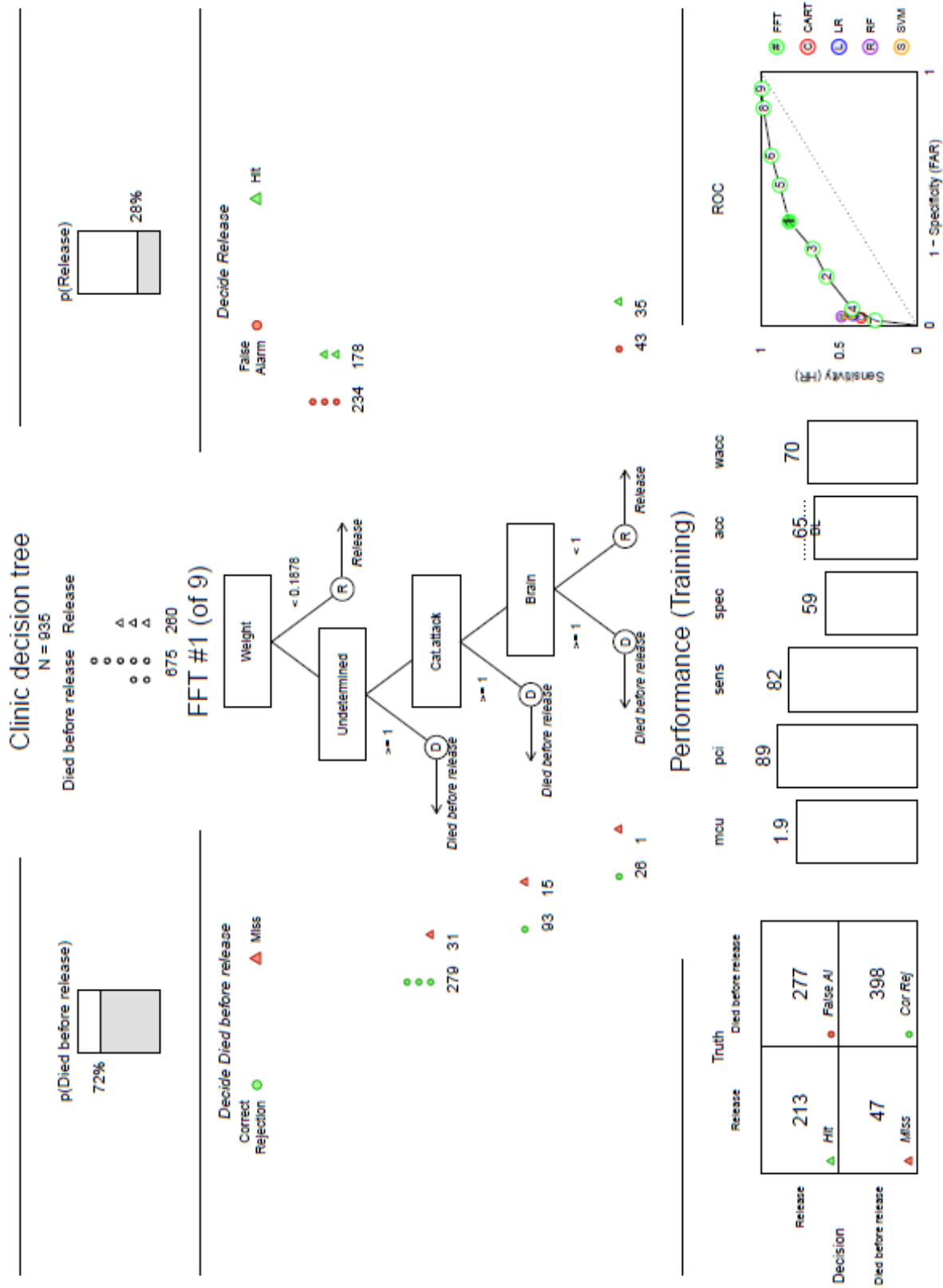
This FFT is consistent with the descriptive analysis, in which a higher body weight within clinic was significantly associated with mortality before release, besides admission causes, such as “undetermined”, and also neurological brain signs were pointed as significantly associated with a poor prognosis. “Cat attack” was considered preponderant for the decision-making process by the FFT, however the association between this cause with the prognosis was not significant, based on the Chi-square test results [$\chi^2(1, N = 1,928) = 5.24, p = 0.02$]. This may be justified by the fact that the dataset used in the Chi-square test included 1,928 cases, while the FFT training subset only included 935 (since all cases with missing information were excluded and the dataset was randomly and equally split), which may have impacted on the results. Nevertheless, when more mature cottontails interact with a cat, there is the possibility of presenting a primary disease which predisposes the inability to evade capture (Richardson, 2016; Schott, 2017) and it should be reminded that less than 20% of the animals admitted with this underlying cause are released, within the clinic facility.

Both decision trees based on eastern cottontails’ data on admission showed a distinct possibility to assess the factors on arrival with the respective outcomes, creating a simple approach to support triage at WRCMN. In both treatment facilities, body weight on admission was identified as the most accurate cue to start the decision tree with, therefore body weight cut-offs could be suggested on admission, for instance. Additively, a readjustment in nursery minimum cut-off concerning body weight on admission based on the FFT (also supported by the Wilcoxon test) could be suggested, however it should be reminded that changing the equilibrium between *sens/spec* would probably change the first cue to look at. Other point to highlight is the fact that not all clinical signs categories were studied, which could change, or

not, the resulting decision tree itself. Additively, in a real scenario, many other factors are counting, sometimes external to the clinical case itself, such as staff, medical materials and facilities availability, besides the perception and experience of the person that is conducting the triage.

FFTs could potentially be applied to other animal species data on arrival, but they could also be useful in other aspects in WRCs, such as diagnosis support to specific diseases that occur at the facility (e.g. infectious disease identification, waterfowl lead poisoning), or aid to choose the treatment/procedure in certain medical situations (e.g. fractures repair, bats hibernation).

Figure 7 - Clinic FFT: on the first row, information about the dataset is presented; the second row shows the decision tree itself, plus how many examples were classified at each level in the tree; on the last row, acc, speed and frugality and other statistical measures can be consulted.



7. CONCLUSION

This study summarizes WRCMN reality in managing eastern cottontails' rehabilitation, focusing on which factors on arrival may be associated with the outcomes, in addition to understand the genesis of the admission causes and respective mitigation measures, especially if they have anthropogenic influence.

Several of the leading admission circumstances identified in the studied period had anthropogenic origin, in which domestic animal interaction excelled all the others, as reported in multiple previous studies in the wildlife rehabilitation field. These results highlight the importance of enforcing measures that protect wildlife from domestic animal impact and, especially, through public education. Other important cause of admission was linked with orphaned rabbit kits, nest destruction/disturbance and inappropriate human possession, which are clear examples of public lack of awareness about this species' life history and reproductive strategies, since people often try to rescue apparently abandoned youngsters, unnecessarily ending up being hand-reared in rehabilitation settings.

Thanks to WRCMN extensive database, it was possible to understand the association between cottontails' age, body condition, body weight, admission causes and clinical signs with the respective outcomes. The PT or length of stay, an important estimator of daily costs and animal welfare, was studied as well. The collected results enable future identification of the most significant factors presented by eastern cottontails on arrival that lead to a better chance of survival and subsequent release, improving animals' welfare and preventing further suffering, apart from easing the triage process and resources management. These results were accomplished based on statistical methods and through the development of clinical decision trees, which may be very useful to employ in this and other rehabilitation centers, based on the respective databases.

This work limitation included the restriction to study only certain categories of clinical signs and the lack of information about injury/illness severity, which may have biased a part of the results. In addition, as secondary and tertiary admission causes were not assessed, some relevant information may have been lost (e.g. data that could lead to classify determined cases as anthropogenic-related). Other limitation, which can be applied to other WRCs, is the fact that animals were frequently rescued nearby human settings, leading to misrepresentative ideas of animal threats/illnesses, besides the anthropogenic influence in the overall wild population. Hereafter, the next step in what regards to the eastern cottontail rehabilitation at the WRCMN would be a more detailed study about possible complications or diseases affecting animals undergoing rehabilitation (e.g. GI disease in rabbit kits and its possible etiologic agents), plus the investigation of enforceable approaches to avoid these issues. The adjustment or creation of cut-offs on admission based on previously studied data would be relevant as well, for example, taking into account that a small proportion of adults thrive to survive, however, clinically healthy adults may have a better chance to be released. Other future challenge would

be the study of the WRCMN database focusing on other animal group species such as birds and reptiles, or possibly concentrating on endangered species, facing distinct threats and illnesses. Other suggestion would be the simulation of FFTs to aid diagnosis establishment or treatment/procedures choices.

Finally, it is essential to emphasize the great value of WRCs database maintenance, improvement and study, not only leading to a better understanding of wildlife threats and subsequent conservation actions implementation, but also to enable disease surveillance and future improvement of rescue, rehabilitation and release procedures.

Preliminary results of this study were presented on the VII FAUNA International Conference, in November 2018, at the Faculty of Veterinary Medicine, University of Lisbon. An open communication was presented (Annex 2) and a poster was submitted and accepted (Annex 3 and 4).

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Annex 1 - Chi-square test results for the associations between all admission causes and outcomes, within each treatment facility.

Admission cause	Chi-square test	
	Nursery	Clinic
Born in captivity	$\chi^2(1, N = 8,561) = 0.05$ $p = 0.82, OR = 0.80$	–
Cat attack	$\chi^2(1, N=8,561) = 61.58$ $p < 0.01, OR = 1.72$	$\chi^2(1, N = 1,928) = 5.24$ $p = 0.02, OR = 1.28$
Collision	$\chi^2(1, N = 8,561) = 1.72$ $p = 0.19, OR = 1.27$	$\chi^2(1, N = 1,928) = 0.05$ $p = 0.48, OR = 1.24$
Confiscation	$\chi^2(1, N = 8,561) = 5.42$ $p = 0.02, OR = 2.81$	$\chi^2(1, N = 1,928) = 0.75$ $p = 0.39, OR = NA$
Dog attack	$\chi^2(1, N = 8,561) = 4.93$ $p = 0.03, OR = 1.12$	$\chi^2(1, N = 1,928) = 0.01$ $p = 0.91, OR = 0.98$
Entrapment	$\chi^2(1, N=8,561) = 31.78$ $p < 0.01, OR = 0.43$	$\chi^2(1, N = 1,928) = 51.72$ $p < 0.01, OR = 0.29$
Environment	$\chi^2(1, N = 8,561) = 0.47$ $p = 0.49, OR = 0.69$	–
External substance contamination	$\chi^2(1, N = 8,561) = 1.60$ $p = 0.21, OR = NA$	$\chi^2(1, N = 1,928) = 0.38$ $p = 0.54, OR = NA$
Inappropriate human possession	$\chi^2(1, N=8,561) = 55.46$ $p < 0.01, OR = 0.50$	$\chi^2(1, N = 1,928) = 157.58$ $p < 0.01, OR = 0.03$
Nest/habitat destruction	$\chi^2(1, N=8,561) = 20.85$ $p < 0.01, OR = 0.66$	$\chi^2(1, N = 1,928) = 13.93$ $p < 0.01, OR = 0.17$
Non-domestic animal attack	$\chi^2(1, N = 8,561) = 3.10$ $p = 0.08, OR = 1.16$	$\chi^2(1, N = 1,928) = 7.44$ $p < 0.01, OR = 0.44$
Orphan	$\chi^2(1, N = 8,561) = 1.91$ $p = 0.17, OR = 1.08$	–
Projectile	$\chi^2(1, N = 8,561) = NA$ $p = NA, OR = NA$	$\chi^2(1, N = 1,928) = 0.60$ $p = 0.43, OR = 2.26$
Referral	$\chi^2(1, N = 8,561) = 5.13$ $p = 0.02, OR = 0.20$	$\chi^2(1, N = 1,928) = 2.66$ $p = 0.10, OR = NA$
Undetermined	$\chi^2(1, N = 8,561) = 0.05$ $p = 0.82, OR = 0.92$	$\chi^2(1, N = 1,928) = 59.87$ $p < 0.01, OR = 2.37$

NA – Not applicable.

Annex 2 – Open communication abstract, submitted and presented on the VII Fauna International Conference, November 10th, 2018.

Title: Decision trees application as triage support in wildlife rehabilitation: the example of the eastern cottontail rabbit (*Sylvilagus floridanus*) in the Wildlife Rehabilitation Center of Minnesota.

Authors: Santos, R.¹, Schott, R.², Nunes, T.¹, Madeira de Carvalho, L.¹ & Reed, L.².

1. CIISA – Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon;
2. Wildlife Rehabilitation Center of Minnesota, nonprofit organization, supported only by private donations.

Introduction: The eastern cottontail rabbit (*Sylvilagus floridanus*) is one of the most frequently admitted species throughout United States rehabilitation facilities and its recovery in captivity is considered a challenging process. About one quarter of the annual admissions at the Wildlife Rehabilitation Center of Minnesota (WRCMN) has been comprised by this species.

Fast and Frugal Trees (FFT) are supervised learning algorithms that allow to make fast and accurate binary classification decisions, based on limited cues of information in a specific, sequential order. A decision tree is non-compensatory, meaning that it uses just a partial subset of all cue information: once a decision is taken based on some subset of the existing information, no additional information is considered or can change the decision. The areas of biomedical and health care are an example of its successful application, in which decision-making is of major importance (e.g. diagnosis process, treatment options selection, prognosis prediction).

Material and Methods: FFTs were studied as a possible support for *S. floridanus* triage, based on age, body condition, body weight, all admission causes and selected clinical signs presented on arrival, in order to predict the respective outcome (dead before release or released), concerning data comprising eastern cottontails' admissions into the WRCMN from 2011 to 2017.

Results and Discussion: Infants and unweaned juveniles (nursery facility) presenting 75g or less of body weight on admission were predicted to die before release; otherwise, the presence of clinical signs such as “clinically healthy” and “trauma” were considered in order to predict the outcome. Lastly, if none of the referred criterion was verified and if the cottontail weighted more than 95g, its release was predicted.

More mature cottontails (admitted into the clinic facility) presenting less than 188g were predicted to be released; if not, the presentation of admission causes such as “undetermined” and “cat attack” were examined to develop the decision tree; otherwise, neurological signs with brain origin were focused – when present, it was predicted that the rabbit would die before release.

Conclusion: The identification of factors on arrival associated with a better prognosis and subsequent release, based on FFTs, may support the triage process and resources management, which are commonly scarce in the wildlife rehabilitation field, enabling the improvement on animals' welfare and stress minimization as well.

Keywords: eastern cottontail rabbit, wildlife rehabilitation, triage, fast and frugal trees, Minnesota, USA.

Annex 3 – Poster abstract, submitted and presented on the VII Fauna International Conference, November 9-11th, 2018.

Title: Eastern cottontail rabbit (*Sylvilagus floridanus*) admission causes, corresponding outcomes and period in treatment at the Wildlife Rehabilitation Center of Minnesota: a retrospective study from 2011 to 2017.

Authors: Santos, R.¹, Schott, R.², Nunes, T.¹, Madeira de Carvalho, L.¹ & Reed, L.²

1. CIISA – Center for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon;

2. Wildlife Rehabilitation Center of Minnesota, nonprofit organization, supported only by private donations.

Introduction: The eastern cottontail rabbit (*Sylvilagus floridanus*) is one of the most frequently admitted species throughout United States wildlife rehabilitation centers (WRCs).

Material and Methods: Data comprising eastern cottontails' admitted into the WRC of Minnesota from 2011 to 2017 was examined to determine which the most relevant admission causes were and the respective outcomes.

Period in treatment (PT) or length of stay at the WRC, an important estimator of daily costs and animal welfare, was also examined regarding the main admission causes.

Results and Discussion: Leading admission causes were linked with domestic animals interactions (37%), followed by “undetermined causes” (32%) and “orphaned” cottontails (15%).

“Euthanized on arrival” was the main overall outcome, whilst 23% of all cottontails were released.

Concerning the PT, admission causes such as “cat attack” and “collision” were associated with more extended lengths of stay for cottontails that were subsequently released.

Conclusion: This study highlights the importance of public education and the establishment of measures to avoid anthropogenic interference in wildlife casualties, which were preponderant in cottontails' admissions. Furthermore, the importance of outcomes and PT study is presented, regarding animal welfare prevention and more efficient resources management.

Keywords: eastern cottontail rabbit, wildlife rehabilitation, outcomes, Minnesota, USA.

Eastern cottontail rabbit (*Sylvilagus floridanus*) admission causes, corresponding outcomes and period in treatment at the Wildlife Rehabilitation Center of Minnesota: a retrospective study from 2011 to 2017

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1. BACKGROUND

The eastern cottontail rabbit (*Sylvilagus floridanus*) is one of the most frequently admitted species throughout United States rehabilitation facilities (Loyd, Hernandez & McRuer, 2017) (Figure 1). About one quarter of the annual admissions at the Wildlife Rehabilitation Center of Minnesota (WRCMN) has been comprised by this species.

Successful cottontail rabbit rehabilitation is considered a challenging process (Bewig & Mitchell, 2009; Cowen, 2016; Schott, 2017) and little is known about cottontails release proportions. Oberly (2015) reported that 34% of the overall eastern cottontails admitted at the Ohio Wildlife Center, during 2014, were released.

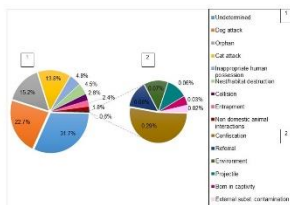
Data regarding eastern cottontails' admissions at the WRCMN between 2011 and 2017 was examined in order to determine which the most relevant admission causes were and the respective outcomes. The period in treatment (PT) or length of stay, an important estimator of daily costs and animal welfare (Molina-López, Mañosa, Torres-Riera, Pomarol & Darwich, 2017), was also examined.



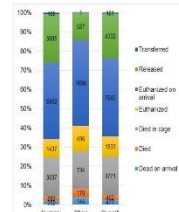
Figure 1: Leading admission causes of the eastern cottontail reported in the literature (Bewig & Mitchell, 2009; Loyd et al., 2017; Schott, 2017), namely: domestic animal attack (1), collision with vehicles (2), nest destruction or disturbance (3) and healthy younglets, frequently misidentified as orphans (4).

2. METHODOLOGY

All eastern cottontail rabbit admissions from 2011 to 2017 (18,985 cases) were exported from the WRCMN database (FileMaker ® Pro) to Microsoft Excel spreadsheets for further data analysis, besides tables and graphs assembly. 3,926 individuals were admitted into the clinic facility (including weaned juveniles and adults) and 15,059 into the mammal nursery (infants and unweaned juveniles). Median (*Mdn*) and interquartile interval (*IQR*) were designed to study the PT at the WRCMN; two-sample Wilcoxon test was chosen to assess the difference between median days in treatment for each outcome, based on The R Project for Statistical Computing.



Graph 1: Admission causes proportions for the overall eastern cottontail population admitted into the WRCMN from 2011 to 2017.



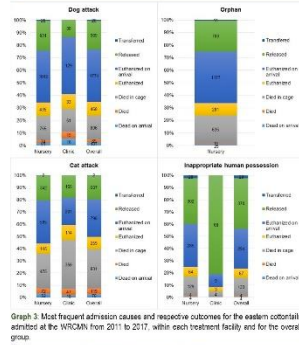
Graph 2: Outcomes for each treatment facility and for the overall eastern cottontail population admitted into the WRCMN from 2011 to 2017.

3. RESULTS AND DISCUSSION

It was verified that the leading admission causes were linked with domestic animals interactions, according to previous results in other wildlife rehabilitation centers, reporting the major impact of cats and dogs attacks, especially concerning the studied species (Loyd et al., 2017) (Graph 1). At least 42% of all the admissions had direct or indirect anthropogenic influence.

The majority of the cottontails were humanely euthanized on admission (41%) and the overall release proportion in the studied period was approximately 23% (Graph 2). Admission causes such as "collision" (13%), "cat attack" (20%) and "dog attack" (23%) were linked with lower release proportions, oppositely to "entrapment" (44%), "inappropriate human possession" (42%) and "nest/habitat destruction" (34%), which were associated with a better prognosis.

Overall, the median PT was significantly wider in released cottontails, compared with the ones which died or were euthanized ($p < 0.01$) (Table 1). Orphaned cottontails, animals which had interacted with cats or presented "collision" as primary admission cause had the longest rehabilitation periods (Figure 2).



Graph 3: Most frequent admission causes and respective outcomes for the eastern cottontails admitted at the WRCMN from 2011 to 2017, within each treatment facility and for the overall group.

Treatment ward	Period in treatment (2nd before release) (days)	Period in treatment (Released) (days)	Wilcoxon rank-sum test
Nursery	1(2 - 16)	12(2 - 10)	$p < 0.0001$
Clinic	2(2 - 3)	12(2 - 22)	$p < 0.01$

Table 1: Period in treatment expressed in days - *Mdn* (IQR) - for each treatment ward and respective outcomes, regarding eastern cottontails admitted at the WRCMN from 2011 to 2017, with Wilcoxon rank-sum test results presented.

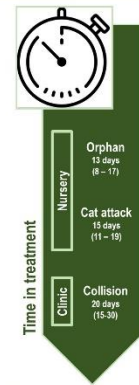


Figure 2: Representation of the most time-consuming admission causes, within the most frequently registered intake categories. Median PT and respective interquartile range - *Mdn* (IQR) - expressed in days. Image source: <https://www.shutterstock.com/file-clipboard/59584629?usqp=ur>

4. CONCLUSIONS

This study reinforces the importance of public education and urgent establishment of measures to avoid anthropogenic interference in wildlife casualties, preponderant in the eastern cottontail admission causes. Furthermore, it highlights the importance of outcomes and PT examination in wildlife rehabilitation, regarding animal welfare prevention and more efficient resources management.

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