UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO DOURO

NECROPSY PROCEDURES IN VETERINARY FORENSIC:

IDENTIFICATION OF CRITICAL POINTS

Dissertação de Mestrado Integrado em Medicina Veterinária

VANESSA CARINA MENDONÇA DELGADO

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DESIGNAÇÃO DO MESTRADO: MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

TÍTULO DA DISSERTAÇÃO DE MESTRADO EM MEDICINA VETERINÁRIA:

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ANO CONCLUSÃO: 2018

DECLARO QUE ESTA DISSERTAÇÃO DE MESTRADO É RESULTADO DA MINHA PESQUISA E TRABALHO PESSOAL E DAS ORIENTAÇÕES DOS MEUS SUPERVISORES. O SEU CONTEÚDO É ORIGINAL E TODAS AS FONTES CONSULTADAS ESTÃO DEVIDAMENTE MENCIONADAS NO TEXTO, E NA BIBLIOGRAFIA FINAL. DECLARO AINDA QUE ESTE TRABALHO NÃO FOI APRESENTADO EM NENHUMA OUTRA INSTITUIÇÃO PARA OBTENÇÃO DE QUALQUER GRAU ACADÉMICO.

VILA REAL, 25 DE OUTUBRO DE 2018

VANESSA CARINA MENDONÇA DELGADO

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RESUMO

A medicina veterinária forense é definida como o uso do conhecimento dos ramos da medicina veterinária para resolver casos criminais e/ou de responsabilidade civil, pela aplicação da lei. Os métodos forenses são aplicáveis a situações fora dos tribunais, como reclamações de seguro, comissões de serviço público, para defender ou apresentar alegações de má conduta profissional ou outras medidas disciplinares, entre outros. Apesar da definição de medicina veterinária forense parecer limitada, a verdade é que a maioria dos casos forenses carecem do conhecimento de outras ciências para além da área da veterinária; uma equipa forense deve ser multidisciplinar e ter um contacto próximo com outras ciências, como a balística, entomologia, genética, e muitas outras. Esta é uma área em fase de crescimento rápido, o que provavelmente se deve ao aumento das leis relacionadas com o bem-estar animal, aumentando assim a tendência para que as pessoas exijam compensações em questões relacionadas com morte e agressões dos seus animais. A patologia forense é um ramo da medicina veterinária forense, em que os seus principais objetivos são documentar, analisar e explicar os achados patológicos de um caso, de forma acessível para aqueles que irão ler o relatório, que presumivelmente não serão da área da medicina, mas sim da lei (juristas, advogados, etc.). A necrópsia forense é, por sua vez, uma subdivisão da patologia forense e é um dos principais componentes em investigações de mortes.

Este trabalho compreende uma revisão bibliográfica dos procedimentos de necrópsia forense e a descrição de três casos de morte violenta não acidental (causa legal da morte), em anonimato, com as seguintes causas de morte: Provável afogamento de dois canídeos, com tentativa de suicídio do seu detentor; (2) Estrangulamento de um canídeo; (3) Disparo de projétil a dois bovinos. Através do seguimento dos diversos casos forenses durante o período de estágio, em conjunto com a revisão bibliográfica, foi elaborada a identificação de pontos críticos nos procedimentos de necrópsia em veterinária forense, que se centram na especialização académica do veterinário patologista; no seu conhecimento jurídico; no exame do local do crime; na manipulação das provas; nos próprios procedimentos de necrópsia; na redação do relatório; na biossegurança; e na cadeia de custódia.

Palavras-chave: Pontos críticos; necrópsia forense; patologia forense; medicina veterinária forense; lei

ABSTRACT

Forensic veterinary medicine is defined as the use of the knowledge of veterinary medicine branches to solve cases of civil and/or criminal liability, supported by the application of the law. Forensic methods are largely applicable to situations outside the courts, such as insurance claims, public service commissions, to defend or propound allegations of professional misconduct or other disciplinary measures, and others. Despite the definition of forensic veterinary medicine seems limited, the true is that most of the forensic veterinary cases require recourse to others sciences knowledge beyond the veterinary area; a forensic veterinary team should be multidisciplinary as well as it should sustain an intimate contact with other departments/sciences such as ballistics, entomology, genetics, and many others. It is a rapidly growing field probably due to the increasing of legislation relating to animal welfare, accruing the tendency for animal owners to seek compensation in matters regarding death and injuries. The forensic pathology is a branch of the forensic veterinary medicine, and its main objectives are to document, analyse, and elucidate the pathological findings of a case in a comprehensible way for those who will read the report, which probably are non-medical people, but people related to the law (jurists, lawyers, etc.). The forensic necropsy is, in its turn, a subdivision of forensic pathology and it is a main component in deaths investigations.

This work comprehends a literature review of the forensic necropsy procedures and the description of three cases of non-accidental violent death (legal cause of death), in anonymity, with the following causes of death: (1) Probable drowning of two canids, with attempted suicide of their caretaker; (2) Strangulation of a canid; (3) Forearm shooting on two cattle. Through following-up the forensic cases presented on the internship, along with the literature review, it was elaborated the identification of critical points regarding to necropsy procedures in veterinary forensic, which focus on the academic specialization of the veterinary pathologist; his/her legal knowledge; the crime scene examination; the evidence handling; the necropsy procedures itself; the report writing; the biosafety; and the chain of evidence.

Keywords: Critical points; forensic necropsy; forensic pathology; forensic veterinary medicine; law

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ABBREVIATIONS, SYMBOLS AND ACRONYMS

3D: Three-dimension

AAN: Animal abuse and neglect

ACVP: American College of Veterinary Pathology

ALS: alternate light source

BCS: Body condition score

BPA: Blood stain pattern analysis

C.L.: Charles Louis

COD: Cause of death

CoE: Council of Europe

CT: Computed tomography

DL: Decree-Law

DNA: Deoxyribonucleic acid

EU: European Union

FVP: Forensic veterinary pathologist

GNR: Guarda Nacional Republicana

INIAV: Instituto Nacional de Investigação Agrária e Veterinária

LCOD: Legal cause of death

MOD: Mechanism of death

MSCT: Multislice computed tomography

NUIPC: Unique process identification number crime / Número único de identificação do processo crime

PMCT: *Post-mortem* computed tomography

PMCTA: *Post-mortem* computed tomography angiography

PMMRI: *Post-mortem* magnetic resonance

SIRCA: Sistema de Recolha de Cadáveres de Animais Mortos na Exploração / Portuguese system of death farm animals collection

SPPA: Sociedade Portuguesa de patologia animal / Portuguese society of animal pathology

WSAVA: World small animal veterinary association

CHAPTER I - LITERATURE REVIEW

Publications related with the subject "Forensic veterinary medicine" were searched in "PubMed" and "ScienceDirect", with the keywords "Forensic veterinary", "Forensic necropsy", "Chain of custody" and "Forensic veterinary pathology".

1. Forensic Veterinary Medicine

1.1. Definition

Forensic veterinary medicine can be summarily defined as "the application of veterinary knowledge to the purpose of the law" (J. Cooper, 1998, p. 161); this means, using the knowledge of the several veterinary medicine branches to solve cases of civil and/or criminal liability, supported by application of the law (Peleteiro *et al.*, 2016). Since this definition is very general, and it can be applied to civil cases *per se*, some authors prefer to name it as legal veterinary medicine rather than forensic veterinary medicine, as the last usually has a direct connection with crime. The studying of the origin of the word forensic is very important to comprehend its generality. It has derived from the Latin word '*forensic'* which means 'public', that in turn was derived from '*forum'* - a place of debate usually in the public arena of court or other legal proceedings that happens in open doors (J. Cooper & M. Cooper, 2007); and it is defined by the concise Oxford dictionary as "relating to, used in, or connected with a court of law" (J. Cooper & M. Cooper, 2007, p. 3).

Furthermore, forensic - originally meaning 'relating to the law'- has broadened even more the generality of the word; it is nowadays implied to be "a detailed investigation and collection of evidence regardless of whether or not there is a specific legal case or enquiry pending" (p. x), so the methods used in conventional forensic work can now be largely applied in situations outside the courts, such as insurance claims, inquiries, environmental impact assessments, public service commissions or when defending or propounding allegations of professional misconduct or other disciplinary measures (J. Cooper & M. Cooper, 2007). In addition, there is an increasing demand by the animal owners to seek compensation in matters regarding death and injuries, especially about deaths, which often triggers an investigation that may lead to a

legal case, disagreements over an insurance claim or allegations of professional malpractice (J. Cooper & M. Cooper, 2007).

Veterinary forensic medicine is not only restricted to animal deaths, as the growth of this science may have an increased acknowledgment in some human forensic cases, as it is already described by Tsokos, Byard, and Puschel (2007), which an human victim succumbed to a dog attack, and veterinary studies assisted on the human investigation, as a necropsy of the offending animal provided information that helped to establish the identity and ownership of the animal, as well as trace evidence linked the dog to the victim; other-like case has been described by Aquila *et al.*, 2014, (p. e1) about "a case study concerning a car accident where both humans and pets were involved; where investigation and reconstruction of the crime scene were conducted by a team consisting of forensic pathologists and forensic veterinarians". Both an autopsy and a necropsy were conducted on the man and the dog, respectively, and the results were compared, and the information was used to reconstruct the collision. "This unusual case was solved through the collaboration between forensic pathology and veterinary forensic medicine, emphasising the importance of this kind of co-operation to solve a crime scene concerning both humans and animals". Therefore, we should be aware that forensic veterinary deals with cases of animal abuse and smuggling, but also with the role of animals in court cases involving people (Ottinger *et al.*, 2014).

After all, "the main objectives of forensic medicine are to document, analyse, and elucidate scientific medical findings in a comprehensible way for courtroom presentation", regardless the type of case (Dirnhofer, Jackowski, Vock, Potter, & Thali, 2006, p. 1306). For instance, we should have in mind that an animal can be witness, victim or perpetrator in a forensic case (J. Cooper & M. Cooper, 2008). When being the perpetrator, the animal can injury the human in a variety of ways such as bites, stings, electrocution, transmission of infectious agents or presentation of allergens - and veterinarians are often called to help understand the case (J. Cooper & M. Cooper, 2008).

1.2. State of the art

Forensic veterinary medicine is not yet a recognized discipline but is rapidly evolving (J. Cooper & M. Cooper, 1998); after all, according to McEwen (2012) as cited in Gerdin and

McDonough (2013): "cases involving insured animals, possible medical malpractice, and alleged animal abuse and neglect are not new; new is the increasing amount of media coverage and degree of public awareness of these cases" (p. 994); mainly due to the changes in laws and their enforcements and because of the public concern about the health, welfare, and conservation of animals, both domesticated and wild, more legal cases relating to such issues are being taken to the courts and a higher standard of expert evidence is expected, becoming widely recognized the need for veterinarians to be more actively involved in forensic work (J. Cooper & M. Cooper, 2008; McEwen, 2012).

Listos, Gryzinska, and Kowalczyk (2015) in their review '*Analysis of cases of forensic veterinary opinions produced in a research and teaching unit*', observed an increase in the demand for the services of veterinary forensic experts at the Department of Pathological Anatomy, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, beginning in 2006 and persisting through 2014 (H. Munro & R. Munro, 2013). Also, recently, Gerdin and McDonough (2013) affirmed the frequent increasing of cases of suspected animal abuse and neglect submitted to veterinary pathologists, as well as Ottinger *et al.* (2014) quoted McEwen (2012) and Salvagni *et al.* (2012), whose reports showed that the number of medico-legal veterinary pathology cases had increased. For instance, Portugal has been witnessing, since 2014, the creation of legal norms designed to protect the animals, either by criminalizing the abuse and neglect of companion animals or by officially acknowledging their status as sentient beings (Moreira, 2017). Harris (1998), declared: "we live in a time in which society is very prone to litigation" (p. 1), and therein lies exciting possibilities for the expansion and refinement of the forensic veterinary pathology knowledge base (H. Munro & R. Munro, 2013).

J. Cooper & M. Cooper at '*Future trends in forensic veterinary medicine'* (1998), early suggested that "one of the main elements that hampers the development of veterinary forensic science and its acceptance as a specialty is the absence of standard systems and protocols" (p. 213), and they recommended the establishment of protocols that would serve the veterinarian well when dealing with forensic cases.

The non-recognized path to forensic veterinary specialization diverges on the growing demand of the forensic veterinary services, and those veterinarians who become involved in this field are usually self-taught or, in a few cases, have attained a qualification based on training for

human medicine (J. Cooper, 1998). Also, the increasing of legislation relating to animal welfare and conservation, makes the demand for specialists higher, thus the continued absence of such a specialty is surprising (J. Cooper, 1998). In Portugal, however, some Universities have already in the Veterinary Medicine course, some subjects of the forensic field. This situation is in stark contrast to human forensic medicine, which is a recognised independent discipline with training opportunities and certified specialists with full-time employment (Ottinger *et al.*, 2014). And while human forensic medicine is a well-recognized and highly developed speciality, the forensic veterinary medicine is a relatively young science (J. Cooper, 1998; Listos *et al.*, 2015). So, much can be gained studying the procedures, methods, and standards used in human forensic cases, especially establishing and using the systems and protocols in a similar way to those used in human forensic medicine, but wisely, because extrapolation from human work of research in the field of forensic science, although necessary, is not always satisfactory (J. Cooper & M. Cooper, 1998; R. Munro, 1998). However, more than grounding the veterinary forensics on the human forensic knowledges, it shall be, instead, linked both areas of practice, as the opportunity to foster collaboration may be seized upon (Byard & Boardman, 2011).

As mentioned before, the growth of the veterinary forensic sciences creates a need to educate and teach and to ensure that the training offered is of a satisfactory standard and objectively assessed and accredited by an independent body (J. Cooper & M. Cooper, 2007); to overcome this, forensic pathologists can contribute in lecturing to veterinary science students on basic injury assessment and evaluation (Byard & Boardman, 2011). The raising number of textbooks and journal articles covering domestic animal and wildlife forensic investigations as well as forensic veterinary lectures are a modern strategic – academic education like the international veterinary forensic science association in 2008, the several presentations in the field of veterinary forensic pathology at national and international meetings at 2011 (such as the C. L. Davis/ACVP symposium on forensic veterinary pathology; the American academy of forensic sciences conference, or the European veterinary conference Voorjaarsdagen (Holland)); and the increasing submissions of forensic cases to veterinary pathology facilities as result of investigations of animal-related crime, are happening world-wide (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013; H. Munro & R. Munro, 2013; Ottinger *et al.*, 2014). Along with that is Portugal, where in 2017 only, had one theoretical-practical course on veterinary forensic pathology by the Portuguese society of animal pathology (SPPA) and one forensic formation of two days by a Portuguese veterinary university.

Essentially, it can be assumed that more and more people care about animal welfare, leading to a greater public concern about a fair and accurate prosecution of the animal cruelty perpetrators (McDonough, Gerdin , Wuenschmann , McEwen , & Brooks, 2015). However this demand is not always linked with society empathy towards animals, but with other factors and various public concerns that are helping to mould a new approach to forensic medicine, such as the recognition of the link between cruelty to animals and violence toward humans, and that animal abuse is often one of the indicators of family violence and child abuse (Balkin, Janssen, & Merck, 2012; J. Cooper & M. Cooper, 2007). McDonough *et al.* (2015) alerted that "more than 70% of battered women who own pets report that their batterer threatened, injured, or killed family pets as a form of revenge or to psychologically control the victim" (p. 5). It is also alarming that multiple studies showed that more than 60% of violent adult offenders have a history of childhood animal abuse (Ascione, 2001). Therefore, the law enforcement community has becoming to recognize that early intervention in animal cruelty cases has a positive and proactive impact on public safety and human welfare (Balkin *et al.*, 2012).

Allen, Gallagher, and Jones (2006) were vigilant about an underestimate collateral fact from domestic violence, that was an important factor to lead the women victim to stay longer under the condition of domestic violence, that was the lack of protection of victim's companion animals at refuges in Ireland, where neither were provided facilities for animal care nor was there an animal foster service, for instance, similar as those operated in the UK by Paws for Kids and First Strike. These types of facilities are starting to appear in certain developed countries as a recognition of the importance of safeguarding the victim's companion animals in the decision of refugeeing; however, in Portugal, there is not yet such services.

1.3. The role of the forensic veterinary pathologist

Forensic veterinary pathology is a diverse discipline, included under the term 'forensic science', that is on an early phase of its development. It walks by side with other forensics sciences, such as crime scene examination, ballistics tests, genetics analysis, toxicology and others that will be described later (H. Munro & R. Munro, 2013). However, in contrast with some forensic sciences, forensic pathology still utilises the time-old, evidence-based methods introduced centuries ago, like the dissection of the cadaver (necropsy), cytology and histopathology and the oral/written description (Bolliger *et al.*, 2008); nevertheless, forensic cases requires

modification of *post-mortem* procedures and written reports compared to those of routine diagnostic cases, as the questions imposed are lightly different, specially since the questions come mostly from the court and are basically to determine the cause, mechanism, manner and time of death and injuries (Gerdin & McDonough, 2013); thus, the duties of the forensic pathologist are to "collect evidence from the body, document injuries or lack of them, deduce how the injuries occurred, (…), determine or exclude other contributory or causative factors to the death, and provide expert testimony if the case goes to trial" (D. DiMaio & V. DiMaio, 2001). The veterinary pathologists play a crucial role in such cases as the identification of lesions and their accurate description and photographic documentation are the key to criminal death investigations (J. Cooper & M. Cooper, 2008; de Siqueira, Cuevas, Salvagni, & Maiorka, 2016; Gerdin & McDonough, 2013; McEwen, 2012).

The veterinary forensic pathologist is a neutral observer, making careful notes of the events leading up to death, the location and position of the animal, environmental considerations, and overall preservation and condition of the animal - specially about the internal normal and abnormal tissues – and collecting samples to perform relevant tests for the case, because, after all, and quoting Brownlie and R. Munro (2016), "the veterinary pathologist is required to act in an independent, objective, and unbiased manner in forensic investigations" (p. 919); and his/her role is "not to pass judgment but to document, interpret, and explain the pathological findings to the investigators and ultimately to the court, thereby assisting the court to reach a decision on the case" (p. 919).

As a matter of fact, veterinarians have for long played a part in legal cases, especially those relating to such matters as the purchase and sale of horses and other livestock, animal welfare, and food hygiene, but nowadays is expected to have a forensic veterinary pathologist, this is, a denote trained and certified veterinary pathologist who have additional forensic qualifications or who have documented, relevant experience in forensic practice to handle forensic cases, since he/she must have a systematic and meticulous approach and should be prepared to present and defend evidence in court (Brownlie & R. Munro, 2016; J. Cooper & M. Cooper, 2008). Although formal training is a part of the necessary specialization, sometimes the courts may place most weight on the experience that the veterinarian has in the field directly related to the case (R. Munro, 1998). Nevertheless, any veterinary practitioner may become involved in a

legal case in countless ways, whether being a "witness of fact", a "professional witness" or an "expert witness" (J. Cooper, 1998).

It is important to note that the veterinary pathologist do not have to accept a forensic case; the forensic necropsy is an intensive labour and requires meticulous documentation and strict maintenance of the chain of custody, thus if the forensic veterinary pathologist (FVP) are not sure if he/she has the time or if it is convenient for him/her, must do not accept the case (McDonough *et al.*, 2015). Another reason for declining a forensic case is the mistaken belief that the veterinary pathologist must determine if a crime was committed; but it should be comprehend that cannot be asked to the FVP if a crime was committed, since animal cruelty is a legal, not medical, determination, therefore, stablishing if acts are criminal is the duty of the members of the court (Benetato, Reisman, & McCobb, 2011; Gerdin & McDonough, 2013; McDonough *et al.*, 2015). The common type of questions made to the forensic veterinary pathologist are to confirm whether suspected inflicted injuries can be confirmed as such, and how the injury may have been caused, or if an injury is consistent with an alleged incident or with the account of the accused, or to confirm if the injuries were sustained *ante-mortem*, and for how long might the animal have survived after the injury and, often the crux of a case, if it was suffering involved. On more specific situations, the questions may be related to if the animal was already dead when thrown into water, or if there was evidence to suggest burial when still alive, or if the animal die in the fire, or if it is possible to estimate the duration of deprivation of food or water (Brownlie & R. Munro, 2016).

As seen before, forensic veterinary pathology is guided in many ways on the knowledge of human forensic pathology, since the pathologic changes in animals are closely parallel to those in humans, whether are electrothermal burns, toxicities or gunshot wounds, the effects on the body of both *Homo sapiens* and *Canis lupus* are similar (Viner, 2016). One of the major differences in human and veterinary forensic pathology is the sheer number of species of animals and birds that the veterinarian might be asked to examine, unlike in human pathology; but is an impossible expectation to be knowledgeable about all of them, thus is important and humbled to establish contacts with specialists, who can provide advice on species identification, feeding habits, anatomy, diseases, etc. (H. Munro & R. Munro, 2008).

1.4. Other forensic sciences

The increasingly widespread use of the term ‘forensic science’ is a reminder that “it is an interdisciplinary subject, par excellence”, where numerous fields such as toxicology, ballistics, entomology (a ramification of parasitology), genetics, anatomopathology, bacteriology, virology, as well as anthropology, biology, botanic, palynology, blood stain pattern analysis (BPA), alternative light source (ALS), as well as justice, bioethics, and many others, find themselves (J. Cooper, 1998; Lima, Ochôa, & Orge, 2016; Peleteiro *et al.*, 2016). There is an excellent citation from J. Cooper and M. Cooper (2007) about this subject showing the immensity of the forensic sciences: “We speak now of ‘forensic scientists’ who, in turn, describe themselves as ‘forensic chemists’, ‘forensic botanists’ or ‘forensic biologists’, and so on” (p. 5).

It is important to recognize when there is a need to resort to other forensic professionals, because all the veterinary forensic cases involve a multidisciplinary approach, that may lead to take assistance from other specialists, and perhaps specialists from human forensic (Touroo & Fitch, 2016). J. Cooper and M. Cooper (2007) wrote that “working with people from other backgrounds is often the key to fruitful investigation and production of sound evidence” (p. 36).

Would be unrealistic extensive describing all the forensic sciences possible to exist. There are some more commonly used nowadays at veterinary forensic, and others, on the other side, that barely take place at the daily veterinary forensic, so the intent here is to summarily describe the ones more common, as well as, in contrast, present the ones innovators in the field.

For instance, in cases of death by killing, blood is the most common body fluid found at crime scenes, and **blood stain pattern analysis** (BPA) may indicate the angle and distance of the blood fall, helping to solve some cases (de Siqueira *et al.*, 2016).

One science regularly used by the forensic pathologists is **toxicology**. Occasionally, the cause and manner of death suspected at the necropsy table is completely changed by the toxicology data (Gill, 2005). The toxic panel asked by the pathologist should be synthetize to the most likely suspicions. And in the end, even though pathologists need the toxicology results for the death investigation, they are the expert ones in interpreting the results in the setting on an entire

case, meaning that the toxicologists give to the forensic pathologists information but not conclusions (Gill, 2005).

The determination of the time of the death or *post-mortem* interval is a major topic in forensic pathology as well it is in forensic **entomology**. The entomologic method is based on the correlation between the developmental stages of arthropods (especially of blowfly larvae) and the time of the death; the major advantage of entomology against the pathological standard methods for the determination of the *post-mortem* interval (body temperature, *post-mortem* lividity and rigidity, and chemical investigations) is that arthropods can represent an accurate measure even in later stages of the *post-mortem* interval when the classical forensic pathological methods fail, due to the advance of putrefaction (Benecke, 2005). But, entomology is much more; it can also give information about the geographic localization of a cadaver - insects that live in restricted areas but are found on a cadaver in a different area can prove that the body had been moved after death – or give toxicological information – drugs that cannot be detected in severely decomposed tissue of a corpse may still be found in the insects that fed on the corpse – or, for instances, about the site of injuries - the location of a stab wound can be determined by unusual feeding sites of beetles and maggots (Benecke, 2005). So, it is easy to perceive that a forensic science team should have a consulting entomologist (a biologist who studies insects) with forensic specialization as a member, and such a specialist should be consulted for the accurate identification and interpretation of insect evidence (Castner, 2009). Although identification of the types and stages of maggots and beetles is outside the competence of most veterinary pathologists, the correct procedures for the collection of entomological evidence must be an inherent skill of those (Byrd, Lord, Wallace, & Tomberlin, 2010; H. Munro & R. Munro, 2013). Forensic entomology can be very valuable for numerous veterinary forensic cases and in Portugal there are some forensic pathology facilities where it is already a common practice.

In the field of **genetics**, **DNA research** for forensic identification prospers at human forensic medicine (Kondo, 2007). Animal DNA results have also been used successfully in cases that come to court. Animals live in close contact with humans and animal-derived trace and DNA evidence is often found at a crime scene or on a suspect (Merck & LeCouteur, 2012a). The application of DNA analysis has a huge potential in cases involving companion animals, and when possible has been used to tie suspects to crime scenes or specific dogs to specific bites, or to identify stolen dogs, among others. Animal DNA also can link a suspect with a crime

scene or victim, being the animal a witness. Transfer of DNA from hair, saliva, blood, urine or faeces will occur during a crime, either from the victim's pet to the suspect or crime scene, or from the suspect's pet to the victim or crime scene, as dictated by Locard's exchange principle, and can be found on wounds, clothing, or property (Gerdin & McDonough, 2013). Likewise, the DNA test take a major importance in animal attacks, at identifying the correct attacker, and preventing innocent animals from being euthanised for aggressive behaviour (Merck & LeCouteur, 2012a). However, we must be realistic and be aware of the different economic power of the countries; in Portugal this field is economic unavailable for veterinary forensic pathology.

An uncommon, or not so known, forensic sciences are the **forensic botany and palynology**. **Forensic botany** refers to the forensic analysis of the plant anatomy, plant growth and behaviour, plant reproductive cycles and population dynamics, and plant classification schemes to species identification (Merck & LeCouteur, 2012a). Plant matter may be found in stomach contents or faeces, and on fur or clothing, and can or cannot be associated with poisoning, and also may be found around a body or area from a weapon (Merck & LeCouteur, 2012a). Plant-derived evidence can be linked to specific locations and certain seasons, which can be useful to verify an alibi, track movements of the suspect or victim, aid in determination of time of death or determine the primary crime scene in cases in which there is a secondary body dump site (Merck & LeCouteur, 2012a).

Forensic palynology is the study of pollen, spores, and other acid-resistant microscopic plant bodies (palynomorphs), and it is valuable because of the microscopic size of the organisms, their large production and high resistance to decay and also because they can be identified in a plant taxon (Milne, Bryant Jr., & Mildenhall, 2005). Pollen is often present on a body or object of a crime scene due to their ubiquitous nature - they can be found on shoes, clothing, fur, rope, or carpet, and inside the nasal cavity or upper airways when inhaled (Merck & LeCouteur, 2012a). Submerged water plants also rely on pollination to reproduce, leading to be a valued evidence in suspicious cases of drowning (Merck & LeCouteur, 2012a). "The recognition and identification of pollen, seeds, diatoms, flowers and plant fibres sometimes play a unique part in providing 'trace' evidence in veterinary and comparative studies" (J. Cooper & M. Cooper, 2007, p. 28).

Imaging techniques take a special importance at forensic work, mainly concerning to *post-mortem* **radiography**, which is getting more and more common its performance prior to the necropsy examination. However, there are other radiological areas - ones recent on the forensic field and others quite recent as a science itself. A modern imaging technique, started as a project founded at the Institute of Legal Medicine of the University of Berne in Switzerland at the turn of the millennium is the **virtopsy** (Flach *et al.*, 2014). The term 'virtopsy' was created from the terms 'virtual' and 'autopsy', and it is based on three groundworks: "3D body surface documentation using photogrammetry-based optical surface scanning, and both multislice computed tomography (MSCT) and magnetic resonance imaging (MRI) to visualise the internal body", giving a resulting data set that contains high-resolution 3D colour-encoded documentation of the body surface and 3D volume documentation of the interior of the body (Bolliger *et al.*, 2008; Dirnhofer *et al.*, 2006).

X-rays are already routinely used in forensic cases, mainly on the detection of the foreign bodies in the cadavers, such as projectiles; but a multislice computed tomography (MSCT) has the advantage of locate with precision the topography of the foreign bodies within the body in a three-dimension (3D) manner, thus facilitating their extraction at necropsy, redirecting pathologists to dissect body parts or areas that are not routinely dissected during a standard necropsy, such as the face bones, shoulder articulation, extremities, outer pelvis, larynx and soft tissue of the back (Flach *et al.*, 2014). "Another advantage of MSCT over conventional X-rays is that MSCT can measure the radiological density" of these objects, as well as having greater precision and definition in detection and demonstration of fractures and, essentially of gas - pneumothorax and gas embolism are difficult to determine and impossible measure the amount of gas at necropsy (Bolliger *et al.*, 2008; Jackowski *et al.*, 2004). According to Jackowski *et al.* (2004), "MSCT sectional images depict the presence of gas immediately, whilst 3D reconstructions display the gas distribution in the blood vessels and the cardiac chambers, and even the precise amount may be determined" (as cited in Bolliger *et al.*, 2008, p. 276).

Although conventional X-rays are often used in forensic practice, and although PMCT and PMCTA are still not considered a quotidian essential in veterinary pathology, nowadays these techniques have gain some supporters, introducing it to improve the *post-mortem* diagnosis, by not destroying the relevant forensic findings on the necropsy (Bolliger *et al.*, 2008; Dirnhofer *et al.*, 2006; Lee *et al.*, 2011; Martinez, Hetzel, Thali, & Schweitzer, 2015; Tahli *et al.*, 2007). Even more, "the 3D reconstructions from computed tomography (CT) are especially clear and

understandable to lay observers and can be rotated on the computer screen to give a graphic view of areas of interest and angles of an attack" at court (Brownlie & R. Munro, 2016, p. 924). Both techniques - PMCT and necropsy - demonstrated injuries one complementing the other, and the inclusion of the PMCT gives important information to the final diagnosis, playing an important role to the veterinary pathology (Pinto *et al.*, 2017). As Franckenberg, Kern, Vogt, Thali and Flach (2015) acclaimed:

> Virtopsy of animals provides not only spectacular insight into the anatomy of the mammals but also aids in the evaluation of hunting-related and veterinary issues and the determination of the cause and manner of death without the need for any manual dissection. (p. 75)

Other imaging techniques, such as **endoscopic**, although not common in forensic necropsy, are already described in human forensic autopsy, as the inspection of the cavities and sinuses, like the eye ground, the external auditory meatus up to tympanic membrane and the naso- and laryngopharynx, can be done without their morphological damage is a massive advantage (Amberg, Lemmen, Pollak, Strutz, & Unsöld, 1992, cited in Ohshima, 2000, p. 154).

1.5. The legal system

Since the notion that cruelty to animals is a symptom of depravity and that the crimes against animals can go beyond the act itself and be a reflection of other types of violence, like the interpersonal, domestic violence and children or elderly abuse, "there has been a recent global increase in the enforcement of laws regarding animal care and the enactment of tougher laws regarding animal abuse and neglect", once it started to be seen by the authorities as a risk factor or a possible indicator of another type of violence, leading to collaboration between several entities with the aim of crossing information between them (Gerdin & McDonough, 2013; Moreira, 2017). On the other side, there is the emotional connection between humans and companion animals, leading to a not surprising growth on the demand of a consistent legal board to protect the last ones, approximating those to the norms applicable to humans (Moreira, 2017).

1.5.1. Governing laws

Any FVP undertaking a forensic case must be familiar with the applicable laws in his/her country, as well as any person involved in animal forensic work should have a sound background in knowledge of the law and be competent in identifying, collecting and preserving evidence, because a thorough knowledge of the relevant legislation is invaluable in the full preparation for a court appearance (J. Cooper & M. Cooper, 1998; J. Cooper & M. Cooper, 2007; Touroo & Fitch, 2016).

As known, the approach to animal forensic work differs between the countries, even within Europe, due to legal variations and different public opinions about the importance of animal welfare (Ottinger *et al.*, 2014); however the European convention for the protection of animals produced by the European Union (EU) and the Council of Europe (CoE), in 1987, comprehends a large body of regulations and directive relating to animal health, farm animal welfare, research animals, wildlife conservation and the international trade in endangered species (J. Cooper & M. Cooper, 2007). This became a law to the Member States countries, being thereby subjected to penalties if there were some failures on the implementation of the directives (J. Cooper & M. Cooper, 2007). In Portugal, this European legislation was approved and ratified in 1993 by the Decree No. 13/93 of 13 April 1993; however, only at 2001 it was become regulated, by the Decree-Law No. 276/2001 of 17 October, and its successive amendments, being the last one the Decree-Law No. 260/2012 of 12 December 2012, which, summarily, declares the prohibition of all violence against animals with the purpose of inflicting death, suffering or injury, being the offenses punishable by a fine (Moreira, 2017). Since this date, Portugal has been having successive law adjustments, for instance, the embracing of the Law No. 69/2014 of 29 August 2014, which criminalizes the abuse and abandon of companion animals, introduced on the Penal Code's Title VI "Crimes against companion animals" (Moreira, 2017). At 2015 emerge the Law No. 110/2015 of 26 August 2015 establishing the framework of accessory penalties applicable to crimes against pet animals; recently, at 2017, the amendment to the Civil Code by the Law No. 8/2017 of 3 March 2017 was introduced, which established a legal status of animals, recognizing their nature as sentient beings (Moreira, 2017).

Regardless all legislation, it should be remembered that any laws, including those of animal protection, can be effortless if not correctly applied. Each legislation is supported by powers of

implementation and enforcement, and this depends on the will of the authority but mainly on the powers provided in the legislation (J. Cooper & M. Cooper, 2007).

1.5.2. Legal description of death

The concepts of cause, mechanism and manner of death are vital to the comprehension of the death of any animal, however these concepts are usually confused between each other (Peleteiro *et al.*, 2016). In forensic pathology the accurate knowledge of each individual ones of these concepts is required since they must be clearly expounded on the necropsy report to be correctly perceived by the law related personnel who will read it (Brownlie & R. Munro, 2016).

1.5.2.1. Cause of death

The cause of death (COD) is the event that produces the physiological alteration that leads to death, this mean "any injury or disease that produces a physiological derangement in the body that results in the death of the individual" (D. DiMaio & V. DiMaio, 2001, p.; Peleteiro *et al.*, 2016); in other words is "the injury or disease that began a sequence of events that ultimately led to the death of the animal" (Merck, Miller, & Maiorka, 2012e, p. 65). Thus, a gunshot wound to the head or to the chest, a run over by a car, a stab wound to the chest, an adenocarcinoma of the lung, a coronary atherosclerosis, or a canine parvovirus are some examples of COD (D. DiMaio & V. DiMaio, 2001); denote that in most cases, euthanasia is not considered as a COD, but indeed is the injury or illness that lead to the euthanasia (Merck *et al.*, 2012e). Determining the COD is often the primary goal of the *post-mortem* investigations, however, sometimes the FVP are not entirely certain about the COD, but are not completely uncertain either, not justifying the use of the term "undetermined"; in such cases it is rather to use "probable" before the cause of death opinion (Gerdin & McDonough, 2013); Merck *et al.* (2012e) claim that this is a safeguard measure to the FVP, who is saying that has some degree of certainty, although his/her opinion may change based on any future information provided.

When stablishing the COD, a concept to keep in mind is the **contributory cause**; it refers to "any condition the animal had that could have contributed to death or injury (Merck *et al.*, 2012e, p. 65). An example is a case of multiple stab wounds in the chest, causing extensions haemorrhages leading to a hypovolemic shock, which the COD is the multiple stab, but maybe a clotting disorder would be a contributory caused for the death due to a massive

exsanguination; other example given by Merck *et al.* (2012e) is a starvation case which an emaciated animal died from hypothermia, the cause of death is the hypothermia but the contributory cause would be starvation, the cause of an severe emaciated state, that limit the ability to regulate the body temperature.

1.5.2.2. Mechanism of death

The mechanism of death (MOD) is defined by Gerdin and McDonough, (2013) as "the pathophysiologic events and pathways that precede termination of brain, heart, and/or lungs function" (p. 996); in other words, is "the physiological derangement produced by the cause of death, and that is responsible by the *exitus"* (Peleteiro *et al.*, 2016). Haemorrhages, septicaemia, and cardiac arrhythmia are some examples of different MOD (D. DiMaio & V. DiMaio, 2001). Must be noticed that a mechanism of death can be produced by multiple causes of death and *vice versa*. For instance, if an animal dies of a massive haemorrhage (MOD), this haemorrhage could have been produced by a cause of death like a gunshot wound, or a stab wound, or a malignant tumour of the lung eroding into a blood vessel, and so forth. The reverse is also true, for example, a gunshot wound of the abdomen (COD) can result in many possible mechanisms of death as haemorrhage or peritonitis (D. DiMaio & V. DiMaio, 2001); a blunt-force injury to the skull (COD) can be deathful by either due a cerebral edema or due penetration of bone fragments on the brain (Brownlie & R. Munro, 2016).

Determine the MOD can be challenging, and "in some cases, the COD may be known with certainty but the mechanism unknown" (Gerdin & McDonough, 2013, p. 996). Thus, the FVP must be kept in mind that the mechanism of death, as well, can be stated as undetermined when the necropsy findings are inconclusive (de Siqueira *et al.*, 2016).

1.5.2.3. Manner of death

The manner of death, synonymous of legal cause of death (LCOD) and medical-legal differential diagnosis, is defined as "the set of circumstances that led to the occurrence of the cause of death", giving a legal explanation of how the cause of death came about (D. DiMaio & V. DiMaio, 2001; Peleteiro *et al.*, 2016). In human forensic, the LCOD is classified in five categories but is grouped in two main ones - **natural death** (from natural causes) and **violent death** (inflicted by an external cause). In the last are included the **accidental**, **homicidal**,

suicidal or **undetermined** deaths (Gerdin & McDonough, 2013). In veterinary forensic, violent deaths only comprises three subtypes: **accidental**, **non-accidental** and **undetermined death**, especially since suicidal is not considered to occur in veterinary (Merck *et al.*, 2012e).

A **natural death** only applies to deaths caused exclusively by a disease or senescence. An **accidental death** is caused by violent means, but not due to an intentional or criminal act, whilst a **non-accidental death** refers to deaths caused by a person which may be a criminal offense under the animal cruelty laws; and an **undetermined death** means that a reasonable classification could not be made because the pathologist could not define what occurred (Merck *et al.*, 2012e).

Just as a mechanism of death can have many causes and a cause can have many mechanisms, a cause of death can also have multiple manners. An animal can die of a massive haemorrhage (MOD) due to a gunshot wound to the heart (COD), with the manner of death being homicide (someone shot the animal), accident (the weapon fell and discharged throw the animal), or undetermined (D. DiMaio & V. DiMaio, 2001).

"The necropsy findings could be directly linked or not to the understanding of how the death occurred" or sometimes the LCOD simply may remain unknow (D. DiMaio & V. DiMaio, 2001). Other times, despite unknowing with certain the COD and the MOD, the circumstances strongly suggest a criminal manner of death, e.g. if a cadaver is found within a container submerged in water, and at necropsy there's no significant lesions and no evidence of euthanasia, and in this situations, the FVP can describe an animal death to have a presumptive abuse or neglect as LCOD, however he/she should be caution of the legal implications this can have on his/her country jurisdiction (Gerdin & McDonough, 2013). Therefore, the FVP should not feel pressure about requests to provide a manner of death merely because the courts may expect one (Brownlie & R. Munro, 2016; D. DiMaio & V. DiMaio, 2001; Gerdin & McDonough, 2013).

1.6. Chain of Custody

In forensic cases, the chronological history of all the evidence has to be exhaustively documented in order to ensure the reliability and tracking of evidence used in court proceedings (Lima *et al.*, 2016); this process is the definition of chain of custody or, by other term, chain of evidence, which its maintenance is emphasized by all forensic books (R. Munro, 1998). It serves as a guarantee and prove of the integrity of the whole process to which the samples were submitted, requiring that "all persons handling or having custody of an evidence can testify that the sample that passed to the next stage of the investigation was the same sample as the one received" (Lima *et al.*, 2016; R. Munro, 1998). Therefore, the information from the field, from the laboratory, and all people who manipulated the sample, should be registered, what requires a close teamwork that involves all parts, both internal and external to the laboratory, including those responsible for sample collection or removal from the body (Lima *et al.*, 2016).

Overall, the chain of custody guarantees the control of all evidences of a case through a nominal identification of the people involved in each phase of the forensic process, discerning their responsibilities (Lima *et al.*, 2016); the first person involved in the case usually is the enforcement officer who went on the field; it is his/her responsibility to maintain custody over the animal or its carcass until it reaches to the laboratory, when it becomes the pathologist's responsibility, and the continuation of possession registration is required to allow an evidence to be acceptable, meaning that whether the live animal, the carcass, the sample or other must be physically accounted for at all times (Green, 1979; Wobeser, 1996).

1.6.1. Case folder

To maintain the chain of evidence, records from the chronological history of the sample, starting on its transportation, to the reception, storage, necropsy, collection of samples and its transfer to other services when needed, must be kept with written and photographic documentation (through logs or diagrams) of the various stages of the process and evidence (Lima *et al.*, 2016). It must be created a securely filed folder for each case, named the case log, where it should be hard copies of significant paperwork such as the cadaver receipt (signed and dated by the person delivering the body and by the recipient), case number and the original labelling of the specimen

(with a link between them), and all the significant events like the below dictated by Brownlie and R. Munro (2016), should be signed and dated in an evidence log:

> The day/date/time the bag was opened, when imaging was performed, when the necropsy started, who was present/ involved, when the examination was temporarily stopped and when it resumed, when the examination was concluded, and when and to where the remains were moved for storage. (p. 921).

1.6.1. Evidence identification

Evidence is "used to prove guilt or innocence, to identify victims, and/or to identify suspects" and it can be the animal himself or his cadaver, the samples collect from him, the photographs and videos token, the radiographs and the tests results and furthermore the case folder of the case itself (Merck *et al.*, 2012e; Touroo & Fitch, 2016). Thus, every cadaver received for a necropsy must be clearly identified and contained with well attached labels or tags at the time of submission, ideally with tamper evident technology (Wobeser, 1996). Tamper evident is anything that provides evidence of unauthorized access, alteration or replacement; it does not necessary resist tampering, but it is determined to leave evidence when someone tamper or try to tamper an evidence (Datagram, 2008). For labelling a cadaver, a braided metal cable seal (Figure 1) or a plastic one (Figure 2) is a good option, and it should be placed on the skin; after the skinning, it should be placed on the body, e.g. attached on the Achille's tendon. This work of identification becomes specially difficult to the forensic veterinary pathologist when dealing with cases involving multiple cadavers, which the maintenance of proper identifications and the continuous control over each specimens and information must be a priority, along with preservation of the chain of custody, so that everyone can testify with certainty as to their identity (R. Munro, 1998; Salvagni *et al.*, 2012; Salvagni, de Siqueira, Maria, Mesquita, & Maiorka, 2014; Salvagni *et al.*, 2016).

Figure 1: Tamper evident braided metal cable seal. Adapted from https://seals.com/

Figure 2: Tamper evident braided plastic cable seal. Adapted from https://seals.com/

The types of evidence most commonly collected at necropsy includes toxicological, entomological, firearm evidence (e.g. bullets) and biological samples, such as tissue, blood, and urine (Touroo & Fitch, 2016). “These samples should be carefully handled, to avoid allegations of adulteration or misconduct that could jeopardize decisions related to the case in question”, and when an evidence needs to be transferred (e.g. a biological sample to a laboratory), it is imperative that the veterinary forensic pathologist have knowledge about the correct way of containing it, in “such a manner that the analytical scientist receives the specimen in a sealed container, as it was packed by the pathologist”, either it was on a tamper evident container such as an plastic envelope (Figure 3) or paper envelope (Figure 4) or covered with tamper evident tape (Figure 5) or by other tamper evident technology (J. Cooper & M. Cooper, 1998; Lima *et al.*, 2016; Wobeser, 1996).

Figure 3: Tamper evident container of plastic envelope. Adapted from https://www.henryschein.com/us-en/Global.aspx

Figure 4: Tamper evident container of paper envelope. Adapted from https://www.beaglelegal.com/tamper-evident-tyvek-envelopes_10x13

Figure 5: Tamper evident tape. Adapted from https://www.aeharris.com/home/76-tamper-evident-tape.html

All the procedures should be followed in order to maintain the chain of custody, as being send along a separate evidence log and with instructions for the receiving laboratory to fill out the chain of custody section and send it back, and the receipt with the name of both parties and the date, time and place of transferral. Also, a covering letter requesting the analytical services in which each specimen is listed, together with the specific test required, in a sealed envelope labelled “Documents”, should be attached to the container, and this set should then be wrapped or placed in a final outer container, identified as “evidence”, “forensic samples”, and/or “criminal investigation” (Green, 1979; Merck *et al.*, 2012e; Wobeser, 1996). Desirable, the FVP who sent the specimen should contact the laboratory who receive it to inform and make the arrangements between the two for the sent (Wobeser, 1996).

The chosen of the laboratory cannot be undermine when managing an evidence sample; thereby according to Wobeser (1996), "it is important to establish in advance that the laboratory chosen has experience with forensic or legal analyses, understands chain of custody, and that the analytical scientists involved are suitably qualified to serve as witnesses" (p. 247).

1.6.2. Secure storage

Even though the necropsy examination is complete, "the body is yet considered evidence and cannot be disposed of without permission from the appropriate legal authorities, either the investigating or prosecuting agency", being held until the legal case is over (Merck *et al.*, 2012e). Some evidence (such as carcasses, tissues, and plasma) should be kept in a locked refrigerator or freezer or in an area with restricted access (if fur and/or feathers) but for many facilities that is not realistic, and when this is not possible, the evidence should be located in an area with limited personnel access, for example, a restricted access box may be placed inside the refrigerator or freezer, such as a metal lockbox, that will allow the evidence to be maintained under acceptable condition, and if the evidence is removed from storage it should be documented in the evidence log, including the purpose, if opened, and any testing or alteration (J. Cooper, 1998)

Notice that when opening a sealed evidence package it should be opened in an area where the original seal can be preserved, with the purpose of providing proof that the original seal was not broken, and at the end of necropsy, the item must be placed back in the original package, sealed with tamper evidence tape, signed and dated (Merck, *et al.*, 2012e).

Remember that all the document evidences, whether they are photographs, radiographs, copies of the necropsy report, photograms, results of ancillary tests and physical specimens, etc., should likewise be kept on the FVP custody until the end of the investigation, advised by the enforcement officer that the case has been closed and the information is no longer required (Touroo & Fitch, 2016; Wobeser, 1996).

2. Necropsy Procedures in Veterinary Forensic

2.1. Introduction to forensic necropsy

The forensic necropsy is a main component in a criminal investigation (Lima *et al.*, 2016); which "an animal's carcass is investigated in a view to determinate the circumstances - cause, mechanism, and manner - of the death" (p. 37), answering to some questions about the dead animal as such: How did it die? Why did it die? When did it die? Where did it die? Who might have been involved? (J. Cooper & M. Cooper, 2008). Information that should be answer with the necropsy includes: (1) the time sequence in which events occurred; (2) the general health of the animal; and (3) the presence of pre-existing conditions that may have influenced its death (Wobeser, 1996). Remember that the necropsy is usually unrepeatable and that the cadaver in a forensic necropsy is evidence (Gerdin & McDonough, 2013; Listos *et al.*, 2015).

A forensic necropsy is performed on the instructions of the legal authority responsible for the investigation of suspicion deaths, like sudden death, suspected poisoning or traumatic death (Lima *et al.*, 2016; Listos *et al.*, 2015; Saukko & Knight, 2004). A forensic necropsy has more extent goals than the clinical necropsies, because not only requires the determination of the cause and mechanism of death but also the determination of the manner of death, any contributory causes, and the legal cause of death, and besides that it has to follow special procedures to ensure that information collected is suitable for use in a court of law (Merck *et al.*, 2012e; Wobeser, 1996). The FVP performing the medico-legal necropsy should have formal training and experience to increase the reliability of the forensic work specially for the court (Wobeser, 1996). Ultimately, after performing the necropsy, the veterinary VFP proceed to the collection of samples, determine the cause of death, and prepare a final report for use in court along with their testimony, as will be described further ahead (Gerdin & McDonough, 2013).

The establishment of protocols for medicolegal investigations in veterinary medicine is unavoidable, in order to standardize the work, helming to objectivity and thoroughness, since it means that each case follows a solid routine, ensuing to reliable documentation. These routines should also be applied to radiography, photography, sample collection, labelling and storage of specimens. However, each veterinarian or group of veterinarians, should adapt the

set of protocols to fit with their working practices (H. Munro & R. Munro, 2008). Thus, the purpose of this subject is to stablish the bases of forensic pathology, the components of a forensic necropsy and its goals in identifying non-accidental injuries (Gerdin & McDonough, 2013).

Ideally, the forensic necropsy would began at the scene (peri-necropsy), instead merely at necropsy room, as it happens in the majority of the countries, as Portugal, because having a FVP escorting the law agents at the field, is not achievable at most times, and so he/she must rely on the background information given by the agents who handled the case (Merck *et al.*, 2012e).

2.2. Reception and labels

The delivery of the body to the pathologist facilities should be done by a law agent or an animal control agent to assure the chain of custody. Once received, the FVP must sign and date the evidence receipt, and keep the shipping label. The packaging of the body arrival should be recorded and held and the information about how the body was conditioned before his arrival should be recorded as well (Gerdin & McDonough, 2013; Merck *et al.*, 2012e; Touroo & Fitch, 2016). The date and time which the cadaver has received should be noted, as well as its destination at the laboratory after being receipt – if immediately necropsied or placed in a cold store or in a freezer for examination later (H. Munro & R. Munro, 2008). Also, note that the FVP sometimes may have to aid the law enforcement officer, giving advises on how to do a proper packaging (Touroo & Fitch, 2016).

All the material removed from or found in the carcass, such as clothes and towels, foreign bodies from the stomach, and even toys, collars and surgical material, bullets, among others, should be collected, labelled and stored appropriately, or passed on to a proper agent, for instance, to ballistics if a bullet is found (Brownlie & R. Munro, 2016).

At the laboratory each cadaver is labelled with a laboratory reference number, that will remain tied to the cadaver for the entire time which is crucial to sustain the chain of custody. These labels should always be on the corpse and appeared at all photographs, and so they must be durable and clearly visible, not reflecting the light for the photos; namely they should be unaffected by freezing, with the laboratory reference number printed permanently in black

letters on a white background or white letters on a blue background (Figure 6). These strips can be placed around the leg above the carpus or hock joint or attached to other exhibits that form part of the evidence (e.g. a wound, a bullet, etc.) (H. Munro & R. Munro, 2008). As mentioned before at 'Evidence identification', the tamper evident braided metal cable seal (Figure 1) or truck/plunger seal (Figure 2) are a good example of label, however these are more commonly used for sealed the package of the cadaver before and after the necropsy.

Each cadaver is an individual item of evidence and in cases of multiple bodies, should be assigned a unique identification number for each one in order to be properly labelled and assured and individual identity label. Each label arrived at the laboratory should include: (1) the general case number; (2) the individual item number; (3) the laboratory identification; (4) and the date and time (Touroo & Fitch, 2016).

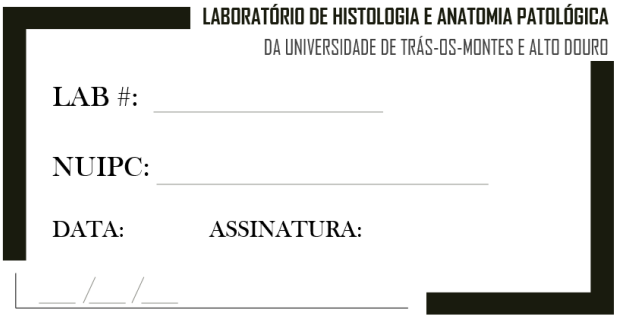

Figure 6: Example of a possible Portuguese laboratory labels that are unaffected by freezing, with the laboratory reference number printed permanently in black letters on a white background. Authored by the own.

2.3. Background information

Ideally, the FVP would be invited to follow the law agents in the field to examine the cadaver on the local where it was found, in every case he or she assists the investigation. Most authors defend that with arguments such the followings:

1. It is crucial to interpreting findings at necropsy and determining the cause of death;
2. It is relevant to place the incident in perspective and aids to interpretation of the *post-mortem* findings;
3. It is advantageous to gain at first hand an insight to the location and to the position in which the animal was discovered;

4. Might be an important opportunity to seek for signs of struggling, attempted escape or restraint (Gerdin & McDonough, 2013; H. Munro & R. Munro, 2008).

However, this barely happens in veterinary forensics, unlike in human forensics, and more usually, the police or other investigators remove the cadaver from the crime scene and submit it for examination some time later (H. Munro & R. Munro, 2008; Touroo & Fitch, 2016). True is that examining the body at the scene can be analogous to taking a medical history, however would requires practice and skill from the FVP attendant (Touroo & Fitch, 2016).

Thereby, the FVP should collect as much information as possible prior to the necropsy, as it is helpful to him/her to obtain information about the circumstances of the crime, that includes details of the animal's environment and items of evidence present on the crime scene, photos of these scene details, police reports including witness statements and statements made by the accused, the weapon that was potentially used to produce the lesions, prior medical history, and treatments including type and method of euthanasia, if applicable (Gerdin & McDonough, 2013). This information is required for an accurate *post-mortem* analysis, as these factors will assist the VFP in the interpretation of necropsy findings, and "should focus particularly on how, when, and where the body was found, by whom, and under what circumstances" (Touroo & Fitch, 2016).

Also, very important is to give information to the FVP about whether the animal was still alive when seized and thus received veterinary attention before dying or being euthanized as the date and hour of the occurred death; as well as information regarding any medical exam prior to death including photographs, diagnostics, test results, treatments, procedures, or any resuscitative measures also should be notified; and if euthanized, information including the route of administration also matters, because it could affect the necropsy (Brownlie & R. Munro, 2016; Peleteiro *et al.*, 2016). Every bit of information is very important to the forensic veterinary pathologist in interpretation of necropsy findings, for instance, prior procedures could be misinterpreted as injuries or altered original injuries, so any medical devices should not be removed until a necropsy is performed (Brownlie & R. Munro, 2016; Peleteiro *et al.*, 2016).

All the pertinent information could be unmistaken obtained if used a pre-made form which includes all the information that should be obtained prior to submission for a forensic examination. Unfortunately, most times there is not much information available (Gerdin & McDonough, 2013).

On the other side, there are authors who defend that pathologists should perform necropsies ‘‘blind’’, this is, the necropsy should be done before any knowledge of the background information, not having access whether to the witness statements, police reports, crime scene, neither other documentation about the case before commencing the necropsy (Brownlie & R. Munro, 2016). The support of this theory is based on the possibility of other “people’s observations and thoughts could skew the view of the veterinarian conducting the necropsy and that only those findings that support the preconceived ideas are observed”; emphasizing that the necropsy work of those FVP could be biased by the information and led them to look for particular features, with the possibility of missing crucial facts (H. Munro & R. Munro, 2008).

2.4. Records and case notes

Records must begin in early stages of the case, and should include the background information, the descriptions, diagrams and photographs produced during the necropsy and the results of subsequent ancillary tests; in the end, it must be assured that all this information is linked together in the record-keeping system used, where is achieved by assigning a unique number to each case and then using that number in all documentation of the case (Wobeser, 1996).

Beginning the necropsy, written notes about the animal’s sex, age, and body condition, should be made, and preferably justifying the statements – e.g. “the animal was judged to be less than 1 year of age based on the presence of deciduous incisors”; or the animal has a body condition score of 2 at 9, based on the world small animal veterinary association (WSAVA), since the ribs are easily visible, there are evidence of other bony prominences, there isn´t palpable fat, and there are already some loss of muscle mass (WSAVA, 2013). Notes about all injuries and/or abnormalities, no matter how trivial they may appear, are essential to record, as well as negative findings must be recorded, so there is no question about whether an organ that was not described was examined and found to be normal or it was not examined at all. Even more, is important to locate these injuries and abnormalities by making a relation between anatomic landmarks (e.g.

located on the caudal surface of the thigh, five centimetres straight line below the ischial tuberosity), along with pre-made outline drawings (Figure 7), and measured them in absolute units (centimetres, grams, centilitres, etc.) rather than in relative terms (enlarged, smaller than normal, the size of an orange, etc.) and describe (Wobeser, 1996).

These rough notes or dictation should be retained by the FVP until all possible court proceedings are completed (Touroo & Fitch, 2016; Wobeser, 1996), because sometimes, "investigators may request submission of the original (bloodsplashed) written notes or the recording of a necropsy as an appendix to the final typed report of the case", which should be provided in clear document sleeves sealed for hygiene purposes or only send a copy, a photograph or a scan of the originals, signed and dated by the pathologist(s) (Brownlie & R. Munro, 2016).

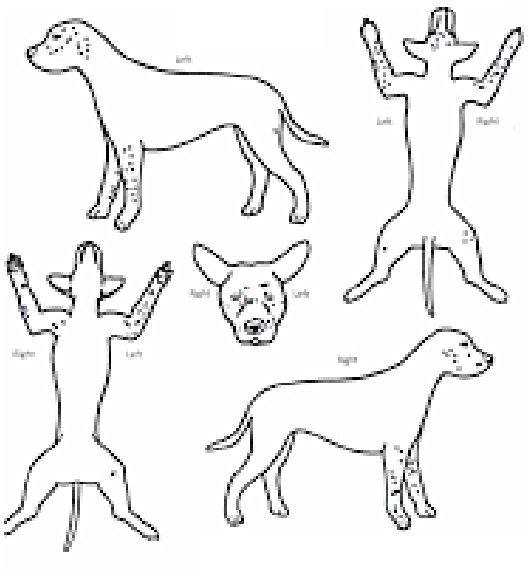

Figure 7: Example of a basic pre-made dog outline drawings. Adapted from http://wiringdiagramblog.today/pet-wound-diagram.html

2.5. Forensic photography

Photography is a fundamental component of a forensic necropsy. It is used to establish identity and document lesions and are useful reminders of the necropsy findings as they are powerful evidence to submit to the court (Gerdin & McDonough, 2013). In the end, forensic photography must have the capacity of provide several purposes: "(1) identify the victim; (2) demonstrate the condition of the evidence at the time of discovery; (3) record and document evidence that cannot be preserved or left unaltered; (4) allow for later review of the evidence; (5) illustrate

and supplement a written report; (6) demonstrate the absence or presence of alleged findings; and (7) present in a court of law, the items of evidence as they were found, thereby validating the testimony being presented" (Sharma, 2003; Touroo & Fitch, 2016). And this photographic documentation should be performed on all necropsy examinations and be incorporated on an individual photo log (see further ahead) (Merck *et al.*, 2012e).

Sooner the case receives its own unique laboratory identifying number (case number), the label for the photographs must be generated and included in every photo (see "reception and labels) (Brownlie & R. Munro, 2016). Thereby, the first photograph must be a picture of the case information, what includes (1) the case number; (2) the date; (3) the animal ID; and the (4) signature of the FVP; all this information written on a card or dry erase board. This first picture can be taken with or without the animal in the photo. Also "a photograph of any paperwork submitted with the carcass at this stage is also of value" (Brownlie & R. Munro, 2016). Then follows "the photographs of the unopened body package, and it must continue shooting each layer of packaging or wrapping around the body" (Merck, *et al.*, 2012e). Oftentimes, the body package bags are sealed with a numbered cable tie, which also should be captured by a closeup photograph along with the case number, including every item accompanying the carcass (such blankets) should be documented photographically (Gerdin & McDonough, 2013; Merck *et al.*, 2012e).

The third step is to photograph the intact corpse after unpacking it and before beginning the necropsy (Wobeser, 1996). This photographic documentation is done by general photos of the entire body of the cadaver, following a six photographic views protocol: **laterals** (right and left sides), **front** (facial), **hind** (rear), **dorsal**, and **ventral**, as seen in Figures 8 to 13, respectively (Merck, Miller, & Maiorka, 2012f). Unusual or identifying marks, such as collars, ear tags, tattoos, brands should also be captured at this stage (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013). Meaning that "all items of evidence during a forensic necropsy must be documented *in situ* via photographs, and its location and measurements should be sketched onto a body diagram" (Touroo & Fitch, 2016, p. 7).

Figure 8: Photographic documentation of the cadaver– Lateral right

Figure 9: Photographic documentation of the cadaver – Lateral left

Figure 10: Photographic documentation of the cadaver– Front/facial

Figure 11: Photographic documentation of the cadaver – hind/rear

Figure 12: Photographic documentation of the cadaver – dorsal

Figure 13: Photographic documentation of the cadaver - ventral

When shooting an injury or other element of evidence, is essential that it is in or near the centre of the image, being in focus, "on a clean, plain background, with minimal background distractions such as utensils, equipment, and personnel", and at least two photos of each and every lesion need to be obtained, first an overall orientation view, in a distant plan, to put the lesion in the context of its surrounding anatomic landmarks (Figure 14), and then, with the label still in place, a closeup view of the particular finding, granting is central piece in the view-finder and that the label is also included (Figure 15) (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013; Lima *et al.*, 2016). When are multiple lesions, it may be placed on the cadaver site markers or symbols (numbers, letters) during photography to clearly indicate the anatomic location of each one (Wobeser, 1996). Note that a photographic registration of absence is as important as a photo of presence of a lesion, as again it may prove the assessment of the organ or tissue.

Figure 14: Example of an overall orientation view, in a distant plan, putting the lesion in the context of its surrounding anatomic landmarks. Photo kindly ceded by Alves, A.

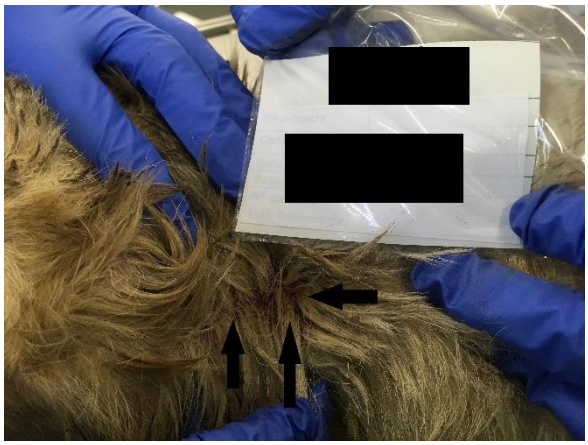

Figure 15: Example of a closeup view of the finding before, granting it is the central piece in the view-finder and that the label is also included. Photo kindly ceded by Alves, A..

Every photograph should include a scale and be rightly framed, which is achieved by using an L-shaped forensic scale (Figure 16) or ruler. The forensic ruler should be placed flat and on the same plane as the item being photographed, to prevent size distortion between the scale and the area being photographed, which can be verified by looking at the circle with crosshairs, that make a 90 degree angle each quadrant when in a correctly plan (Touroo & Fitch, 2016). If the ruler is not levelled, the circle will appear elongated, rather than round. The ruler has other functions as verifying proper colour and exposure and provide measurement (Touroo & Fitch, 2016). In small lesions, where the scale and case number could obscure the area of interest, two

close-up photographs, one with the scale and case number included and one without, are recommended (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013).

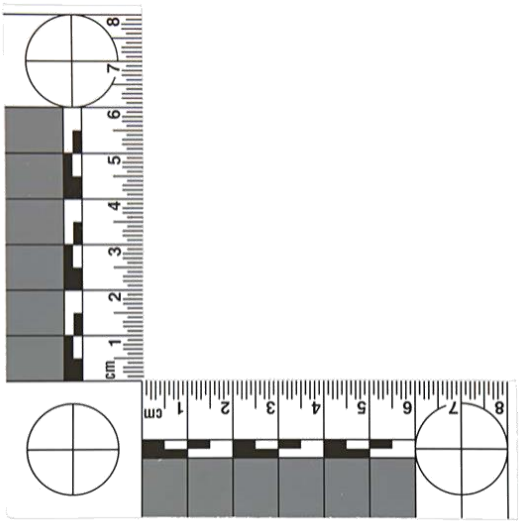

Figure 16: L-shaped forensic scale. Adapted from http://www.bvda.com/en/rulers-5-cm-2-inch

The use of knives, scalpels, and other necropsy instruments for scale purposes is discouraged and outdated by the L-shape forensic scale (Gerdin & McDonough, 2013). However, some instruments can be used as pointers (e.g. closed forceps, aluminium gun-cleaning, surgical catheter, etc.) (Figure 17) for "identifying small lesions or making a time connection between them, such as the path of a bullet through the body, but it must be clean and not distract from the feature being illustrated" (Gerdin & McDonough, 2013; Wobeser, 1996).

Figure 17: Example of the use of a blunt instrument – surgical stylet - as pointer, to identifying the track of a gunshot injury at the scalp of a dog.

An important detail is that all photographs following the initial animal ID picture should only be of that animal and should be taken in a continuous numbered series, and "if additional photographs are needed of an animal that has already been examined and photographed, the sequence starts again with the case and animal ID information card" (Merck*et al.*, 2012e). These measures will help to ensure the consistency of the photographs, and especially in cases involving multiple animals may reduce the chance of errors when working (Brownlie & R. Munro, 2016; Merck *et al.*, 2012e).

Remember that "every photograph should contain information that clearly identifies the subject animal, the date, a size reference scale, and the identity of the pathologist(s)" on the case, therefore, it is convenient to have cards prepared in advance that have a reference scale ruler, and ideally also spaces for the appropriate information (Figure 18) and be several of them of various sizes (Figure 19). These cards should be made of matt paper and of a mid-range colour such as medium grey, green, or brown, because white cards are usually over-exposed and illegible (Wobeser, 1996). For instance, small identification labels may be used for closer views and should contain at minimum the animal ID (Merck, *et al.*, 2012e). This variety of pre-made labels is important to ensure that the label does not obscure, distort or cover the lesions, and this is achieved by using the right label size (R. Munro, 1998).

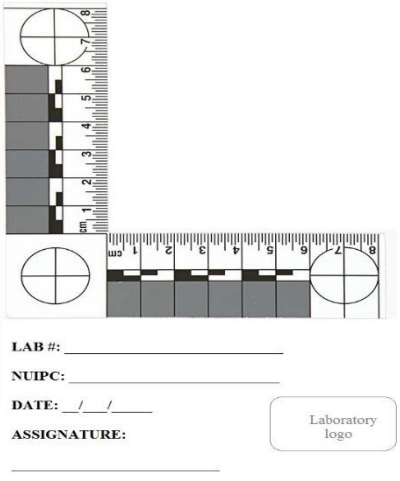

Figure 18: Example of a reference scale ruler, in a L-shape, prepared for serve as well as identification card. Authored by the own, based on http://www.bvda.com/en/rulers-5-cm-2-inch

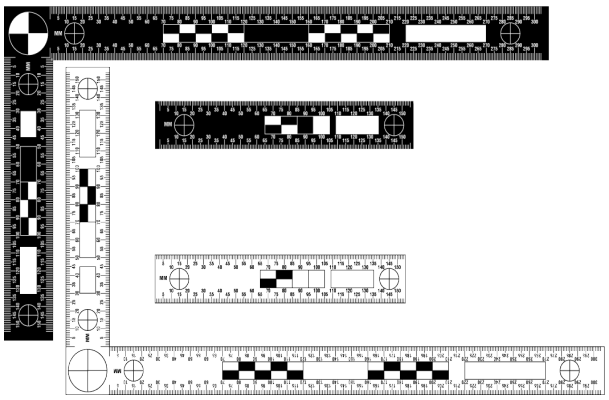

Figure 19: Several sizes of reference scale rulers. All of them can be used in one case, depending on the size and type of the lesion. Adapted from http://www.bvda.com/en/rulers-5-cm-2-inch

Every photograph is evidence, so its concordance in the chain of custody must be certified. Therefore, each photograph should have a clear description of what is illustrated in a common document. This document is named as photo log and assumes a list format which contains all the photos number taken in a specific series, with the specific photographic and forensic information needed about each photo. This log along with the photographic documentation must be kept in a secure storage, such as the lab server or transferred to a compact disk and stored in a secure location (Lima *et al.*, 2016; Wobeser, 1996). "Altering or amending copies of images, such as adjusting the contrast and adding arrows and labels, may be permissible in some jurisdictions, but only if the original image is maintained and the content of the image is not altered" (Gerdin & McDonough, 2013). Also, all taken photos should be incorporated in the photo log, even when images are of poor quality, out of focus, inadvertent irrelevant shots or were taken by mistake, because deleting photos can jeopardized the integrity of the photographic documentation because the disruption of the sequence can raise questions of doubt and mistrust from the court to the FVP (Brownlie & R. Munro, 2016; Touroo & Fitch, 2016). Remember – one clear media card to start a necropsy photography, and once this is full, or no further photographs are needed, all the images should be downloaded to a ''Master'' file located on a secure digital storage device (Touroo & Fitch, 2016). The best format used to take the images is the RAW format, once it is impossible to be enhanced, and so they must be retained in this format, although they could be converted to a readable format such as JPEG or TIFF but should be stored in a ''copy'' file (Touroo & Fitch, 2016). When dealing with numerous deaths of a case, photographs should first be downloaded in the original sequence to correspond to the photo logs and only then can be created folders for each animal ID and copy the respective photos into the folders (Merck, *et al.*, 2012e).

Ultimately, the photographic documentation would be ideally performed by a professional photographer, with the guidance of the FVP, however, this hardly ever happens, and the pathologist or his/her assistant is the one who made the photographic record. As so, it´s important to have in mind that the photos will be seen by members of the court (non-medical personnel) and must be understandable but knowing that they aren´t accustomed to see carcasses and the photos should convey an air of methodical and thorough examination and not a haphazard and messy dissection, as that images ruled too graphic can be barred from court (Gerdin & McDonough, 2013).

With the advance of technology, it is possible to watch experimental systems being done with the goal of, in the future, replace the standard 2D photography with the reference ruler by a 3D photogrammetry-based optical scanning, using the TRITOP/ATOS III system to document lesions in a 3D fashion (Bolliger *et al.*, 2008). According to Bolliger *et al.* (2008):

> This system consists basically of one central projection unit and two digital cameras mounted beside the projector. A fringe pattern is projected onto the surface of the object, which is recorded by the two cameras. Based on the principle of triangulation, 3D coordinates are calculated by the scanning software ATOS. Thus, a 3D surface can be recalculated. (p. 274)

2.6. Forensic radiography

Before begging a forensic necropsy, a whole body skeletal radiological survey is recommended (J. Cooper & M. Cooper, 2007; Heng *et al.*, 2009; Merck, 2007; Saukko & Knight, 2004). The radiographs should be obtained even before removing the body from the materials it was received in, for many reasons – first, for practicability, as the cadaver sealed bag is received and goes right in that way to the radiological room; second, for hygiene purposes, as this allow to keep the radiological table clean; and third, for evidence management, as it ensures that items that may have become dislodged from the body, but are still in the shipping materials, are detected oh the X-rays (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013).

Radiography is a very valuable tool in the *post-mortem* evaluation of a carcass as it can easily reveal bony lesions, such as fractures and/or bone reparative processes (e.g. bone calluses), suggesting serial beatings or neglect of an injury, likewise discovering the presence of metallic objects such as bullets and microchips, that even could be missed in the necropsy, thus a hole-body radiographs taken in two views - lateral and dorsoventral/ventrodorsal – has been advocated as standard practice (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013; H. Munro & R. Munro, 2008; Viner, 2016).

The interpretation of radiographs obtained can be done by the FVP, who at least should denote the presence or absence of displaced fractures and radio-opaque foreign material; however, the *post-mortem* radiographs is not equal to a live animal radiograph, because the changes seen on the body after death, and literature describing of animals is yet not very available, so, ideally

the interpretation of these *post-mortem* X-rays should be done by an imaging specialist, with some complementary pathology training, for more detailed interpretation about "the weapon used, the position of retained missiles, fragments or gunshot residues within or under the skin, and the tracts caused by the shot or dislodged bone fragments", which has to be credible as it is evidence (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013; Heng *et al.*, 2009).

The X-ray results – both images and respective description – are sent within the case information – photographic documentation, necropsy report, ancillary tests results, etc. – via portable data storage or central files to the court, where the images can be viewed as hard copies or via a computer connected to a large screen, what emphasize the importance of the correct and distinguishable identification of each X-ray (Brownlie & R. Munro, 2016).

2.7. General necropsy technique

The general necropsy technique here described has not the intend to describe in detail how to conduct a necropsy, since it is assumed to be well known by the veterinarian pathologists, but instead summarize the stages of a forensic necropsy and highlighting the critical ones which differs from the clinical necropsy.

The necropsy examination must be initiated as soon as possible after the animal death occurred, not exceeding 24 hours, because then the tissue autolysis is accentuated; when not possible, according to the literature, the body should be refrigerated, never frozen, especially because it can produce changes similar to trauma-induced lesions and *ante-mortem* inflammatory edema, which can lead to misperception (Alves, Pires, Gama, & Prada, 2016; Stroud, 2012).

Before anything else, the carcass should be weighed, and preferably record the number appearing at the balance by photograph (Wobeser, 1996). When the carcass is incomplete, it should be weighed as well, and additional information about the portions that are present and the ones that are absence should be made (Wobeser, 1996).

The second step is describing any coverings around the cadaver, such as plastic bags, blankets, towels, papers or other materials, as they are removed - from the more external to one closer to the body (H. Munro & R. Munro, 2008). When opening the package, any odours from the body (decomposition, smoke, motor oil, insecticides, feces, etc.) also should be described (Merck *et*

al., 2012e). From the body, the first analysis is to observe and describe the presence and the location of *rigor mortis*, lividity and the level of decomposition (Merck *et al.*, 2012e). At this point of the beginning of the necropsy, leakage of fluids should also be noted, and any entomology evidence should be collected and preserved in 70% ethanol (Merck *et al.*, 2012e; H. Munro & R. Munro, 2008). Always must be taken special care to prevent cross contamination, which could ruin eventual toxicological and DNA analyses, and so it must be used clean instruments for each cadaver only, and each tissue must be placed in a separate containers (Wobeser, 1996).

The focal point for a complete necropsy is to always follow a systematic necropsy, because no matter what the order that each FVP follows (e.g. on internal examination, the order of each cavity are open – thoracic or abdominal – is irrelevant), the important is to be consistent by following the same necropsy procedure each time, because it decrease the probability of some pathological condition be missed (Johnson, 2001; Peleteiro *et al.*, 2016). However, it must be kept in mind that a necropsy is an unrepeatable procedure by nature (Peleteiro *et al.*, 2016); it can be done a second necropsy in a cadaver, but it won't have the value as the first one, as the organs already have been moved from its original location and cut, and cross contamination has been done. The three main phases of a forensic necropsy technique are: (1) external examination; (2) skinning; and (3) internal examination (Allen *et al.*, 2006; Peleteiro *et al.*, 2016).

2.7.1. External examination

The external examination is the first stage of the necropsy, and it takes a distinct importance in forensic necropsies, especially in trauma deaths (Gerdin & McDonough, 2013; Listos *et al.*, 2015; Saukko & Knight, 2004).

As mentioned before, the external examination begins with inspection of the materials in which the body was received (Gerdin & McDonough, 2013). After the removal of all coverings, follows the weighing of the cadaver on an accuracy validated weighing machine and then the measuring of the body length from the back of the head to the base of the tail; these two parameters are especially important in cases of starvation and neglection of innutrition (H. Munro & R. Munro, 2008). Then, it should be evaluated the *post-mortem* changes – *pallor*

mortis/body lividity; *algor mortis*/body cooling; *rigor mortis*/stiffness; body evaporation; *livor mortis*/hypostasis (Peleteiro *et al.*, 2016). It is also imperative to evaluate carefully the skin and coat condition, looking for lesions, ectoparasites, sutures, marks like tattoos, and search for microchip with an appropriated reader (Peleteiro *et al.*, 2016). Next is the examination of the oral and nasal cavities, the ocular mucosa, the outer ear and the external genitalia (in males the penis should be exteriorized for inspection) (Peleteiro *et al.*, 2016).

2.7.2. Skinning

Reflecting the skin over the entire cadaver is essential for forensic necropsies because important features such as the trajectory of penetrating wounds (e.g. gunshot wounds) or the extent of subcutaneous haemorrhages, in cases of animal abuse, and determining the amount of subcutaneous fat and condition of the musculature, in cases of neglect, often are obscured by pelage or by cloaked by the colour and/or the tough elastic hide (R. Munro, 1998; Wobeser, 1996). The skin should be ideally removed in one piece as entirely as possible to maintain "the orientation and juxtaposition of anatomic structures and landmarks so that the physical location of lesions can be shown" (Viner, 2016, p. 640); in order to this to happen an incision is made through the skin on the midline from the tip of the chin to the rectum and dissected towards the back; the limbs are dissected from medial to lateral by an incision along the inner face (Peleteiro *et al.*, 2016). However, some authors prefer to leave a portion of the skin of the body rather than removed it as one piece, in order to keep some 'identity' of the animal on the cadaver.

In cases of suspected gunshot wounds, the skinning must be done carefully, since the bullet can be captured just under the tough hide in the opposite side to the entrance wound, where usually it will be found a subcutaneous haemorrhage with no skin perforation, and it can be easily lost during the skinning (Wobeser, 1996).

2.7.3. Internal examination

In order to begin the internal examination, the cadaver should be placed in supine, because is the position that allows to preserve the typographical relation between the organs that most resemble to once in life, and also avoids the leakage of fluids if is the case (e.g. pleural effusion, ascites) (Peleteiro *et al.*, 2016); then, it must be sectioned the limbs, for a correct stabilization. Nevertheless, every step of the protocol should be adjusted according to the background history,

and in cases that the union between the limbs and the upper body have some injury or it is suspected to have, this step is not recommended to do (Merck *et al.*, 2012e).

There are many techniques of necropsy and the VFP should use the one he/she prefers; a basic and common technique is the Rokitansky's technique, which involves some dissection *in situ*, and then an outside examining of the organs of the animal's body, removed in blocks, to ensure that the anatomical and pathological continuity of organs is kept and assessed in a first place, and only then their extraction (Alves *et al.*, 2016; Connolly, Finkbeiner, Ursell, & Davis, 2015; Ludwig, 2002). The basic clinical necropsy principals such as opening and inspection the lumen and content of all hollow organs and incisioning the parenchymatous organs, should be made and record as it is being done, as well as the photographs, to capture the real appearance of tissue and lesions, before changes happen during the time necropsy procedure, for example, the integrity of the diaphragm should be noted right after the opening of abdominal cavity and before the thoracic cavity, and then it should be punctured and listened for the presence of negative pressure, as this will be altered if forgotten and proceeded to the opening of the thorax (Merck *et al.*, 2012e; Wobeser, 1996). During the examination of all the internal organs, samples should be collected for histopathology, microbiology, virology, parasitology, clinical pathology, toxicology, and/or any of them or other when considered needed (Merck *et al.*, 2012e).

The injuries seen in the internal examination of forensic necropsies, although not very commonly reported, can be sever and even fatal, given that a blunt abdominal trauma can result in ruptures of the liver, spleen, kidney and bladder, which usually results from kicks or punches (H. Munro & Thrusfield, 2001a).

2.7.4. Opening of the skull

In forensic necropsies, regardless of whether nervous symptoms were related, or head trauma is suspected, the necropsy must include the opening of the skull, and sometimes the rachis also should be opened (Lima *et al.*, 2016). This step comes after the extraction and examination of the internal organs of the cadaver. The head should be disarticulated from the remaining body, and then removed the skin dorso-cranially over the skull and the muscular layer, exposing the

calvarium, and the opening is equal to a clinical necropsy (Merck *et al.*, 2012e). The brain is examined and, if necessary, fully or identified sections are sent for complementary tests.

Ideally, at the end of a necropsy, all organs would be replaced inside the body, and the skin sutured, as a manner of respect for the cadaver and for the owner, who may wish to have the body returned at the end if the investigation (H. Munro & R. Munro, 2008). Till then, the cadaver must be stored in a security freezer, and records of where, when, and under what conditions it occurs should be in *post-mortem* notes, signed by the responsible FVP and, if required, corroborated by the second pathologist (H. Munro & R. Munro, 2008).

2.8. Estimation the time of death

An important feature at forensic necropsy is to estimate the time of death, this is, the time between death and the *post-mortem* examination, giving a period of which death may have occurred (Erlandsson & R. Munro, 2007). This is a crucial point in investigations both in criminal and civil cases to link the suspected and the victim in a correct chronological set of events, for instance, it can set the time of the murder, eliminate or suggest suspects, confirm or disprove an alibi, in criminal cases, and can determine who inherits property or whether an insurance policy was in force, in civil (D. DiMaio & V. DiMaio, 2001). Thus, is required from the FVP to estimate this time based on he/she experience of interpreting the *post-mortem* changes, such as *livor mortis*, *rigor mortis*, body temperature, degree of decomposition (development of odour or colour), dermal and ocular changes and alterations to the internal organs, in blocks of time such as <24 h, 1 to 3 days, 3 to 7 days, 7 to21 days, weeks, months or years (D. DiMaio & V. DiMaio, 2001; Erlandsson & R. Munro, 2007; H. Munro & R. Munro, 2008).

Other resources used to enhance the accuracy of the determination of the time of death are "chemical changes in vitreous, flow-cytometry, stomach contents, insect activity, scene markers (papers, letters, clothing, televisions, TV schedules, etc.)" (D. DiMaio & V. DiMaio, 2001). The entomological evidence, is quite used in forensic necropsies in Portugal, and it is promising, since the insect activity - puparia, insect larvae, and eggs – present in the cadaver can provide valuable information, and shall be collected, preserved and analysed by the entomologist (Touroo & Fitch, 2016).

However, it must be kept in mind that there is an accepted body of knowledge and experience, and there are limitations and errors, and the FVP does not should overestimate his/her knowledge. Erlandsson and H. Munro (2007) cited James and Knight (1965) which alleged that "experienced forensic pathologists frequently underestimate the *post-mortem* interval" (p. 150). D. DiMaio and V. DiMaio (2001) said: "the longer the *post-mortem interval*, (…), the less precise the estimate of the interval".

2.9. Necropsy approach to animal abuse and neglect (AAN)

In forensic necropsies, the recognition of suspected animal abuse and neglect (AAN) is a requirement of the FVP. There is a classification scheme, based on the same of human child abuse, that comprises four basic categories: physical abuse, sexual abuse, emotional abuse, and neglect (Almeida, Torres, & Wuenschmann, 2018; H. Munro & R. Munro, 2008). At necropsy the physical and sexual abuse as well neglection can be detected, and sometimes identified the way of killing, the weapon, the time and/or the perpetrator through the type and characteristics of the injuries.

Physical abuse is when the "perpetrator subjects the animal to a variety of actions that cause bodily injury", which includes kicking, punching, throwing, beating with an instrument, stabbing, burning, micro-waving, drowning, asphyxiation, shooting and also the administration of drugs (e.g. narcotics or alcohols) or poisons (e.g. the slug bait, metaldehyde) (Allen *et al.*, 2006; H. Munro & R. Munro, 2008). Summarizing, at forensic necropsies, the cases of AAN fall into trauma, projectile wounds, neglect and starvation, alone or more than one in one case (Gerdin & McDonough, 2013). **Sexual abuse** is seen as the use of an animal for sexual gratification, and "**neglect** is a failure to provide food, water, shelter, companionship or veterinary attention" (Allen *et al.*, 2006).

In forensic necropsies it is possible to determine three main wound patterns according to the object used in the physical trauma, which induce a specific manner of the mechanical force to the skin: blunt injury, sharp weapon injury and gunshot wound; blunt injuries are sub-classified into abrasion, laceration, bruise, intradermal bleeding, etc.; sharp weapon injuries are also sub-classified into incised wound, stab wound , chop wounds, and the conjugation of this patterns, like incised-stab wounds, and so on (Ohshima, 2000; Peleteiro *et al.*, 2016). The wound

examination is “one of the most important and indispensable areas for forensic pathologists” because by the discrimination of *ante-mortem* wounds from *post-mortem* damage, how long before death the *ante-mortem* wounds were sustained, and also by the description of the type of wound, it can be match a weapon and maybe a suspect (Kondo, 2007). “This means, in order to exactly diagnose the cause and manner of death, it is essential to describe the findings of wounds correctly and objectively as much as possible” (Ohshima, 2000). For instances, age estimation of bruises can also allow the FVP to determine how long a victim has been abused, in cases of plural bruises of different ages (Ohshima, 2000).

Note that the appliance of mechanical forces on the body will have a different damage depending on the nature of the target tissue, “for instance, violent compression (as in an explosion) may do little harm to muscle, but may rupture lung or intestine, while torsion may leave adipose tissue unaffected, yet cause a spiral fracture in a femur” (Saukko & Knight, 2004). Also, it “should be realized that a wound might display more than one type of injury, for instance, one may have a laceration with abraded margins lying in the centre of an area of contusion” (D. DiMaio & V. DiMaio, 2001).

2.9.1. Blunt force trauma

A wound or injury is define as the “damage to any part of the body due the application of mechanical force”, and despite of being generally considered to be a skin injury, it may be extended to deeper tissues, and sometimes the external forces may injure (or wound) deeper tissues without necessarily breaching the skin; this is defined as blunt force injuries, which is caused by trauma, and is “often the primary focus in a forensic investigation” (H. Munro & R. Munro, 2008; H. Munro & R. Munro, 2013; Saukko & Knight, 2004). “Blunt force injuries are more commonly encountered in veterinary medicine than wounding by sharp implements”, and they are the result of the “impact of an animal’s body against a blunt surface or the impact of an object with a blunt surface against an animal’s body such as by kicking, punching, or by using objects like hammers, sticks, baseball bats, billiard cues and bricks (Merck, Miller, Reisman, & Maiorka, 2012g; H. Munro & R. Munro, 2008). “The severity, extent, and appearance of blunt trauma injuries depend on the amount of force delivered to the body, the time over which the force is delivered, the region struck; the extent of body surface over which the force is delivered, and the nature of the weapon” (D. DiMaio & V. DiMaio, 2001).

The blunt force injuries in the skin can be divided into "abrasions, bruises (contusions) and lacerations (split wounds); and in the deeper tissues include bruising and laceration of muscles, damage to internal organs, and fractures of the skeletal system" (H. Munro & R. Munro, 2008).

2.9.1.1. Bruising/contusions

Bruising and contusions are generally terms used to describe areas of haemorrhage into soft tissue due to rupture of blood vessels typically caused by a blunt force impact; however, more specific, "bruising occurs deep to an intact dermis while contusions are collections of escaped blood anywhere in the body", such as in internal parenchymal organs such as the spleen, lung, heart, brain and muscle (Brownlie & R. Munro, 2016; Merck *et al.*, 2012g). A large focal collection of blood in the subcutaneous layer – bruise - can also be seen referred as hematoma or ecchymosis, which is really a small bruise (D. DiMaio & V. DiMaio, 2001; Saukko & Knight, 2004).

"Assessing the validity of what appears to be bruising in carcasses in the context of suspect animal abuse cases is vital" (Stroud, 2012). The FVP can be able to differentiate an area of bruise and contusion from *livor mortis*; in the last the blood can be wiped or squeezed out; also, bruising are more easily occurred in areas with thin, lax skin and in fatty areas, e.g. over the eyebrow, rather than where the skin is more strongly supported (D. DiMaio & V. DiMaio, 2001; Vanezis, 2001). However, distinguishing an *ante-mortem* bruising from lividity becomes harder as the time of death goes by, because of the increase of decomposition (Merck *et al.*, 2012g).

A pure bruise implies an intact epidermis, however it is often combined with abrasions or lacerations (Saukko & Knight, 2004). "The true extent of the bruising, which is usually larger than what was apparent on the skin surface" and is only revealed by skinning, and moreover, to detect deeper lesions, is needed to reflect the muscle layers (Merck *et al.*, 2012g). The precise understanding of bruising at necropsy is essential to a forensic necropsy, as can give information about how a victim has been injured and reconstructing the events leading to death (Vanezis, 2001). For instances, bruising can support or deny the weapon suspected to be used, by linking the bruise pattern with the shape and type of a weapon (Brownlie & R. Munro, 2016). Thus, the FVP always should question if the discolouration seen is really a bruise and when and how it was caused (Vanezis, 2001). The description of a bruise should include their shape (the

contour, pattern, and degree of swelling), size (width and length), colour and site; and it must be photographic documentation (Vanezis, 2001). Nevertheless, a bruise never should be examined singly because accompanying lacerations or abrasions would be useful pointers in assessing the force of an impact (Vanezis, 2001). Shaving the coat of the cadaver may be a practical and useful way to identify bruises. Furthermore, the FVP should be aware that the absence of a bruise does not indicate that there was no blunt force to that area (D. DiMaio & V. DiMaio, 2001).

2.9.1.2. Abrasions

Blunt trauma also can involve tangential forces, result in superficial wounds to the skin, named as abrasions (Brownlie & R. Munro, 2016; Gerdin & McDonough, 2013); an abrasion consists in the removal of the epidermis due to friction against a rough surface or its destruction by compression upon impact (Brownlie & R. Munro, 2016; D. DiMaio & V. DiMaio, 2001). The restritive definition refers abrasions as the "most superficial of injuries not penetrating the full thickness of the epidermis", thus, not bleeding, as blood vessels are into the dermis (Saukko & Knight, 2004); therefore, some authors extent the definition as "a superficial injury to the skin, which would allow penetration of the upper dermis rather than only the epidermis", contemplate the possibility of an abrasion to bleed (Saukko & Knight, 2004).

Ante-mortem abrasion is seen as a reddish-brown appearance (Figure 20) and heal without scarring, while *post-mortem* abrasions are pale yellow with a translucent ''parchment'' appearance, and the last are even common due to the movement of the cadaver (Brownlie & R. Munro, 2016; D. DiMaio & V. DiMaio, 2001). It is important to be aware that the animal's hair coat protects the skin and minimizes the surface injury (unless it is in areas of glabrous skin), diminishing the severity of the abrasion, which can make the FVP underestimate the underlying trauma (Merck *et al.*, 2012g).

Post-mortem/artefactual abrasions are more common than bruises, due to facility to be befall - dragging the cadaver or even, in cases of drowning or when the cadaver is left in moving water, by its buffeting; the insect bites, specially by ants, may also induce artefactual abrasions (Saukko & Knight, 2004).

Abrasions can be classified into three types, according to the characteristics of the injuries. "There are the scrape or brush abrasions; the impact abrasions; and the patterned abrasions" (D. DiMaio & V. DiMaio, 2001).

Figure 20: An *ante-mortem* abrasion on a cat that died in a clothes dryer. Note the reddish-brown appearance - impact abrasions - on the bony prominences. Adapted from Merck *et al.* (2012g).

Scrape or brush abrasions occurs when the "blunt object scrapes off the superficial layers of the skin, leaving a denuded surface", causing a serosanguineous fluid covering on the abrasion (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012g). When cutting the area of a scrape or brush abrasion, is not found haemorrhage in the underlying soft tissue, indicating the injury is confined to the epidermis (Merck *et al.*, 2012f). "One of the most common types of scrape abrasions is the linear abrasion known as the scratch", which are caused by a sharp edge or finger nails (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012g); other scrape-like abrasions are caused when the body slides across a rough surface such as pavement, leaving an extensive abrasion, termed as graze abrasions, which may be able to see embedded in it gross or microscopic debris related to the object or surface that caused the injury; graze abrasions also may be seen when an object, such as a bullet, sideswipes the body (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012g). Some authors referes the "evidence of bunching of the epidermis at one end of the injury as an indication of the direction of the body or object movement", however this theory is not so easy to apply in practice (Merck *et al.*, 2012g).

Impact abrasions are caused when the blunt force is directed perpendicular to the skin, resulting in crushing injuries, and frequently affect areas of bony prominences, as they have less underlying protective tissue (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012g). "The

lesion is slightly depressed below the surface unless an underlying bruise or local oedema bulges the tissues", and usually reproduce the pattern of the injuring object, leading to a pattern abrasion (Saukko & Knight, 2004, p. 140)

"A **patterned abrasion** is a variation of an impact abrasion", in which the weapon is imprinted or stamped as characteristic mark on the skin by the crushing effect of the blunt object (D. DiMaio & V. DiMaio, 2001). The impression can be the object itself (e.g. a pipe) or be an intermediary material, such as clothing, or likewise be a patterned imprint of ligature injuries, which give the revealing type of ligature used (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012g).

2.9.1.3. Lacerations

Lacerations results on a blunt trauma of shearing or crushing forces, due to the impact of a blunt instrument or a fall against a hard surface in which the full thickness of the skin is penetrated (D. DiMaio & V. DiMaio, 2001; Saukko & Knight, 2004). There are skin lesions deeper than abrasion, extended into the dermis and often the subcutis, with irregular wound margins that are also often abraded and/or bruised (Figure 21) (D. DiMaio & V. DiMaio, 2001). They can be confused with incised wounds (mentioned ahead at sharp-force injuries), however the last distinguishes from having "clean, straight margins with no or minimal abrasion and bruising", and the lacerations have an unique feature that is the presence of bridging, made by the deeper vessels and nerves (Gerdin & McDonough, 2013). In a brief definition, lacerations are "full-thickness breaches of the skin that are caused by blunt trauma, as opposed to incision by sharp object (…) and tend to occur with forceful impact over bony prominences, such as hammer blows to the skull" (Brownlie & R. Munro, 2016).

Lacerations also may affect internal organs, even that is more usual to occur in the skin over bony prominences, such as in the scalp (D. DiMaio & V. DiMaio, 2001). True is that it can either occur on muscles (commonly seen in predator attacks), mucous membranes (e.g. in the gingiva) or internal organs (e.g. a disruption of the liver parenchyma); tissue bridging also may be seen in internal organs with some quantity of interstitial tissues, indicating crushing injuries, such as motor vehicle impacts or falls from significant heights, or even non- accidental cases from deliberate, directed force such as a kick or punch (Merck *et al.*, 2012g).

Lacerations do not easily reflect the shape of the injuring instrument, though some injuries can have a recognizable shape – e.g. hammer blow to the head, which reproduce a circle or an arc of a circle in an underlying depressed skull fracture -however, the size of the hammer may be minor than the laceration (Saukko & Knight, 2004). Generally, long and thin objects (e.g. pipes and pool cues) produce linear lacerations, while objects with flat surfaces produce irregular, ragged, or Y-shaped lacerations (D. DiMaio & V. DiMaio, 2001).

Figure 21: An *ante-mortem* laceration of the skin in the internal left thigh of a female dog due to road trauma. Denote that the skin lesion is deeper than an abrasion, extended into the subcutis, and with adjacent bruising; it is also possible to observe the bridging tissue in the depth of the laceration, as multiple white fascia (arrows).

2.9.1.4. Avulsion injuries

A blunt force impact at a tangential or oblique angle can "ripping skin and/or soft tissue off the underlying fascia or bone", or even occur deeply, torn the internal organs from their attachments, leading to avulsion injuries (D. DiMaio & V. DiMaio, 2001). Sometimes, an avulsion injury can be hidden in an appeared normal skin, and the only thing seen is a pocket of blood or blood-tinged fluid underneath the skin, which ordinarily occur "when an animal is picked up by the scruff of the neck and shaken" (Merck *et al.*, 2012g). In this case, the blood pocket may appear due to a tracheal avulsion near the bifurcation of the mainstem bronchi (H. Munro & R. Munro, 2008).

2.9.1.5. Fractures of the skeletal system

A fracture can be caused directly or indirectly by blunt force trauma and it is defined as a catastrophic disruption of bones and teeth, by one or more of five basic forces - tension, compression, bending, shear, and torsion (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012g). In the overlying tissue of a fracture is often found associated injuries such as contusions, abrasions, or lacerations (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012g).

A fracture can be classified on oblique, transverse, comminute, spiral, longitudinal split, segmental, tension wedge, and compression wedge, which depends on the type of force applied to (Merck *et al.*, 2012g). When there is a fracture site distant from the applied force, it is called an **indirect fracture** (Merck *et al.*, 2012g).

To determine the time of a fracture, it should be evaluated the around tissue; a recent fracture has fresh haemorrhage around the fracture site and adjacent soft tissues, as well as "clot formation between the fractured pieces and fibrin strands over and between the fractured ends of the bone, and absence of obvious repair; whereas an slightly-older fracture has "accumulation of yellow, serum-like fluid around the site or in the soft tissues distal to the fracture", which suggests that haematoma retraction has already occurred; in older fractures there is the formation of callus (H. Munro & R. Munro, 2008)

2.9.1.6. Specific types of cruelty associated with blunt force injuries

2.9.1.6.1. Fall injuries

Fall injuries are included on blunt force trauma, and assumes a special importance given that the FVP should take into account that such injuries may be non-accidental, as they can be due to throwing or dropping the animal (Merck *et al.*, 2012g).

The lesions found "should be distributed over bony prominences and have a pattern of injuries along one plane of body impact"; and in contrast, when found on recessed or protected parts of the body it should rise suspicion of non-accidental causes (Merck*et al.*, 2012g). Thereby, it must, whenever possible, have information about the occurred fall - how the animal fell, the distance, the mass of the animal, the species, and age - and the surface on which the animal

landed and if could have landed on any objects or surface protrusions, in order to the FVP be able to know what injuries to expect and discern those suspicions (Merck *et al.*, 2012f).

It is imperative that the FVP have some knowledge regarding on the characteristic injuries of some species; for instances, "dogs are more likely to have extremity injuries than cats", while cats have more tendency to suffer severe facial trauma; yet they are more likely to survive falls from heights greater than six floors than dogs (Merck*et al.*, 2012g, p. 108). Distinguished falls from jumping is perhaps achievable; Merck *et al.* (2012g), claim that dogs that jump are more likely to have forelimb, head, thoracic and spinal injuries and those that fall are more likely to have hindlimb injuries.

2.9.1.6.2. Swinging/ dragging injuries

Swinging and dragging an animal either by the tail or limbs, is somehow common in AAN, which results in multiple blunt force injuries such as contusions, abrasions, lacerations, avulsion injuries, fractures, dislocations, ligament injuries, muscle tearing (Merck *et al.*, 2012g). Being alert to some specific lesions may help to recognize cases of swinging and dragging. These lesions can be a dislocation of the elbow joint, without fracturing the anconeal process, which is caused by traction and torque forces created by pulling and twisting the forearm, and it can happen in cases which the animal is swing or suspend by the leg; a caudal displacement of the femoral head relative to the coxo-femoral articulation indicates a tearing of the muscle attachments as a result of force applied caudally to the hindlimb, which occur in swinging or suspension of the animal by the hind leg; or a luxation or even a fracture of the cranial coccygeal vertebrae, if the tail is pulled away or forced dorsally or laterally, respectively (Merck *et al.*, 2012g).

"Abrasions from dragging an animal usually are more circular or elongated in shape, typically located over the points of the body that had ground surface contact" (Merck *et al.*, 2012g, p. 108).

2.9.2. Sharp force injuries

Sharp force injuries are characterized as being wounds resulting from skin penetration as well as the underlying tissues with a relatively well-defined traumatic separation of them, that are

caused by a mechanical force against the skin, through pointed or sharp-edged objects (de Siqueira *et al.*, 2016; Merck *et al.*, 2012f). They are classified according to the lesion pattern as **incised wounds** (cuts or slashes), **stab wounds** (puncture or penetrating wounds), **chop wounds** (produced by heavy instruments, such as cleavers, axes, and machetes) and **therapeutic/diagnostic wounds** (resulting from veterinary intervention) (de Siqueira *et al.*, 2016; D. DiMaio & V. DiMaio, 2001; H. Munro & R. Munro, 2008).

The analysis of the edges of the wound is crucial, thus sometimes the coat may have to be shaved to better assessment; for instances, the correct assessment of a wound edges may discern a laceration (blunt trauma injury) from a incised wound (sharp force injury) (de Siqueira *et al.*, 2016). Thus, upon necropsy, the "overall wound length, width, depth, and directionality should be described, including the wound's margins and angles", as it may provide information about the instrument involved, the relationship between the animal and the perpetrator, and the force of the stab (de Siqueira *et al.*, 2016). The FVP should be aware of the changes that a wound appearance can ensue due to elasticity or laxity of the skin, either increasing or decreasing the size, thus it should be re-approximated the wound margins, by using clear tape over the wound or superglue to bind the subcutaneous tissues together, in order to identify weapon characteristics and accurate measurements (Merck *et al.*, 2012f). It should be described the direction of the long axis of a sharp force wound - vertical, horizontal, or angled – merely referring to the orientation of the long axis and not to the direction of the cutting (Merck *et al.*, 2012f).

2.9.2.1. Incised wounds

Incised wounds are inflicted by sharp or pointed instruments originating slashes or cuts, which mean that the defect is generally longer than it is deep, and as all sharp injuries, have a clean division of the skin and underlying tissues with almost any damage of the margins, being them sharp and clean with no abrasions, contusions, or tissue bridging in the base of the wound, and they can be superficial - cuts (Figure 22a) - or deep – slashes (Figure 22b) (Brownlie & R. Munro, 2016; D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012f; Saukko & Knight, 2004). The wound typically begins very superficially, become deeper, and then become superficial again, and likewise lacerations, the edges of the wound can reflect the angle that the object impacted on the skin, or instances, with an oblique angle, the wound will present a bevelled or

undermined edge; and with an extreme angle, it will be produced a skin flap (D. DiMaio & V. DiMaio, 2001). Other rare lesions can be produced by a single slash with a sharp, edged weapon, and they are called as "wrinkle wounds", which happen when the skin is not flat, but in folds, making "the cutting edge skips from crest to crest of the skin, leaving a string of cuts, all of which have resulted from a single slash" (Merck *et al.*, 2012f).

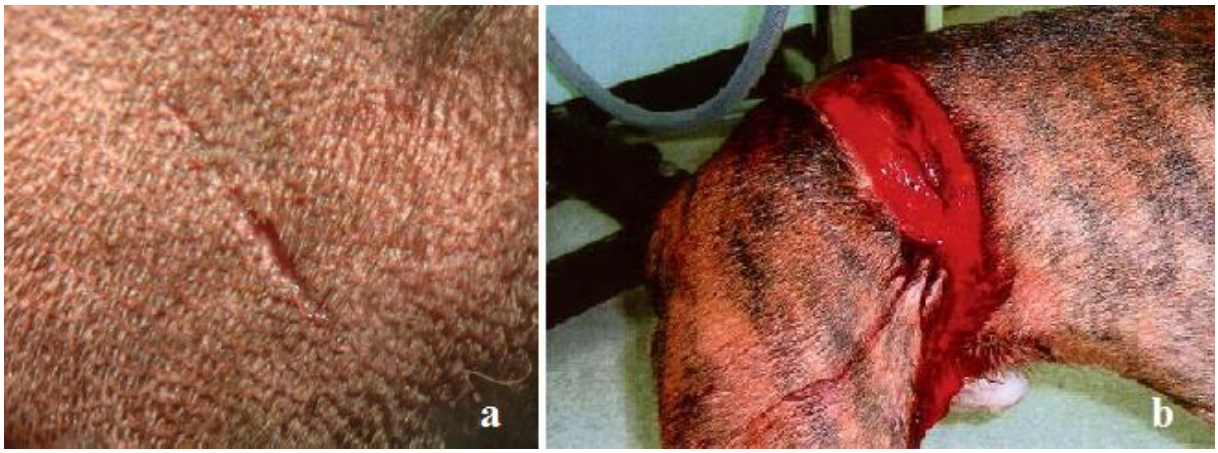

Figure 22: Examples of *ante-mortem* incised wounds. Denote that the defect is generally longer than it is deep, and as all sharp injuries. 22a. A superficial incised wound (cut); 22b. A slash wound caused by a sharp blade in a dog. Adapted from Merck *et al.* (2012f) and H. Munro and R. Munro (2008).

Sharp-edge instruments or weapons that created incised wounds includes knives, glass and metal implements, however, other instruments with dull, irregular, or nicked cutting edge can also produce incisive wounds, which can be very similar to lacerations, and can be differentiated by the absence of tissue bridging in the depth of the wound on the incised wounds (Merck *et al.*, 2012f). The examination of the edges of the slash may reveal important information about the object used, for example, the serration of a knife may be seen along the wound margins and are even more visible when the edges are re-approximated (Merck *et al.*, 2012f).

Slashed wounds of the neck are the most dangerous, with the most serious complication being bleeding (Saukko & Knight, 2004); however, death may not always be only due to exsanguination, but to massive air embolus (D. DiMaio & V. DiMaio, 2001). "Slashed wounds are less dangerous than stabs, as the relative shallowness of the wounds is less likely to affect vital organs" (Saukko & Knight, 2004, p. 154).

2.9.2.2. Stab wounds

Just like incised wounds, stab wounds are also produced by pointed instruments (D. DiMaio & V. DiMaio, 2001); in fact, a stab wound is an incised wound that is deeper than it is wide, this is, the depth of the wound exceeds its length in the skin, and its edges in the skin are typically sharp, without abrasion or contusion (Figure 23) (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012f; Saukko & Knight, 2004).

Figure 23: A characteristic stab wound, from a single-edged blade knife. Notice the depth of the wound exceeds its length in the skin, and its edges in the skin are sharp, without abrasion or contusion. Adapted from Merck *et al.* (2012f).

Stab wounds are originated by sharp or pointed objects, which includes a variety of instruments as knives, scissors, screwdrivers, pens, forks, broken glass, ice picks, etc. According to Merck *et al.* (2012f), "the most common weapon used is a knife, primarily a single-edged blade, which also can produce incisive wounds" (p. 124).

The surface and internal appearances of a stab wound may give information about the dimensions and the type of the weapon, the amount of force used, the taper of the blade and its movement in the wound, the depth and the direction of the thrust, among others (Saukko & Knight, 2004).; however, the appearance of the stab wound depends on a diversity of factors such as the skin properties, the nature of the blade and knife, the movement of the victim, and the movement of the blade in the wound, which makes the interpretation of the surface and internal appearances of a stab wound to be sometimes very challenging (Merck *et al.*, 2012f). The better way to examine a stab wound it is reconnecting its edges and then measured, to obtain an estimation of blade width (de Siqueira *et al.*, 2016). The depth of the stab wound in the skin can be equal, less or greater than the length of the blade - it may be lesser if the blade

is not inserted all the way, and it may be greater if the "knife is plunged deeply into the body with such force as to indent the abdominal or chest wall, so that the length of the knife track exceeds the length of the knife blade" - whereas the width of the wound may be lesser or greater than the width of the blade – it may be lesser because of the elasticity of the skin, which contracts, and it may be greater if the cutting edge of the blade is drawn against the skin, slicing through it and enlarging the wound (de Siqueira *et al.*, 2016; D. DiMaio & V. DiMaio, 2001). Thus, in cases of numerous stab wounds, it must be all examined and make a length and width average (D. DiMaio & V. DiMaio, 2001). It must not be forgotten to be traced the track of the stab (de Siqueira *et al.*, 2016).

2.9.2.3. Incised-stab wounds

When a stab wound is converted to an incised (slashing) wound, it is called an incised-stab wound (D. DiMaio & V. DiMaio, 2001). This happens in cases which the knife is plunged into the body, stabbing it, but instead of withdrawing the blade, it is pulled along the body, cutting through tissue and creating a wound that is longer than it is deep, in one continuous flowing movement (D. DiMaio & V. DiMaio, 2001). "The only way to determine the direction is if there are markings at one end indicating where the blade was withdrawn" (D. DiMaio & V. DiMaio, 2001).

2.9.2.4. Chop wounds

"A chop wound represents a combination of sharp and blunt force injuries" as they are produced by the impact of heavy instruments with a cutting edge, such as machetes, axes, meat cleavers, or by sharp objects wielded with a tremendous amount of force, which creates a sharp force injury (cutting of the skin and underlying tissues) through the edge of the object, and in the same way creates associated abrasions, lacerations, contusions and/or fractures by the intensity of the force or the relative bluntness of the object (Figure 24) (D. DiMaio & V. DiMaio, 2001; Merck *et al.*, 2012f). Thus, commonly it is seen as an incised wound of the skin with an underlying comminutes fracture or a deep groove in the bone (D. DiMaio & V. DiMaio, 2001).

Figure 24: Multiple chop wounds in the head of a dog made from a meat cleaver. Notice the sharp force injury through the edge of the object along with abrasions, lacerations, contusions and/or fractures. Adapted from Merck *et al.* (2012f).

The appearance of chop wounds can also support the determination of the type of weapon used, since "the weapon can leave unique tool mark striations on the bone" (Merck *et al.*, 2012f). For instance, meat cleavers macroscopically create clean, narrow wounds without fractures and microscopically creates sharp, distinct, fine striations in the bone, whereas "machetes produce wider, less-clean wounds with fractures in the bed of the cut and small fragmented bone at the entrance site" (Merck *et al.*, 2012f).

2.9.2.5. Specific types of cruelty associated with sharp force injuries

2.9.2.5.1. Mutilations, predator attacks and dog attacks

Mutilation of live animals, predator or dog attacks are specific types of cruelty associated with sharp force injuries and are often seen in veterinary forensic as they may be cases of criminal investigations or potentially involve eventual civil litigation (Merck *et al.*, 2012f).

Mutilations are frequently done by "predators, such as foxes and coyotes, which prey on pets that are allowed outside", but it can also be done by human hand, for instance, a decapitated body, with the head missing, may be evidence of a ritualistic crime, animal cruelty or human opportunistic action for a trophy (Merck *et al.*, 2012f).

Is each the above case, “it is important to conduct a complete forensic odontological exam to include, identify, or exclude certain dogs accused of the crime” (Merck *et al.*, 2012f). Also, at the exam of the victim the it should be searched for foreign hair/fur and evidence of saliva, where the use of UV light or alternate light source (ALS) may be of value (Merck *et al.*, 2012f). The injuries found on the victim must be determined as *ante-mortem*, *peri-mortem* or *post-mortem*, and usually include “abrasions on the head or muzzle from dragging and impact injuries, dirt or debris in the mouth, and other findings typical of head trauma such as fractured teeth and ocular injuries” (Figure 25) (Merck *et al.*, 2012f).

Figure 25: Chin abrasions on a cat from dragging in a predator attack. Adapted from Merck *et al.* (2012f).

Bite marks are found on the skin surface, and they should be carefully photographed taken at 90° angles, first without and then with the ABFO (American Board of Forensic Odontology) no. 2 photo scale, in order to compare them with the suspected attacking animal. The injury provoked by the canine teeth typically is characterized by elliptical puncture wounds and may be deep punctures or tears in the muscle and/ or internal organs due to the struggle during the attack and/or shaking of the victim by the predator, thus the skin must be reflected to reveal these characteristic injuries (Merck *et al.*, 2012f).

Sometimes, the FVP, expert in forensic odontology, may be called to aid on the investigation of dog and predator attacks on humans (Merck *et al.*, 2012f).

2.9.3. Thermal injuries

"Thermal wounds are relatively uncommon in veterinary practice" and they can be classified into **burn-, electrical-, and fire-related injuries** (Merck & Miller, 2012d; Pavletic & Trout, 2006). The burn-related injuries includes scalds, hot stove surfaces, radiators, hot air dryers, hot water bottles, chemical (exothermic) hot packs, heat lamps; electrical-related injury comprehends electrical heating pads, electrical cords, and improperly grounded electrocautery units; and fire injuries are all those that cause flame burns (Pavletic & Trout, 2006).

Obviously, not all injuries are of non-accidental character, and through the examination of the lesions patterns, the FVP should reach to a suspicion of deliberated infliction when the injuries seem to indicate it. In burns, this suspicion is raised when the owner history does not match to the presentation of the burn, the environment, or the expected animal behaviour that could result in accidental burns (Merck & Miller, 2012d). Other times, especially at the veterinary practices, the burns ae truly accidental, either be by an excessive appliance of supplemental heat to treat or prevent hypothermia in patients under or recovering from general anaesthesia or by radiation burns of radiotherapy for oncology treatment (Pavletic & Trout, 2006).

Burns are generally classified according to its depth and the body surface area involved, thus they a **first degree burn** – when is superficial, only involving the outermost epidermis - a **second degree burn** – affecting partial thickness, involves the epidermis and a portion of the dermis – and a **third degree burn** - reaching the full-thickness epidermis and dermis (Pavletic & Trout, 2006).

A type, apparently common, of non-accidental burns in veterinary forensic are the cigarette burns (H. Munro & Thrusfield, 2001b). There are no morphological description of these burns in animals, but the same lesions in children are well described, and summarily they appears to be a red, circular mark, with a diameter of 0,5 to 1,0 cm, and they are full thickness and cratered, leaving circular, depressed, paper-thin scars in abuse situations (Figure 26), but are superficial and eccentric, with a tail from the brushed contact, if accidental (H. Munro & Thrusfield, 2001a).

Figure 26: A red, circular mark, with a diameter of 0,5 to 1,0 cm, cratered, appearing to be a non-accidental burn lesion, in a dog. Adapted from http://hopedogrescue.blogspot.com/2014/05/puppy-bruno-1st-update.html

2.9.4. Firearms injuries

According to J. Cooper and M. Cooper (2008), in *"Forensic veterinary medicine: a rapidly evolving discipline*", "most firearm injuries to domesticated animals (…) are caused by shotguns, airguns, or rifles" (p. 79), therefore, the FVP needs to have some knowledge of how to investigate injuries associated with firearms, or if he/she does not have the knowledge, should be willing and able to consult an appropriate expert.

When performing a necropsy of a suspected firearm injury, the main objectives are to identify of the entry and exit wounds, to recuperate the projectile(s) if there is not the exit wound, and to determine the direction and range of fire (Gerdin & McDonough, 2013). It is also advisable to reconstruct the track of the projectile, using malleable objects such as urinary catheters or insemination pipettes, which may indicate the relative position of time animal and the shooter (Gerdin & McDonough, 2013; Green, 1979; Wobeser, 1996). If there is a entry or exit wound and on radiographs is not seen no projectile, it is necessary to search for the corresponding exit/entry wound, which sometimes it is only possible by skinning and examining the subcutaneous side (J. Cooper & M. Cooper, 2007).

The diverse damage that a firearm injury can have on a body lean on the "characteristics of the projectile, the kinetic energy absorbed on impact, and the tissues struck by the projectile" (Pavletic & Trout, 2006, p. 873). For instance, they may be perforating, this is, passing along

the completely body, or penetrating, keeping the projectile on the body (D. DiMaio &V. DiMaio, 2001). However, the presence of bullets, shotgun pellets or any other metallic fragments in the body of an animal, does not necessarily indicate that they were responsible for its death; otherwise they can be merely from a previous, nonfatal, shooting or similar incident, or be ingested and be within the gastrointestinal tract, or be acquired after the animal death (J. Cooper & M. Cooper, 2008).

An entry wound is often characterized by their perfectly circular to oval shape, with finely abraded margins, and in general its diameter are smaller than exit wounds (Figure 27a) (Pavletic & Trout, 2006; Wobeser, 1996); but, as all rules, there are exceptions, and in cases which the bullet fragments, the individual exit wounds may be very small (Wobeser, 1996). Exit wounds are generally irregular and can have many shapes (round, oval, stellate, crescent, etc.) due to the deformation and flattening of the bullet during its passage through the tissues, but usually don't have abrasion associated of the skin margins (Figure 27b) (V. DiMaio, 1998; R. Munro, 1998). Depending on the angle that a projectile srikes the skin, it will create a certain characteristic – if perpendicular, an abrasion margin will either not be appreciable or form a complete ring, while if angled, a bevelling will indicate the direction from which the bullet came and, hence, the position of the shooter (V. DiMaio, 1998). This information is severely relevant to affirm or refute the shooter's account of events – the shooters may claim self-defence against an attacking dog, but if bevelling margins are seen it indicates the dog was shot from the side or behind, contradicting his/her self-defence theory (Gerdin & McDonough, 2013). The direction from which gunshot wounds were created are sometimes able to determine the direction of the shot, e.g. in bone injuries, cone-shaped (outward bevelling) defects show the direction in which the projectile passed through the bone (Dirnhofer *et al.*, 2006).

Figure 27: A gunshot injury. 27a. The entry wound in the right flank of a cat. 27b. The matching exit wound in the left flank, observe that his hole is marginally larger than the entrance hole and shows a halo of bruising. Adapted from H. Munro and R. Munro (2008).

The distance that the firearm is shot from the animal's body can, in some situations, be estimated; if the gun is fired in close contact range (<0.6 meters), it may be seen a stippling, this is, a punctate black spots of unburned gunpowder surrounded the entrance wound that cannot be wiped away the skin; but a soot, a black powder composed mainly from carbon that comes out from the muzzle and can be wiped off the skin is only seen in contact range, where the firearm is held up against the body when fired (Gerdin & McDonough, 2013). Nevertheless, finding soot and stippling on the pelage of animals can be challenging, thus it is recommended a close inspection and photography of the entry wound, and for detection of soot, a careful wiping with a white paper towel may be done, and for identify stippling, shaving may be the best method (Gerdin & McDonough, 2013).

During the necropsy, if a projectile is found in the cadaver, primarily it should be photographed in situ and then removed using gloved fingers or plastic instruments as rubber-covered forceps; they never should be handled with metal forceps as "instruments can ruin microscopic patterns on the bullet, confounding ballistic testing" (Gerdin & McDonough, 2013; Wobeser, 1996). After its recovery the bullet must be washed free of blood and tissue, by holding it in the hand under running water over a strainer, and after it is dried it should be placed in a padded paper envelope container (to prevent corrosion), clearly labelled with the case number, sealed, and signed (Sinclair, Merck, & Lockwood, 2006; Wobeser, 1996).

2.9.5. Asphyxia and drowning

Asphyxia is a wide-ranging term defined as an interference with uptake and utilisation of oxygen and elimination of carbon dioxide and can be caused for situations leading to: (1) lack of oxygen in the inspired air; (2) obstruction of the air passages; (3) restriction of chest movements; or (4) histotoxic anoxia (H. Munro & R. Munro, 2008). The situations that commonly instigated asphyxia are: **strangulation** (includes ligature strangulation, manual strangulation and hanging), **suffocation** (includes choking and smothering), **mechanical asphyxia** and **drowning** (Merck & Miller, 2012c; H. Munro & R. Munro, 2008). Strangulation leads to asphyxia by the closure of air passages as well as blood vessels of the neck due to an external pressure on the neck, as they being by ligature strangulation, manual strangulation and hanging the animal; chocking is a type of suffocation which asphyxia occur by obstruction of the air passages below the epiglottis, blocking the upper airways; while the obstruction in

smothering occurs above the epiglottis; **mechanical asphyxia** is either due to the position of the body or to external chest compression which restricts the respiratory movements; and finally, drowning leads to asphyxia by the immersion in a liquid (Merck & Miller, 2012c; H. Munro & R. Munro, 2008).

Deliberated **strangulation** on animal victim's usual occur by hanging them to a tree branch, a loft hatch or other convenient high point (H. Munro & R. Munro, 2008). Hanging is a common "form of strangulation in which the pressure on the neck is applied by a constricting band tightened by the gravitational weight of the body or part of the body" (Merck & Miller, 2012c). At the external examination there may be limited evidence of ligature marks in fatal strangulation, particularly because the coat and the looseness of the skin around the neck can protect against skin abrasion, and sometimes only an indentation of hair (Figure 28), patchy loss of hair or reddening or mild bruising of the skin (Figure 29) is observed (H. Munro & R. Munro, 2008). Other external signs may include cyanosis in the teats, perineum and vulva, a generalised congestion of the skin of the ventral neck, thorax or abdomen, or in cases of hanging, the skin of the posterior abdomen and immediately cranial to the ventral vulva may present focal intradermal haemorrhages, and congestion of the sclera of one or both eyes (H. Munro & R. Munro, 2008).

Figure 28: A situation of a dog strangulation, being possible to see the indentation of hair in the neck. Adapted from (H. Munro & R. Munro, 2008).

Figure 29: A situation of a dog strangulation, being possible to see a mild bruising and reddening of the skin on the ventral surface and both sides of the neck. Adapted from (H. Munro & R. Munro, 2008).

Examination of the subcutaneous tissue, after the skinning, may be shown a subcutaneous bruising present over the larynx at the angle of the jaw, or at the area of where the knot in the ligature has tightened against the neck in cases of hanging (H. Munro & R. Munro, 2008); it also may be features as "epiglottic congestion, fresh ecchymotic haemorrhages over the laryngeal cartilages or in the glossoepiglottic fold" (H. Munro & R. Munro, 2008, p. 65). Opening the internal cavities, at the lower trachea, commonly it is seen a pinked froth, which sometimes may be extended into the larynx (H. Munro & R. Munro, 2008). The gross finding at the thorax of a strangulated animal is severe congestion of the diaphragmatic lobes as well as the lungs, with collapsed areas, and in some cases, with the rib markings are clearly defined due to over-inflation of the lungs (H. Munro & R. Munro, 2008).

Suffocation by choking is a blockage of the upper airways usually by a foreign body, in example, in a non-accidental live burial may lead to a death by chocking on the mud, sand or other material in which the animal was buried by its inhalation (H. Munro & R. Munro, 2008). The changes on the lungs in choking, as well in smothering cases, are congestion with intra-alveolar flooding and haemorrhage and alveolar over-distension (H. Munro & R. Munro, 2008).

Non-accidental **drowning** is also typically found in veterinary forensics, being the animal immersed in a water or other liquids, inhibiting the breathing of available oxygen, and even though may seem obvious, the coat of the cadavers are often wet at necropsy; however it may be taken into account that other times the coat may be partially dried, as the perpetrator may attempt to dry it to cover the suspicion of abuse (H. Munro & R. Munro, 2008). Consistent pathological changes are congestion of the lungs with variable quantities of pink frothy fluid in the bronchi and the trachea (Figure 30) (H. Munro & R. Munro, 2008).

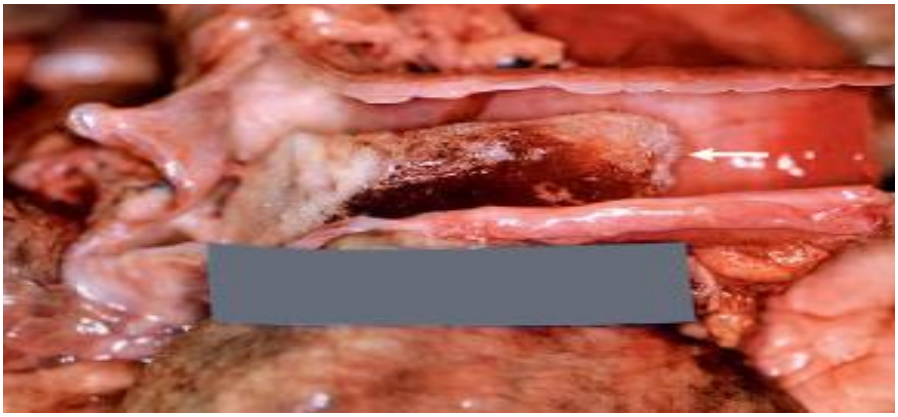

Figure 30: A frozen pink frothy fluid in the lumen of the trachea of a drowned dog. Adapted from (H. Munro & R. Munro, 2008).

External injuries may occur when the animal fights to survive, in the form of bruising; if the animal was pressed and hold by the head or by the whole body, bruising may be seen develops over the side of the neck and the back where the animal was gripped; "when held by the legs and inverted, bruising may be found on the distal limbs where the legs were gripped or tied together" (H. Munro & R. Munro, 2008).

2.9.6. Poisoning

Regarding to poisoning would be a lot to discuss; the FVP knows there are unlimited amounts of malicious toxic agents, hence he/she knows that challenge that toxicology can be if not be rightly oriented for a suspicion narrow range of agents. Gwaltney-Brant (2012) performed an informal survey for toxicologists at some veterinary diagnostic laboratories, concluding that the primary poisons of choice in intentional poisonings are anticoagulant rodenticides, ethylene glycol, organophosphate insecticides, carbamates, strychnine, caffeine, and methylxanthines (Gwaltney-Brant, 2012). In a general way, the perpetrator commonly uses the poisons more accessible found, which is directly related to the geographic laws regarding to the legitimacy of the poisons; also, may be related to the his/her labour, e.g. more probably a farmer may use organophosphate insecticides and an industry worker may use, e.g. ethylene glycol.

Towards suspicion of poisoning it is imperative the collection of samples for confirmatory tests by toxicology (Gwaltney-Brant, 2012); and it must be recalled that these samples must be more than is thought necessary rather less, because can always be thrown away the unneeded samples but it cannot be re-collected (Gwaltney-Brant, 2012). A start point is closely examining the stomach contents for evidence of foreign objects, such as granules and pellets, plant material, foods, pill casings, tablet fragments, or illicit drugs, and send for the toxicology (Gwaltney-Brant, 2012). The toxicology department or laboratory must be contacted in order to know what organs to collect (usually samples of heart blood, liver, kidney, urine, stomach/intestinal contents, hair, and feces; if organophosphates or carbamates are suspected, samples of brain and retina (entire eyeball) are required) and how to send it, according to the suspected narrow range of toxics. The range of suspected toxics are usually determined based on the clinical, historical, and environmental findings in the case (Gwaltney-Brant, 2012). Generally, "glass containers are preferred over plastic because plastic can leach contaminants into samples over time", but if plastic containers must be used, it must be opted for harder plastic as it pose less

risk of sample contamination; syringes should never be used for sample store, because of the risk of leakage and of the risk of needles to receiving personnel (Gwaltney-Brant, 2012). The "Table 1" gives some general information about the shipping conditions of the various organic tissues.

Table 1: General packaging and shipping conditions of toxicological samples

Organic tissue	**Packaging**	**Container**
Blood	Refrigerated, never frozen	Preferably glass containers; hard plastic containers are acceptable
Urine	Refrigerated or frozen	
Gastric contents, and feces	May be frozen	
Hair samples	Dry damp hair	Paper envelope

2.9.7. Neglect

Neglect is defined as the failure to provide the basic physical necessities of life - food, water and shelter – as well the veterinary attention needed for injury and naturally occurring illness of the animal in custody (H. Munro & R. Munro, 2008). Neglect also includes abandonment and the practice of allowing a collar to tighten and constrict the neck of a growing animal (H. Munro & R. Munro, 2008).

Dealing with neglected cases at the necropsy, the main goal is to "document the condition of the body and to rule in or out concurrent disease that accounts for it" (Gerdin & McDonough, 2013). The FVP should be alert to the physical manifestations of inadequate husbandry, which are indicative of neglect: unkempt and/or dirty hair coat, overgrown nails, reduced body condition, and external and/or internal hyperparasitism (Gerdin & McDonough, 2013). Characteristic findings of neglect must always be documented, recorded and kept as evidence, such as the hair coat (if severely matted), the type and relative number (severity) of external parasites, and dental lesions such as missing teeth, fractures, and advanced or abnormal wear, etc. (Gerdin & McDonough, 2013).

The greatest impression in a neglect case by **starvation** is the markedly reduced body condition, many times already with loss of skeletal muscle; and when the reduction of body condition is extreme, it is called emaciation (Gerdin & McDonough, 2013); some authors call this state as cachexia, while others, like Gerdin and McDonough (2013), prefer reserved the term cachexia for the cytokine-mediated wasting that occurs in conjunction with diseases such as cancer or organ failure. In order to have clarity on the used terms, there is the need of using a published body condition scoring (BCS) system worldwide; A 1 to 9 scoring system made by WSAVA (Annex I) for dogs and cats has been shown to be repeatable within observers and consistent between observers (Gerdin & McDonough, 2013). In suspected cases of emaciation, bone marrow should be collected to histopathology, since it is the last anatomic location to undergo serous atrophy of fat (Gerdin & McDonough, 2013). At necropsy the body weight and also the internal organs weight must be recorded, because it will be reduced due to the loss of subcutaneous adipose tissue and adipose tissue surrounding internal organs, atrophy of internal organs, and atrophy of muscles (Madea, 2005); "the gallbladder is commonly distended as a result of the absence of food as the natural stimulant of bile excretion" (p. 7), and usually the stomach and small bowel are empty, and may be present gastric ulceration and indigestible materials (plastic bags, rubber, cloth, wood) at the stomach; the colon characteristically have extremely dry stool (Madea, 2005). "All organs atrophy as emaciation progresses, with the changes being most obvious in the liver, spleen, pancreas, thymus and salivary glands" (H. Munro & R. Munro, 2008, p. 19). Therefore, if a forensic necropsy is conducted on an emaciated body, it should be looked for diseases that could account for emaciated states (e.g. neoplasia), and if not found, the cause of death could be considered as being starvation (Madea, 2005). Besides that, fatal starvation may be accompanied by dehydration as immediate cause of death, which is characterised by "classical autopsy findings as loss of body weight, sunken eyes, poor skin turgor, tinting of skin, and dry organ surfaces on cut sections" (Madea, 2005, p. 19).

2.9.8. Sexual abuse

Sexual abuse is defined as "the use of an animal for sexual gratification" (H. Munro & R. Munro, 2008). Formerly it was used the terms 'bestiality' and 'zoophilia', which nowadays are replaced by the term 'animal sexual abuse', increasing the idea of the animal as a victim of a physical harm (H. Munro & R. Munro, 2008).

The nature of sexual interaction between a human and the animal can be expressed of many ways, as the following examples: masturbating the animal, receiving oral sex, performing oral sex, performing vaginal intercourse, performing anal intercourse, receiving anal intercourse, sodomy with objects, etc. (Merck & Miller, 2012b).

The necropsy findings will depend on the type of sexual abuse practice, but a very fact is that it will almost always involve the sexual organs or rectum, and the severity of injuries found depend on what was used to commit the assault, the type of contact, the size of the penetrating object, and the size of the animal, perhaps being fatal for the animal's life – e.g. fatal peritonitis due to rectum perforation (H. Munro & R. Munro, 2008).

2.10. Sample collection and ancillary tests

The ancillary tests commonly used on forensic necropsies includes histology, toxicologic, microbiologic and entomologic analyses, ballistics and, recently in some laboratories, DNA tests to establish identity of individual animals, etc.; the great difference to those used on clinical necropsies is that in forensic all the collected samples are evidence, thus they must be handled, collected, stored and sent following strict rules in order to maintain the chain of custody (Wobeser, 1996). Denote that a large part of the ancillary tests will be useless if the necropsy was performed late of the death (Peleteiro *et al.*, 2016).

The samples to be sent for posterior analysis, either on the same laboratory or for others, must go along with the following information:

1. Identification and contacts of the performing FVP and laboratory;
2. Identification of the cadaver, including the case number;
3. Information about the death;
4. Date and time of the sample collection;
5. What ancillary tests are required;
6. Type of sample sent;
7. The suspicion (Peleteiro *et al.*, 2016).

If sent more than one sample of the same animal to the same department, each one of them must be properly identified and its matching identification be described in the animal form, sometimes this can be marked by colour pins or suture stitches on the samples (Vala & Pires, 2016).

Herewith, quickly can be noticed that the "forensic autopsy is not just the act of anatomical dissection"; instead it encompasses a wide variety of specialist investigations (O'Donnell & Woodford, 2008). The right collection of the samples can determine the success of the case, or otherwise, botch the entire case (Vala & Pires, 2016). The FVP should carefully manage the samples in a rapid and proper way according to the type of sample, choose the most adequate recipient to its storage, do a correct and complete identification on the container and guarantee that the one is on a convenient packaging for transport to the laboratory (Vala & Pires, 2016).

2.11. Forensic necropsy report

Saukko an Knight (2004) alleged that the forensic necropsy report provided by the FVP for whoever commissioned the examination was as much important as the necropsy itself, and further it was an integral part of the necropsy procedure, wherein it should have been received as much attention as any necropsy physical procedure. In 2012, Merck, Miller, & Maiorka, corroborate with that, affirming that "the reports generated for any legal case are of utmost importance" as they "are legal documents and are examined throughout the legal process of the case" by the "entire community of law enforcement, prosecutor, defence attorney, defendant, judge, and jury".

It is advisable to write the report right after, or even during (through recording notes), the necropsy; it must be written in present tense, in careful language, without slang or abbreviations, with a minimum font size of 12 point and double spaced (H. Munro & R. Munro, 2008; Pires, 2016). Sentences must be clear, concise and transparent and focused on the description of the pathological element, without issuing personal non-medical opinions or value judgments (Pires, 2016).

It is highly recommended that the necropsy report be writeen, as far as possible, with a non-technical language, this is, with a limited use of medical terms and jargon, because although may be convenient for the FVP to use specific anatomical and pathological terms, it is entirely

unhelpful for the lawyers, judges and other non-veterinarians who are required to read the report; thus is advisable formulated sentences in which the lay term first, and the medical term after, in brackets, e.g. 'the long narrow bones in the hind foot (metatarsal bones)' (H. Munro & R. Munro, 2008). However, there are terms that because of its common use on human forensic medicine are already understood by lawyers and judges, and therefore are familiar to the court, as the following examples: abrasion, laceration, incised wound, puncture wound and perforating wound (R. Munro, 1998; H. Munro & R. Munro, 2008); the use of these known terms in veterinary cases simplifies reports and makes veterinary cases more understandable to the legal profession and to juries (H. Munro & R. Munro, 2008).

After elaborated, the necropsy report should be double-checked, not only regarding to its pathological substance, but also for typographical and clerical errors, and, if possible, by someone other than the person who wrote it, "because simple mistakes are easy ways for attorneys to call into question a pathologist's aptitude and ability" and this document will be dissected in a court of law months or even years afterwards, and probably many details won't be remembered by the FVP, therefore they should be clearly expressed in this vital permanent record of the necropsy findings (Gerdin & McDonough, 2013; Saukko & Knight, 2004). It is never too much to remember the importance of a clear, transparent and well written legal report, since a poorly written report may not be correctly understood by the target-audience (police, the prosecution and ultimately the court) and results to be detrimental for the case (Merck, 2013).

Being the purpose of the forensic report the provision of a clear understanding of the veterinary evidence, it must gather all the information provided by the investigating authorities, the exam findings, the procedures, the samples collected, test submissions and results (medical and forensic) (Merck *et al.*, 2012e); a provisory report may be sent out when waiting for lab results or others (ballistics, entomology, …), including "at least a preliminary cause of death (where possible), a summary of the major findings and details of what has been retained", and only after their arrived, the complete and final report should be sent, taking into account that may be the need to modify the conclusions and cause of death based on the results received (The Royal College of Pathologists, 2002).

The forensic necropsy report should follow a structured systematic format, similar to those of a diagnostic necropsy report, with the greater difference that in a forensic report "all organs or

tissues examined should be noted, even if just to say that there were no significant gross abnormalities" (Brownlie & R. Munro, 2016). Yet, there is no strict rules about the report conception, even though it should follow the same technique as used on clinical necropsy (Pires, 2016); it can be performed in a "free-style" – the pathologist is free to expand on various aspects according to his evaluation of their importance - or in a "printed proforma"/necropsy protocol – in which the various sections of the examination and organ systems are already set out by title; this last has the advantage of aid the FVP not forgetting any steps of the necropsy (Saukko & Knight, 2004). Diagramming numbered injuries in the written report is encourage, since they are a clear and easy way of presenting findings in court, giving a better uptake of the number and location of injuries as well finding their corresponding description in the report; they must be dated and signed in order to be considered part of the official report (Gerdin & McDonough, 2013). Photography is highly advised and encouraged on a forensic report, as it preserves visual evidence (Wobeser, 1996).

The terminology used in the forensic report is crucial to its credibility, thus the FVP should guard him/herself by using terms such as: "undetermined" (less than 50% certainty); "reasonable medical or investigative probability" (50%– 70% certainty); "preponderance of medical/investigative evidence" (70%– 90% certainty); "clear and convincing medical/investigative evidence" (90%–99% certainty); and if medical or scientific proof that something occurred, statements as "beyond any reasonable doubt" (99% certainty) and "beyond any doubt" (100% certainty) can be done (Gerdin & McDonough, 2013; Merck, *et al.*, 2012e).

The report should have different sections with the goal of facilitate the access of information, as the following example of general description of a complete report, based on the recommendations of Merck *et al.* (2012e) and H. Munro and R. Munro (2008):

Title page

"The report reference number and/or the animal's name together with the name and address of the author of the report should be displayed prominently on the front cover" (H. Munro & R. Munro, 2008).

Index

Here, the various sections and subsections of the report are listed and page numbered (H. Munro & R. Munro, 2008).

Heading

The top of the report should contain all legal information, including the case number, but also the veterinarian information - name, address, phone number - and date of the exam (Merck *et al.*, 2012e).

Subject of exam

This section must contain the complete description regarded to the cadaver in a sentence such as the example: the carcass is that of an given age five-year-old, 30 kg, neutered female, brown and black German shepherd dog, with microchip number [000000000], in good nutritional condition (score 5 in WSAVA scale of 9) received from [submitter's name] on [date] to which is affixed a label with the following information: Specimen A-93-102, and bearing the signature of [enforcement officer] (King, Roth-Johnson, Dodd, & Newsom, 2013; Merck *et al.*, 2012e; Peleteiro *et al.*, 2016; Wobeser, 1996). The exception for this is when multiple cadavers are in the same case, in which the number of animals and species should be listed in this section, but the animal IDs together with the rest of the information should be in the "exam findings" section (Merck *et al.*, 2012e).

Reason for exam

The reason for the necropsy examination, along with information about who requested it, must be elucidated in this section (Merck *et al.*, 2012e; H. Munro & R. Munro, 2008). For example: "General attorny [name], of Lisbon district, requested a *post-mortem* examination with a view to: (1) establishing the cause of death; (2) providing an opinion on whether the animal would have suffered pain and distress as a result of any injuries or disease before death; (3) determining if the injuries observed are compatible with those capable of being inflicted by the suspected weapon identified by the local policemen's at the scene (H. Munro & R. Munro, 2008).

Method of arrival and description of packaging

In this section shall be a brief documentation regarding to the arrival of the animal (Merck *et al.*, 2012e). It should include the date and time of the cadaver receipt, where and by whom it was received, and in what conditions it arrived – e.g. received frozen; in fresh, etc. - and the conditions it has been placed before the necropsy began - in chill at 4ºC; allowed to thaw in an *ante-room* off the *post-mortem* room for 24 hours; etc. – and the details of the packaging, seals or identity tags (H. Munro & R. Munro, 2008).

Crime scene/forensic findings

Every pertinent crime scene or forensic findings that help on the context of the case of the should be here described or exposed, if photographs, videos, reports or witness statements. Sometimes the information is provided verbally by the investigator, and in these cases, it should be conveyed by making sentences as: "According to Officer [name], on the [date], …" (Merck *et al.*, 2012e).

Medical history

Equally to crime scene information, the known medical history also should be documented. It includes all medical records related to the victim's injuries pertinent to the case, such as if the animal died or was euthanized (Merck *et al.*, 2012e).

Abbreviations

The report can be written using medical abbreviations, however is necessary to exist an abbreviation list, that can come in this section (Merck *et al.*, 2012e)

Definitions

This section has the purpose to facilitate the understanding of medical terms used in the report for the non-veterinarian readers. The body condition scoring system used should be here defined, in a document attached to the report (Merck *et al.*, 2012e).

Examination findings

This section may have several subsections fitted for each case, therefore may be section headings not used, and those must be deleted (Merck *et al.*, 2012e).

It is recommended the separation in subsections of external and internal *post-mortem* findings, because it bring clarity to the report, for instance, for understanding whether particular lesions are visible on the surface of the body or located internally (H. Munro & R. Munro, 2008).

2.11.1. External exam

All the findings during the external examination came well documented in this section. In cases with multiple cadavers, the findings for each one may be listed in alpha-numeric order by the animal ID (Merck *et al.*, 2012e). Here, mention about the presence and the degree of cadaveric phenomena is crucial to setting and justifying the *post-mortem* interval (Peleteiro *et al.*, 2016).

2.11.2. Evidence of medical and/or surgical intervention

This section should contain any findings of medical and/or surgical intervention on the animal such as intravenous catheters or chest tubes" (Merck *et al.*, 2012e).

2.11.3. Radiographic interpretation

Every radiographic documentation should be here attached, and their proper interpretation made (Merck *et al.*, 2012e).

2.11.4. Internal exam

All the internal findings, as seen at "internal examination" of "general necropsy technique" should be described here. It is recommended to number the paragraphs and always make mention on the organ described, to facilitate reference to particular findings, as the examples: "3.2.2. Liver: there are not observe significant findings in the liver"; "2.2.12. eyes: the right eye is not present" (H. Munro & R. Munro, 2008).

2.11.5. Evidence of injury

This section is optional and is most helpful with complicated injuries such as multiple stab or gunshot wounds, in which may be placed here the gunshot trajectory (Merck *et al.*, 2012e).

2.11.6. Photographs and diagrams

In this section must be the photographic and/or diagram documentation. Denote the importance of this section, since photography and diagrams are an extremely beneficial tool for the understanding of the injuries (Merck *et al.*, 2012e).

2.11.7. Time of death

There is a section discuss the estimation of time of death, based on the investigation findings, witness statements, *post-mortem* exam findings, and/ or forensic (Merck *et al.*, 2012e).

Procedures and results

This section takes a list format to formally identify every samples or evidence (e.g. bullet fragments) retained or sent to further examination, by means of serial numbers and the name of the person to whom they were handed (Saukko & Knight, 2004). Collected samples that are kept for possible future testing should also be listed (Merck *et al.*, 2012e).

Summary of findings

Here begins the second part of the report, in which all the pertinent findings - exam findings, materials reviewed, and the crime scene/forensic findings - can be brought together, yet without comment on their significance, like the following example given by Merck *et al.* (2012e): "Blunt force trauma to the head, a minimum of three blows, consistent with a cylindrical object."

Beginning in this section there is uppermost the use of lay terms instead of medical terms, because is this second part of the report in which target public will focus on reading. Here is where the FVP can connect the injuries with a particular weapon, by statements as "consistent with" or "not consistent with" the weapon type or the alleged weapon (Merck *et al.*, 2012e).

Comments and conclusions

In this section, the FVP ties all the exam and crime scene/forensic findings together and provides the medical and legal opinion (Merck *et al.*, 2012e). Some authors, as H. Munro and R. Munro (2008) defend that this section should be placed at the front of the report rather than at the end, since this is the section of the greatest and most immediate interest to the person receiving the report, thus it should be read first and then, the reader could pass to the detail of the *post-mortem* findings in the following sections. This is debatable and therefore each FVP should done it according to his/her own preferences.

Here, the FVP states clear explanations of how the conclusions were reached and the basis for each, by conjugating the necropsy findings with the information provided by the police report and crime scene (de Siqueira *et al.*, 2016; Merck *et al.*, 2012e). "It should contain factual findings as well as the veterinary opinion of all the information documented in the report" (Merck *et al.*, 2012e, p. 66).

The legal descriptions of the death – mechanism, cause, contributory cause and manner of death – are here contemplated by adding subsections.

References

When references are cited in the "comments section" they must be listed here, at the end of the report (H. Munro & R. Munro, 2008).

Finalization

The finalization of the report is accomplished with the FVP name(s), signature(s), and the date of the report (Merck *et al.*, 2012e).

CHAPTER II: AIMS

The main objective of this work is to identify the general critical points present in veterinary forensic necropsies.

As more specific aims:

1. Perceive the reality of the forensic veterinary medicine in Portugal;

2. Understand the breadth of forensic veterinary medicine to the other sciences;

3. Recognize the errors that can put at risk the chain of custody.

CHAPTER III: PRESENTATION OF CASES

Three forensic cases were selected from all of those followed during the internship to illustrate different causes and mechanisms of death. These cases are presented anonymously – hiding the NUIPC, the laboratory number, the date, the identification of the criminal police department (OPC) and the owner/veterinarian identification, and any other parameters considered relevant to not be exposed – for protection of confidential information. The conclusion of each case made in this work does not represent a direct copy of the real necropsy report.

1. Case 1

1.1. Subject of the exam

Cadaver 1A: The carcass is that of an adult, unknown age, spayed female dog, undetermined breed, 8,670 Kg, average long coat, of light brown colour with a white ventral band from the chin to the pubis and white colour in the distal third of the limbs (Figure 31), in a good nutritional condition, score 5 in WSAVA scale of 9, since the ribs are palpable without excess fat covering, the waist is observed behind the ribs, when viewed from above, and the abdomen is tucked up when viewed from side. There is not detected any microchip identification through the proper reader.

Cadaver 1B: The carcass is that of an adult/senior, unknown age, neutered male dog, undetermined breed, 6,480 Kg, average long wavy coat, of white colour with brown spots on the head, lumbar region and dorsal coccygeal (Figure 42), and with a nutritional score of 4 in 9 of the WSAVA scale, since the ribs are easily palpable, the waist is easily noted viewed from above, and the abdominal tuck is evident. There is not detected any microchip identification through the proper reader.

1.2. Reason for exam

It was asked for a necropsy examination of both cadavers in order to establish the cause of death, as well as the suffering that may have been inflicted upon them.

1.3. Method of arrival and description of packaging

Cadaver 1A: The cadaver is refrigerated and packed in a black plastic bag, in which is attached the laboratory identification (Figure 32).

Cadaver 1B: The cadaver is refrigerated and packed in a black plastic bag, in which is attached the laboratory identification (Figure 43).

1.4. Crime scene/forensic findings

Two dead dogs were delivered to the pathology laboratory by the Guarda Nacional Republicana (GNR), with the chain of custody form containing the follow description: "The canines were killed by the owner, followed by his failed suicide attempt". After talking with the officer, a clearer description was made: these two dogs, who belonged to the man unconscious lying in bed, were found lifeless also lying in bed next to him. No photographs of the crime scene were made available to the pathologist.

1.5. Examination findings

1.5.1. Radiographic interpretation

Cadaver 1A: A latero-lateral projection of the entire body of the cadaver in right recumbency was made, in which there is no evidence of any radiographic abnormalities.

Cadaver 1B: A latero-lateral projection of the entire body of the cadaver in right recumbency was made, in which the only anomaly observed was some degree of atlanto-occipital luxation.

1.5.2. External examination

Cadaver 1A: Presence of *rigor mortis* in the temporomandibular joint (opening of the mouth) and in the limbs. The fur is in a good general appearance. Perioral hair with bloody soiling and the hair of the head and ears are found wet, mainly on the right side (Figure 33). The plantar face of the limbs extremities has presence of blood. The oral mucosa has presence of blood and the remaining are in cyanotic colour. Multifocal dark-red coloured petechiae and haemorrhagic

suffusions, with a diameter varying from 0,2 to 3,5 cm, more than 20 in number, in the thorax and ventral abdomen, extending to the medial aspect of the right posterior limb (Figure 34). Presence of a circular chop wound in the right temporo-parietal area, with associated haemorrhage, which reddish the surrounding skin (Figure 35 and 36). Buccal cavity contaminated with blood, and gingiva with discrete lesions of gingival hyperplasia. There are no putrefying or transformative cadaveric alterations.

Cadaver 1B: Presence of *rigor mortis* in the temporomandibular joint (opening of the mouth) and in the limbs. The fur is in a good general appearance. In the oral cavity it is observed pronounced gingivitis, periodontitis and tartar in all teeth and the lack of 2 upper and lower incisor teeth. Also, there are erosions on the oral mucosa, on the lip. Multifocal haemorrhagic suffusions (more than 10) distributed ventrally on the neck and thorax, with diameters ranging from 1 to 2 cm (Figure 44). Presence of a chop wound of 1 cm of diameter, on the caudal nasal area. There are no putrefying or transformative cadaveric alterations.

1.5.3. Eyes and ears examination

Cadaver 1A: The right pinna is wet. No macroscopic changes are seen in the eyes.

Cadaver 1B: Bilateral hyposphagma - Subconjunctival hemorrhage - (Figure 45).

1.5.4. Skinning

Cadaver 1A: Presence of generalized subcutaneous fat. After skinning, it is possible to observe suffusions in the corresponding areas of the lesions detected on the external examination – thorax, ventral abdomen, medial aspect of the right posterior limb and temporal muscles under the right temporo-parietal area. Disarticulating the limbs, the spilled blood is dark and partially coagulated.

Cadaver 1B: Presence of diffuse haemorrhage of the subcutaneous tissue – bruising - of the entire ventral and lateral region of the neck (Figure 46). Lymphadenomegaly of the lymph nodes of the head and neck. Small subcutaneous haemorrhage of 1 cm of diameter on the penile region, at the prepuce. Disarticulating the limbs, the spilled blood is dark and partially coagulated.

1.5.5. Internal examination

1.5.5.1. Bone marrow

Cadaver 1A: Bone marrow without significative macroscopic changes.

Cadaver 1B: Bone marrow without significative macroscopic changes.

1.5.5.2. Endocrine and exocrine glands

Cadaver 1A: Thymus with presence of reddish, haemorrhagic areas. Remaining glands without significative macroscopic changes.

Cadaver 1B: No significative macroscopic changes of the endocrine glands.

1.5.5.3. Thoracic cavity

Cadaver 1A: The lungs did not collapse into the opening of the thoracic cavity. All organs of thoracic cavity in their proper anatomic typography.

Cadaver 1B: The lungs did not collapse into the opening of the thoracic cavity. All organs of thoracic cavity in their proper anatomic typography.

1.5.5.4. Abdominal cavity

Cadaver 1A: All abdominal organs are found in their proper anatomic typography.

Cadaver 1B: Gastric dilatation promoting diaphragmatic bulging. Remaining abdominal organs in their anatomic typography.

1.5.5.5. Urinary system

Cadaver 1A: Left kidney weight: 30 grams; right kidney weight: 25 grams. Both kidneys present a dark red coloration of the renal medulla (congestion/haemorrhage). The ureters, bladder, and urethra show no significant pathological changes.

Cadaver 1B: Left kidney weight: 25 grams; right kidney weight: 15 grams. Both kidneys have poor cortico-medullary distinction. The ureters, bladder, and urethra show no visible relevant changes.

1.5.5.6. Genital system

Cadaver 1A: Absence of ovaries, uterus and cervix. The vagina and vulva do not macroscopically present pathological changes.

Cadaver 1B: Absence of testicles. Remaining genitalia with no significant pathological changes.

1.5.5.7. Iliac lymph nodes and abdominal aorta

Cadaver 1A: The iliac lymph nodes and the abdominal aorta do not present significant macroscopic changes.

Cadaver 1B: The iliac lymph nodes and the abdominal aorta do not present significant macroscopic changes.

1.5.5.8. Heart

Cadaver 1A: Heart weight: 90 g. The heart has the presence of dark, uncoagulated blood in the four cardiac chambers. Neither the pericardium, the myocardium, the valves or the large vessels have significant macroscopic changes.

Cadaver 1B: Heart weight: 85 g. The heart has the presence of dark, uncoagulated blood in the four cardiac chambers. At opening it is evident an myxomatous degenerative valvular disease of the mitral and tricuspid valves, evidenced by a nodular thickening of the valves.

1.5.5.9. Vasculature

Cadaver 1A: The blood is not completely clotted inside the vessels.

Cadaver 1B: The blood inside the vessels is dark and uncoagulated.

1.5.5.10. Respiratory system

Cadaver 1A: Presence of abundant fine bubbles of white foam in the tracheal lumen. The lungs are humid, shiny and heavy, with heterogenous coloration, alternating congested dark red areas with inflated pale areas. The bronchi present a colourless fluid and a white foam in their lumen, predominantly of fine bubbles, but also with larger bubbles (the largest with 1 cm in diameter), (Figure 37).

Cadaver 1B: Presence of fine bubbles of white foam in the cervical portion of the tracheal lumen and larynx lumen. At opening of the trachea, it shows on its cervical portion, located immediately caudal to the larynx, haemorrhage on its wall (from the adventitious to the muscular layer), with 3 cm of extension (Figure 47 and 48). On pulmonary palpation can be felt diffuse hard consistency throughout the parenchyma, resembling sand, compatible with calcification of the bronchioles.

1.5.5.12. Liver

Cadaver 1A: Weight of liver with gallbladder: 300 grams. Liver with a dark reddish colouration – congested liver – and increased in size – hepatomegaly.

Cadaver 1B: Liver weight with gallbladder: 225 grams. Liver dark, congestive.

1.5.5.13. Pancreas

Cadaver 1A: Pancreas without significant macroscopic changes.

Cadaver 1B: Pancreas with red areas, congestive-haemorrhagic.

1.5.5.14. Spleen

Cadaver 1A: Spleen weight: 25 grams. Spleen without significant macroscopic changes.

Cadaver 1B: Spleen weight: 15 grams. Spleen without significant macroscopic changes.

1.5.5.15. Gastrointestinal tract

Cadaver 1A: Buccal cavity filled with blood, teeth with some tartar and gingiva with some degree of gingival hyperplasia. Stomach with solid food compatible with formulated dry dog food, wrapped in mucus. The gastric mucosa with numerous linear ulcerative lesions, dark on the depth, with random multifocal distribution, being the largest with 4 cm of extension. Intestine with pasty content throughout its length.

Cadaver 1B: Stomach dilated, with air and a brown pasty substance. Intestine with pasty content throughout its length.

1.5.5.16. Non-vertebral bones and joints

Cadaver 1A: Linear fracture in the parietal/temporal bone, with well-co-opted borders, of 1 cm long, sited on the area corresponding to the chop wound injury observed on the head in the external examination (Figure 38).

Cadaver 1B: Elliptical shape fracture in the nasal bones, with 1 cm of larger diameter, located in the middle of an imaginary line between the eyes (Figure 49), corresponding to the external lesion found on the skin.

1.5.5.17. Head

Cadaver 1A: After skinning, it is found a haemorrhagic injury – a contusion - in the temporal muscles under the external wound observed and described above (Figure 39); under that lesion, at the parietal/temporal bone it is seen a linear fracture of 1 cm long, with well-co-opted borders. After opening of the scalp, it is observed haemorrhage in the right hemisphere of the brain, in the frontal, parietal and occipital lobes, being the most exuberant, depressed, injury in the parietal lobe, 0.5 cm from the longitudinal fissure (Figure 40 and 41), due to repercussion of the external injury. Meninges with haemorrhage in the same area corresponding to brain haemorrhage (Figure 38).

Cadaver 1B: Congestion and haemorrhage of the brain (Figure 50), that represents the repercussion of the external lesion. Meninges with haemorrhage in the same corresponding area to brain haemorrhage.

1.5.5.18. Nasal cavity and sinuses

Cadaver 1A: The nasal cavity and sinuses have no significant macroscopic changes.

Cadaver 1B: Presence of dark yellow material inside the sinuses, compatible with chronic sinusitis.

1.5.5.19. Spine

Cadaver 1A: The spine does not show any significant macroscopic changes.

Cadaver 1B: Instability of the atlanto-occipital joint.

1.5.5.20. Spinal canal

Cadaver 1A: Spinal canal with no significant macroscopic changes.

Cadaver 1B: Spinal canal with no significant macroscopic changes.

1.5.5.21. Peripheral nerves

Cadaver 1A: Peripheral nerves with no significant macroscopic changes.

Cadaver 1B: Peripheral nerves with no significant macroscopic changes.

1.5.6. Photographs and diagrams

Cadaver 1A:

Figure 31: View of the cadaver 1A in a left lateral decubitus.

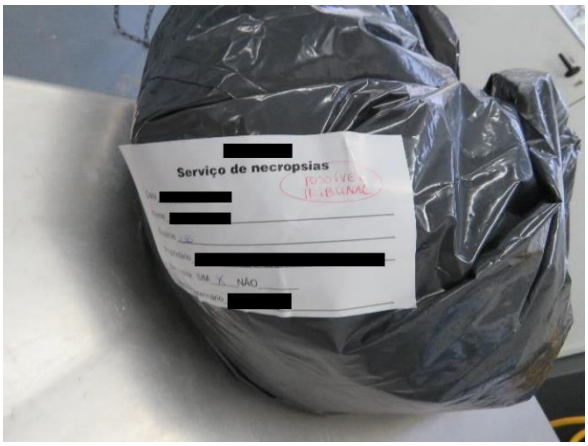

Figure 32: Packaging of the cadaver 1A.

Figure 33: A closer view of the Figure 31; denote the wet fur around the right pinna (black arrow) and the bloody soiling fur perioral (white arrow).

Figure 34: Petechiae and haemorrhagic suffusions in the ventral region of the cadaver 1A.

Figure 35: Circular chop-wound in the right temporo-parietal area of the cadaver 1A.

Figure 36: Closer view of the previous s figure.

Figure 37: Pulmonary edema - presence of foam in the trachea and bronchi – in the lungs of the cadaver 1A.

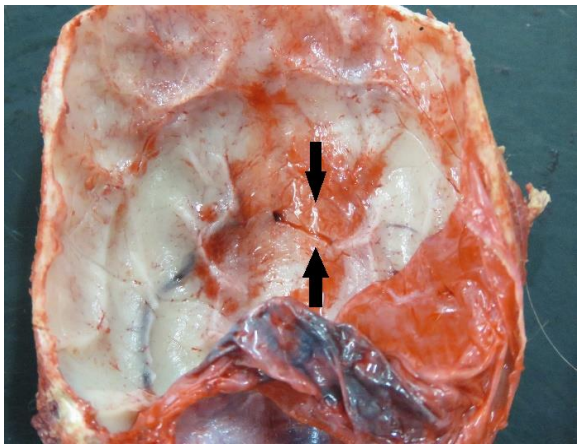

Figure 38: Linear fracture in the parietal/temporal bone (black arrows) of the cadaver 1A.

Figure 39: Contusion in the temporal muscles under the external wound observed and described above.

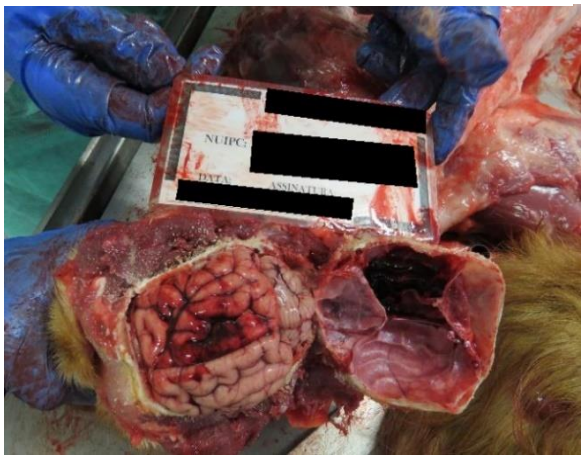

Figure 40: *In situ* view of the haemorrhage in the right hemisphere of the brain (frontal, parietal and occipital lobes) under the external injuries described above.

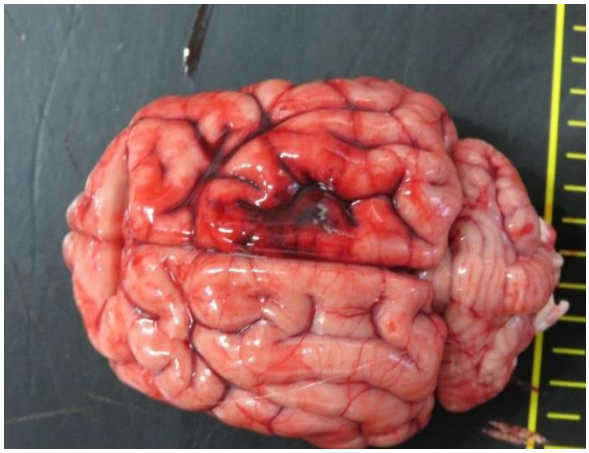

Figure 41: Ex situ view of the previous injury in the cadaver 1A.

Cadaver 1B:

Figure 42: View of the cadaver 1B in a left lateral decubitus.

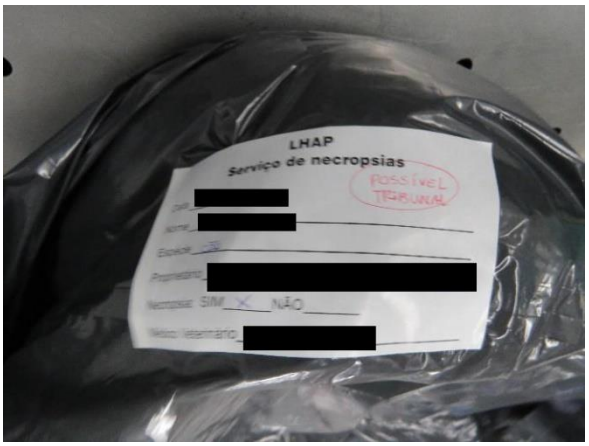

Figure 43: Packaging of the cadaver 1B.

Figure 44: Petechiae and haemorrhagic suffusions in the ventral region of the cadaver 1B.

Figure 45: Bilateral subconjunctival haemorrhage of the cadaver 1B.

Figure 46: Ring bruise in the neck muscles, found after the skinning on the cadaver 1B.

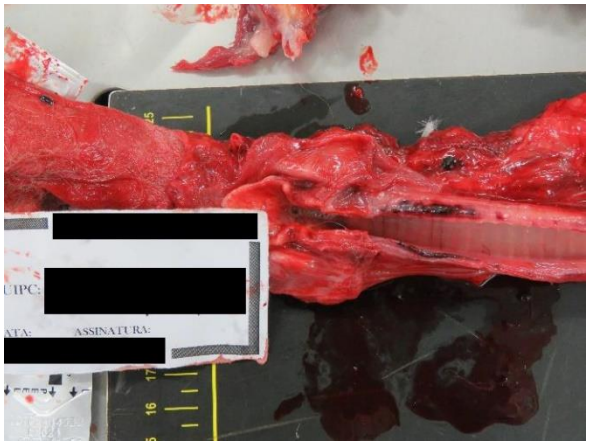

Figure 47: Presence of foam in the trachea lumen and haemorrhage in the ventral trachea wall of cadaver 1B.

Figure 48: Closer view of the previous figure.

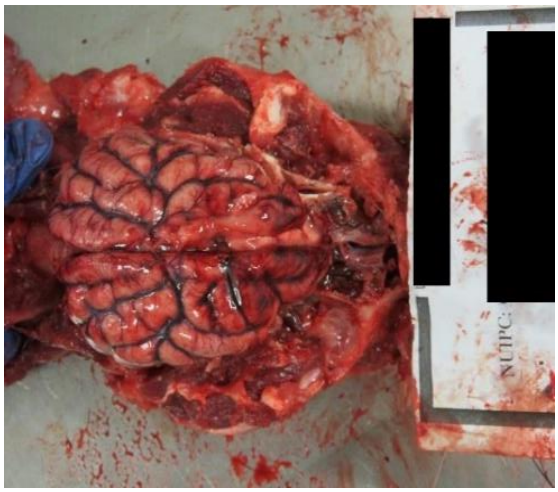

Figure 50: Generalized congestion and haemorrhage of the brain of the cadaver 1B.

Figure 49: Elliptical shape fracture in the nasal bones corresponding to the external chop wound of the cadaver 1B.

1.5.7. Time of death

Cadaver 1A: The observed *post-mortem* changes suggest that the death occurred between 24 and 48 hours prior to necropsy.

Cadaver 1B: The observed *post-mortem* changes suggest that the death occurred between 24 and 48 hours prior to necropsy.

1.6. Procedures and results

Cadaver 1A: The noble organs were collected to histopathology.

Cadaver 1B: The noble organs were collected to histopathology.

1.7. Summary of findings

Cadaver 1A: The wet coat on the surrounding of the pinna, along with the generalized visceral congestion, the cyanosis of the oral mucosa and with the acute lung edema (without any cardiac change) - the lungs are humid, shiny and heavy, did not collapsed into the opening of the thoracic cavity, and presence of abundant white foam in the tracheal an bronchial lumen – are compatible with asphyxia by drowning.

The multifocal dark-red coloured petechiae and haemorrhagic suffusions, with a diameter varying from 0,2 to 3,5 cm, more than 20 in number, in the thorax and ventral abdomen, extending to the medial aspect of the right posterior limb may indicate a struggle between the animal and the perpetrator.

The chop wound in the right temporo-parietal area originated a contusion in the temporal muscles and a 1 cm long linear fracture in the parietal/temporal bone, which had repercussion in haemorrhages in the right hemisphere of the brain - in the frontal, parietal and occipital lobes. The multifocal ulcerative lesions on the gastric mucosa represents a chronic feature and does not have medico-legal importance.

Cadaver 1B: The findings of erosions on the lip, the bruising distributed ventral and laterally on the neck and thorax, the instability of the atlanto-occipital joint, the haemorrhage of the tracheal wall, and the bilateral subconjunctival haemorrhage (hyposphagma) are suggestive of asphyxia by strangulation. However, this cadaver also presents acute lung edema, compatible with drowning or instead may be a sequel of a possible heart disease, since evidenced of myxomatous degenerative valvular disease of the mitral and tricuspid valves were seen.

This cadaver also presents a chop wound of 1 cm of diameter, on the caudal nasal area, originating an elliptical shape fracture in the nasal bones, pursuant to congestion and haemorrhage of the meninges and brain.

The diffuse hard consistency throughout the palpation of pulmonary parenchyma, compatible with calcification of the bronchioles, there is no medico-legal value, since it is common in older dogs and have no pathological meaning. The presence of dark yellow material inside the sinuses is compatible with chronic sinusitis and also there is no medico-legal value.

1.8. Comments and conclusions

Cadaver 1A: The cranio-meninge-encephalic traumatic injuries and the injuries compatible with drowning have *ante-mortem* characteristics, thus the cause and mechanism of death cannot be exactly defined, since any of both could lead to death. Thus, may have two closures to this case:

1. Cause of death: a blunt force trauma on the right temporo-parietal area of the head, in a chop wound. Mechanism of death: Brain haemorrhage.
2. Cause of death: asphyxia by drowning. Mechanism of death: lack of oxygen by immersion in water.

Both theories have the same manner of death: violent, non-accidental death.

Cadaver 1B: As well as the cadaver 1A, the cadaver 1B does not have an exact cause and mechanism of death. Thus, the two probable closures to this case are:

1. Cause of death: a blunt force trauma on the caudal nasal area of the head, in a chop wound. Mechanism of death: Brain haemorrhage.
2. Cause of death: asphyxia by strangulation. Mechanism of death: lack of oxygen by compression of the trachea.
3. Cause of death: asphyxia by drowning. Mechanism of death: lack of oxygen by immersion in water.

Here, also both theories have the same manner of death: violent, non-accidental death.

To conclude this case conclusion, it should be remembered that neither the law enforcement officer nor the pathologist, should made any assumptions on the criminal events, unless they have concrete evidence or facts, therefore, the description of the chain of custody form: “The canines were killed by the owner, followed by his failed suicide attempt”, is improper to a forensic case. He/she should have described what were seen, maybe some like: Two canines were found death lying in the bed next to their unconscious owner, who appears to attempt suicidal.

It is seen that both deaths are very similar, and it seems to be tried several ways of violent deaths in the same time.

2. Case 2

2.1. Subject of the exam

The carcass is that of an adult, more than 5 years of age, male dog, not neutered, undetermined breed, small size of 5,830 Kg, short-medium coat of light brown colour (Figure 51), in a heavy body condition, score 6 in WSAVA scale of 9, since the ribs are palpable with slight excess fat covering, the waist is discernible from above but is not prominent, and the abdomen tuck is apparent. There is not detected any microchip identification through the proper reader.

2.2. Reason for exam

It was asked for a necropsy examination of the cadaver in order to establish the cause of death, as well as the suffering that may have been inflicted.

2.3. Method of arrival and description of packaging

The cadaver is frozen and packed in a black plastic bag, in which is attached the NUIPC and sealed with adhesive tape (Figure 52). Following the cadaver are two tamper evident transparent plastic bags, being one identified as containing a bait found at crime scene and the other with a plastic bag inside but without identification (Figure 53).

2.4. Crime scene/forensic findings

The cadaver was found outdoors being a poisoning suspicion since in the perimeter surrounding it was found food remains similar to those used on baits.

2.5. Examination findings

2.5.1. Radiographic interpretation

A latero-lateral projection of the cranial 2/3 of the body of the cadaver in left recumbency was made (Figure 54). It is seen the presence of microchip (although it was not identified by the reader) and some subcutaneous emphysema in the neck region. The remaining does not show evidence of any radiographic abnormalities.

2.5.2. External examination

Presence of indentation of the fur in the neck, which in a closer view it is possible to see a bruise of the corresponding tissues in a ring shape around the neck (Figure 55 and 56). The remaining fur in good presentation. Mouth defiled with soil and the oral mucosa is congestive (Figure 57). The penile and rectal mucosa, as well as the skin of glabrous areas (axillae and groin) are pale. There are no putrefying or transformative cadaveric alterations.

2.5.3. Eyes and ears examination

Bilateral hyposphagma - subconjunctival haemorrhage – (Figure 58 and 59). The outer ear mucosa is congestive.

2.5.4. Skinning

After skinning, it is possible to observe extensive haemorrhagic contusion in the neck region (ventral and dorsal) in the shape of a ring with 3 cm width, dorsally extended to the frontal-parietal region of the head (Figure 60 and 61), and also an extensive laceration of the ventral cervical muscles, exposing the trachea (Figure 62).

2.5.5. Internal examination

2.5.5.1. Bone marrow

Bone marrow without significative macroscopic changes.

2.5.5.2. Endocrine and exocrine glands

Thyroid is congestive-haemorrhagic. The remain endocrine glands without significative macroscopic changes.

2.5.5.3. Thoracic cavity

All organs of thoracic cavity in their proper anatomic typography.

2.5.5.4. Abdominal cavity

All abdominal organs are in their proper anatomic typography.

2.5.5.5. Urinary system

Kidneys weight: 15 grams and 20 grams. Kidneys, adrenal glands, ureters, gallbladder, and urethra without significant pathological changes.

2.5.5.6. Genital system

Testicles and remaining genitalia with no significant pathological changes.

2.5.5.7. Iliac lymph nodes and abdominal aorta

The iliac lymph nodes and the abdominal aorta do not present significant macroscopic changes.

2.5.5.8. Heart

Heart weight: 55 grams. The heart presents some clots of blood in the right ventricular cavity but does not show any significant macroscopic changes.

2.5.5.9. Vasculature

There is not any significant macroscopic alteration of the vasculature.

2.5.5.10. Respiratory system

Presence of a contusion in a ring shape on the cervical region of the trachea, with 5 cm length (Figure 63). Lungs without significant macroscopic changes.

2.5.5.12. Liver

Weight of liver with gallbladder: 205 grams. Liver and gallbladder without significant macroscopic changes.

2.5.5.13. Pancreas

Pancreas without significant macroscopic changes.

2.5.5.14. Spleen

Spleen weight: 20 grams. Spleen without significant macroscopic changes.

2.5.5.15. Gastrointestinal tract

Oesophagus with a circular haemorrhage of 3 cm length on its cervical region. Stomach without content. The remaining gastrointestinal organs do not show any significant pathological changes.

2.5.5.16. Non-vertebral bones and joints

Complete fracture of hyoid bone.

2.5.5.17. Head

On the brain is seen a generalized congestion of the meninges and the brain.

2.5.5.18. Nasal cavity and sinuses

The nasal cavity and sinuses have no significant macroscopic changes.

2.5.5.19. Spine

The spine has no significant macroscopic changes.

2.5.5.20. Spinal canal

The spine canal has no significant macroscopic changes.

2.5.5.21. Peripheral nerves

The peripheral nerves have no significant macroscopic changes.

2.5.6. Photographs and diagrams

Figure 51: View of the weight (5,830 Kg) of the cadaver. Photo kindly ceded by Alves, A.

Figure 52: View of the cadaver along with the black plastic bag in which was conditioned and with the transparent bag of the suspected bait. Photo kindly ceded by Alves, A.

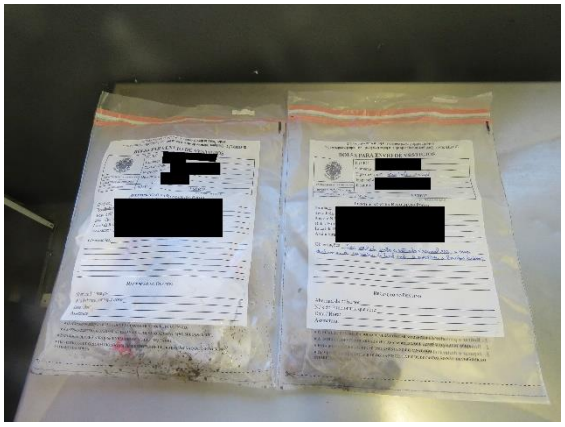

Figure 53: Both tamper evident bags, one containing the suspected bait and the other unidentified. Photo kindly ceded by Alves, A.

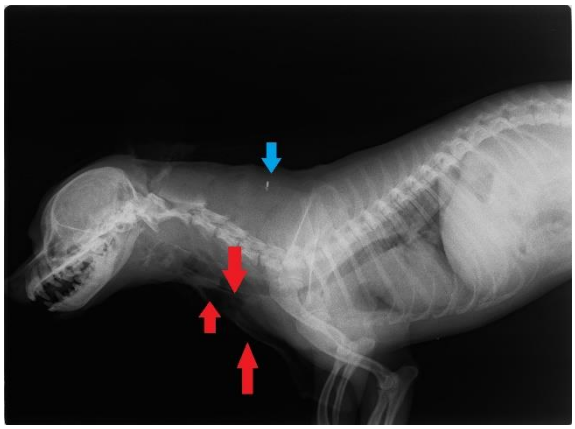

Figure 54: Radiograph showing the microchip (blue arrow) and the subcutaneous emphysema (red arrows). Photo kindly ceded by Alves, A.

Figure 55: Ring shape indentation of the fur around the neck. Photo kindly ceded by Alves, A.

Figure 56: Closer view of the previous photo, being possible to see a bruise of the corresponding tissues (black arrow). Photo kindly ceded by Alves, A.

Figure 57: Mouth defiled with soil and congestion of the oral mucosa. Photo kindly ceded by Alves, A.

Figure 58: Subconjunctival haemorrhage of the right eye. Photo kindly ceded by Alves, A.

Figure 59: Subconjunctival haemorrhage of the left eye. Photo kindly ceded by Alves, A.

Figure 60: Extensive contusion on the ventral neck region. Photo kindly ceded by Alves, A.

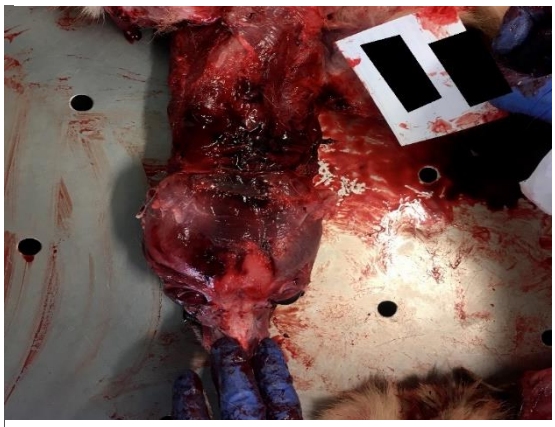

Figure 61: Extensive contusion on the dorsal neck region, extended to the frontal-parietal region of the head. Photo kindly ceded by Alves, A.

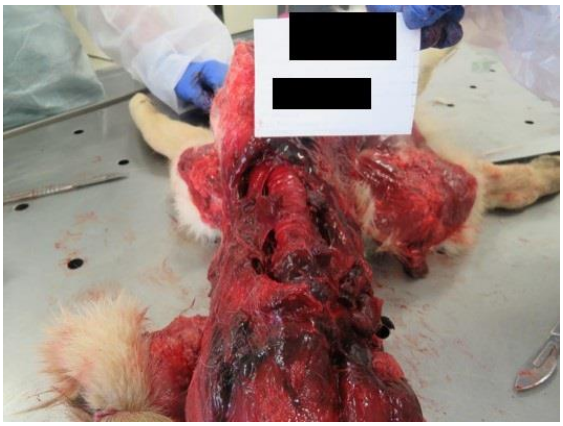

Figure 62: Extensive laceration of the ventral cervical muscles, exposing the trachea. Photo kindly ceded by Alves, A.

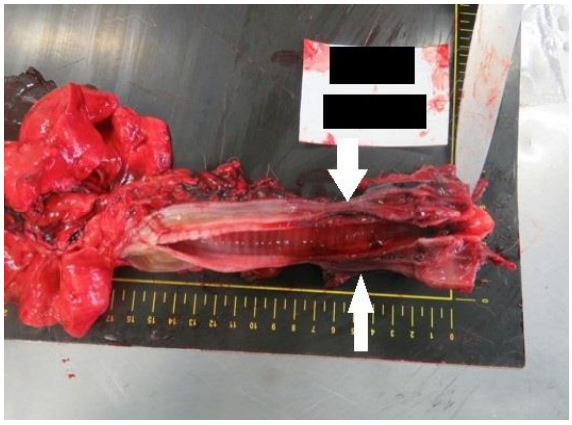

Figure 63: Ring shape contusion on the cervical region of the trachea, with 5 cm length (white arrows). Photo kindly ceded by Alves, A.

2.5.7. Time of death

The observed *post-mortem* changes suggest that the interval between death and the collection of the cadaver will not have been longer than 24 hours.

2.6. Procedures and results

The noble organs were collected to histopathology. The cadaver and the tamper evident bags with their content are stored frozen at the necropsy room, waiting for the decision of the Public prosecutor's office.

2.7. Summary of findings

The heavy body score along with the presence of a microchip (not identified by the proper reader but seen at the X-ray) suggest that this dog have or had an owner.

The findings of food similar to bait lead the law enforcement officers to suspect of poisoning, however the pathological findings did not directly match with suspicion of poisoning as they match with a strangulation by hanging or waggling using a ligature. The cervical injuries of bruising and contusion, laceration of the ventral cervical muscles and fracture of the hyoid are compatible with a strangulation of a brute force. The subcutaneous emphysema seen at the X-ray may be due to a laceration on the trachea (compatible with the injuries found) or else it can

be due to putrefactive phenomena, although it was not observed other signs of putrefying or transformative cadaveric alterations.

2.8. Comments and conclusions

Strangulation by hanging or waggling with a ligature/rope of a brute force (giving the ventral cervical muscles and fracture of the hyoid) are the cause of death. However, the attempted of poisoning should not be excluded and tests should have been made.

The more probable cause of death is asphyxia by strangulation, being the identified injuries suggestive of great suffering, and compatible with the lack of oxygen as the mechanism of death. The legal cause of death is considered to be a violent and non-accidental death.

3. Case 3

3.1. Subject of the exam

Cadaver 3A: The carcass is that of a young male bovine, of typical phenotypic characteristics of "Maronesa" breed, with a good nutrition score (Figure 64).

Cadaver 3B: The carcass is that of a young female bovine, of typical phenotypic characteristics of "Maronesa" breed, with a good nutrition score.

3.2. Reason for exam

It was asked for a necropsy examination in the field of both cadavers in order to establish the cause of death, as well as the suffering that may have been inflicted upon them.

3.3. Method of arrival and description of packaging

Cadaver 3A: The necropsy was performed in the field, in the presence of elements of the GNR, on April 3, 2018, at 3:25 p.m.

Cadaver 3B: The necropsy was performed in the field, in the presence of elements of the GNR, on April 3, 2018, at 3:25 p.m.

3.4. Crime scene/forensic findings

Two young "Maronesa" bovines were found death, with exterior evidence of being victims of gunshot injuries.

3.5. Examination findings

3.5.1. External examination

Cadaver 3A: Presence of *rigor mortis* in the temporomandibular joint (at the opening of the mouth) and in the anterior and posterior limbs. Good overall coat appearance. All the mucosas are pale. In the right scapular region there is a perforating circular wound, of 1 cm diameter,

with no contour halo (Figure 65). In the left scapular region, is observed a cutaneous perforation, with contusion associated, measuring the larger diameter 2,5 cm, with everted and irregular edges (Figure 66). In the right dorsal thoracic region, it is evidenced some predation marks with no hemorrhage associated. There are no putrefying or transformative cadaveric alterations.

Cadaver 3B: Presence of *rigor mortis* in the temporomandibular joint (at the opening of the mouth) and in the anterior and posterior limbs. Good overall coat appearance. Presence of ectoparasites of *Ixodes* genus distributed for the whole body and fly larvae around the left outer ear. It is seen congestion of the vaginal mucosa and a not accentuated vaginal and rectal prolapse considered to be due to putrefactive changes. In the frontal region, the hair is stained with blood (Figure 73). There are some signs related with putrefaction as tympanism and post-mortem subcutaneous emphysema.

3.5.2. Eyes and ears examination

Cadaver 3A: Eyes and ears without significative macroscopic changes.

Cadaver 3B: Eyes and ears without significative macroscopic changes.

3.5.3. Skinning

Cadaver 3A: After the skinning, it is observed contusion and laceration of the scapular muscles corresponding to the external perforating circular wound of the right side of the scapular region, with extrusion of pulmonary tissue outside the thoracic cavity (Figure 67). When dissecting the scapula is observed that the lesion continues in depth until the thoracic wall, with fracture of the scapula and laceration of the underlying intercostal muscles (Figure 68).

Cadaver 3B: In the right frontal region, adjacent to the cornual process of the frontal bone, there is a circular perforated wound, without contusion halo (Figure 74 and 75), corresponding to the external wound.

3.5.4. Internal examination

3.5.4.1. Bone marrow

Cadaver 3A: Bone marrow without significative macroscopic changes.

Cadaver 3B: Bone marrow without significative macroscopic changes.

3.5.4.2. Endocrine and exocrine glands

Cadaver 3A: No significative macroscopic changes of the endocrine glands.

Cadaver 3B: No significative macroscopic changes of the endocrine glands.

3.5.4.3. Thoracic cavity

Cadaver 3A: Presence of a blood clot in the thoracic cavity – haemothorax - rupture of the pleura serosa and an injury of the left lung (Figure 69). On the right side is observed a fracture of the 6^{th} rib (Figure 70), with presence of bone fragments in the right lung. On the left side, it is seen rupture of the intercostal muscles, between the 4^{th} and 5^{th} ribs left and comminuted fractures of the same ribs (Figure 71).

Cadaver 3B: In the thoracic cavity, all organs are in their physiological typography.

3.5.4.4. Abdominal cavity

Cadaver 3A: All abdominal organs are found in their proper anatomic typography.

Cadaver 3B: All abdominal organs are found in their proper anatomic typography.

3.5.4.5. Urinary system

Cadaver 3A: Both kidneys with pale coloration. The ureters, bladder and urethra without any significative macroscopic changes.

Cadaver 3B: The kidneys have a pale coloration and a pulpy consistency. The urethra, bladder and ureters do not show relevant macroscopic changes.

3.5.4.6. Genital system

Cadaver 3A: Presence of testicles. All genital organs without significative macroscopic changes.

Cadaver 3B: Presence of a female fetus on the uterus of about six months, meaning that the animal was pregnant when was killed (Figure 76 and 77).

3.5.4.7. Iliac lymph nodes and abdominal aorta

Cadaver 3A: The iliac lymph nodes and the abdominal aorta do not present significant macroscopic changes.

Cadaver 3B: The iliac lymph nodes and the abdominal aorta do not present significant macroscopic changes.

3.5.4.8. Heart

Cadaver 3A: Presence of blood in the pericardial sac – hemopericardium. I tis observed a traumatic rupture of the left atrium with irregular borders (Figure 72). The ventricular myocardium is pale. No other relevant morphological changes of the valves nor of the great vessels are seen.

Cadaver 3B: The heart contains dark blood in the four chambers. Neither the pericardium, myocardium, the valves nor the greater vessels have significative macroscopic changes.

3.5.4.9. Vasculature

Cadaver 3A: There is not any significant macroscopic alteration of the vasculature.

Cadaver 3B: There is not any significant macroscopic alteration of the vasculature.

3.5.4.10. Respiratory system

Cadaver 3A: The pulmonary parenchyma is damaged due to the bone fragments and the blunt trauma (Figure 72). A right pulmonary lob is extruded through the rib and scapular fracture until the exterior.

Cadaver 3B: Lungs without significative macroscopic changes.

3.5.4.11. Liver

Cadaver 3A: Liver with a soft consistency and pale colour.

Cadaver 3B: Live of increase size – hepatomegaly - and with a soft consistency and pale coloration, with gas bubbles under the Glisson capsule, compatible with the gaseous phase of putrefaction.

3.5.4.12. Pancreas

Cadaver 3A: Pancreas without significant macroscopic changes.

Cadaver 3B: Pancreas without significant macroscopic changes.

3.5.4.13. Spleen

Cadaver 3A: The spleen is bloodless.

Cadaver 3B: The spleen does not show any relevant macroscopic changes other than those attributed to putrefaction, such as gas.

3.5.4.14. Gastrointestinal tract

Cadaver 3A: Gastric compartments with abundant food content consisting of green vegetable food (pasture), without changes of consistency and odour. Intestine with pasty contents. The rumen, reticulum, omasum and abomasum mucosas, do not present changes with medico-legal significance.

Cadaver 3B: Gastric compartments with abundant food content consisting of green vegetable food (pasture), without changes of consistency and odour beyond those attributed to advanced putrefaction. The mucosa of the rumen is easily detached. Intestine with pasty contents. Remaing organs with no relevant macroscopic changes.

3.5.4.15. Non-vertebral bones and joints

Cadaver 3A: Fracture of the right scapula, the 6th right rib and the 4th and 5th left rib.

Cadaver 3B: Presence of a circular fracture of the frontal bone, described on the subsection "1.5.4.16. Head".

3.5.4.16. Head

Cadaver 3A: Head without any significative macroscopic changes.

Cadaver 3B: In the upper right quadrant of the frontal bone it is observed a circular perforating wound, of 1 cm diameter, with contusion of the surrounding tissues with an extension of 2 cm laterally and 6 cm rostrally, with muscle laceration of the trapezium (cervical part), splenius, semispinalis capitis, longissmus capitis and longissmus atlantis (Figure 78). The lesions continue to the third cervical vertebra on the right side, with muscular laceration and associated hemorrhage. During the dissection of the muscles injured are detected metal fragments, compatible with projectile (Figure 79). Deeper, is seen smash fractures of the right parietal and occipital bones, and other fracture on the right wing of the atlas bone, in which is detected a bigger metal fragment (Figure 80). Hemorrhage with extensive laceration in the right hemisphere of the brain and meninges.

3.5.4.17. Nasal cavity and sinuses

Cadaver 3A: The nasal cavity and sinuses have no significant macroscopic changes.

Cadaver 3B: Presence of blood on the sinuses (Figure 81).

3.5.4.18. Spine

Cadaver 3A: The spine does not show any significant macroscopic changes.

Cadaver 3B: The spine does not show any significant macroscopic changes.

3.5.4.19. Spinal canal

Cadaver 3A: Spinal canal with no significant macroscopic changes.

Cadaver 3B: Spinal canal with no significant macroscopic changes.

3.5.4.20. Peripheral nerves

Cadaver 3A: Peripheral nerves with no significant macroscopic changes.

Cadaver 3B: Peripheral nerves with no significant macroscopic changes.

3.5.6. Photographs and diagrams

Cadaver 3A:

Figure 64: Cadaver 3A in lateral view.

Figure 65: Right scapular region with a perforating circular wound, of 1 cm diameter, with no contour halo (red arrow).

Figure 66: Left scapular region with a cutaneous perforation and contusion associated, measuring the larger diameter 2,5 cm, with everted and irregular edges (red arrow).

Figure 67: Contusion and laceration of the right scapular muscles, with extrusion of pulmonary tissue outside the thoracic cavity (white arrow).

Figure 68: Fracture of the scapula and laceration of the underlying muscles and extrusion of the pulmonary tissue.

Figure 69: Presence of a blood clot in the thoracic cavity – haemothorax - rupture of the pleura serosa and an injury of the left lung.

Figure 70: Fracture on the 6th right rib.

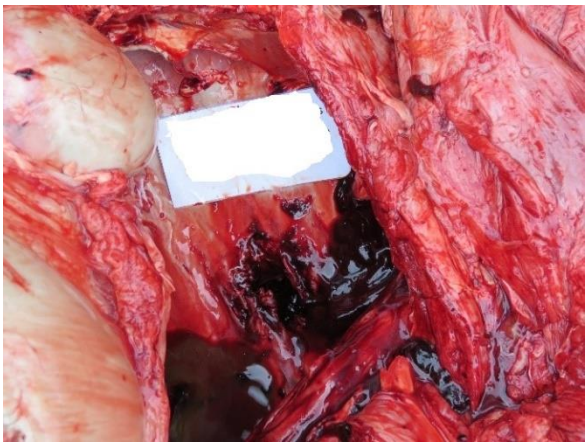

Figure 71: Laceration of the intercostal muscles between the left 4th and 5th ribs and comminuted fractures of the same ribs.

Figure 72: Blood in the pericardial sac due to a traumatic rupture of the left atrium with irregular borders.

Cadaver 3B:

Figure 73: Frontal region of the head with the hair stained with blood.

Figure 74: Circular perforated wound, without contusion halo, adjacent to the cornual process of the frontal bone.

Figure 75: Circular perforated wound, without contusion halo, adjacent to the cornual process of the frontal bone, seen after better dissection.

Figure 76: Female foetus on the uterus of about six months, meaning that the animal was pregnant when was killed.

Figure 77: Female foetus from the previous figure, exteriorized from the uterus.

Figure 78: Laceration and contusion of the neck muscles.

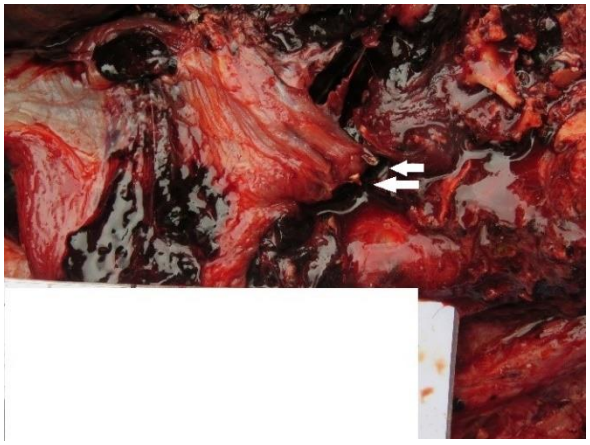

Figure 79: Metal fragments (white arrows), compatible with projectile, detected during the dissection of the injured muscles.

Figure 80: The bigger metal fragment (white arrow), detected on deeper dissection, in the fracture of the right wing of the atlas bone.

Figure 81: Blood clot in the sinuses.

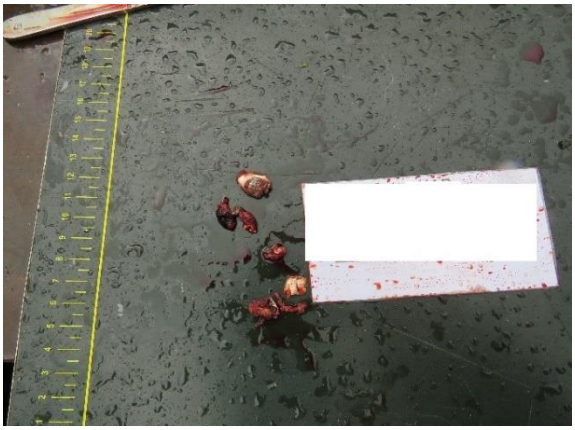

Figure 82: Metal fragments collected from the Cadaver 3B.

Figure 83: Trajectory of the projectile, using a surgical instrument.

3.5.7. Time of death

Cadaver 3A: The cadaveric phenomena observed are in agreement with the date of death (about 48 hours), taking into account the animal species and atmospheric conditions, namely local temperature and humidity.

Cadaver 3B: The advanced cadaveric phenomena observed are in agreement with the date of death (about 48 hours), taking into account the animal species and atmospheric conditions, namely local temperature and humidity.

3.6. Procedures and results

Cadaver 3B: The metal fragments were stored by the laboratory and will be delivered in the hand of the prosecutor (Figure 82).

3.7. Summary of findings

Cadaver 3A: The perforating circular wound found in the right scapular region corresponds to the entry wound, since there hasn't a haemorrhagic halo and is minor in diameter (1 cm) than the corresponding perforating circular wound on the left scapular region (2,5 cm of diameter), which have a halo of bruising and everted and irregular borders, corresponding to the exit wound. Furthermore, the projection of the bone fragments indicates that the direction of the shot was from the right to the left side. The predation marks right dorsal thoracic region are considered to be *post-mortem* since there is not hemorrhage associated. The presence of blood in the pericardium cavity was due to the perforation of the right atrium, and the bloodless spleen is a cause of that massive blood loss.

Cadaver 3B: The perforating circular wound found in the right frontal region, adjacent to the cornual process of the frontal bone, which passed through the head bones and the brain until the cervical bones, correspond to the entry wound of a gunshot injury. The trajectory occurs from the frontal region to the occipital, in rostro-caudal direction (Figure 83), without the presence of an exit wound. The blunt trauma extended to the surroundings tissues, namely the sinuses.

3.8. Comments and conclusions

Cadaver 3A: The traumatic thoracic injuries, with pulmonary and cardiac rupture are an adequate cause of death. The lesions are compatible to have been produced by a firearm projectile, since it is seen injuries corresponding to entry and exit wounds, and to a trajectory. However, no projectiles or fragments thereof have been recovered. Therefore, the cause of death is a shotgun wound to the thorax; and despite of the lung injuries, the more probable mechanism of death is a massive haemorrhage due to a rupture of a heart chamber. The legal cause of death is a violent, non-accidental death.

Cadaver 3B: The traumatic cranio-meninge-encephalic injuries are, with clear and convincing pathological evidence, the mechanism of death, being the cause of death a gunshot to the head. The legal cause of death is a violent, non-accidental death. The cadaver was sutured in order to be collected by the Portuguese system of death farm animal collection (SIRCA).

CHAPTER IV: CRITICAL POINTS OF NECROPSY PROCEDURES IN VETERINARY FORENSIC

Through the cases seen during the internship and through the literature review, were identified the general critical points regarding to necropsy procedures in veterinary forensic. The critical points were not made case-by-case since a generalist approach was considered to be more valuable and ethical.

Critical points can be defined in this situation as being the most important stages and skills of the forensic necropsy procedures, and usually is in which more easily the errors are made. When a stage of the procedure is a critical point means that it must be unavoidable performed and with the minimal errors possible; when a skill (e.g. expertise of the FVP) or consideration (e.g. biosafety) is a critical point means that theses faculties must be highly controlled, either for the success of a well-done necropsy procedure or for other purposes, such as public healthy factors. Thus, the identification of critical points is essential to the FVP be aware of them and therefore improve them, achieving the accomplishment of the veterinary forensic necropsy.

1. Academic specialization

As mentioned before at "state of the art", exists a need for education and training in the field of veterinary forensic pathology. The court expect specialized FVP for achieve a plausible level of credibility of the cases. The true is that many, if not most, of the mistakes that are made stem from the fact that non-specialized or non-experienced pathologists are so often unaware of some of the important objectives of the forensic necropsy and are not familiarized from the fussy procedures that distinguished a medicolegal necropsy from a clinical necropsy (Moritz, 1981).

This lack of academic specialization also reflects in an insufficient knowledge on how to interpret lesions; also, will reflects in fewer studies in the area, which will slow the development of the forensic veterinary medicine, for example, the lack of species-specific reference data are common problems (J. Cooper & M. Cooper, 2008).

Beck the academic specialization is to beck the forensic veterinary medicine evolution, and nowadays, due to the increase demand of the forensic work, it is important that the veterinary

pathologists can undertake the cases and executed them in a routinely, not exhaustive way, which could be a breaking point to not want to accept a case.

2. Legal knowledge

With academic specialization accrue the legal knowledge. Since forensics deals with legal, criminal cases, as well as many others (mentioned at "Definition" of "forensic veterinary medicine"), being aware of the governing laws regarding to animal cruelty in force on their country it is imperative for any FVP. It is particularly important for the report writing, in which the FVP should know how to write the sentences to be judged as he/she intended.

The legal descriptions of the death are often mistaken and each one of the definitions are extremely important to transparently expression the sequel of events that lead to death.

3. Crime scene examination

Accessing the crime scene is very important, and for the FVP not being able to examine the body at the death scene has been regarded as one of the classical mistakes in forensic pathology (Dolinak, Matshes, & Lew, 2005; Moritz, 1981). Gerdin and McDonough (2013) affirmed that examining the body at the scene can be analogous to taking a medical history. However, due to lack of personnel and funding, it is very unusual to the have supporting forensic specialists available to assist on scene, along the law enforcement officers.

The examination of the cadaver at the crime scene requires practice and skill, and most time are the law enforcement officers by themselves responsible to analyse and collect the evidences (cadaver and others) and then deliver it to the laboratory. The problem is that many times the law enforcement officer stationed to the crime scene do not possess the specialized training required to investigate an animal abuse scene, which may result into overlooked information (Touroo & Fitch, 2016).

However, be aware that, as everything, this subject will depend on the countries and regions legislative procedures, and in some places, having the FVP may be a reality, but the literature in general is consistent in this issue, this is, in the lack of the FVP assisting the crime scenes.

4. Evidence handling

Evidence is anything collect of a case, which can be used to prove guilt or innocence, to identify victims, and/or to identify suspects. Therefore, in forensic necropsy, evidence can either be the cadaver itself, the samples collect (from the cadaver or from the crime scene), the photographs and videos token (during the necropsy or before, at the crime scene), the radiographs, the tests results, and furthermore the case folder of the case itself (Merck *et al.*, 2012e; Touroo & Fitch, 2016).

Here, it will be identified the critical points prior to the necropsy examination, regarded with the handling of the evidence when it arrived at the laboratory.

4.1. Conservancy of the cadaver

The conservancy of the cadaver is one of the utmost critical points of forensic necropsy. It begins at the transportation between the crime scene and the laboratory. The conservancy in which the cadaver arrives and how it is thereafter preserved influence the outcome of the necropsy.

If the cadaver is not subjected to any conservancy method and is left at ambient temperature, the advanced *post-mortem* changes, such as putrefaction, will sabotage the necropsy material whether for the macroscopic examination or for the ancillary tests; also, less severe *post-mortem* changes can mimic some injuries, e.g. advanced *livor mortis* can be jumbled with bruising. If the cadaver is subjected to an incorrect conservancy method, as freezing, many ancillary tests, such as histopathology, will be impaired, and many internal changes will be difficult to analyse because the freezing can produce changes like trauma-induced lesions and *ante-mortem* inflammatory edema, as mentioned at “general necropsy technique”.

The cadaver should always be conserved in refrigeration, in temperatures between 0ºC- 5ºC as soon as it is collected from the crime scene.

4.2. Evidence reception

The reception of any type of evidence, whether they are the full cadaver itself or pieces of it, or samples – arthropods, bullets, blood, animal tissues for histopathology, etc – must follow rules, mentioned before at "reception and labels" of "necropsy procedures in veterinary forensic", and often the errors or missteps occurred in this stage, probably because there are a wider range of people which could have contact with the samples.

Although it should be the FVP to receive the evidence and sign it, most times, due to work management, are the laboratory receptionists who do it; thus the personnel working at the laboratory which eventually may have to deal with this kind of work, even if only by receiving it, must to do formation in this field, in order to do a well-done reception of the evidence, as summarized below:

1. Sign and date the evidence receipt;
2. Keep the shipping label (with the name of the agent who delivered it);
3. Register the date and time of the reception;
4. Assign a laboratory reference number and correlate it with the unique process identification number crime (NUIPC) of the case;
5. Attach the lab number to the evidence;
6. Carriage the evidence to the correct destination at the laboratory;
7. Register the evidence destination at the laboratory.

4.3. Evidence labelling and identification

Labels are essential from the reception of the evidence until the end the of the case, to the maintenance of the chain of custody. Labels must always follow the cadaver and every samples taken from it. They shall appear on every record, such as photographic and radiological. For instances, a frequent difficulty encountered in the laboratory of pathology is the incorrect identification of the container (Vala & Pires, 2016).

However, labels are not limited to the attribution of a laboratory number; the identification of the received cadaver or sample is very important, and it is one basic fact that is often neglected (Green, 1979).

The true is that most times laboratories are not prepared with the most suitable material, and that could be an explanation to labelling be sometimes forgotten or improper. The follows are examples of laboratory material and methods which could improve the labelling:

1. Possess tamper evident containers with writing fields for the lab number and identification;
2. Possess tamper evident cable seals able to be engraved the lab number and adapted to the areas of the corpse;
3. Having an extensive variety of rulers – L-scaled and others – of several sizes, with writing fields to be simultaneously used as identification cards; or instead, have several sizes of referenced rulers and as well some sizes of identification cards (to fit on close and distant photo views).

5. Necropsy procedures

The necropsy is a stage entirely concerning to the FVP. The critical points detected in this stage comprehends: (1) the use of systems and protocols; (2) the performing of records and case notes; (3) the collection of the samples; and (4) the expertise of the FVP.

5.1. Systems and protocols

Forensic necropsy systems and protocols are a substantial critical point. The existence of defined and tested necropsy procedures systems and protocols are imperative to the veterinary forensic medicine, and it "needs to be rectified as a matter of urgency" (J. Cooper & M. Cooper, 1998).

A systematic necropsy procedure decreases the probability of an organ to be examined and a pathological condition to be missed. Wobeser (1996) stated that most errors could be avoided by "careful thought prior to the necropsy and by following a protocol with strict attention to detail during the necropsy". Consistent necropsy reports, which follows the same protocol,

increase the competence of the judges to be familiar to the reading of the forensic veterinary medicine reports.

5.2. Records and case notes

Records and case notes encompasses much more that written notes; it can be anything which can be recorded – a photograph, a radiograph, a receipt of receiving the cadaver, the result of an ancillary test, etc.

The attainment of every form of records and case notes are a critical point of forensic necropsy, since they are the grounding of a case – it turns the theoretical words into the reality of the case, exposing the animal and/or evidences crude photos and radiographs; and it puts the case in a contemporary mode, by showing the real bloodsplashed written notes made during the necropsy, proving that adulteration was not made to the report.

Both records and case notes must begin beforehand to the necropsy, including the background information, the descriptions, diagrams and photographs produced prior to the necropsy (e.g. about wrapping). At necropsy the FVP should not underestimate any lesion or disregard the examination of any organ, as he/he should record everything by photography and written; every photography must be identified by the identification card adjusted to the plan of the camera. After the necropsy, results of subsequent ancillary tests also must be kept as a record. Every record must be retained by the FVP until all possible court proceedings are completed.

5.3. Sample collection

The collection of the samples during the necropsy also are a critical point because the wrong collection and/or conservancy of the samples can abolish the possibility of obtaining essential results to the conclusion of the case. The FVP shall be well informed of the know-how of each type of sampling collection, and if not, he/she should not be shy to ask to the receiving laboratory. Every single sample must be recorded.

The samples always must to be identified and go along with the information stated at "sample collection and ancillary tests", here transcribed:

1. Identification and contacts of the performing FVP and laboratory;
2. Identification of the cadaver, including the case number;
3. Information about the death;
4. Date and time of the sample collection;
5. What ancillary tests are required;
6. Type of sample sent;
7. The suspicion (Peleteiro *et al.*, 2016).

5.4. Expertise of the forensic veterinary pathologist

The experience of the veterinary pathologist is one of the primary mode of the court evaluate his/her expertise on the case. It is preferred an experienced pathologist and/or a medicolegal specialized, to take a forensic case, because he/she will be better prepared to the challenges of this kind of cases and will be less likely to make basic mistakes; also, there is a multitude of yet unanswered questions that can confound the inexperienced veterinarian, thus his/her knowledge could be brought to devalue the findings and the conclusion that was made.

In forensic cases, the signs of animal abuse and neglect must be very well recognized, and not be undervalued and/or misjudged, thus a formation on this area to those not specialized is opportune.

The FVP, as anyone else, does not knows everything about all the subjects, and especially due to the huge variety of species that is presented to veterinarians and to the scarce detailed forensic examination of many of these species, the uncertainties often arises. The pathologist should appeal to other specialists (e.g. biologist) for elucidation. Other cases, is when the doubt is a forensic pathology matter for which do not exists recorded data at all; H. Munro and R. Munro (2008) presented some of these situations: "What circumstances lead to fracture of the hyoid in badgers?" or "Do resolving bruises in dogs show the same changes at the same time as those in cattle?". And then emphasized the importance of an honest and humble report conclusion, in

which any lack of knowledge about some aspect of the pathology should be stated clearly (p. 13).

6. Report writing

The forensic necropsy report is the document which provides a clear understanding of the case to the non-medical personnel, by focusing all the findings of the necropsy into a conclusion of cause, mechanism and manner of death. Therefore, its writing – **when**, **what** and **how to write it** – represents a critical point.

It is crucial to start the report writing as soon as the necropsy end. Most times, only a matter of hours or even minutes can influence the memory of the pathologist, even with the guiding of his/her rough notes.

The information on the report does not concerns the necropsy findings alone; instead, it gathers all the information provided by the investigating authorities, the exam findings, the procedures, the samples collected, test submissions and results (medical and forensic) (Merck *et al.*, 2012e). The second part of the report (summary of findings and conclusion) are the one that most weigh to the non-medical personnel who read the report, and many times the pathologist can feel some pressure to have a conclusive report, however it must be reminded that is not always possible and the best outcome is to be humble and honest in the conclusion, and use terms as "undetermined" in situations with less than 50% certainty.

As mentioned at "forensic necropsy report", it must be written in present tense, in careful language, with clear, concise and transparent sentences, without the use of slang or abbreviations, and with a minimum font size of 12 point and double spaced (H. Munro & R. Munro, 2008; Pires, 2016). On the second part of the report, the use of lay terms is imperative.

7. Biosafety

Some thoughts should be given to the hazards to the pathologist and other laboratory personnel at the necropsy and regarded to public health issues. For instances, "the danger of injury posed by a slippery floor, the sharp corner of a table, the blade of a knife or saw, or the point of a needle represents a hazard" to the pathologist, and as well as the risk of disease spreading, must

be considered before beginning the necropsy examination (Burton, 2003; H. Munro & R. Munro, 2008). “Pathogens may be acquired by inhalation (of aerosols), ingestion, direct inoculation, entry though pre-existing breaks in the skin, and through the mucous membranes of the eyes, nose, and mouth”; thus in cases which there are possible risks from contagious diseases, the use of a safety cabinet may be appropriate to safeguard the health of laboratory personnel (Burton, 2003; H. Munro & R. Munro, 2008).

8. Chain of evidence

The maintenance of the chain of evidence is undoubtedly the main critical point of a forensic case. The chain of custody encompasses all the steps of the chronological history of the evidence, from the cadaver transportation, to its reception, storage, necropsy examination, collection of samples and its transfer to other laboratory or services. So, the registers from the necropsy are only a parcel of this process. The FVP is responsible for guarantee that no compromise, contamination, alteration, or substitution of the evidence, or any part, occurred during the entire process of the pathological examination, since the arrival at the pathology laboratory until the end of the case (Brownlie & Munro, 2016).

The case folder as well as the cadaver and/or its samples should be held by the FVP in a secure storage in the laboratory until the case’s ended, and the timing and method of disposal of the cadaver or it remains should be dictated prior to necropsy by the court and be documented in the final report. Most time it is required that the remains be held until the case is adjudicated in the event the defence requests a second necropsy, which often is not practical for most facilities (Gerdin & McDonough, 2013). Yet, until then the cadaver or its remains must be storage as safely as possible, e.g. should not be storage in a common freezer chest, or if only one freezer chest exists, it must be placed in a corner and covered with a tamper evident mechanism. The case folder must as well be storage in a privately place with a security mechanism, e.g. in a lockable locker that only a limited number of personnel have access to the keys.

CHAPTER V: CONCLUSIONS

The purpose of this work was to arouse attention for forensic veterinary medicine, focusing on the necropsy work of such cases, identifying its critical points in order to improve this discipline.

The conclusions of this work are the followings:

1. Forensic veterinary medicine is a rapidly developing subject, assuming special importance from the moment when violence against animals start to be considered as an indicatory parameter of social violence, recognizing the link between animal cruelty and violence toward humans, mostly family violence and child abuse;
2. The laws regarding to animal cruelty varies by country; while some have strict laws on this subject, many others may not give the matter significant attention;
3. The veterinary pathologists should have specialization in the field and act as forensic pathologists, becoming more involved in investigations, prevailing the combined roles of police, forensic veterinary pathologists and prosecutors in the fight against animal cruelty;
4. Forensic veterinary medicine is strongly interdisciplinary, involving biologists, agriculturalists, animal behaviourists, DNA technologists, etc.;
5. A good forensic veterinary pathologist is the one that, other than having ability and willingness to give evidence and to appear in court, have professional skill and integrity;
6. Forensic necropsy is immensely important because it provides documented observation of injury, natural disease, neglect, and decomposition, which may provide unique insights into events occurring both before and after death;
7. Forensic necropsy must follow protocols to minimize mistakes;
8. The chain of custody is the main critical point of a forensic case and it is mostly of the forensic veterinary pathologist responsibility, before, during and after the necropsy.
9. The forensic medicine in Portugal, despite being increasing each year, is yet very behind from some developed countries and manifold critical points are executed with errors mostly due to poor economic power of the pathological laboratories.

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ANNEX I

WSAVA Body condition score (BCS) system for dogs and cats