UNIVERSIDADE DE TRÁS-OS-MONTES E ALTO-DOURO

# SMALL ANIMAL MEDICINE AND SURGERY PRACTICE

FINAL INTERNSHIP REPORT ON VETERINARY MEDICINE MASTER DEGREE

**SARA CRISTINA MACEDO SANTOS**

**Supervisor:**

Professora Doutora Maria Isabel Ribeiro Dias
*Universidade de Trás-os-Montes e Alto-Douro*

**Co-supervisor:**

Professor Doutor José Manuel de Melo Henriques Almeida
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**Vila Real, 2015**

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Professora Doutora Maria Isabel Ribeiro Dias

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I declare that this Master's report is the result of my own research and personal work and guidance of my supervisors

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**(signature)**


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I owe my gratitude to Dr. Nuno Paixão and Dr. Richard Leonard for giving me this internship opportunity and for all the transmitted knowledge. I also thank the whole team of both hospitals (HVC and MediVet) for the experience, knowledge and joy shared with me.

I take this opportunity to thank the entire faculty, Universidade de Trás-os-Montes e Alto Douro, including its professors, staff and colleagues, for giving me all the support to reach this final stage.

Most importantly, none of this could have happened without my family. Words cannot express how grateful I am to my parents and sisters for their love, support and motivation throughout my life.

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I am indebted to my friends, Steven Sybenga and Miguel Caldeira, for their constant support and encouragement during the development of this internship report.

Last but not least I want to express my gratitude to one and all who, directly or indirectly, have lent their helping hand in this venture.

# Abstract

This report describes four clinical cases I attended during my undergraduate internship.

Laparoscopy is a minimally invasive technique with numerous applications. It is a great alternative to conventional open procedures and widely considered the method of choice to diagnose many conditions and take biopsy samples. The minimal trauma to soft tissues is associated with a lower incidence of intraoperative and postoperative complications. The biggest drawback of this technique is the high costs of the specialized instruments required to perform it.

Sino-nasal aspergillosis is one of the most common causes of chronic nasal disease in dogs. *Aspergillus* spp. are opportunistic pathogens that can cause severe rhinosinusitis. The process can extend to adjacent tissues and to the lungs, causing many different clinical signs. In very severe cases, intracranial signs can arise. Disseminated aspergillosis is also possible but is generally caused by different species of Aspergilli. Sino-nasal aspergillosis can be very difficult to diagnose and to treat. Its diagnosis rely on multiple modalities as hardly any single diagnostic method is foolproof. There are different treatment methods available, including invasive and noninvasive techniques and the use of systemic or topical antifungal drugs, which are sometimes combined. Early detection and treatment are important for the treatment success. The pet may respond well to therapy but the condition may be recurrent and difficult to cure in some cases.

Ibuprofen is one of the drugs most commonly involved in dogs and cats poisonings. It is widely used in human medicine with relative safety, however for pets it is not recommended as it can easily cause clinical signs that can lead to gastric perforations and acute renal failure. In turn, metaldehyde is a pesticide mainly used to control slugs and snails in gardens and croplands and is between the toxic agents most commonly involved in pet poisoning episodes. Metaldehyde has the potential to disturb their major body systems. Common clinical signs involve CNS excitation, GI upset and respiratory disturbances. Animals can die within a few hours of exposure, generally as a result of respiratory failure or a few days later due to liver failure. Both toxicosis are generally diagnosed based on history of exposure and compatible clinical signs but differential diagnosis must be considered when the available information is not enough to diagnose. The prognosis depends on the ingested

dose and the time passed since exposure until treatment is initiated. Prompt and aggressive symptomatic therapy and supportive care accompanied by continuous monitoring until the patient fully recovers are perhaps more important than the ingested dose for positive outcomes. Besides, the best way of treatment is to prevent it. To do so, proper owner education is the key, as owners are commonly unintentionally involved in their pet poisonings. Ibuprofen can be accidentally ingested or administered by the owner in an attempt to help his sick pet. In turn, metaldehyde is commonly placed on the house premises by the owner and not protected from the pets. Thus, it is crucial that veterinarians alert their clients about potentially toxic substances.

**Keywords:** Aspergillosis; Ibuprofen; Laparoscopy; Metaldehyde.

# Resumo

Este relatório descreve quatro casos clínicos a que tive oportunidade de assistir durante o estágio curricular.

A laparoscopia é uma técnica minimamente invasiva com inúmeras aplicações. É considerada uma boa alternativa à cirurgia convencional e um excelente método de diagnóstico de várias patologias. O menor traumatismo tecidular está associado a uma baixa incidência de complicações intra e pós-operatórias. A principal desvantagem relaciona-se com o custo elevado do equipamento.

A aspergilose nasal é considerada uma das causas mais comuns de doença nasal crônica em cães. Os fungos do gênero Aspergillus são patógenos oportunistas que podem causar uma rinossinusite grave. O processo pode estender-se aos tecidos adjacentes e pulmões, causando sinais clínicos muito diversos. A aspergilose disseminada, por sua vez, é geralmente causada por diferentes espécies de *Aspergillus*. A aspergilose nasal pode ser muito difícil de diagnosticar e de tratar. É geralmente necessário combinar vários métodos de diagnóstico para obter um diagnóstico definitivo. Métodos de tratamento incluem técnicas invasivas e não invasivas e uso de medicamentos antifúngicos sistémicos ou tópicos, por vezes combinados. O diagnóstico precoce e um tratamento imediato são provavelmente os aspetos mais importantes para um bom prognóstico. O animal pode responder bem ao tratamento, mas a condição pode ser recorrente e difícil de curar.

O ibuprofeno é um dos fármacos mais frequentemente envolvidos em intoxicações de cães e gatos. Embora amplamente utilizado em medicina humana, não é recomendado para animais de companhia, nos quais tem o potencial de causar perfurações gástricas e insuficiência renal aguda. Por sua vez, o metaldeído é um pesticida usado para controlar lesmas e caracóis em jardins e áreas de cultivo e é um dos agentes tóxicos mais comumente envolvidos em intoxicações de animais de companhia. Este moluscicida tem o potencial de afetar os principais sistemas orgânicos. Os sinais clínicos mais comuns incluem excitação do sistema nervoso central, distúrbios gastrointestinais e respiratórios. A morte dos animais afetados pode ocorrer dentro de algumas horas após a exposição, geralmente como resultado de uma insuficiência respiratória. Pode também ocorrer uns dias mais tarde devido a uma insuficiência hepática. O diagnóstico de ambas as intoxicações baseia-se geralmente na história de exposição ao tóxico e em sinais clínicos compatíveis. Quando a informação

disponível não é suficiente para estabelecer um diagnóstico, diagnósticos diferenciais devem ser considerados. O prognóstico depende da dose ingerida e do tempo que passa desde a exposição até que o tratamento é iniciado. A brevidade e agressividade do tratamento de suporte e a monitorização contínua dos animais são provavelmente mais relevantes para o prognóstico do que a dose de tóxico ingerida. Como sempre, a melhor forma de tratamento é a prevenção. Para que isso seja possível é imprescindível que se promova a educação dos proprietários, uma vez que estes estão frequentemente envolvidos nas intoxicações dos seus animais. Isto acontece quando o tóxico é colocado/deixado em locais de fácil acesso para os animais ou até administrado pelos proprietários. Torna-se assim fundamental que os veterinários informem os seus clientes sobre as substâncias que são potencialmente tóxicas para os seus animais.

**Palavras-chave:** Aspergilose; Ibuprofeno; Laparoscopia; Metaldeído.

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# Abbreviations

| | | |
|---|---|---|
| ARF | - | Acute Renal Failure |
| AGDD | - | Agar - Gel Double Immunodiffusion |
| ALT | - | Alanine Aminotransferase |
| ALP | - | Alkaline Phosphatase |
| AST | - | Aspartate Aminotransferase |
| BPH | - | Benign Prostatic Hyperplasia |
| BID | - | *Bis In Die* (every 12 hours) |
| BUN | - | Blood Urea Nitrogen |
| BCS | - | Body Condition Score |
| BAL | - | Bronchoalveolar Lavage |
| C-section | - | Caesarean Section |
| CRT | - | Capillary Refill Time |
| CNS | - | Central Nervous System |
| CSF | - | Cerebrospinal Fluid |
| CD ($4^+$ or $8^+$) | - | Cluster Of Differentiation |
| CT | - | Computed Tomography |
| CHF | - | Congestive Heart Failure |
| CK | - | Creatine Kinase |
| COX | - | Cyclooxygenase |
| DJD | - | Degenerative Joint Disease |
| DIC | - | Disseminated Intravascular Coagulation |
| ECG | - | Electrocardiography |
| ET | - | Endotracheal |
| ELISA | - | Enzyme - Linked Immunosorbent Assay |
| FHM | - | Feline Hemotropic Mycoplasmosis |
| FHV-1 | - | Feline Herpesvirus-1 |
| FIA | - | Feline Infectious Anemia |
| FIP | - | Feline Infectious Peritonitis |
| FLUTD | - | Feline Lower Urinary Tract Disease |
| FHO | - | Femoral Head Ostectomy |
| FB | - | Foreign Body |
| GABA | - | Gamma - Aminobutyric Acid |
| GI | - | Gastrointestinal |
| GIT | - | Gastrointestinal Tract |
| GA | - | General Anaesthesia |
| HVC | - | Hospital Veterinário Central |
| IMHA | - | Immune-Mediated Hemolytic Anemia |
| Ig | - | Immunoglobulin |
| IBD | - | Inflammatory Bowel Disease |
| IBD | - | Inflammatory Bowel Disease |

| | | |
|---|---|---|
| IL(17 A) | - | Interleukin |
| IVDD | - | Intervertebral Disk Disease |
| IM | - | Intramuscular |
| IP | - | Intraperitoneal |
| IVFT | - | Intravascular Fluid Therapy |
| IV | - | Intravenous |
| ILE | - | Intravenous Lipid Emulsion |
| JPS | - | Juvenile Pubic Symphysiodesis |
| LDH | - | Lactate Dehydrogenase |
| LD50 | - | Lethal Dose 50 |
| MRI | - | Magnetic Resonance Imaging |
| MHC | - | Major Histocompatibility Complex |
| MAO | - | Monoamine Oxidase |
| NSAID | - | Nonsteroidal Anti - Inflammatory Drug |
| OVE | - | Ovariectomy |
| OVH | - | Ovariohysterectomy |
| PCR | - | Polymerase Chain Reaction |
| PCV | - | Packed Cell Volume |
| PO | - | *Per Os* (Oral Administration) |
| PG | - | Prostaglandin |
| PGE | - | Prostaglandin E |
| R.T.A. | - | Road Traffic Accident |
| SID | - | *Semel In Die* (every 24 hours) |
| SNA | - | Sino - Nasal Aspergillosis |
| SC | - | Subcutaneous |
| Th (1;17) | - | T - helper |
| TID | - | *Ter In Die* (every 8 hours) |
| TPLO | - | Tibial Plateau Leveling Osteotomy |
| TTA | - | Tibial Tuberosity Advancement |
| TP | - | Total Protein |
| TS | - | Total Solids |
| USG | - | Urine Specific Gravity |
| WNL | - | Within Normal Limits |

# Chapter I - Introduction

This report is the culmination of a training period in the area of Small Animal Medicine and Surgery. This traineeship had the duration of six months, three months in Hospital Veterinário Central (Charneca da Caparica, Portugal) and, subsequently, three months in Medivet Hendon Veterinary Hospital (London, UK).

During this six-month internship, I had the opportunity to integrate teams full of professionals and to participate in various activities of the different areas that make up the everyday of the veterinary practice.

I've contacted directly with all the services provided by these hospitals, such as consultation, pet hospitalization, with separate wards provided for dogs, cats and small exotic pets, anesthesiology, orthopedic and soft tissue surgeries, including laparoscopic surgery, intensive care and emergency services. I also had contact with many diagnostic procedures, including blood tests (hematocrit, blood count, blood smears, biochemical panels and electrolytes determination), cytological analysis, ultrasound, echocardiography, endoscopies (GI, respiratory tract), radiology, CT, MRI, among others. Performing a rotation by the different services, allowed me to attend and actively participate in a wide variety of situations and respective means of diagnosis.

Relying on the overwhelming support of my co-workers and mentors, this experience allowed me to strengthen and expand the knowledge I have already acquired during the curricular phase of my studies in the university. It also gave me a comparative insight about the veterinary reality in Portugal and the United Kingdom and made me improve my skills and knowledge well beyond my academic prior knowledge.

In addition, I had access to a series of lectures on a range of topics, including some conducted by us trainees.

In conclusion, the undergraduate internship is undoubtedly a practical experience essential to our training.

In the present report, I decided to describe four clinical cases from all the cases I had the opportunity to follow during the traineeship. Each clinical case is followed by the respective theoretical discussions based on a literature review. This report also includes a list of other clinical cases attended, not detailed due to space limitation.

# Chapter II - Performed Activities

I completed my undergraduate internship at HVC (Charneca da Caparica, Portugal) from 1th of September 2014 to 30th of November 2014 and at Medivet Veterinary Hospital and Emergency and Critical Care Centre from 15th of January 2015 to 15th of April 2015.

During this time I have participated in a wide range of activities. These included being present during many consultations with different veterinarians and in many different situations during both normal daytime hours as well as out-of-hours emergency periods.

I assisted during diagnostic procedures, including Cardiac and Abdominal Ultrasound, Cystocentesis, Rhinoscopy, Cystoscopy, Bronchoscopy and BAL, GI endoscopy, X-rays (see Figure 1), CT and MRI. Also FNA's and biopsies of many different organs and masses, such as skin, lymph nodes, prostate, nasal passages, lungs, kidneys, liver, spleen, pancreas, stomach, duodenum, etc., were performed. I also helped with some laparoscopic-assisted biopsies of abdominal organs.

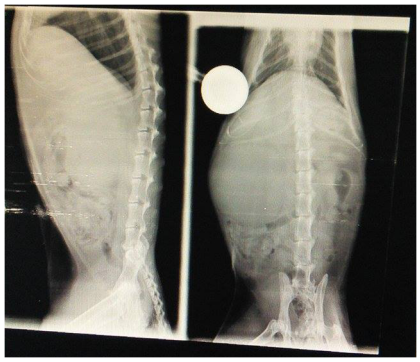

Figure 1: X-rays of a cat with a massive right kidney (US revealed total loss of corticomedullary distinction and huge perirenal (probably subcapsular) fluid. accumulation).

I observed a broad range of surgeries and scrubbed in on several (See Table 1, Appendix A). These ranged from Orthopaedic (see Figure 2) and Spinal procedures to Soft tissue surgery (see Figures 3, 4, 5 & 6). I performed anaesthetic monitoring for many of them. I also performed some dog castrations and cat castrations and practiced suturing during some procedures under supervision.

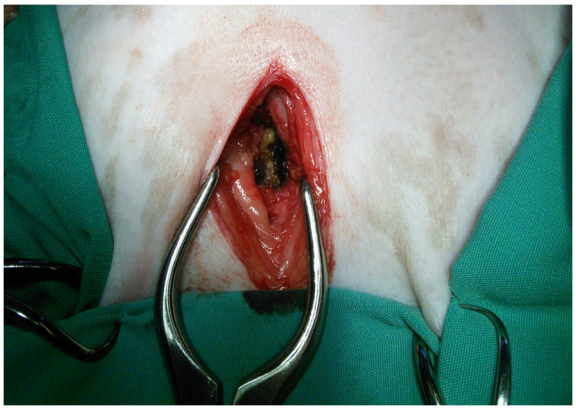

Figure 2: JPS in a dog.

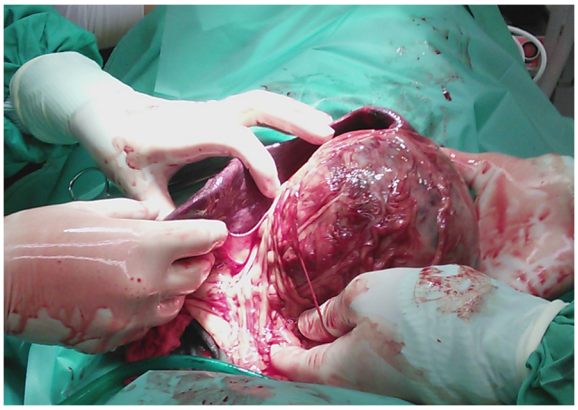

Figure 3: Splenectomy in a dog with a massive tumor attached to the spleen.

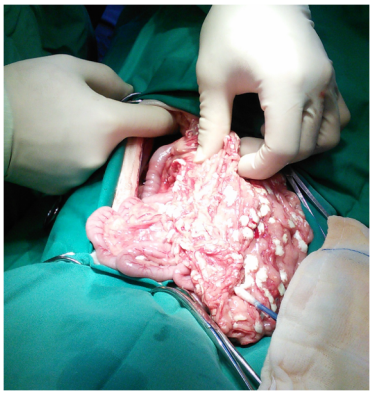

Figure 4: Ex-lap in a cat with severe peritonitis (probably secondary to pancreatitis).

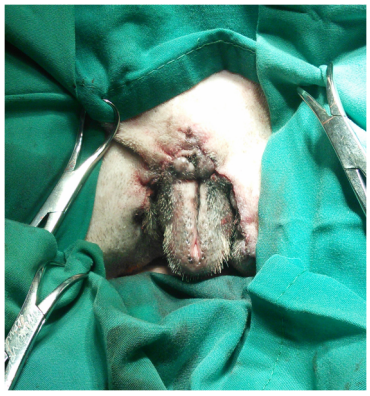

Figure 5: Vaginoplasty.

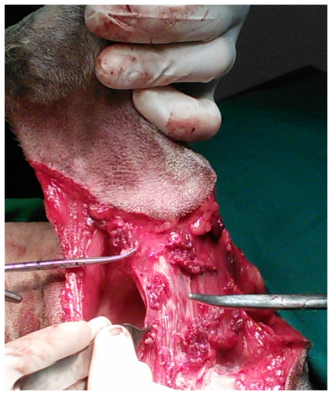

Figure 6: Amputation.

I practiced placement of IV catheters, taking of blood samples (jugular and both limbs), placement of nasogastric tubes and ultrasound-guided FNA's and free fluid sampling. I also practiced laboratory procedures such as preparing many types of smears (blood, ear, joint fluid, etc.), urine sediments, biochemistries, pcv/tp, haematology's, antigen test kits, microscopic evaluation of smears (see Figure 7), etc.

Figure 7: Microscopic 7: Microscopic evaluation of an ear smear from a cat with ear mites (*Otodectes cynotis*).

I had the opportunity to attend and assist in many treatment procedures, including hemodialysis, hyperbaric oxygen therapy (see Figure 8) and acupuncture, apart from routine procedures.

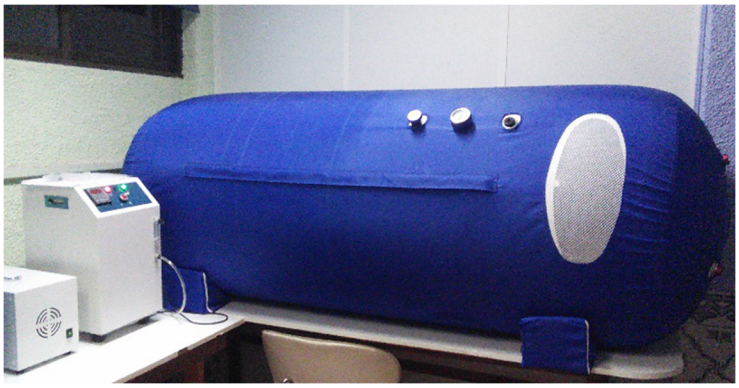

Figure 8: Hyperbaric oxygen therapy chamber in HVC-Portugal.

Each day I participated in case discussions during ward rounds, performed physical examinations and follow-up of inpatients in dog and cat ward and ICU and took part in resuscitation of many different emergencies presented from day to day (see Table 2, Appendix A).

In addition, I had contact with other animal species, such as hamsters, rats, ferrets, chinchillas, guinea pigs, rabbits and birds (parrots, parakeets, pigeons, etc.). I attended many consultations involving these species. The most common problems reported were inappetence, lethargy, weight loss, lack of defecation (gut stasis) and teeth overgrowth/malocclusion. Some of them needed ongoing monitoring and treatment, so they were admitted to the hospital. During their hospital stay, I performed physical examinations and helped with their supportive care and treatment. I also observed surgical drainages of tooth abscesses (see Figure 9) and spay in rabbits and castrations in both rats and rabbits.

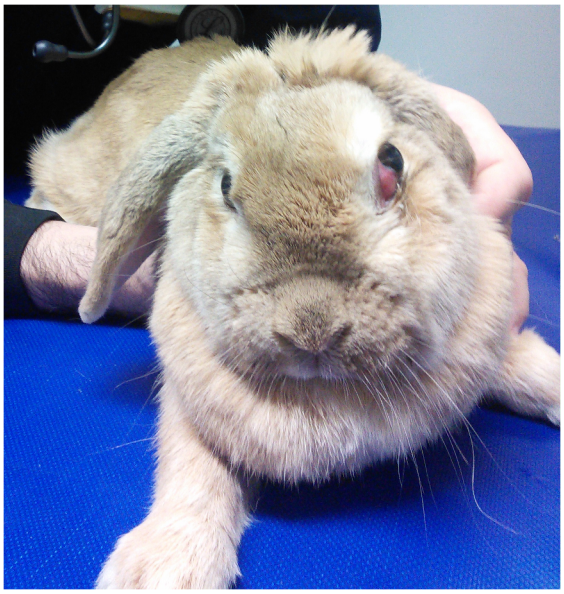

Figure 9: Retrobulbar (molar-associated) abscess leading to evident exophthalmos.

# Chapter III - Clinical cases

# Laparoscopic spay (ovariectomy)

## Clinical Case

### Animal identification

| | | |
|---|---|---|
| **Pet name:** Michka<br>**Species:** Canine<br>**Breed:** Dachshund miniature smooth haired | **Colour:** Black<br>**Gender:** Female<br>**Age:** 0 y 6 m 11 d | **Weight:** 5.9 kg/13 lbs<br>**Insured status:** Insured (Petplan) |

**Clinical history:**

No relevant information.

**Clinical examination prior to surgery:**

All parameters were within normal limits (WNL).

**Surgical procedure:**

Premedication: medetomidine hydrochloride 0.01 mg/kg IM and methadone 0.3 mg/kg IM;

Induction: propofol (2 ml) IV;

Maintenance: Isoflurane;

Animal positioning: dorsal recumbency;

Duration of procedure (from initial skin incision until final suturing): 40 min.

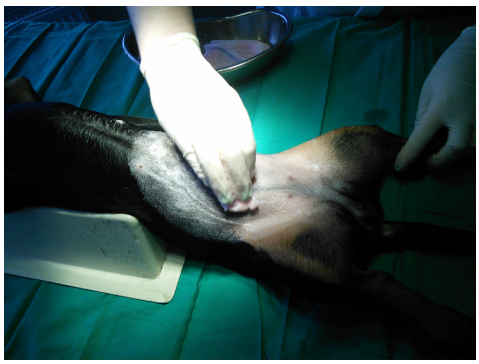

Figure 10: Aseptic surgical field preparation.

After the surgical field (see Figure 10), the surgeon and his assistant were properly prepared, the surgeon placed the surgical drapes on the animal and the procedure began. The first incision was made in the skin 2-3 cm caudal to the umbilicus on the midline. The

abdominal wall at this level was pulled up and a needle with suture (3/0 Supramid®) passed through the abdominal wall, entering around 2 cm distance from one side of the incision to approximately the same distance in the opposite side. This suture facilitates the elevation of the abdominal wall for the Veress needle introduction.

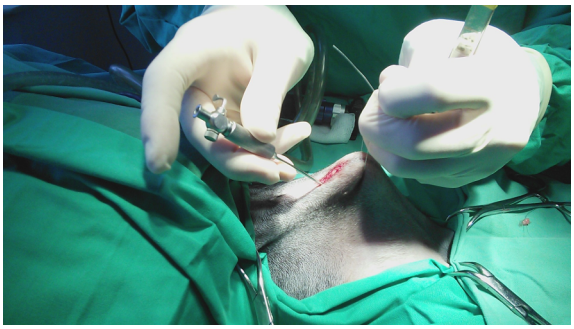

Figure 11: Veress needle introduction.

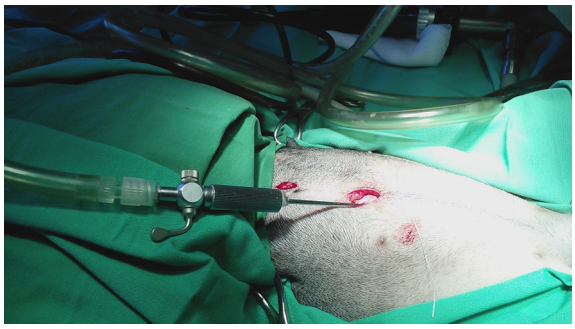

Figure 12: Insufflation of the abdomen and second incision made.

The surgeon inserted the Veress needle (see Figure 11) and, after verifying its correct location, he connected it to the insufflation tube. When the pressure reached 12 mmHg, the second incision was made (nearly 1 cm long and 1 cm cranial to umbilicus) paramedian to the midline (to avoid the falciform ligament) and an EndoTIP™ cannula (Karl Storz) was inserted with a clockwise rotatory movement (see Figure 12 & 13). The pressure was increased up to 12 mmHg for instruments placement but otherwise kept between 7 and 9 mmHg.

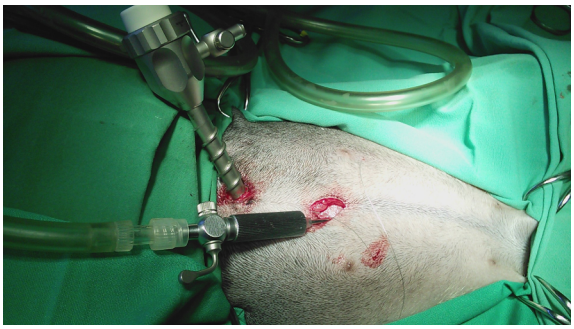

Figure 13: First EndoTIP™ cannula inserted.

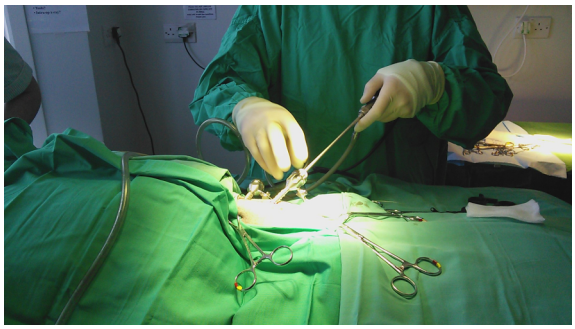

Figure 14: Introducing laparoscope.

The insufflation tube was transferred from the Veress needle to the cannula, the Veress needle removed and a second cannula was introduced in its place. The rigid laparoscope (5 mm diameter, 0 degrees angulation) was connected with the light source and introduced through the caudal portal (see Figure 14). The abdominal cavity was inspected for any hemorrhages or other traumas, especially under portal placements. Since no complication occurred, he was able to continue. The table was rotated 30 degrees toward the surgeon, who was positioned on the right side of the table, to allow access to the right ovary. While

visualizing the ovary, the surgeon introduced the grasping forceps through the cranial portal (see Figure 15) and grabbed the ovary (see Figure 16 & 17).

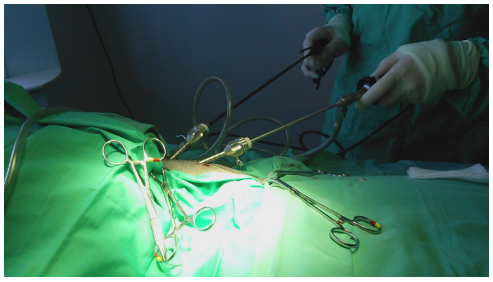

Figure 15: Grasping forceps being introduced.

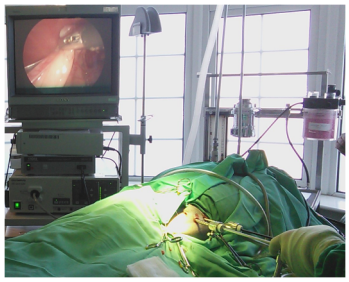

Figure 16: Grasping forceps grabbing right ovary with video camera visualization.

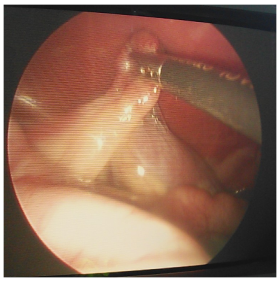

Figure 17: Ovarian pedicle being grasped by forceps (monitor visualization).

The grasped ovary was pulled up against the abdominal wall and, with the support of a needle holder, a needle (with suture) was introduced through the abdominal wall in order to fix the ovary to the abdominal wall after externally palpating its position inside the abdomen (see Figure 18).

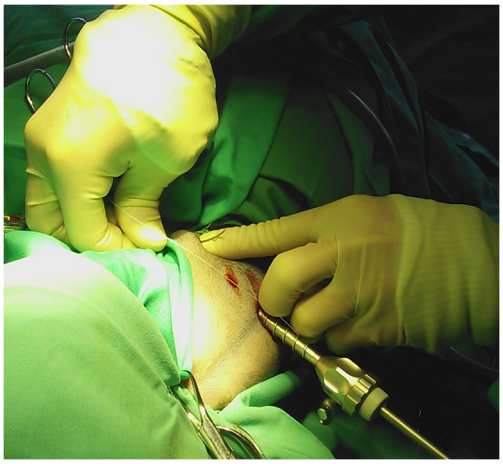

Figure 18: Needle introduction to fix ovary to abdominal wall.

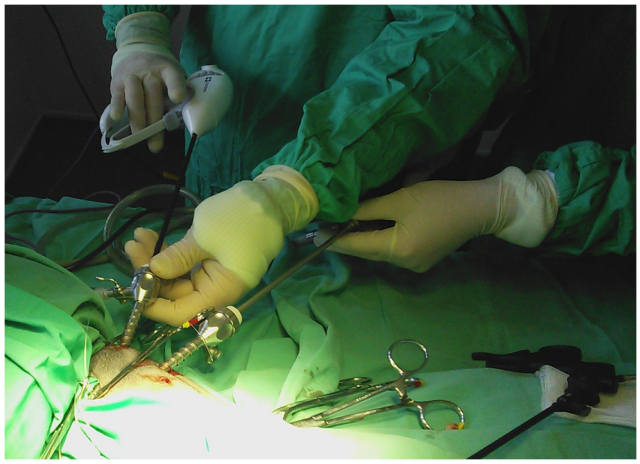

Figure 19: Bipolar forceps being introduced through cranial portal.

With the ovary in position the grasping forceps could be removed and the bipolar cauterizing forceps introduced instead (see Figure 19). These were used to cauterize the ovarian pedicle, the proper ovarian ligament and oviduct/proximal uterus (see Figure 20).

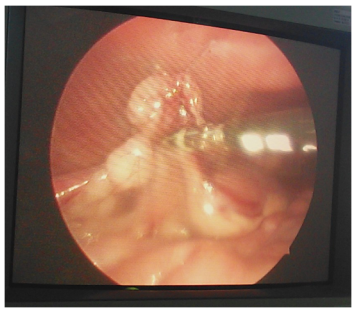

Figure 20: Ovarian pedicle cauterization visualized on monitor.

Afterwards, the grasping forceps are introduced once again to remove the ovary out of the abdominal cavity. So, the ovary was grabbed and pulled out through the cranial portal,

together with the cannula (see Figure 21). The cannula was placed back (see Figure 22) and the animal tilted for the opposite side and the same procedure for the left ovary was carried out (see Figure 23).

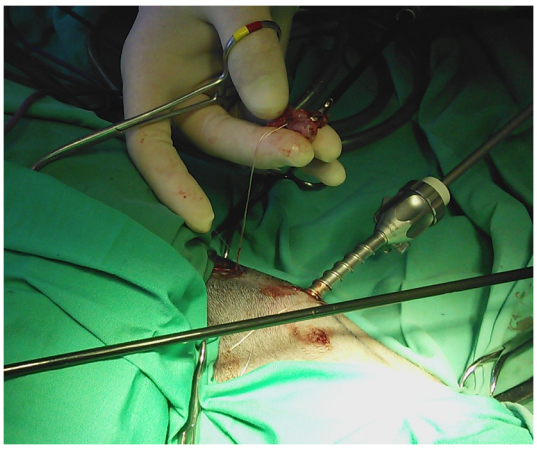

Figure 21: Ovary removed through cranial portal.

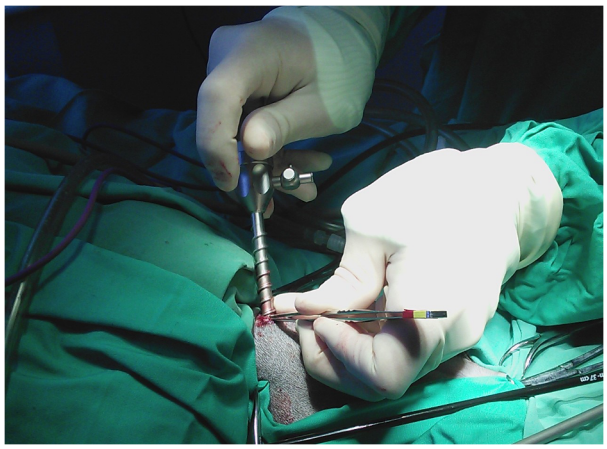

Figure 22: Cranial cannula being reinserted after ovary removal.

The abdomen was again checked for any abnormality and, since nothing emerged, all the instruments were removed and a mild pressure applied to the abdomen to release the remaining amount of intra-abdominal CO2. The abdominal incisions were closed in three layers: peritoneum/muscles/fascia with interrupted stitch, subcutaneous with a continuous suture pattern and skin through an intradermal suture, all using 3/0 biosyn (monofilament synthetic absorbable suture).

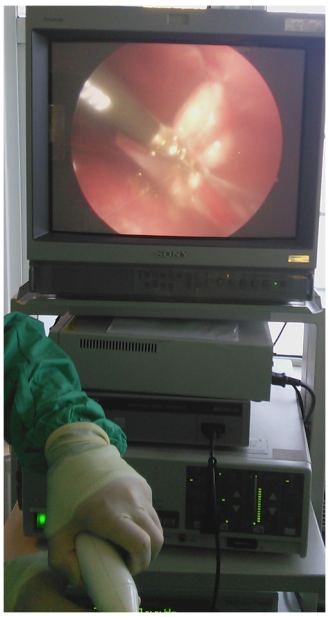

Figure 23: Left ovary cauterization.

**Post-operative evaluation:**

Michka had a good and painless recovery from anesthesia and returned home later on the same day. There were no complications in the hospital or later at home reported by the owner.

# Literature review

## Introduction

Laparoscopy is a minimally invasive surgical technique used in Veterinary Medicine either as a diagnostic method, as an alternative to invasive exploratory laparotomy, or in the surgical treatment of many conditions or even elective procedures such as ovariohysterectomy (OVH) and ovariectomy (OVE) (Lhermette & Sobel, 2008). It is considered the preferred technique in the diagnosis of various internal diseases, especially hepatobiliary diseases (Rothuizen, 1985).

It is a safe procedure with several advantages over other alternatives, such as being less invasive and associated with low incidence of intraoperative and postoperative complications and low mortality (Matyjasik, Adamiak, Pesta, & Zhalniarovich, 2011). It allows direct visualization of the abdominal structures with minimal trauma to the soft tissues and reduced incidence of postoperative infections, as the incisions are smaller if compared with the conventional technique. As a general rule, the smaller size of the incisions are linked with reduced pain/discomfort after surgery, faster healing and fewer sutures, which saves time of surgery (Lhermette & Sobel, 2008). It is generally associated with faster recovery and shorter hospital stay, leaving owners very satisfied (Lhermette & Sobel, 2008; Matyjasik et al., 2011). Laparoscopy enables rapid collection of larger and more precise biopsies when compared with the ones taken percutaneously, once the direct visualization of the target organ allows selection of the areas of greatest interest for sampling. Moreover, it enables direct control of any intraoperative hemorrhage (Lhermette & Sobel, 2008).

The success and safety of laparoscopy largely depends on patient selection and the surgeon's experience, closely related to the correct performance of the technique and proper use of instruments (Rothuizen, 1985). The quality of the equipment used is of equal importance (Tobias & Johnston, 2013).

Therefore, laparoscopy entails acquiring a set of specialized instruments. These are costly, which makes it the biggest drawback of the technique. However, the equipment can be used, at least partially, for numerous types of endoscopy such as thoracoscopy, arthroscopy, urethroscopy, rhinoscopy and otoscopy (Rothuizen, 1985; Lhermette & Sobel, 2008). The initial training period and costs associated with it can also be considered a disadvantage of the

technique, although this is something inherent to most new procedures (Pope & Knowles, 2014).

It is very important that, before the procedure starts, owners are informed about the possibility of the laparoscopic procedure be converted into an open surgical technique in case some complication arise, such as persistent bleeding or presence of a mass bigger than expected to remove. In any case, the equipment necessary to carry out a conventional laparotomy should be available (Lhermette & Sobel, 2008; Tobias & Johnston, 2013).

**Indications**

Laparoscopy is a minimally invasive technique with numerous applications and is widely considered as the preferred method for some procedures. Some examples are mentioned hereinafter.

Laparoscopy is used as a diagnostic method of pathologies that involve direct visualization and/or histopathological evaluation of the intra-abdominal structures. It is considered the method of choice for obtaining biopsies of organs and/or abdominal masses, such as the liver, kidney, pancreas and gastrointestinal tract, allowing the selection of different areas of the organ/structure to obtain a sample. For this reason it has a higher diagnostic value than a blind biopsy. In addition, laparoscopy allows immediate detection of bleeding or any other iatrogenic trauma, enabling early resolution of it (Rothuizen, 1985). Laparoscopy is also used for placing feeding tubes (stomach, duodenum or jejunum), which is possible through exteriorization of the respective segments of the gastrointestinal tract, introduction of the feeding tube and, at the end, segment fixation to the abdominal wall (pexia). It is also indicated to perform preventive gastropexy in animals at high risk of developing gastric dilation and/or volvulus and to remove foreign bodies from the stomach (Lhermette & Sobel, 2008; Matyjasik et al., 2011). Laparoscopy can even be used for cystopexy, for example in male dogs with perineal hernia to prevent retroflexion of the bladder (Matyjasik et al., 2011). It is highly suitable for carrying out elective procedures such as OVE and OVH and also for the removal of remnant ovarian tissue and testicles retained in the abdominal cavity (cryptorchidectomy). Laparoscopy is the method of choice also for cystoscopy of male dogs and cats with large calculus to remove. It can be used for cholecystectomy, which is the method of choice in human medicine for any condition that require removal of the gallbladder, however, it is a technique that requires a surgeon with

high experience and ancillary equipment (Lhermette & Sobel, 2008). Research studies involving repeated intra-abdominal assessments in the same animal can benefit from the use of laparoscopy as it can be conducted multiple times at short intervals (Rothuizen, 1985).

**Contraindications**

Laparoscopy is contraindicated in patients with diaphragmatic hernia, due to the required pneumoperitoneum, extensive intra-abdominal adhesions, pyometra, severe cardiac dysfunction and when all the suspected conditions entail an open surgical procedure (Rothuizen, 1985).

**Equipment**

The laparoscopes used in dogs and cats usually range between 2.7 mm and 10 mm diameter. The 10 mm scope provides greater visual field but implies larger incisions and therefore, is only used in animals with a minimum of 10 kg body weight, while the narrower laparoscope entails less trauma (Rothuizen, 1985; Matyjasik et al., 2011). The 5 mm one is probably the most versatile of all (Tobias & Johnston, 2013), and is suitable for most pets and a variety of circumstances. The narrower is recommended for the smaller animals and imply the use of a protective sheath as it can be easily damaged (Rothuizen, 1985; Matyjasik et al., 2011). The smaller the diameter, the stronger the light source should be (Matyjasik et al., 2011). Although the telescope length is variable, the 29 cm one is generally suitable (Tobias & Johnston, 2013). The angle of the scope defines the direction of the endoscopic image field and can range between 0 and 180°, although the most commonly used vary between 0 and 45°. The non-angled scopes allow the visualization of the structures that are just in front of the scope's tip ("straight-on" view), which probably provides the easiest viewing for the surgeon, but are also more easily contaminated. In turn, the angled scopes give a wider field of view, allowing the inspection of the structures right below the incision area but also others more difficult to access, such as around the hepatic lobes, which can only be observed due to the angulation. However, in an initial stage, the oblique view provided may hinder the surgeon's handling (Rothuizen, 1985; Tobias & Johnston, 2013). The laparoscope is inserted into the abdomen through a cannula, whose trocar might have a conical or pyramidal end, this last one allowing an easier perforation. Laparoscopes with working channels allow the insertion of other instruments through the same cannula, such as biopsy forceps, enabling visualization and simultaneous removal of material (Rothuizen, 1985).

Xenon is the recommended light source and considered to provide the most realistic colors of the abdominal viscera. Alternative light sources that can be used are halogen or metal halide light sources (Lhermette & Sobel, 2008).

A pneumoperitoneum should be established before the insertion of the laparoscope or any other instruments used during the laparoscopic procedure. It creates a working space between the abdominal wall and the abdominal organs and structures, which prevents the trauma caused by perforation of these organs. A Veress needle is commonly to induce pneumoperitoneum. It consists of a spring-loaded cannula that contains a blunt-end mandrel that protrudes beyond the cannula when entering the abdominal cavity so it doesn't injure the organs and structures below. An adapter is used to connect the needle to the insufflator. Some trocars have an extension that allows insufflation of the abdominal cavity without the use of the Veress needle (Matyjasik et al., 2011). Insufflation may be accomplished either manually or using an automated gas insufflator, which is most suitable as it can regulate and record the volume of gas introduced, the intra-abdominal pressure, the gas flow rate and gas losses during the procedure, replacing these losses automatically. The recommended gases are carbon dioxide (CO2) or even nitrous oxide (N20). These are much safer than air, which is totally contraindicated as it is relatively insoluble and, under some pressure, would cause air embolism and, consequently, sudden death of the animals (Rothuizen, 1985).

The basic equipment for a laparoscopic procedure is usually housed in the laparoscopic tower, and includes the monitor, the camera control unit, the light source, the insufflator and the recording data apparatus. In the past, for the abdomen to be visualized, the surgeon had to look through the eyepiece of the laparoscope. This revealed to be very impractical, therefore the use of a video camera has become an essential part of the procedure. The camera, which binds to the head of the scope, captures the image transmitted through the scope's lens and sends the information to the camera control box that will project the image as a real-time video on the monitor (Tobias & Johnston, 2013). Besides, this has the great advantage of allowing the surgeon to move around the animal and the rest of the team to follow the different steps of the procedure and synchronize their activities (Matyjasik et al., 2011).

There is a wide variety of instruments available for many different laparoscopic procedures, which includes an ample range of forceps and scissors, and also suction and irrigation cannulas, retractors, dissectors, suture material, staples and clips, trocars and

hemostatic devices. The retractors are atraumatic instruments that facilitate the laparoscopic technique as it provides better exposure of the target structures by pushing away other structures that come between the camera and the target organ. The irrigation and aspiration equipment allows clearness of the operative field by removing fluids, tissue debris, clots and the smoke resulting from electrocoagulation. To ensure effective hemostasis, mono- or bipolar electrodes are routinely used. The electrocautery devices are often combined with instruments such as forceps and laparoscopic scissors, which acquire a mono or bipolar current allowing simultaneous electrocoagulation. A set of trocars of different lengths and tips and its accessories are available. These trocars allow us to access the peritoneal cavity and are used as an extension of the surgeon's hand. Thus, the cannula/trocar unit allows the surgeon to manipulate the intra-abdominal structures, introducing and removing working tools without intra-operative gas loss. This is only possible due to the inherent valve system, either manual or automatic (Prisco, 2002; Matyjasik et al., 2011). There are cannulas with smooth surface and thread-like cannulas, the latter being recommended as it prevents the cannula from sliding off when it is moved or when other instruments are being introduced (Tobias & Johnston, 2013). EndoTIP™ cannulas are also commercially available and are safer than the previously described units since it does not require a trocar and has no sharp tip. It consists of a thread-like cannula that can be connected to the laparoscope, allowing simultaneous visualization of the different layers transposed during the introduction and removal of the cannulas. Therefore, it decreases the risk of iatrogenic lesions to the underlying viscera and solves the major concern of the primary trocar blind insertion. After the skin incision is made and the subcutaneous tissue dissected, a small anterior rectus fascial incision is performed and the EndoTIP™ cannula inserted perpendicular to the tissues and with a clockwise rotation (Ternamian, 2002; Lhermette & Sobel, 2008). In the clinical case reported two EndoTIP™ cannulas were used according to the technique aforementioned, although not benefiting from its potential connection to the laparoscope and, consequently, from the incremental video-monitoring during introduction and removal of the portals.

**Anesthetic considerations and creation of pneumoperitoneum**

A laparoscopic procedure involves placing the animal under general anesthesia (GA). Local anesthetic blocks are recommended in the regions where each cannula will be placed, contributing to a balanced anesthesia. By introducing CO2 into the peritoneal cavity the intra-abdominal pressure will increase, therefore, interference with the ventilation should be taken

into account. Compression of the caudal vena cava and/or liver is also possible, which would contribute to a decreased venous return and even to a reduced diaphragm movement. Although the consequences of increased pressure are usually minimal and easily overcome by homeostatic mechanisms, a complete physical examination of the animal must always be performed and any condition that can compromise the animal's respiratory function must be ruled out prior to laparoscopy. In any case, assisted ventilation may be required. Insufflation should be started at the lowest possible rate, allowing the animal to adapt. Later on, when the insufflation is complete, the rate can be increased to compensate for any gas losses during instrument's insertion (Lhermette & Sobel, 2008).

Prior to cannulas placement, a meticulous palpation of the abdomen should be performed in order to avoid puncturing the spleen. Although this is generally not life-threatening and has spontaneous resolution, it should always be avoided, especially because the consequent bleeding can make visualization harder, which might disrupt the entire procedure (Lhermette & Sobel, 2008).

The most common method of insufflation implies the use of a Veress needle. Using a scalpel blade number 11 or 15, a skin incision with a length similar to the diameter of the needle is performed. Subsequently, the skin around the incision is slightly lifted with forceps and the Veress needle is introduced pointing caudally towards the pelvis. This angulation minimizes the risk of injuring the spleen and avoids the falciform ligament (Lhermette & Sobel, 2008). The inherent mechanism of this needle allows its blunt end to retract once the wall is perforated and to immediately protrude when entering the abdominal cavity, thus avoiding any damage to the abdominal structures (Matyjasik et al., 2011). To further reduce the risk of perforation, fasting should be respected and the bladder emptied before surgery (Lhermette & Sobel, 2008). This was the abdominal access technique chosen in the clinical case reported. The surgeon followed the procedure described in the literature, although the performed skin incision was bigger (about 1 cm) than the diameter of the Veress needle. Since an EndoTIP™ cannula was going to be inserted afterwards, the incision was longer to avoid skin dystocia.

An alternative technique for abdominal access, which prevents the blind insertion of the needle and facilitates the introduction of the first cannula when adhesions are present, is the Hasson technique. This technique consists of a mini-laparotomy, in which a 1 cm skin incision is performed, followed by blunt dissection and a 3-4 mm incision in the *linea alba*,

caudal to the umbilicus. The cannula/trocar unit is introduced through the incision into the peritoneal cavity and the insufflator connected to the cannula (Matyjasik et al., 2011; Tobias & Johnston, 2013). A suture around the cannula is applied to avoid gas leakage and prevent it from being pulled out or excessively moving when instruments are being introduced. The disadvantage of this technique is the implementation of an incision larger than required for the insertion of the cannula, which also entails a larger post-surgical wound. Besides, the risk of subcutaneous emphysema is higher once the possibility of gas leakage around the cannula is also greater (Lhermette & Sobel, 2008; Matyjasik et al., 2011).

After the introduction of the Veress needle, a syringe must be connected to it and its plunger pulled back to check for blood or other typical material from several intra-abdominal organs, such as faeces or urine, which should not emerge in the syringe. Then a slow injection of sterile saline should be carried out without showing any resistance. The aspiration can result in a small amount of fluid in the syringe, however, larger amounts indicate that the lumen of some abdominal organ has been reached (Matyjasik et al., 2011). It is extremely important to ensure that the Veress needle is correctly positioned before insufflation, otherwise the gas will be directed to other tissues, such as subcutaneous tissue, omentum and round ligament, or even to the lumen of vessels or viscera. A subcutaneous placement of the needle can result in subcutaneous emphysema, which generally resolves spontaneously within about 48 hours but will probably hamper the repositioning of the needle to the correct location and, consequently, all the following steps. On the other hand, when positioned within the omentum or falciform ligament, these structures will inflate and the remaining structures may become hard to view. This may require disinsufflation and a restart of the procedure. If the needle tip is inside a vessel or viscera, it can result in fatal gaseous embolism (Lhermette & Sobel, 2008).

When the Veress needle is correctly positioned inside the peritoneal cavity, the insufflator must be connected and the abdomen insufflated to a suitable pressure. For animal safety, the pressure should always be kept below 15 mmHg. Usually 8 to 10 mmHg is enough to get good laparoscopic images of the intra-abdominal structures. When inserting the instruments, this pressure can be slightly raised to reduce the risk of injury of intra-abdominal organs (Lhermette & Sobel, 2008). If any structure is blocking the gas flow into the abdominal cavity or the needle is incorrectly positioned, the insufflation control box will

indicate no gas flow. Therefore, the needle will need to be repositioned or even reinserted (Matyjasik et al., 2011).

**Insertion of instruments and inspection of the abdominal cavity**

After establishing pneumoperitoneum, a second skin incision is made for the insertion of the cannula. To maintain control and avoid entering the abdominal cavity too deeply or quickly, the cannula is held in the palm of the hand with a finger along its shaft. It is inserted by performing a rotary movement. Once inside the abdominal cavity, the trocar is removed and the cannula can be further advanced into the abdomen. Immediately after trocar removal, the cannula valve closes, preventing gas leakage. The laparoscope, previously connected to the video camera, is then inserted through the cannula. The insufflator is disconnected from the Veress needle and connected to the laparoscope portal instead, so the Veress needle can be removed (Lhermette & Sobel, 2008; Matyjasik et al., 2011). If the lens becomes too foggy due to the cold gas, the CO2 inflow can be transferred to another portal. In addition, to prevent the images from becoming blurred, the application of an anti-fog commercial solution to the distal end of the endoscope is advised. A povidone-iodine solution can be used for the same effect. Placing the endoscope in a previously heated saline solution for one or two minutes before its introduction can assist with obtaining a clearer image. It can also be helpful to touch a serosal surface with the lens during the procedure (Lhermette & Sobel, 2008).

Once we are able to visualize the peritoneal cavity, it should be carefully inspected for any signs of bleeding or iatrogenic trauma, especially under the portal locations. Depending on the laparoscopic purpose, two or more portals may be required. The insertion of the subsequent cannulas is carried out by following the same procedure described above, this time aided by internal image monitoring. This allows us to choose the best location for trocar introduction, reducing the risk of hemorrhage and injury to internal organs. After perforation, trocars are removed and the necessary instruments introduced through the cannula (Matyjasik et al., 2011).

When inspecting the abdominal viscera, indirect palpation of the different structures is achieved by using a palpation probe, which also enables us to move the organs during the procedure. The use of an adjustable table can be very useful for some procedures, such as laparoscopic OVE/OVH. When tilting the patient, the viscera are moved down via gravity permitting a clearer field of view. This facilitates the evaluation of the target structures and

the advance of the procedure. To avoid unnecessary iatrogenic lesions, it is recommended to follow all the instruments and their movements by the laparoscope (Lhermette & Sobel, 2008).

**Procedure conclusion**

When the laparoscopic procedure ends, the laparoscope and other instruments are removed and the CO2 influx stopped. The valves are opened and the cannulas removed. A slight pressure must be applied on the abdominal wall so that the remaining gas can exit. Incisions are then routinely sutured in two or three layers (Lhermette e Sobel 2008; Matyjasik et al. 2011).

**Potential post-laparoscopic complications and ways to prevent it**

There are very few cases where complications associated with laparoscopic procedures arise. When it occurs, it is usually due to the surgeon's inexperience in performing the technique, which may be associated with an erroneous conduct of the procedure or inadequate use of the equipment. Other complications can result from the pathology itself and/or anesthetic risk of individual patients, or even from technical faults with the equipment used. For this reason, preoperative assessment of the animal and equipment functionality must always be checked. Additionally, the surgeon should be confident in performing this technique. The possibility of an anesthetic complication should always be considered, so everything should be prepared to reverse and/or ventilate the animal if needed. Some problems may also occur during the introduction of the Veress needle or a trocar. These may include puncture, laceration or perforation of any intra-abdominal organ, structure or blood vessel. The trauma is usually minimal and of no consequence and will eventually heal without surgical intervention. However, in severe cases, it may require lesion repair or hemorrhage control through conventional laparotomy. Once again, to prevent this from happening, it is important that the surgeon is properly instructed on the technique, manipulation and positioning of the instruments. The hemorrhage resulting from a biopsy is rarely a reason for major concerns. It is also important to rule out diaphragmatic hernia before the procedure, as the pneumoperitoneum would lead to a secondary pneumothorax. The same problem can occur if the diaphragm is accidentally punctured. This reinforces the importance of video-monitoring any advance of the instruments within the peritoneal cavity. If this situation is detected, positive-pressure ventilation should be started straightaway. With adequate

monitoring (identical to that for open-chest surgery), the procedure can usually be continued, but a chest drain must be placed at the end of the procedure. Gas embolism is a rare but possible complication. If the gas is introduced into an organ, especially the spleen, or blood vessels, by improper positioning of the Veress needle, the embolism may be fatal. When small amounts of gas are introduced, the carbon dioxide will usually dissolve quickly. However, every time gas embolism is suspected, the animal should be positioned on left lateral recumbency with the head tilted downwards and ventilated with oxygen. This will move the gas bubbles away from the right ventricle flow, in order to help relieving the obstruction. On the other hand, if the CO2 is introduced subcutaneously, subcutaneous emphysema may arise. This usually resolves spontaneously but can complicate the ongoing procedure (see Anesthetic considerations and creation of pneumoperitoneum). Correct location of the needle tip must be tested by syringe aspiration and saline instillation as previously described in order to avoid these complications. When a neoplastic mass is suspected and it will be removed or biopsied, laparoscopic retrieval bags should be used to avoid spreading neoplastic cells through the abdominal cavity. It allows the isolation of tissue during its transport to the outside, thereby avoiding the occurrence of metastasis. These specialized endoscopic networks (retrieval bags) are very practical but costly, so, as alternative, the finger of a sterile surgical glove can be used (Lhermette & Sobel, 2008).

**Laparoscopic surgical contraception in bitches - Ovariectomy**

Surgical sterilization is routinely performed in many practices to control sexual behavior and conception. It also helps preventing some diseases such as pyometra and mammary gland tumor. It can be accomplished by OVE or OVH, either by open or laparoscopic method (Shariati, Bakhtiari, Khalaj, & Niasari-Naslaji, 2014). One of the main applications of laparoscopy is elective sterilization of bitches, either through an OVE or OVH. It has been demonstrated that the risk of complications, such as pyometra or uterine neoplasm, does not increase when only the ovaries are removed, compared to when an ovariohysterectomy is performed (Bojrab, Waldron, & Toombs, 2014). Therefore, many surgeons choose to perform an OVE, because it is a simpler and less time consuming procedure (Lhermette & Sobel, 2008, p. 169). In addition, OVH has been associated with higher postoperative morbidity due to increased handling and intraoperative trauma and larger incision, when compared to OVE (van Goethem, Schaefers-Okkens, & Kirpensteijn, 2006). Moreover, the absence of data demonstrating that removal of the uterus is advantageous,

makes bilateral ovariectomy the preferred method for bitch sterilization (Lhermette & Sobel, 2008).

The laparoscopy is nowadays the method of choice for spaying female dogs. When compared with the traditional procedure, laparoscopy has several advantages. The smaller incisions facilitates wound healing and reduces the risk of wound infection, contributing to lower adhesions development and postoperative pain. This reduces the need for analgesics in the postoperative period and leads to a faster and less stressful recovery of the animal (Devitt, Cox, & Hailey, 2005; Bojrab et al., 2014; Shariati et al., 2014). Besides, the animal activity after surgery has been demonstrated to decrease significantly less after a laparoscopic procedure, in comparison to the analogous open surgery (Culp, Mayhew, & Brown, 2009). Laparoscopy is also indicated for the management of ovarian remnant, by removing the ovarian tissue that subsisted after an incomplete ovariectomy. Moreover, the magnification and light consented by laparoscopy allows better visualization of the genitourinary tract, which avoids leaving behind any ovarian tissue. So the risk of ovarian remnant is much lower in a laparoscopic procedure (Lhermette & Sobel, 2008). According to a study by Shariati et al. (2014), the duration of the laparoscopic sterilization of bitches is significantly shorter than the analogous open surgery but is not consistent between authors. The surgical time will depend on the surgeon experience, position of the patient, extent of fat surrounding the ovarian pedicles, which is correlated with the animal body condition score (BCS), and different vessel sealing devices (Van Goethem, Rosenveldt, & Kirpensteijn, 2003; Fröhlich, 2008; Shariati et al., 2014). The biggest drawbacks are the high costs of the laparoscopic equipment, the specific training required to use it, the surgeon's limited precision and the need for an assistant surgeon (Shariati et al., 2014). According to Pope & Knowles (2014), once the surgeon achieves surgical aptitude in laparoscopic ovariectomy (around 80 procedures), the intraoperative complications rate will be lower (Pope & Knowles, 2014). Over the years, techniques have been improved and more qualified instruments developed, which makes laparoscopy an increasingly attractive option for surgeons and animal owners. This progress has allowed the reduction of the surgical time and the number of portals used (Moore & Ragni, 2012). According to a study based on 18 healthy dogs, the laparoscopic ovariectomy with two cannulas placement is the preferred technique, since it is less time consuming and entails no more postoperative pain when compared with the procedure using one cannula. On the other hand, the use of three instrument cannulas resulted in a significantly higher total pain score, when compared with the two cannulas procedure (J Brad Case, 2011).

**Equipment and technique used for laparoscopic ovariectomy**

To accomplish this procedure, all the main tower components (video camera control box, monitor, insufflator, carbon dioxide gas cylinder, light source, etc.) and an adjustable surgical table must be accessible for use. Besides, the surgical towels and paper drape, an insufflation hose, a camera head, a light cable, a laparoscopic suction/irrigation device, a general surgery pack, an adequate blade, a Veress needle, trocar-cannula units, grasping forceps, a vessel sealing device and a laparoscopic spay hook (or large curved needle), a needle holder and suture material are the most common and basic instruments used in this technique and must be available as well. The size of the laparoscope used will change depending on the animal size. For cats and small breed dogs a rigid scope with a diameter of 2.7 mm is advised. In turn, the most appropriate option for dogs with less or more than 25 kg, is a 5.0 mm or a 10 mm laparoscope, respectively (Moore & Ragni, 2012; Bojrab et al., 2014).

When the bitch is under GA, she should be placed in dorsal recumbency on the adjustable surgical table. Afterwards, the surgical field is aseptically prepared from xiphoid process to pubis and the animal covered by surgical drapes (Shariati et al., 2014).

Laparoscopic ovariectomy is most widely performed using a two portals technique. According to Lhermette & Sobel (2008), the first skin incision should be made midway between the umbilicus and the xiphoid process. The ventral abdominal wall around it can then be elevated to distance it from the underlying viscera and safely introduce the Veress needle, which should be slowly inserted angled towards the pelvis (Lhermette & Sobel, 2008). At this point, the needle should be advanced and withdrawn softly without difficulties and the saline test performed to make sure its tip is in the right location. The saline should flow easily and almost no fluids be aspirated back to the syringe as explained before (Matyjasik et al., 2011). Subsequently, the insufflation tube is connected to the Veress needle and the insufflation should start at a low gas flow. If the intra-abdominal pressure becomes too high, the needle should be slightly moved to achieve the correct position and if it doesn't work it may need to be removed and the procedure restarted. When correctly placed, the flow can be increased up to an 8-10 mmHg pressure or even slightly higher for instruments insertion. A second skin incision 1 to 2 cm caudal to the umbilicus is performed, followed by the primary cannula placement. Then the laparoscope is introduced through it and a complete evaluation of the abdominal cavity should be performed. The insufflation hose is disconnected from the Veress

needle and connected to the laparoscope portal. The Veress needle is removed and a second cannula introduced in its place under laparoscopic visualization. Afterwards, the table is tilted to one side (right or left lateral recumbency) and the grasping forceps are introduced through the cranial portal to grasp the proper ligament of the contralateral ovary (left or right ovary, respectively) and pull it up against the abdominal wall (Lhermette & Sobel, 2008). At this point, the spay hook or needle with suture are used transcutaneously to hold the ovary in position. When it is fixed, the grasping forceps are removed and the electrocautery device is introduced to crosscut/cauterize the ovarian pedicle and suspensory ligament first and the proper ligament of the ovary and fallopian tube or proximal part of the uterine horn next. If no hemorrhage is detected, the bipolar cutting forceps are removed and the grasping forceps introduced to grasp the ovary. By simultaneously releasing the hook/suture, the ovary is removed through the portal together with the cannula. Then the cannula is replaced and the patient tilted to the opposite side to remove the second ovary following the same procedure. If neoplastic tissue is suspected a retrieval bag should be used to remove it, in order to avoid any intra-abdominal spillage. In the end, the abdominal cavity is again scanned for any lesion and if no complication has emerged, the CO2 flow is discontinued, all the instruments and cannulas removed and a gentle pressure applied to the abdomen to set free all remaining gas. At this moment, the port entries can be closed in a routine manner with two or three layers. It is advised to apply a 5% lidocaine patch over the incisions and to give pain relievers to decrease post-operative discomfort (Lhermette & Sobel, 2008; Moore & Ragni, 2012; Bojrab et al., 2014). In the clinical case presented, the surgeon followed a similar approach to the one described above but the order of portals placement was reversed.

# Sino-Nasal aspergillosis

## Clinical case:

**Animal identification**

| | | |
|---|---|---|
| **Pet name:** Radley<br>**Species:** Canine<br>**Breed:** Staffordshire bull terrier | **Colour:** Blue/cream<br>**Gender:** Male - Neutered<br>**Age:** 3 y 5 m 25 d | **Weight:** 21.60 Kg/47.62 lbs<br>**Insured status:** Insured |

**Clinical history:**

*May 2012*

Radley was brought to the clinic on May 2012 because he had recurring epistaxis from the right nostril. He had nothing relevant reported until that date and, at this time, further investigation of the nostril was advised to the owner. Under sedation, both nasal cavities were examined and, on the right side, the mucosa was found to be inflamed and a blood clot was spotted. Although no wound or cut was detected, it was diagnosed as traumatic epistaxis because Radley had been playing/fighting with other dogs and no other reason, such as foreign body (FB), mass, etc., was found. Nonsteroidal anti-inflammatory drugs (NSAIDs) were prescribed for 7 days. A swab was taken for culture and sensitivity and, according to the results, there was moderate *Pseudomonas luteola* growth.

*March 2014*

In March 2014, the owner brought him to consultation because he was concerned about the watery nasal discharge presented by Radley for the previous two weeks. He had discharge from both nostrils and was also sneezing. At that time, it was associated with possible allergic reactions since he had some skin reactions as well. Apoquel trial and allergen testing were advised but denied.

*From November 2014 to January 2015*

In November 2014, Radley was brought back because he had a watery nose (mainly the right nostril) for a few weeks and was sneezing again. The following work-up was

discussed with the owner, who chose a trial with an anti-inflammatory dose of prednisolone instead of further diagnostic tests (x-rays, endoscopy of nose, biopsies). This was a 15 days trial with gradual withdrawal of the steroids and it cleared the nasal discharge. However, during the steroid therapy course, Radley developed some respiratory episodes (possibly reverse sneezing).

At the end of December 2014, the owners brought Radley back to the hospital to investigate the respiratory problem since it wasn't resolved, even after the steroid therapy was ended. Some unilateral (right) bloody mucous nasal discharge was detected at this time. Visualization through an otoscope of the nasal passages revealed inflammation of the right nasal mucosa. Swabs for *Aspergillus* and culture and sensitivity were taken as well as slides for cytology. The x-rays of the chest were clear and the skull x-rays showed increased opacity of the right nasal passage. A CT scan of the skull was advised and performed to assess the extent and to get closer to a diagnosis (see Figure 24). The analysis of the swabs/slides taken concluded a *Pasteurella multocida* growth sensitive to clavulanate-potentiated amoxicillin (bacteriology), an intense predominantly neutrophilic inflammation with moderate hemorrhage with the remainder cells being macrophages, lymphocytes and occasional eosinophils but without bacteria (cytology), and the *Aspergillus* PCR was negative (mycology). A bacterial infection, an FB, a non-exfoliative neoplasia or other infections (e.g. fungal) were possibilities considered by the Clinical Pathologist.

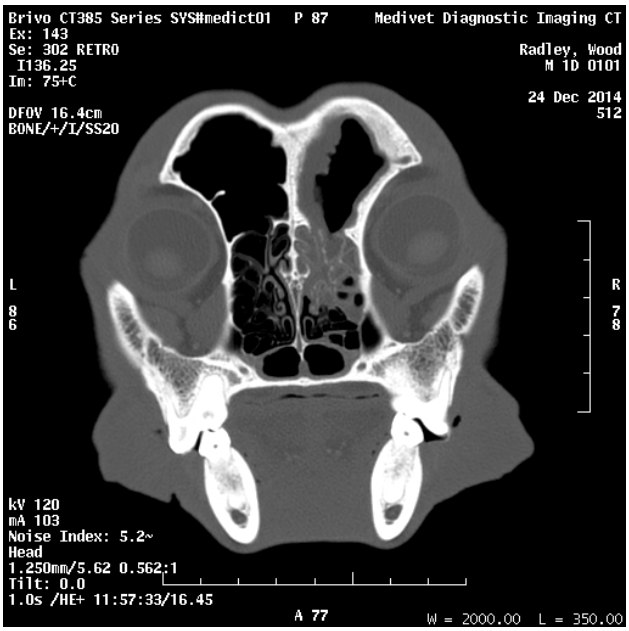

Figure 24: CT scan, transverse section.

The diagnostic interpretation of the pre- and post-contrast CT images by the CT specialist concluded the existence of an extensive turbinate destruction along the right nasal passage, creating multiple cavitation surrounded by thickened soft tissue material. The right frontal sinus revealed irregular, peripheral soft tissue thickening with marked endosteal proliferation in the wall, which were consistent with a frontal sinusitis. The mandibular and medial retropharyngeal lymph nodes were mildly enlarged and, therefore, likely reactive. In turn, the left nasal passage and frontal sinus were normal. These abnormalities were considered indications of a destructive rhinitis and the multiple cavitation and endosteal proliferation in the frontal sinus typical of a fungal rhinitis (aspergillosis). The soft tissue thickening could represent fungal colonies and/or mucus accumulation. The specialist advised to take samples from the frontal sinus, as the nasal cavity samples were negative. Therefore, an endoscopy of the nose and sinuses with biopsies was suggested to the owner. However, the owner decided to go ahead with topical noninvasive treatment instead. So, a nasal infusion with 2% enilconazole was performed. An endotracheal (ET) tube was placed in the trachea and well cuffed and a 24-French diameter Foley catheter was placed in the nasopharynx and its balloon inflated. Some swabs were placed around the Foley catheter to hold it in place and to absorb the fluid from the nasal flush and also around the top incisors to stop leakage into oral cavity. For the nasal infusion, a 10-French diameter catheter was introduced into each nostril. This was followed by the introduction of a 12-French diameter Foley catheter (one in each nostril) and inflation of its balloons in order to hold the antifungal drug in place. All the Foley catheters were occluded by towel clamps. Afterwards, the antifungal preparation was slowly instilled (over 1 hour) into both nasal cavities through the infusion catheters and the dog rotated in order to stay 15 min in each position (dorsal, right lateral, ventral, left lateral recumbency). At the end, the head was tilted downwards, all the catheters removed and the oral cavity examined and cleaned to remove any wash residues. The animal remained in that position for a while to allow the antifungal agent to drain through the nostrils. After ensuring that there was no leakage into trachea, the ET tube was removed as well. The owner was warned that the procedure might need to be repeated 3 weeks later and the antibiotic therapy (clavulanate-potentiated amoxicillin) should be continued. Radley responded well to the therapy as nasal discharge was greatly reduced. However, two weeks later Radley presented with increased nasal discharge, but this time watery and not bloody. The nares were discolored where the discharge was passing. Furthermore, the owner mentioned that Radley had developed mild muscle spasms/twitching on the face/cranial aspect of the neck. The

owner noticed it even before the CT scan was performed but only reported it later when the twitches got stronger. On palpation of the neck, the spasms increased. Otherwise, Radley was eating well, was lively and even gained weight after the nasal flush was performed. The parameters of his physical examination were also WNL. The specialist advised to do an MRI and CSF to rule out meningitis (infiltration to the meninges/brain) and to hold the second nasal flush.

*27th January 2015*

Radley was brought back to the hospital to perform the MRI (see Figure 25) and to collect a CSF sample (see Figure 26). The owner accepted to do the endoscopy with biopsies under the same GA, so a rigid endoscopy of the nose was performed and biopsies taken and sent to the histopathology laboratory (see Figure 27).

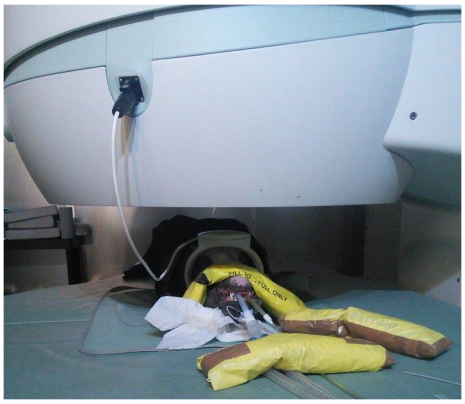

Figure 25: Radley placed in the MRI scanner.

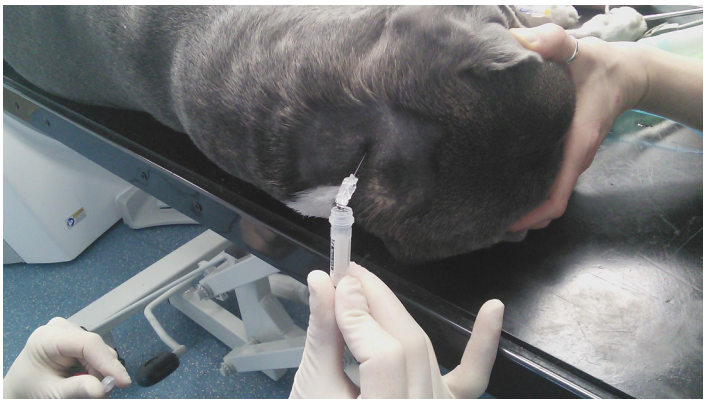

Figure 26: CSF collection.

Results of the MRI, CSF analysis and histopathological evaluation of the biopsies taken:

Figure 27: Rhinoscopy and biopsies.

On the MRI of the head and cervical spine, there was no evidence of meningitis or masses (see Figure 28 & 29). The brain parenchyma didn't present any abnormalities, there was no evidence of a disc herniation or spinal cord compressions and the musculature was normal. Multiple cavitation and moderate (contrast enhancing) soft tissue thickening were seen in the mucosa of the right nasal passage. It was also observed a marked contrast enhancement in the mucosa of the right frontal sinus and, adjacent to the mucosa, an

irregular, hypointense thickening in the frontal sinus and nasal cavity. These changes were consistent with the reported destructive rhinitis and frontal sinusitis. The hypointense material could represent mucous or fungal colonies.

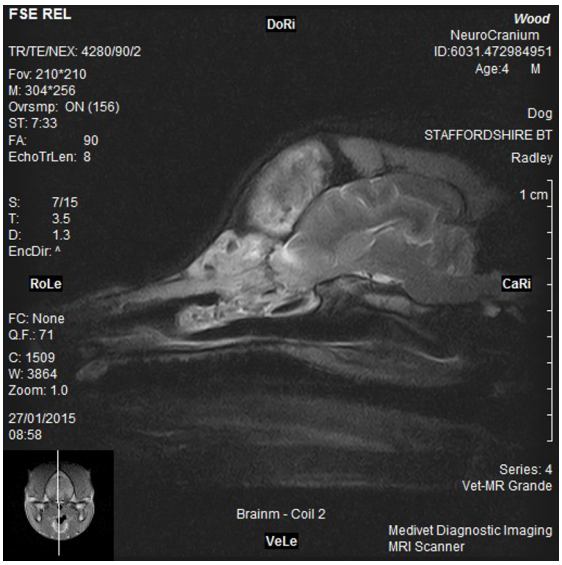


Figure 28: MRI, sagittal section- we can see the extension of the affected area, from the frontal sinus to the nasal passages.

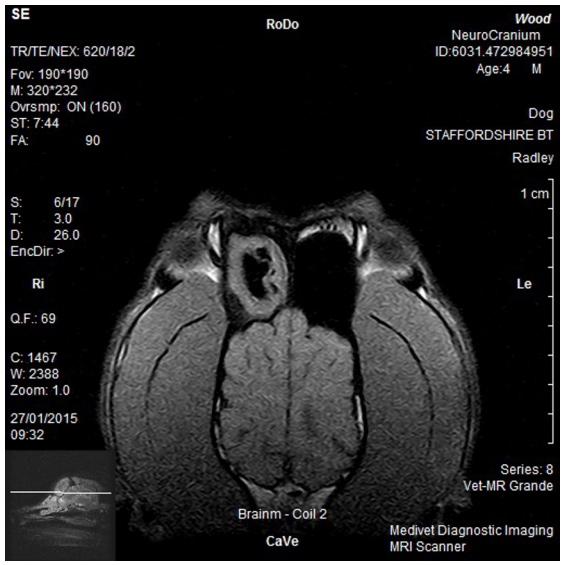


Figure 29: MRI, dorsal section - we can notice the brain remains intact.

The CSF culture had no bacterial growth and its cytological analysis was unremarkable. According to the histopathology report, the nasal cavity showed multifocal ulceration of the mucosa and a submucosa with dense aggregates of plasma cells, neutrophils, lymphocytes, eosinophils and macrophages surrounding mildly hyperplastic glands. Besides, large aggregates of fibrin admixed with degenerate and viable neutrophils, eosinophils, erythrocytes and karyorrhectic debris were found on the surface of the ulcerated epithelium. Abundant exudate was also observed. The histological diagnosis was ulcerative, neutrophilic and eosinophilic, multifocal and marked rhinitis. According to the pathologist, these findings were suggestive of an underlying fungal infection, although no fungal organisms were observed in the examined sections. However, other causes, such as bacterial infection or nasal FB, couldn't be totally ruled out. The presence of eosinophils could also indicate an underlying or concurrent hypersensitivity process. Since fungal invasion was not detected, a second topical treatment was suggested, this time by frontal sinus flush and antifungal treatment after trephination.

*30th January 2015*

Radley was admitted to the hospital for the antifungal topical treatment on the 30th of January. He was pre-medicated with medetomidine and methadone, induced with propofol and maintained with isoflurane. The cuff of the ET tube was inflated and a Foley catheter was placed in the nasopharynx and its balloon inflated (See Figure 30). Swabs (attached to strings to allow easier removal) were placed around the catheter until the nasopharynx was packed with it, to avoid any leakage, swallowing or aspiration of the antifungal cream and debris.

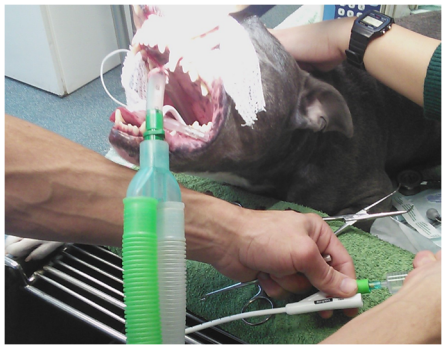

Figure 30: Filling cuff of Foley catheter with saline.

The animal was placed on sternal recumbency and the skin over the frontal sinus aseptically prepared. After placing the sterile drape, a 1 cm skin incision over the right frontal sinus was performed (see Figure 31). Finally, the frontal sinus was trephined with an intramedullary pin (see Figure 32). A wide bore needle (14 gauge) was placed through the hole into the sinus (see Figure 33) and the sinus was flushed with 500 ml of warm saline over a 5-minutes period (see Figure 34). The patency of the nasal ostium was confirmed by exit of the solution through the nostril. Then, a syringe filled with 50 ml of a 1% clotrimazole solution was connected to the needle and the solution instilled over a five-minute period (see Figure 35). In the end, a syringe with 20 g of a 1% clotrimazole cream was connected to the needle and the cream was introduced into the frontal sinus.

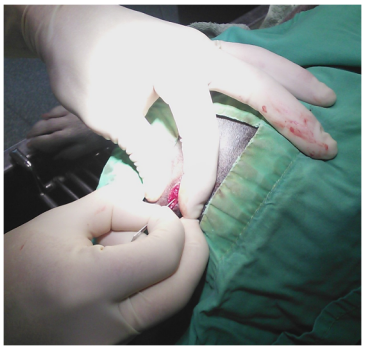

Figure 31: Incision.

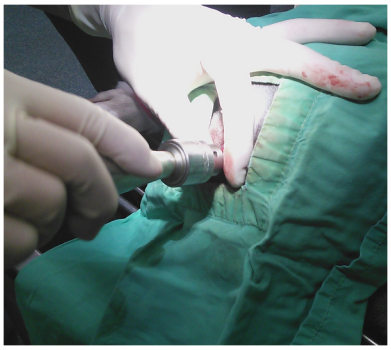

Figure 32: Right Frontal sinus trephination.

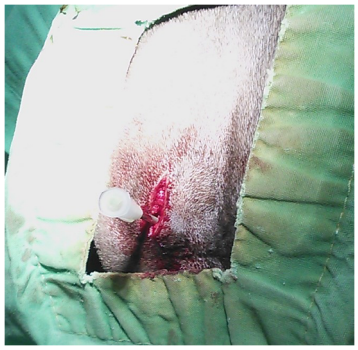

Figure 33: Wide bore needle insertion into right frontal sinus.

The needle was removed, the skin around incision flushed to clean any fluid debris and the incision sutured. The swabs and Foley catheter's removal were delayed for a little bit to allow any excess of fluid to drain through the nostril. Afterwards, everything was removed

and Radley was monitored until fully recovered (see Figure 36). Radley went home with a buster collar, tramadol, clavulanate-potentiated amoxicillin and meloxicam.

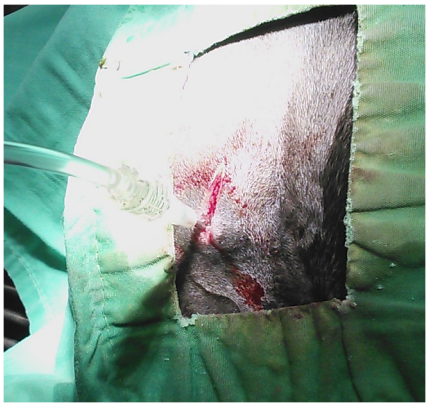

Figure 34: Flushing Frontal sinus with saline.

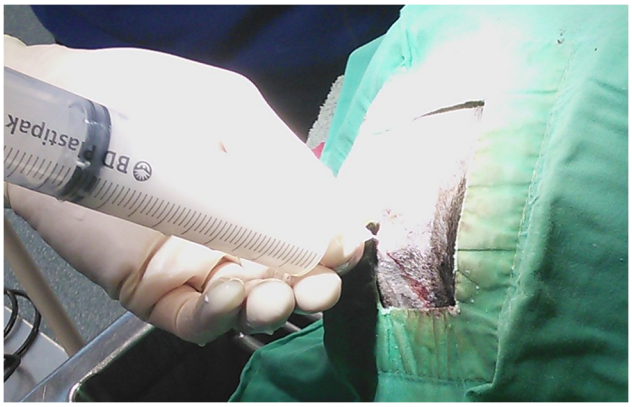

Figure 35: Clotrimazole 1% solution (Canesten®) instillation into the right frontal sinus.

*Follow-up consults*

On the 3th of February, Radley was brought back for a check-up. He had developed subcutaneous emphysema of the head and neck (more pronounced on the right side), which, after one day, slightly extended to the right hind leg. However, Radley was still with great appetite and normal physical parameters. The drill hole (into the frontal sinus) was suspected to be the origin of the subcutaneous emphysema. Therefore, the dog was sedated, the skin incision opened and the hole closed with bone wax. In the end, the skin was sutured again and two Penrose drains were placed to release emphysema. In the following four days, the emphysema was greatly reduced and, a few days later, it was totally gone. Eight days after being placed, the first Penrose drain was removed and it took 14 days before the second one and the sutures were removed. At this time, Radley had no more nasal discharge or sneezing. The owner was advised to bring Radley back in about 4 weeks for a follow-up rhinoscopy. In the end of March, Radley was back to normal, no discharge or sneezing had returned and the right nasal mucosa was free of inflammation.

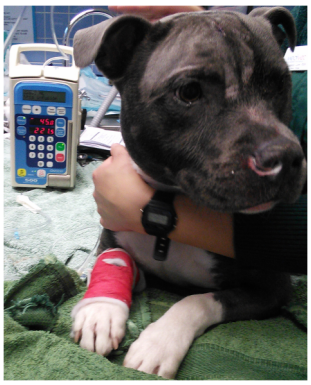

Figure 36: Radley recovering from anesthesia. We can notice some cream drainage through the right nostril.

Literature Review

# Sino-Nasal Aspergillosis

## Etiology and epidemiology

Chronic nasal disease, mainly characterized by nasal discharge, sneezing and difficulty breathing through the nose, is a commonly reported problem in the canine practice. Fungal infection is one of the most common underlying causes of chronic nasal disease and fungi of the genus *Aspergillus* spp. are often involved. Within this genus, *Aspergillus fumigatus* is the most prevalent species reported (Meler, Dunn, & Lecuyer, 2008; Vanherberghen et al., 2013). This is a ubiquitous and saprophytic fungus, whose infection can take the form of severe rhinosinusitis in otherwise systemically healthy dogs (Vanherberghen et al., 2013). According to a study by Talbot et al. (2014), *A. fumigatus* represented 96.7% of the 91 fungal isolates from dogs with Sino-Nasal Aspergillosis (SNA). In turn, *Aspergillus tubingensis* and *Aspergillus uvarum* were also etiological agents involved in SNA, although far less frequently (Talbot et al., 2014). Other species of the same genus are also potentially pathogenic and sporadically involved, such as *A. flavus*, *A. nidulans* and *A. niger* (Mathews & Sharp, 2006). The *Aspergillus* species can survive in the environment for a long period of time and are mainly distributed in the soil and decaying vegetation. It produces small conidia (asexual reproduction) that spread through the air and withstand different environmental conditions. *Aspergillus* species, particularly *Aspergillus fumigatus,* are highly resistant to heat, surviving at temperatures above 50 degrees Celsius (Dagenais & Keller, 2009).

SNA can affect dogs with any age but is more common in dogs with less than 7 years old and roughly 40% are 3 years old or less (Benitah, 2006). It seems to be more prone to affect dolichocephalic and mesocephalic breeds, with relatively high incidence in German Shepherds and Rottweilers by comparison with others. Also Border collies, Retrievers and Rhodesian ridgebacks are predisposed breeds, however it can affect dogs of any breed (Benitah, 2006; Sykes, 2013). SNA occurs worldwide and no sex predisposition has been described (Sykes, 2013).

## Pathogenesis

*Aspergillus* fungi are considered to be opportunistic pathogens, therefore it is thought that SNA occurs when the local immune system is impaired, which could be due to any

intrinsic (genetic) or acquired debilitating cause. However, other conditions (local neoplasia, FB, etc.) are not common findings in dogs with SNA and these dogs are otherwise usually immunocompetent. In rare occasions, affected dogs have concomitant debilitating systemic diseases (hyperadrenocorticism, diabetes mellitus, etc.). It remains unknown if the defective cell-mediated immunity (sometimes detected in affected dogs) is due to a debilitating underlying cause, which predisposes the dog to aspergillosis, or if this is a result of the infection (Sykes, 2013; Vanherberghen et al., 2013).

Dogs become infected through inhalation of airborne spores (conidia). To invade the host animal, the fungus must adhere and penetrate the ciliary respiratory epithelium, while resisting the mucociliary mechanism of expulsion and phagocytosis. This is accomplished probably due to metabolites and enzymes (proteases and phospholipases) produced by the fungus. Gliotoxin is one of the toxic metabolites produced by *A. fumigatus* and is known to act as an immunosuppressant metabolite, causing ciliostasis and apoptosis and protecting the fungus against phagocytes. Some other metabolites also inhibit its expulsion by the ciliary epithelium contributing to the permanence of the fungus. The invasion of the nasal mucosa is supported by the production of proteolytic enzymes by the fungus, which will destroy the surrounding cells. This fungus may cause osteolysis of the underlying bones, probably due to the production of an endotoxin that has a necrotic and hemolytic action. However, the turbinates are usually the only bones affected (Mathews & Sharp, 2006; Sykes, 2013).

The fungus is considered to motivate a local inflammatory response, involving different inflammatory cells that are actively recruited, such as blood monocytes and neutrophils, and even lymphocytes, plasma cells, macrophages and dendritic cells. This inflammation has generally a chronic character and can be severe causing osteolysis of the nasal and sinuses bones (Day, 2009). As its name says, the paranasal sinuses, especially the frontal sinuses, are often affected in dogs with SNA (Johnson, Drazenovich, Herrera, & Wisner, 2006).

The reason why affected dogs are unable to clear the aspergillosis infection is still not fully understood. According to a study by Vanherberghen et al. (2013), the production of IL-17A by Th17 cells triggers an uncontrolled pro-inflammatory reaction, which probably debilitates the antifungal immune response, allowing the fungal growth (Vanherberghen et al., 2013).

The clinical signs of SNA are mostly nonspecific and can include lethargy, decreased appetite, sneezing and nasal discharge, including epistaxis. In most dogs, the nasal discharge is initially unilateral but progresses to bilateral as a result of fungal invasion. The nasal discharge presented by the animal can be profuse and change from mucoid (or mucopurulent) to hemorrhagic. Typical clinical signs also include depigmentation and ulceration of the nostrils, which is often associated with scabs formation. Affected dogs may also show signs of pain or discomfort in the sino-nasal region and surrounding tissues (pawing at the face, hiding, stepping backwards when the affected area is palpated, etc.). Moreover, the ongoing process can extend to adjacent regions, as paranasal sinuses and orbital region, causing different injuries, which can be associated with facial swelling and even exophthalmos. In very severe cases even intracranial signs can arise. In cases where the nasolacrimal duct is harmed or blocked, ocular discharge (epiphora) may be seen. Other clinical findings, such as stertor, stridor or even open-mouth breathing are less common but have been reported. In rare situations in which the fungus spreads to the lungs, clinical signs may also include fever and cough, beyond the mucopurulent discharge. A disseminated modality of *Aspergillus* infection can also occur in dogs, but usually involves other species of *Aspergillus* such as *A. terreus* and it is most commonly reported in German Shepherds. With this systemic infection, a huge variety of signs can be seen as a result of multiple organic systems affected, being the more common signs vertebral pain and limb lameness, paraparesis and paraplegia (Benitah, 2006; Mathews & Sharp, 2006; Greene, 2013).

Haematological and biochemical profiles from dogs with SNA usually reveal no abnormalities. However, possible haematological disorders may include mild nonregenerative anemia, neutrophilia and eosinophilia. The serum biochemical profile may show mild hypoalbuminemia (Sykes, 2013).

**Diagnosis**

Firstly, the dog's history and a list of all the presented problems must be present and as complete as possible. This, along with a thorough physical examination and cautious detection of all the clinical signs presented by the affected animals, are an indispensable step to achieve a correct diagnosis. Furthermore, the order of the following diagnostic steps should be well thought out, to avoid spending unnecessary time and money. Besides, a wisely chosen sequence of steps to achieve a specific diagnosis will allow us to provide the animal a suitable treatment and a more accurate prognosis (Cohn, 2014).

The characteristics of the nasal discharge are different between nasal diseases with different etiologies but that does not give us a definitive diagnosis. However, these features, along with the history and other clinical signs, can guide us toward the next diagnostic step. A retrospective study of 105 dogs with nasal disease suggested that a mucous discharge arises more frequently in dogs with non-specific rhinitis or nasal neoplasia. In turn, a hemorrhagic discharge mainly occurred in cases of nasal neoplasia in which the discharge was present for more than two weeks, but also occurred in cases of nasal disease due to FBs or nasal mycosis. A purulent discharge was associated with FBs and non-specific rhinitis, especially in cases of prolonged discharge period. With nasal mycosis the purulent discharge was the second type of discharge more common, occurring mainly in a chronic phase. This study also suggested a younger median age for dogs with FBs and nasal mycosis when comparing with dogs with nasal neoplasia and even non-specific rhinitis. Moreover, the duration of nasal discharge at presentation was much shorter in dogs with FB than in dogs with other nasal diseases, whereas the nasal stridor was more common in dogs with nasal neoplasia (Plickert, Tichy, & Hirt, 2014). As the infection becomes chronic, the exudate present in the nasal cavities and sinuses often becomes more consistent (known as cheesy or claylike exudate) and will mainly be detected during surgery or endoscopy (Mathews & Sharp, 2006).

The oral cavity should always be examined in order to discard any possible dental origin of the problem. Dental x-rays may be taken as needed. Some blood tests should be performed as part of an initial investigation to assess the general health of the patient. In cases of epistaxis, it is advised to perform blood clotting tests to exclude any coagulation defects. Besides, in cases of persistent epistaxis, some level of anemia can be found, reinforcing the importance of performing at least base-line blood tests. This basic steps should be carried out before more expensive and/or invasive diagnostic methods are (Cohn, 2014).

It should be borne in mind that sino-nasal aspergillosis can be difficult to diagnose and treat. Its diagnosis will usually rely on multiple modalities, as hardly any single diagnostic modality is foolproof. Many diagnostic techniques are currently available, such as radiography, rhinoscopy, cytological/histopathological evaluation of affected tissues, advanced imaging methods (CT and MRI), fungal culture, serology and PCR. However, results must always be interpreted with caution and compared with the dog's history, clinical signs and other performed procedures. For example, a diagnosis can't be made based on culture results alone since Aspergilli are commonly part of the nasal flora of healthy dogs and

will often result in a positive culture (Meler et al., 2008; Sharman & Mansfield, 2012; Greene, 2013).

The conventional radiography may reveal increased opacity, due to the presence of fluid or masses, and bone lysis, but it may also appear normal even if nasal disease is present. Therefore, radiography is not the diagnostic method of choice. Due to the presence of several overlapping bone structures on nasal x-rays, this modality revealed to be unreliable to distinguish differential causes of nasal diseases. In turn, rhinoscopy is a diagnostic modality that provides visualization of the nasal passages by introducing an endoscope camera through the nose of the animal. This noninvasive procedure is performed with the intent of detecting abnormalities, such as fungal plaques, tumors and FBs, and taking tissue biopsies. On the other hand, the large amounts of discharge and induced hemorrhage sometimes present has proven to be an obstacle to an appropriate visualization of the nasal cavity. Besides, rhinoscopy is insufficient to evaluate the extent of the injury (Sullivan, 2008; Kuehn, 2014). According to some authors, the most effective way to diagnose SNA remains the direct visualization of the fungal plaques by rhinoscopy and the identification of fungi by cytology or histopathology (Saunders et al., 2004; Peeters & Clercx, 2007).

In a study by Peeters et al. (2005), the nasal cavity and frontal sinuses of 15 dogs with SNA were examined through rigid endoscopy. Duplicate biopsies were taken close to the junction between the nasal cavity and the frontal sinus. The most common histopathological findings were severe mononuclear inflammation plus an ulcerated mucosa with a plaque of necrotic material mixed with fibrin on its surface. The lamina propria presented mainly infiltrated by lymphocytes and plasma cells, but also by some macrophages and rarely other cell types, such as eosinophils and neutrophils. Fungal hyphae were found in the necrotic material adjacent to the mucosa or even on the surface of the mucosa, but there was no evidence of mucosal invasion. In some samples, necrotic and hemorrhagic foci deep into the mucosa were observed, while in others the mucosa was relatively intact and only smoothly infiltrated. Foci of granulation tissue were also sighted in some areas (Peeters, Day, & Clercx, 2005). The histopathological evaluation of the biopsies taken from Radley (the clinical case described in the present report) revealed findings similar to the ones mentioned above. Even though no fungal hyphae were detected, the findings were considered suggestive of an underlying fungal infection.

In the same study, the immunohistochemical evaluation of the biopsy samples revealed that the different samples were qualitatively similar. The mucosal immunological response in SNA was suggested to be mainly regulated by Th1-cells due to the predominance of $IgG^+$ (over $IgA^+$ and $IgM^+$) plasma cells, MHC class $II^+$ macrophages and dendritic cells, $L1^+$ cells and also several $CD4^+$ and $CD8^+$ T cells (Peeters et al., 2005).

Fungal elements, such as hyphae and even conidia, can also be detected on cytological preparations. However, the sensitivity varies with the chosen technique. A study by De Lorenzi et al. (2006) was carried out to test the diagnostic value of different cytological sampling techniques. Samples were collected from 15 dogs with clinical and radiographic signs suggestive of aspergillosis. In compliance with this study, the samples collected under direct endoscopic visualization are endowed with high diagnostic value, either by brushing suspected areas (fungal hyphae detected in 93.3%) or using a squash technique of mucosal biopsies (fungal hyphae detected in 100%). On the other hand, the blind swab technique and the direct smear from the nasal discharge revealed poor sensitivity, with fungal hyphae detected in 20% and 13.3% of the cases, respectively (De Lorenzi, Bonfanti, Masserdotti, Caldin, & Furlanello, 2006; Sykes, 2013).

The advanced imaging modalities are being used more and more over the last years, which greatly contributes to determinate the extent and severity of the undergoing disease. For this reason, both computed tomography (CT) and magnetic resonance imaging (MRI) are considered the diagnostic imaging methods of choice. Each method has its own strengths and there is still no evidence that one has superiority over the other on the diagnosis of nasal aspergillosis (Kuehn, 2014). However, it seems to be consensual that both techniques are more sensitive detecting nasal aspergillosis lesions than radiography and more efficient in delimiting the extent of injuries compared to rhinoscopy (Saunders & Van Bree, 2003; Saunders et al., 2004). While the CT provides better evaluation of the bone structures, allowing easier detection of bone lysis, the MRI is better for assessing the soft tissues. On a study undertaken on 15 dogs diagnosed with nasal aspergillosis, the CT revealed to be the best option to expose cortical bone lesions while the MRI allowed to distinguish the mucosal thickening from secretions or fungal plaques, but not between secretions and fungal material (Saunders et al., 2004). One study mentions that MRI may have some advantage over CT regarding the distinction between SNA and idiopathic rhinosinusitis. The T1-weighted images of the MRI often revealed turbinate bones hyperintensity in dogs with SNA, in contrast to

dogs with idiopathic rhinosinusitis, where the turbinate bones were hypo or isointense comparing with the muscle tissue. This is likely related to the bone destruction and hemorrhage, which are commonly involved in the aspergillosis process and absent in the idiopathic nasal inflammatory disease. The increased local levels of iron, resulting from hemoglobin degradation, are probably responsible for the increased tissue intensity on the MRI images since iron is a paramagnetic substance. Conversely, the CT has the advantage of being cheaper and less time-consuming, which enables a shorter duration of anesthesia. It also allows easier identification of bone lysis, which is important to select the most appropriate method of treatment (Kuehn, 2014). In the clinical case presented, both imaging procedures (CT and MRI) were performed, although at different times. Turbinate destruction was better assessed through the CT images, although multiple cavitation in the right nasal passage was evident with both imaging techniques. The CT images also revealed a marked endosteal proliferation in the right frontal sinus. A contrast enhancing soft tissue thickening in the mucosa of the right nasal cavity and frontal sinus were observed, mainly through the MRI images. An irregular, non-contrast enhancing material adjacent to the mucosa was also detected. According to the imaging specialists, the CT or MRI studies were indicative of a destructive rhinitis and frontal sinusitis and typical of a fungal rhinitis (aspergillosis).

*Aspergillus* often extends to the frontal sinuses of affected dogs. Sometimes fungal plaques can be found in those cavities and absent from the nasal cavity. Thus, dogs with SNA may benefit from frontal sinus trephination and sinuscopy, especially when rhinoscopy suggests destructive rhinitis and the CT images reveal sinus involvement but no fungal plaques are detected in the nasal cavity (Johnson et al., 2006).

Serologic diagnosis may involve either antigen or antibody detection in the serum. The sensitivity and specificity vary between different techniques (Sykes, 2013). A study undertaken to evaluate the most effective serological technique to diagnose SNA, concluded that Platelia™ test (for detection of serum galactomannan antigen) is unreliable (low sensitivity and specificity). The agar-gel double immunodiffusion (AGDD) test revealed better specificity (100%) than the anti-*Aspergillus* IgG ELISA test (96.8%) but the sensitivity was higher for ELISA test (88.2%) than for AGDD (76.5%). So both ELISA and AGDD are considered to have great specificity and fairly good sensitivity (Billen et al., 2009; Greene, 2013; Sykes, 2013). Another study (based on 58 dogs with nasal discharge plus 26 healthy dogs) showed that the fungal culture has even higher sensitivity and specificity (81% and

100%, respectively) comparing with the AGDD test (67% and 98%, respectively). In any case, negative results do not totally exclude the diagnosis of aspergillosis because no test is a 100 per cent sensitive (Pomrantz, Johnson, Nelson, & Wisner, 2007; Greene, 2013). In turn, the use of Polymerase Chain Reaction (PCR) for the diagnosis of canine SNA has been demonstrated to have either really low specificity or sensitivity, being less valuable than serologic techniques (Sharman & Mansfield, 2012; Greene, 2013; Sykes, 2013).

As previously mentioned, chronic nasal disease is a common finding in dogs but there are several different etiologies and it can be hard to distinguish it. Therefore, other differential diagnosis must be considered and ruled out before treatment protocols are initiated. According to a study (based on 42 dogs with persistent nasal disease), the most common underlying etiologies were neoplasias (33%), particularly adenocarcinoma, and non-specific rhinitis (24%), followed by sino-nasal disease secondary to dental disease (10%), aspergillosis (7%) and FBs (7%) (Tasker et al., 1999). In another report, based on 80 dogs, 23.7% had non-specific rhinitis, 15% neoplasias, 8.7% SNA, 8.7% cleft palate, 4% periodontal disease, 1.3% parasites, 1.3% FB and 1.3% primary bacterial disease but in 36.3% of the dogs a definitive diagnosis couldn't be achieved (Meler et al., 2008).

SNA can occur alongside, secondary to another condition or alone. Different types of rhinitis or rhinosinusitis, such as lymphoplasmacytic, bacterial or allergic, should be considered as differential diagnosis. Other fungi, namely *Penicillium* or *Cryptococcus neoformans*, can also cause chronic nasal disease but occur much less often (Mathews & Sharp, 2006). On the other hand, *Cryptococcus neoformans* is the fungus most commonly found in cats with nasal disease (Wolf, 1992). Once SNA and nasal penicilliosis are clinically identical, these conditions are easily confused with one another, thereby to differentiate the two fungi a microscopic evaluation is required (Mathews & Sharp, 2006). From time to time, many other fungi genus, such as *Alternaria*, *Blastomyces*, *Exophiala*, *Histoplasma* and *Trichosporon*, are reported to be involved in the nasal disease of both dogs and cats. The alga *Prototheca* has also been associated (Wolf, 1992). Bacterial infections are rarely the primary cause of the disease but can occur secondarily to the underlying disease (Meler et al., 2008).

## Treatment

Regardless of the treatment method chosen, early detection and treatment of the disease are extremely important to achieve a positive outcome. It has been challenging to find

a curative treatment for sino-nasal aspergillosis. However, different drugs and surgical techniques have been used with varying success. The several therapeutic recommendations available include the use of systemic and/or topical antifungal drugs and more invasive surgical procedures (Greene, 2013; Sykes, 2013).

The oral administration of antifungal drugs is a noninvasive medical therapy, but has been considered less effective in comparison with topical treatment protocols. Since the systemic therapy requires prolonged administration (minimum 4 weeks), it should always be borne in mind that side effects, such as anorexia, vomiting and hepatotoxicity problems, can arise. Moreover, this entails high treatment costs. Amphotericin B is no longer recommended for the treatment of SNA. It has many potential side effects and its use in the past revealed lack of efficacy, probably because it fails to achieve adequate local concentrations. The systemic antifungal agents most commonly used to treat SNA in dogs are thiabendazole, ketoconazole, itraconazole and fluconazole. It has been reported that the first two compounds cured half of the animals treated with it. On the other hand, the success rate was up to 70% in dogs treated with itraconazole and fluconazole (Greene, 2013). In a study based on 47 dogs treated with thiabendazole (20 mg/kg orally for 6 weeks), only less than half of the dogs achieved clinical improvement, so this therapy was considered not effective (Ce, 1984). In another study, only three out of seven dogs treated with oral ketoconazole (40 mg/kg), either combined or not with surgery, achieved clinical cure. In the other four cases, the therapy was not effective. All the seven dogs revealed inappetence and hypoalbuminemia and only one didn't show elevated levels of alanine aminotransferase (ALT) and alkaline phosphatase (AP) (Sharp, Burrell, Sullivan, & Cervantes-Olivares, 1984). In another report involving 15 dogs treated with oral ketoconazole (5 mg/kg, q 12 h for 2 to 18 weeks), only 47% achieved cure during a follow-up period of 6 or more months (Sharp & Sullivan, 1989). According to a study involving 10 dogs, with either SNA or penicilliosis, treated with oral fluconazole (2.5 to 5.0 mg/kg/day), six of them were cured over a median follow-up period of 27 months (Sharp, Harvey, & O'Brien, 1991). Terbinafine is another antifungal agent that has been used in refractory cases of SNA, either together with azoles or by itself, but further investigation about its effectiveness is needed (Greene, 2013).

The topical antifungal therapy is considered to be the treatment of choice for SNA as it is more effective than the systemic alternative. However, the success of treatment will depend on the antifungal agent and technique chosen, in addition to the individual response from case

to case. The best results occur when the fungal material is dissolved and removed by a sequence of curettage, washing and suction, prior to the application of the antifungal agent. A CT scan of the skull should always be performed prior to any topical infusion to detect any cribriform plate's destruction, in order to avoid any neurologic complication due to the drug contact with the meninges (Greene, 2013).

Clotrimazole and enilconazole are the topical antifungal agents most commonly used to treat dogs with SNA. Clotrimazole is readily available on the market as a 1% formulation in a polyethylene glycol base. The common dosage is a 30 ml vial per side in small-sized dogs and two vials of 30 ml per side in mid to large-sized dogs. It should be taken into account that irritation and/or swelling of the pharynx may arise due to propylene glycol compound. On the other hand, enilconazole has been associated with less side effects. The topical agent chosen (enilconazole or clotrimazole) can be introduced into the nasal cavities and frontal sinuses of affected dogs through catheters placed either surgically or non-surgically, entailing more or less invasiveness (Greene, 2013).

For several years, dogs with SNA were commonly treated with an enilconazole emulsion that was instilled into the nasal cavities and frontal sinus through surgically implanted indwelling catheters (Greene, 2013). In a study by Sharp et al. (1993), 24 dogs with SNA were treated with topical enilconazole (10 mg/kg q12 h for 7-14 days), which was instilled through catheters placed after trephination of the frontal sinuses. These dogs were followed up for a period of about 18 months. From the 24 dogs, 19 were cured, four were not cured and one passed away, although this could not be related to the fungal infection according to the post-mortem examination. From another group of dogs treated with the same topical therapy combined with oral ketoconazole for 6 weeks (5 mg/kg q12 h), six out of seven dogs were successfully treated over a follow-up period of 35 months, while the seventh dog needed repeated enilconazole instillation. According to the authors, the topical therapy with enilconazole was more effective than the systemic antifungal therapy with either ketoconazole, thiabendazole or fluconazole. Furthermore, the combined use of topical and systemic therapies was beneficial in cases with extranasal tissues involvement (as periorbital tissues). In this study, some side-effects of enilconazole, such as inappetence and hypersalivation, were described (Sharp, Sullivan, Harvey, & Webb, 1993). Other complications with the same method were reported and included catheter dislodgment and aspiration pneumonia. Besides, this technique entailed prolonged hospitalization and repeated

sedation of the dogs that did not tolerate well the regular handling of the catheters (Sharman & Mansfield, 2012). As a result, over the years, the aforementioned use of enilconazole has been replaced by cheaper and less invasive methods, which are more plausible for owners (Greene, 2013).

To overcome the disadvantages of the indwelling catheters, an alternative technique, consisting of a one-time infusion of clotrimazole (lasting 1 hour), was developed. This treatment method still included frontal sinus trephination but the catheter was removed immediately after the clotrimazole infusion. Thus, it did not entail the potential complications related to the fixation and maintenance of the catheters. Consequently, it was better tolerated by the animal and more readily accepted by the owner. This technique was associated with good outcomes after the first treatment (Sharman & Mansfield, 2012; Greene, 2013).

Non-invasive techniques were also developed to avoid the potential complications (infection of the incision area, emphysema, etc.) associated with the trephination of the frontal sinus of affected animals. Both enilconazole and clotrimazole have been used in different dosages and administered through catheters inserted through the nose blindly or assisted by an endoscope. In some cases, a second or third topical infusion may be beneficial and can be performed at 3-week intervals. Different success rates have been obtained with different protocols. However, these methods have reached an overall success rate of up to 80 to 94% (Peeters & Clercx, 2007; Sharman & Mansfield, 2012; Greene, 2013). According to a study carried out to compare the administration of clotrimazole through surgically versus nonsurgically placed catheters in 60 dogs, both techniques were successful but the one using nonsurgically placed catheters had fewer complications (Mathews et al., 1998). The instillation of clotrimazole (for 1 hour) through nonsurgically placed catheters was the first non-invasive technique described. In this procedure, an adequate endotracheal tube is placed on the trachea and cuffed. Foley catheters of different diameters are introduced into the nasopharynx and into each nostril and its balloons inflated to occlude the respective structures and prevent the leakage of the antifungal agent. The use of gauze sponges is helpful to hold the pharyngeal Foley catheter in place and to prevent leakage into the trachea. Before the Foley catheters are introduced through the nostrils, a 12-French diameter catheter is inserted into each nostril. Afterwards, these catheters are used to infuse the antifungal agent. The animal is rotated 360˚ and stays 15 min in each position (dorsal, right lateral, left lateral and ventral), which allows contact between the agent and all the nasal surfaces. Subsequently, the

animal is positioned with the head tilted 30 degrees down and all the catheters are removed. The animal stays in this position for 20 min to allow drainage. At the end of the procedure, the larynx and pharynx are carefully inspected. This technique has been improved by placement of the catheters under endoscopic guidance. The downside of these procedures is the extensive time that the animal is under GA (Greene, 2013). In the clinical case reported (Radley), the treatment technique initially performed was identical to the one described by Greene (2013). However, a 2% enilconazole infusion was used instead of the 1% clotrimazole. The similar procedure using 2% enilconazole has been described by Billen et al. (2010), in a study that is mentioned below (regarding the efficacy of bifonazole cream, alone or combined with a 2% enilconazole infusion), and by Sykes (2013).

Experimental studies have demonstrated a better distribution of the infusate (through the nasal cavity and paranasal sinuses) when nonsurgically placed catheters were used, instead of the ones placed after trephination of the frontal sinus. However, further studies based on clinical cases are needed to confirm that fact (Sharman & Mansfield, 2012; Greene, 2013). A study (based on nine dogs with SNA) was carried out to describe the distribution and retention of a 1% clotrimazole or 10% enilconazole solution throughout the nasal cavities and sinuses. The respective antifungal agent was applied through catheters surgically placed into the frontal sinuses (temporary trephination). With both treatments, the distribution through the different sino-nasal regions was achieved but variable from dog to dog and the frontal sinuses retention was poor. According to the authors, this fact might be related to different variants (turbinates destruction, persistence of fungal granulomas, etc.). One out of five dogs treated with the 10% enilconazole solution showed ataxia and seizures, which lead owners to decide for euthanasia. The post-mortem examination of this dog revealed a subacute meningoencephalitis but no relationship was found between the meningoencephalitis and the higher (when compared with other studies) enilconazole concentration used. However, that possibility cannot be ruled out (Sharman, Lenard, Hosgood, & Mansfield, 2012). The techniques involving trephination of the frontal sinus are undeniably more invasive but also provide some advantages over the noninvasive alternatives. In particular, it allows debridement of the lesions within the frontal sinuses and guarantees that the antifungal agent reaches these cavities. In addition, the patency of the nasal ostium can be tested with these procedures (Sharman & Mansfield, 2012).

A surgical procedure that looks promising is the trephination of the frontal sinus with a combined clotrimazole irrigation and depot therapy. After trephination, a 5 min flush with a 1% clotrimazole solution is performed and followed by instillation of a 1% clotrimazole cream (10 to 20 g per sinus), which stays inside the sinus allowing prolonged contact with the drug. This is an invasive procedure but quite less time-consuming than the ones previously mentioned (either invasive or noninvasive) and that seems to be equally effective. A study involving 14 dogs with SNA revealed that 86% of the dogs had no clinical signs or only mild signs of rhinitis after this therapy (Sissener, Bacon, Friend, Anderson, & White, 2006). Since clinical cure was not obtained with the first treatment option, Radley was submitted to the aforementioned treatment.

The use of more viscous creams is thought to increase its retention in the frontal sinuses, the contact time and the treatment success. Bifonazole, a more viscous antifungal agent, has been used as a depot therapy (Billen et al., 2010; Sharman & Mansfield, 2012). After perendoscopical debridement, the instillation of 1% bifonazole cream, alone or combined with a 2% enilconazole infusion, through catheters inserted perendoscopically into the frontal sinuses, is a procedure with high success rates. This is particularly true after a second infusion and in dogs with moderate disease. From 12 dogs treated by the combined topical therapy (1 hour infusion with 2% enilconazole, followed by a 1% bifonazole cream instillation), seven became disease-free after the first procedure, three after a second procedure, and the other two after a second procedure consisting of a 1% bifonazole cream alone. Another five dogs were only treated with a 1% bifonazole cream (after debridement). After the first instillation, three of them (with moderate fungal infection) were cured. However, in the other two dogs (older, with systemic disease and severe SNA) cure could not be confirmed during the follow-up period. According to Billen et al. (2010), the treatment failure could be explained by the incomplete debridement performed in those two dogs. Besides, further information about the stability and retention time of bifonazole is required. In conclusion, the protocol using bifonazole alone is less time consuming than the enilconazole protocol alone or both combined. This leads to a reduction in the duration of anaesthesia and to a shorter period of hospitalization (Billen et al., 2010; Greene, 2013).

The goal of surgical procedures such as rhinotomy or sinusotomy is to remove fungal elements and necrotic material from the nasal cavities and frontal sinuses by surgical debridement. The removal of the turbinates (turbinectomy) should be avoided as much as

possible, as it has been associated with deterioration and has no proven benefit on the control of nasal discharges. These more invasive surgical procedures should only be considered in debilitated dogs that didn't improve with noninvasive techniques, in dogs with abundant fungal plaques and necrotic material difficult to debride and in dogs with damaged cribriform plate (Greene, 2013). The use of povidone-iodine dressings (replaced every 2-3 days during 15-21 days) after rhinotomy and surgical debridement has been described (Sharman & Mansfield, 2012; Greene, 2013). In another study, regarding seven dogs with SNA, a 2% enilconazole solution was topically administered after rhinotomy and surgical debridement. Four of them were also treated with oral itraconazole during 1 month. According to the results of this study, the removal of the bone flap during rhinotomy had better results than when preserved. The three dogs in which the bone flap was initially preserved had recurrence of the disease. One was euthanized and the other two had the bone flap removed during a second surgery. From the six dogs (four dogs in which the bone flap was initially removed plus the two dogs in which the flap was removed in the second surgery), five were free of fungi and one had a small aspergilloma, according to the rhinoscopic examination. During the follow-up period, one of the dogs had intermittent serous discharge and sneezes, one had intermittent epistaxis and the others were asymptomatic (Claeys, Lefebvre, Schuller, Hamaide, & Clercx, 2006). According to Sharman & Mansfield (2012), the success obtained with the aforementioned invasive procedures was probably more related to the meticulous debridement involved than to the topical agent and technique used (Sharman & Mansfield, 2012).

It is not uncommon that treatment methods, either topical or systemic, invasive or noninvasive, have side-effects on the treated animals. Therefore, it is extremely important to perform a proper and continuous monitoring of the animals, particularly taking into account the potential complications of the protocol chosen. For example, potential side-effects of indwelling catheters (placed after trephination of the frontal sinus) are anorexia, incision site infection and subcutaneous emphysema (Mathews & Sharp, 2006; Sharman & Mansfield, 2012). Topical administration of enilconazole have been reported to cause inappetence and hypersalivation (Sharp et al., 1993). In turn, topical clotrimazole (propylene glycol base) can potentially lead to irritation and edema of the pharynx (Greene, 2013). On the other hand, systemic antifungal drugs have been associated with inappetence, nausea, hepatopathy (elevated liver enzymes) and GI disturbances (vomit, diarrhea). There are also some reported cases of dogs that developed cataracts after being treated with ketoconazole. Hepatotoxicity is

usually only recognized when using high doses but, in any case, it is advised to routinely monitor liver function when using this drugs (Ramsey, 2011; Greene, 2013).

**Outcomes**

Animals with SNA may respond well to therapy, but the condition may be recurrent and difficult to cure in some patients. The outcomes are likely to be influenced by multiple factors, including the severity of the disease at the time of diagnosis, the technique and antifungal agent chosen, the experience of the clinician and the extent of debridement. In some cases, a second or third infusion of topical antifungal drug and/or repeated period of systemic therapy is required. In other cases, changing or combining the type of therapy may be adequate. Therefore, the owners should be advised to monitor and immediately report any signs of possible disease recurrence (Sharman & Mansfield, 2012; Greene, 2013; Sykes, 2013).

A study revealed that 3 out of 15 dogs, that seemed successfully treated, had late recurrences. According to Pomrantz & Johnson (2010), this was a relatively high level of reinfection when compared to previous studies of dogs treated with topical clotrimazole (Pomrantz & Johnson, 2010). In another study, three out of 27 dogs treated with topical enilconazole, fungal reinfection emerged up to 36 months after resolution of clinical signs (Schuller & Clercx, 2007). Another study describe the case of a 2 years old Australian cattle dog that was diagnosed with recurrent nasal aspergillosis after 4 years of resolution of the clinical signs (during which remained asymptomatic). This dog was initially treated with topical clotrimazole but the clinical signs recurred two months later. Therefore, the topical treatment was repeated and oral itraconazole added to it. The therapy with itraconazole was then stopped due to a febrile episode. During the following 4 years, the dog didn't present any clinical signs. Later on, the clinical signs (mucopurulent nasal discharge) reappeared and the dog was again treated with oral itraconazole. Once more, he developed an adverse reaction (pyrexia) to it, so the itraconazole dose was decreased from 5 mg/kg BID to SID. This resolved the fever but, since the nasal discharge persisted, an additional topical infusion with clotrimazole was performed and daily itraconazole administered (Schochet & Lappin, 2005).

The success of the therapy should be observed by resolution of the clinical signs (nasal discharge, local pain, nostrils ulceration). However, in some aspergillosis-free dogs, a milder nasal discharge continues. This may be due to the restructuring of the nasal cavities and

sinuses, or, if more profuse, due to inflammation or bacterial infection, which emerge secondarily to the prior destruction. The bacterial infection is usually resolved by an antibiotic therapy based on culture and sensitivity (Schuller & Clercx, 2007; Greene, 2013).

Since the presence or absence of clinical signs, particularly of nasal discharge, are often not consistent with the disease status, a follow-up rhinoscopy is advised to detect fungal plaques and monitor the response to therapy (Zonderland et al., 2002; Pomrantz & Johnson, 2010; Greene, 2013). The severity of the lesions observed on the CT images does not seem to be related with the treatment success, although dogs with low scores (limited injuries) seem to respond successfully to the first treatment. However, further research with larger number of animals is required to confirm these findings (Saunders et al., 2003). Serology also tells us very little about the response to treatment since it can take years until the serological titers decrease, so it is not recommended for monitoring the response to treatment (Pomrantz & Johnson, 2010; Greene, 2013).

**Public health considerations**

Even though the *Aspergillus* infection is generally acquired from exposure to environmental sources and transmission between animals and humans has not been reported, infected animals should be handled with care. Whenever possible, the contact between immunosuppressed individuals and affected dogs should be avoided. If the contact is necessary, proper hand washing and use of gloves are advised (Greene, 2013; Sykes, 2013).

# Ibuprofen poisoning

## Clinical case:

### Animal identification

| | | |
|---|---|---|
| **Pet name:** Mimi<br>**Species:** Feline<br>**Breed:** Domestic short-hair | **Colour:** Black and white<br>**Gender:** Female - Neutered<br>**Age:** 1 y 7 m 20 d | **Weight:** 3.6 Kg/7.94 lbs<br>**Insured status:** Not insured |

**Clinical history:**

No relevant information prior to March 2015.

*28th March 2015*

In the morning, Mimi's owner found a chewed capsule of Ibuprofen 200 mg on the floor. During the day, the owner noticed that Mimi wouldn't eat even if stimulated and reported that when tried to feed Mimi, she spat the food. For this reason, he decided to bring Mimi to the consultation at night.

On consultation, Mimi presented bright, alert and responsive, with good general condition and hydration, BCS 3/5 and chest clear. Heart and respiratory rates were considered WNL taking into account that Mimi was a bit stressed for being in the consulting room. The mucous membranes were pink and moist, the CRT was less than two seconds, the pulse was strong, symmetric and synchronic, the abdomen was soft and not painful during palpation and the rectal temperature was 39 °C. Eyes, ears, oral cavity, skin and lymph nodes were examined and nothing abnormal was detected.

The main risks of ibuprofen intoxication were explained to the owner and he was advised to leave Mimi at the hospital for blood and urine monitoring, clinical signs monitoring and intravenous fluid therapy and other supportive therapy as needed. A budget was presented to the owner, who declined it, only agreeing to run a blood test, whose results would serve to further decisions.

The results of the in-house hematology only indicated a slight dehydration and all the parameters of the biochemistry profile were WNL. He was again advised to leave Mimi in the hospital for continuous monitoring because the parameters could change at any time. The importance of urine monitoring and USG assessment was made clear. Still, the owner decided to bring Mimi home. Therefore, Mimi returned home with ranitidine (one fourth of a 75 mg tablet BID) and misoprostol (one eighth of a 200µg tablet TID) to protect against NSAID-induced gastric ulceration. The owner was told to bring Mimi back if any concerns would arise and, in any case, to bring her for re-visit in 3 days.

*30th March 2015*

Mimi's owner brought her back for re-evaluation. He was worried because Mimi was not eating and, when trying to eat, dropped the food every time. Besides, Mimi did not pass any faeces since the 28th March and had vomited this morning yellow foam. Even though previously advised to pay attention to urine production, the owner was not sure about it. He also mentioned that Mimi was more lethargic on this day.

In the consulting room, Mimi vomited white foam. On physical examination, Mimi was quiet but alert and responsive and all the physical parameters were WNL. This time, the owner agreed to let Mimi stay in the hospital for further testing, monitoring and supportive therapy. The plan was to admit Mimi to the hospital, repeat in-house blood tests, proceed with in-house urine tests, and initiate IVFT and parenteral medications.

On palpation, the urinary bladder was felt to be medium size, so a cysto sample was taken for urine multistix, USG and sediment. The results were: USG 1020; pH 6.5; protein ++; non-hemolysed blood ++; all the other parameters were WNL.

According to the blood tests, haematology was unremarkable and several biochemistry parameters were altered with increased values of BUN- 37, 2 mmol/l (3.6-10.7), creatinine- 1058 µmol/l (27-186), phosphate- 3.86 mmol/l (1.1-2.74) and potassium- 5.9 mmol/l (3.7-5.8).

Intravenous fluid therapy was started and ranitidine (2.5 mg/kg slowly IV BID), misoprostol (one eighth of a 200 µg tablet TID), omeprazole (one third of a 10 mg tablet SID) and maropitant citrate (0.94 mg/kg SC SID) added to her hospital sheath. Her hydration, weight, bladder size and urine production were constantly monitored and a urine output chart filled. Many attempts to feed Mimi were done, but were unsuccessful, so mirtazapine (3.75

mg PO every 72 h) was added to her medications to stimulate appetite. During all day, she didn't pass urine.

Mimi was considered to be in acute kidney failure (ARF) with azotaemia and oliguria/anuria. The owner was often updated and clarified about the situation.

*31th March 2015*

Mimi passed urine (about 100 ml) at 2 am for the first time since arrival. She was alert but grumpy and not happy. Furthermore, she ate only a really small amount of food in the middle of the day and didn't eat anything else after it. Some drooling was noticed. Yet she had put on some weight, so the fluid rate was reduced. Mimi didn't pass any faeces and didn't vomit. The chest auscultation was clear and the physical parameters WNL. Supportive therapy and monitoring were maintained and the owner informed about Mimi's progress.

*1st April 2015*

Overnight, Mimi showed evident drooling and looked uncomfortable. Buprenorphine (0.02 mg/kg IV TID) was added to Mimi's daily medication.

Figure 37: Mimi at the hospital- We can notice her drooling in the picture.

During day, Mimi remained quiet, uncomfortable and drooling a lot (See Figure 37). She didn't pass any urine (> 24 hours), so she was considered to be anuric. Her bladder was still small. Mimi gained even more weight (from 3.87 kg on the 31th March to 4.02 kg on the 1st of April), so fluid rate was reduced to maintenance. The possibility of pulmonary edema was considered but the chest was clear with no crackles detected. No obvious ulcers were found in her mouth but abdominal palpation was now painful for her.

A blood sample was taken and the relevant results were: BUN- 64 mmol/l (previously 37.2), creatinine- 1664 µmol/l (previously 1058), phosphate- 3.29 mmol/l (previously 3.86) and potassium- 6.7 mmol/l (previously 5.9). In conclusion, renal parameters became worse.

The owners came to the hospital to see Mimi and discuss her situation. It was explained to them that her prognosis was poor due to the increased renal parameters and the fact that Mimi was now anuric and painful as well. After discussing it, the owners opted for euthanasia.

Literature Review

# Ibuprofen Poisoning- cats

## Introduction

According to a study by Mahdi & Van der Merwe (2013) in the USA regarding pet exposure to potentially harmful substances over a period of 3 years, oral exposure was the most common route of exposure, followed by dermal, inhalation and parenteral routes. The biggest group of substances involved were therapeutic drugs, followed by others such as household chemicals, human food, pesticides, plants, fertilizers, industrial products, cosmetics and other toxins. From the reported therapeutic drugs, drugs intended for human use were accounted for 91.2% of drug exposures with non-steroidal anti-inflammatory drugs (NSAIDs) being the most common group of drugs involved, including ibuprofen, acetaminophen and acetylsalicylic acid. This was followed by other human medications, such as supplements, antibiotics, and anxiolytics, among others. On the other hand, veterinary drugs were reported in 8.8% of cases regarding therapeutic drugs poisoning and included mainly parasiticides and insecticides. Even though in most countries there are a higher number of pet cats over dogs, it has been demonstrated that dogs are the predominant group exposed, with cats being involved in 15.3% of cases in this study. Previously published data show similar results (Forrester & Stanley, 2004; Hornfeldt & Murphy, 1997; Mahdi & Van der Merwe, 2013; Medeiros, Monteiro, Silva, & Nascimento Júnior, 2009; Xavier, Kogika, & de, 2002). This is most likely due to the particularly picky nature of the cats when compared to dogs, which, on the other hand, are natural scavengers and commonly known for eating anything and everything, including things they shouldn't (Mahdi & Van der Merwe, 2013). Human drugs were involved in about 30-35% of total pet poisoning cases reported from USA and some European countries (Cortinovis, Pizzo, & Caloni, 2015; Mahdi & Van der Merwe, 2013). According to many animal poison control center records, either from USA or Europe, ibuprofen was the NSAID most commonly involved in pet poisoning (Jones, Baynes, & Nimitz, 1992; Poortinga & Hungerford, 1998; Khan & McLean, 2012; Cortinovis et al., 2015).

Ibuprofen, a propionic acid derivate, is a NSAID used in human medicine for its anti-inflammatory, anti-pyretic and analgesic properties but is not recommended for animals, especially due to its potential to cause gastric perforations and acute renal failure. It is an

over-the-counter drug often used by human beings and widely available in many different formulations and strengths. Some of it even have a sweet cover making it more attractive to animals. Ibuprofen poisoning can happen through accidental ingestion or administration by the owner. One can do it by mistake or in an attempt to help his sick pet. Besides, signs can arise after excessive ingestion or repeated administrations of therapeutic or high doses of ibuprofen. Although humans can safely take ibuprofen in a broad range of doses, these drug has a narrow safety margin in dogs and cats. Cats have been considered two times more sensitive to ibuprofen than dogs, due to their deficient glucuronidation (Villar, Buck, & Gonzalez, 1998; Richardson, 2000; Dunayer, 2004; Fitzgerald, Bronstein, & Flood, 2006; Bischoff, 2007; Cortinovis et al., 2015). According to a study by Shrestha et al. (2011), the lack of the enzyme responsible for the glucuronidation of some drugs in the *Felidae* family, and particularly in cats, are most likely related to the fact that present-day felines and their ancestors are hypercarnivores (higher than 70% animal matter in their diet). This implies a low exposure to plant-derived phenolic compounds, which allowed the pseudogenization of the gene *UGT1A6* (the base of glucuronidation expression) without adverse effects on species survival. It only became a problem when humans started challenging them with phenolic drugs, such as acetaminophen (N-acetyl-p-amino-phenol) (Shrestha et al., 2011). Cat's poor glucuronidation is well known, however, according to some reported data this fact doesn't justify the toxic effect caused by all the glucuronidated drugs. There is evidence that some glucuronidated drugs are indeed cleared at a slower rate when compared to other mammalian species but sometimes this is due to other reasons. For example, slower acetylsalicylic acid clearance is mainly caused by poor glycine conjugation. There is also evidence that ibuprofen is glucuronidated with comparable efficiency in cats and other species, such as humans and dogs (Magdalou, Chajes, Lafaurie, & Siest, 1990; Court, 2013).

**Pathogenesis**

Ibuprofen's anti-inflammatory action is achieved by inhibiting cox (cyclooxygenases) enzymes, thus blocking the conversion of arachidonic acid to prostaglandins. It is used in human medicine due to its COX-2 inhibition. This enzyme's activity is induced by inflammatory stimuli, otherwise is generally inactive. The main side effects of ibuprofen, and many other NSAIDs, occur due to the unwanted inhibition of COX-1 enzymes. On the other hand, this enzyme's activity is naturally present in most body cells and is responsible for the synthesis of important prostaglandins. The prostaglandins synthetized by COX-1 in the

stomach and intestine increase the mucosal blood flow and stimulate the crypt cells proliferation, thus allowing mucosal repair and integrity. Therefore, the risk of ulceration and perforation, especially of the stomach and small intestine, is increased when using NSAIDs. The role of the prostaglandins synthetized in the kidney is mainly maintenance of blood flow, especially if the animal is hypovolemic or his kidneys are already compromised. Thus, when PGs production is blocked, papillary and tubular necrosis in the kidney may arise as a result of ischemia, leading to acute renal failure. COX-1 is also responsible for the production of thromboxane A2, so platelet aggregation can also be affected when using ibuprofen and other NSAIDs. Therefore, the use of ibuprofen has been associated with many of the mentioned side effects (Prescott, 1979; Vane, Bakhle, & Botting, 1998; Richardson, 2000; Dunayer, 2004).

About 60 to 86% of the ibuprofen ingested by a dog is rapidly absorbed from gastrointestinal tract, with its maximal plasma concentration being reached within 30 min to 3 hours. Different formulation and simultaneous ingestion of food can affect the time to reach the peak plasma concentration. Thus, it takes less time with liquid formulations than with solid formulations or with food (Dunayer, 2004; Fitzgerald et al., 2006). About 90 to 99% of the ibuprofen reaching the plasma is bound to plasma proteins in humans (Dunayer, 2004; Bushra & Aslam, 2010). According to studies *in vitro*, 99% of the ibuprofen was bound to plasma proteins in dogs as well (Mills, Adams, Cliffe, Dickinson, & Nicholson, 1973). Its plasma half-life has been reported to be about 2.5 to 5.8 hours in dogs and cats, while in humans it is 1.8-3 hours and in rats 1 hour (Fitzgerald et al., 2006; Bushra & Aslam, 2010; Bischoff & Mukai, 2012). According to distribution studies, there was no evidence of ibuprofen (or its metabolites) accumulation in dog tissues after repeated exposure to ibuprofen, contrary to other animal species, in which accumulation was evident in many tissues, such as adrenals, ovaries, fat, thyroid and skin. However, dogs revealed high levels of ibuprofen in the bile. Furthermore, only ibuprofen was detected in dog's plasma, while in other species, such as rabbits and rats, ibuprofen metabolites were also detected in plasma (Adams, Bough, Cliffe, Lessel, & Mills, 1969). In dogs, ibuprofen has been reported to be excreted through urine and bile in a proportion of about 70 and 30%, respectively (Adams et al., 1969; Dunayer, 2004).

The lower tolerance of dogs and cats to ibuprofen, in comparison with other species, might be related to the longer half-life of this drug in dogs and cats. It has been reported that

the clearance of ibuprofen from the plasma of dogs is slower than in rats, which probably contributes to the higher susceptibility to ibuprofen toxicity in dogs comparing to rats. According to a study involving dogs, rats and rabbits, the lowest dose of ibuprofen at which gastrointestinal ulcers emerged in dogs was 8 mg/kg/day administered orally (in the post-mortem evaluation of animals without clinical signs of toxicity), while in rats was 180 mg/kg/day or 20 mg/kg/day in pregnant animals. Thus, pregnancy have shown to affect animal sensitivity as also pregnant rabbits revealed to be highly susceptible with ulcerogenic effect evident at 7.5 mg/kg/day. In this study, erosions and ulcers were more commonly found in the gastric antrum and pylorus in dogs and in the intestine in rats. Ibuprofen ulcerogenic effect was present either when administered orally or parenterally. Besides, ibuprofen has not revealed any hepatotoxic effect in rats or dogs even when high doses were administered (Adams et al., 1969; Fitzgerald et al., 2006).

The first clinical signs arising are commonly the result of gastrointestinal upset, such as vomiting, diarrhea, abdominal pain, hypersalivation, nausea and anorexia. Lethargy, polyuria and polydipsia are also common signs. Single doses as low as 25 mg/kg body weight but more commonly exceeding 50 mg/kg body weight have been reported to originate gastrointestinal signs in dogs. In cats, also single exposure to 50 mg/kg body weight or higher dose of ibuprofen have been associated with clinical signs. However, with repeated exposures, doses as low as 16 mg/kg per day have been reported to cause gastrointestinal signs in dogs that were receiving it for 8 weeks. With doses exceeding 175 mg/kg body weight in dogs or even 87 mg/kg in cats, signs of acute renal failure may also arise, including oliguria/anuria and uremia. Occult or evident digested blood on faeces (melena) have also been detected in many cases as a result of bleeding ulcers in the upper gastrointestinal tract. Similarly, blood in the vomit is also a possibility. With serum ibuprofen concentrations of 138 µg/ml, a dog presented faeces with blood and increased BUN on the biochemistry profile. Central nervous system signs, such as depression, ataxia, seizures and coma have also been reported. In dogs, these signs can be seen when the ingested dose is equal to or higher than 400 mg/kg body weight. Other signs, such as hypotension, respiratory depression and metabolic acidosis have also been described. In humans, cardiac arrhythmias and hepatic dysfunction have also occurred with ibuprofen overdoses. On the other hand, the lowest dose reported to cause death in dogs is 600 mg/kg body weight. For cats, there is no such information available. With increasing doses of ibuprofen, dogs usually have renal or CNS signs in addition to the gastrointestinal signs already present, however, in other species, it

doesn't always happen. Renal signs have been reported in cats without any gastrointestinal signs and CNS signs have been seen in ferrets without gastrointestinal disturbances. According to a study regarding three dogs exposed to ibuprofen, serum ibuprofen concentrations lower than 31 μg/ml caused no clinical or laboratorial disturbances on the affected animals (Adams et al., 1969; Jackson, Costin, Link, Heule, & Murphy, 1991; Richardson, 2000; Dunayer, 2004; Fitzgerald et al., 2006; Bushra & Aslam, 2010; Osweiler, Hovda, Brutlag, & Lee, 2011; Bischoff & Mukai, 2012).

**Diagnosis**

Ibuprofen toxicosis is diagnosed according to history of exposure to ibuprofen and compatible clinical signs. A detailed history and thorough physical examination of the animal is extremely important to achieve a correct diagnosis and treatment planning. Sometimes the ingestion is witnessed, sometimes tablets are found near the animal or in the middle of gastric contents after vomiting. However, there are also many cases in which the owner has no idea of what happened with his pet (Richardson, 2000; Grave & Boag, 2010; Bischoff & Mukai, 2012).

To confirm ibuprofen ingestion, animal's serum can be sent to some laboratories, where ibuprofen presence and concentration can be measured. Sometimes, it can also be detected through a urine sample. Radiographies or abdominal ultrasound can be performed to detect signs of GIT perforation or consequences of it, such as signs of peritonitis. In these cases, peritoneal effusion and presence of free gas on the abdomen can be found on x-rays. If ulcers are present, thickened and disrupted gastric mucosa can be observed on the ultrasound images. With perforations, free fluid can additionally be seen. Contrast radiographies may detect extensive ulcers, however, if perforation can't be ruled out, barium shouldn't be used as the contrast medium because of its potential to worse peritonitis. Upper GIT endoscopy is a more sensitive diagnostic method to detect ulcers and allow us to evaluate its extension and risk of perforation. Baseline haematological and biochemical profiles should always be obtained at admission and repeated daily to check for any disturbances, including renal dysfunction and anemia. PCV/TP can be below normal limits due to gastrointestinal bleeding ulcers. BUN and creatinine may be above reference levels, electrolytes may be variably altered and acid-base disturbance is possible if renal involvement is present. In this case, urine samples may reveal low specific gravity. Abdominal ultrasonography may be useful to rule out some differential diagnosis of renal affection, such as pyelonephritis and hydronephrosis

among others. In humans, papillary necrosis has been confirmed by computed tomography (Bischoff & Mukai, 2012; King & Boag, 2007; Osweiler et al., 2011; Richardson, 2000).

If ibuprofen ingestion cannot be confirmed, other differential diagnosis should be ruled out. For example, when gastrointestinal signs are present, other NSAIDs (aspirin), ethylene glycol, masses/neoplasias or foreign bodies in the GIT, gastroenteritis, IBD, among other causes, should be considered. On the other hand, nephrotoxic signs can emerge with the ingestion of other NSAIDs, ethylene glycol, lilies, cholecalciferol, zinc and other substances or also with some other conditions, for example, urinary tract obstruction, uroabdomen and pyelonephritis. CNS signs can have many different underlying etiologies, including inflammatory, infectious, metabolic, vascular and neoplastic. Other toxicants can also cause neurological disturbances. Besides, we should always keep in mind that the important is to treat the patient and not the toxic (Osweiler et al., 2011; Richardson, 2000).

The postmortem evaluation in ibuprofen poisoning has revealed mainly gastrointestinal lesions, such as edema, petechiae, hemorrhages, erosions, ulcers and perforations either on the stomach, small intestine or even colon. Lesions in the kidneys, including papillary, tubular or cortical necrosis and interstitial nephritis, have also been reported (Bischoff & Mukai, 2012; Richardson, 2000).

**Treatment and Monitoring**

The initial approach, as for any other toxic substance, involves stabilization of the patient as needed and decontamination (Grave & Boag, 2010; Richardson, 2000).

As in any emergency, our priority is to stabilize the animal. The ABC (airway, breathing and circulation) approach should be followed. The airways should be checked for any obstruction and the animal intubated if necessary. Oxygen support must be given to dyspneic animals and in any case where aspiration pneumonia is a possibility, intubation and assisted ventilation must be considered. For example, if the animal has neurological signs and is vomiting, there is increased risk of aspiration so the animal should be intubated. The respiratory rate and breathing effort must be closely monitored. In many intoxications, as with ibuprofen, pets can potentially develop respiratory depression or coma, thus respiratory support must always be available and provided. The third but not least step regards circulation, thus heart rate and rhythm, pulse quality and mucous membranes color and CRT must be promptly assessed and often monitored. If the animal is considered hypovolemic,

fluid therapy must be initiated according to the individual needs of each patient. Too aggressive fluid therapy must be avoided in cats as fluid overload have been commonly reported in this animals. However, if intravascular volume need to be expanded, it is advised to give multiple crystalloid boluses, which must be given slowly and in small amounts, such as 10 ml/kg bolus over 30 min. Animals with bleeding ulcers can present hypovolemic and anemic upon arrival in the hospital and a blood transfusion may be needed depending on its severity. It is also extremely important to measure animal's temperature and keep them normothermic. On the other hand, if the pet is seizuring, anti-convulsant agents should be administered. Diazepam is widely recommended as the first-line agent and should be given as a bolus of 0.5 to 1 mg/kg of body weight intravenously or intrarectally. It can be repeated every 10 min and up to three times if no effect seen. In refractory cases, other anti-seizure drugs should be administered, such as barbiturates or propofol CRI. A standard haematological and blood biochemistry profile should be carried out in any pet presented with suspected intoxication, including at least PCV, TP, electrolytes, BUN, creatinine, glucose and acid-base status. This will allow us to detect any concurrent disturbance and ensure appropriate intervention as quickly as possible. For example, in case of severe metabolic acidosis, intravenous sodium bicarbonate solution must be slowly administered. Also, some electrolytes imbalances may require intervention (Richardson, 2000; Dunayer, 2004; Grave & Boag, 2010; Ramsey, 2011; Osweiler et al., 2011; DeClementi, 2012).

In cases where the dose ingested can potentially cause renal failure (>175 mg/kg in dogs or >50 mg/kg in cats), fluids must be given at twice the daily maintenance rate (5 ml/kg/h) during at least 24-48 hours to induce diuresis with the main goal to preserve renal function more than improve excretion of ibuprofen. Due to the high degree of plasma protein binding, induced diuresis is not very effective in ibuprofen clearance. Renal parameters, such as BUN, creatinine and phosphorus (blood), urine output and specific gravity (urine), should be measured daily. In oliguric animals (<1 ml/kg per hour), the urine output control will be more accurate if an indwelling urinary catheter is placed and a closed collection system connected to it. When the animal remains oliguric/anuric even though fluid deficit has already been replaced, furosemide or mannitol may be used to stimulate diuresis. Furosemide can be used in a dose of 2 mg/kg IV and, if there is no response after one hour, a higher dose (4 mg/kg) can be given. A third dose of 6 mg/kg IV can also be administrated if there is no response in the next hour. As an alternative, mannitol can be used and the recommended dose is 0.25 to 0.5 g/kg slowly IV (over 10 min). If after 48 h of fluid diuresis those values are still

abnormal, then diuresis should be continued. Once the results are back to normal, the fluid rate can be decreased to maintenance (2.5 ml/kg/h) for 24 h and then interrupted if renal values are still WNL (Richardson, 2000; Dunayer, 2004; King & Boag, 2007; Osweiler et al., 2011; Ramsey, 2011; Bischoff & Mukai, 2012).

Within about two hours post ingestion and if no neurological signs are seen, emesis should be induced through the use of emetic agents, such as xylazine or even hydrogen peroxide or sodium carbonate crystals (washing soda). Apomorphine hydrochloride has been used in dogs as the first-line emetic agent. However, it can cause behavioral changes and is less effective in cats, and therefore its use is not recommended. On the other hand, xylazine is the agent of choice to induce emesis in cats. It has been effectively used at a dose of 0.44 mg/kg IM. Hydrogen peroxide has also been used with success to induce vomit in small animal's practice. The empirical dose for cats is 1-3 ml/kg of body weight of a 3% solution, but a total dose of 10 ml per cat should not be exceeded. In addition, activated charcoal should be administered orally to all the animals known or suspected to have ingested ibuprofen. Activated charcoal should be given at 0.5 to 4 g/kg of body weight PO and diluted with water (1 g to 5 ml of water). It should be repeated every 6 to 8 hours for three administrations once ibuprofen undergoes enterohepatic recirculation. This compound adsorbs many toxics, such as ibuprofen, thus preventing its gastrointestinal absorption and facilitating its faecal excretion. It is also often recommended to be combined with a cathartic agent, not only to stimulate evacuation in general, which is beneficial in general poisoning, but also to counteract the coprostatic effect of activated charcoal. Sorbitol is a commonly used cathartic agent and can safely be given at a dose of 3 mg/kg. It is already mixed with activated charcoal in commercial products but can also be added manually. Also, some saline cathartics, such as sodium sulfate or magnesium sulfate, can be given 20-30 min after the activated charcoal administration. If the animal recently ingested ibuprofen and presents any reflexes deficit (or neurological signs) or the induction of emesis failed, a gastric or enterogastric lavage may be considered. In uncooperative patients, the gastric tube can also be used to administrate the activated charcoal. However, since this procedure requires GA, we should consider whether it is beneficial for each case individually (Richardson, 2000; Dunayer, 2004; Grave & Boag, 2010; Ramsey, 2011).

Gastrointestinal protectants have an important role on ibuprofen poisoning treatment. It will help preventing or treating potential gastric ulcers. Misoprostol has been used for this

purpose as it inhibits gastric acid secretion, increases bicarbonate and mucous secretion and stimulates epithelial cells repair on the gastric mucosa, protecting its integrity. It is recommended at a dose of 5µg/kg PO every 8 h for cats. Ranitidine is a $H_2$-blocker that can be used alongside misoprostol at a dose of 2.5 mg/kg IV slowly or 3.5 mg/kg PO every 12 h as it blocks histamine receptors and inhibits gastric acid secretion. Cimetidine is also a $H_2$-blocker used for management of gastrointestinal ulcers but is less potent than ranitidine and its use is controversial in ibuprofen poisoning. A study performed in rats concluded that cimetidine can increase the gastrointestinal absorption of ibuprofen. Besides this potential problem, cimetidine decreases the action of the microsomal enzymes on the liver, which can affect ibuprofen glucuronidation on cat liver microsomes. Sucralfate is also helpful at a dose of 250 mg per cat PO every 8 to 12 h. It protects ulcers from further damage due to gastric acid and stimulates bicarbonate and PGE production, thus enhancing mucosa protection. However, it can decrease the bioavailability of some drugs, such as $H_2$-blockers, so it should be administered 2 hours before other drugs. Proton pump inhibitors, particularly omeprazole, have also been successfully used in the management of gastroduodenal ulcers. Omeprazole inhibits gastric acid secretion and this effect lasts at least 24 hours after administration. Its recommended dose is 0.5 to 1.5 mg/kg IV or PO for dogs or 0.75 to 1 mg/kg PO for cats, given every 24 h. Bismuth subsalicylate-containing products are totally not recommended as it can interact with ibuprofen and increase the risk for side effects. If the cat doesn't present any gastrointestinal signs and the ingested dose was very low, perhaps antacids, such as magnesium or aluminum hydroxide, will help. The administration of gastrointestinal protectants should be maintained for at least 7 days and it may be prolonged for more than 14 days depending on severity and progression of each individual case. If the cat is vomiting, metoclopramide may be used as an antiemetic agent. It is recommended at a dose of 0.25-0.5 mg/kg IV, IM, SC or PO every 12 hours, or 0.17-0.33 mg/kg IV, IM, SC, PO every 8 h or 1-2 mg/kg/day CRI. In any case, if ulcer perforation occurs immediate surgical intervention and intensive supportive care must be provided (Richardson, 2000; Dunayer, 2004; Ramsey, 2011; Bischoff & Mukai, 2012; Court, 2013). In the clinical case reported, emesis was not attempted since Mimi was brought to the hospital many hours after exposure to ibuprofen. However, the importance of gastrointestinal protectants administration in these cases was explained to the owner and misoprostol and ranitidine prescribed in accordance with the literature.

It was recently reported a case where ILE (intravenous lipid emulsion) was used to treat ibuprofen toxicosis in one dog. This dog received supportive treatment and ILE after ingesting 180 tablets of ibuprofen and was recovered after a few days. ILE has been successfully used in human medicine to treat toxicity from fat-soluble compounds. Also in veterinary medicine, it has been used effectively in multiple lipid-soluble drug toxicosis, such as moxidectin, ivermectin, baclofen and diltiazem toxicosis in dogs. In both cases, an initial bolus of a 20% sterile lipid solution was given and followed by a constant rate infusion of the same solution. It is also reported the use of this intravenous lipid therapy in cats with permethrin and lidocaine toxicosis. Intravenous lipid administration has shown to result in great clinical improvement and no significant adverse effects. Therefore may be a promising option for treatment of any lipophilic drug toxicosis. The most likely hypothesis regarding its mechanism of action involves a lipid-sink mechanism, where the lipid-soluble drugs are removed from affected tissues and trapped in the plasma lipid phase created by the lipid emulsion administrated, thus reversing signs of toxicity. However, the exact mechanism of action remains unknown. Further investigation about the efficacy, dosage and safety of ILE treatment on ibuprofen toxicosis in dogs and cats is needed (Crandell & Weinberg, 2009; O'Brien, Clark-Price, Evans, Di Fazio, & McMichael, 2010; Clarke, Lee, Murphy, & Reineke, 2011; Haworth & Smart, 2012; Bates et al., 2013; Maton, Simmonds, Lee, & Alwood, 2013; Bolfer, McMichael, Ngwenyama, & O'Brien, 2014; Cortinovis et al., 2015). There is still no specific antidote available for ibuprofen poisoning.

Patients should be kept under appropriate and regular monitoring until a full recovery is achieved. In the meantime the symptomatic treatment needed should be provided (Richardson, 2000; Grave & Boag, 2010).

In conclusion, prompt decontamination and aggressive supportive therapy together with close monitoring are the key for a successful management of ibuprofen poisoning. This has long been known and should always be kept in mind. The early intervention has shown to have even more influence over outcomes than the reported dose ingested. This, in turn, can easily be inaccurate as it depends on what the owner observed, reported and can remember. It is also commonly unknown by the pet caretaker. Therefore, the reported dose may differ from the actual amount ingested (Poortinga & Hungerford, 1998).

**Prevention and Outcomes of ibuprofen toxicosis**

Unsafe storage of drugs and animal's curious nature make this and other kinds of accidental poisoning possible. Many poisonings happen because pet owners are unaware of what is or is not dangerous for their own pets. They don't know that many foods, plants, medications and other household products are among the most toxic substances for animals. Thus, dogs and cats commonly have easy access to potentially hazardous substances because caretakers have no idea it can be harmful for them, and sometimes they even administrate human medication in an attempt to help. Thereby, owners are often unintentionally involved in their pet poisonings (Berny et al., 2010; Mahdi & Van der Merwe, 2013; Cortinovis et al., 2015).

Proper pet owner education is the key to prevent the occurrence of these incidents. Thus, one of the most important roles of veterinarians is to give their clients essential information about how to handle and maintain their pets and alert them about some precautions they must take. Tips should be given on how to keep their furry friends safe, including a list of substances to avoid and information about proper storage of potentially harmful products. Owners should be aware that cats are curious animals and have great ability to reach spaces even through small holes and can easily hide items so that exposure is not immediately detected. Thus, any drug or potentially toxic product must be safely stored in child and animal proof facilities. To keep the pet entertained and avoid playing with unsafe items, proper and safe toys should always be available for them. Besides, owners should be advised to immediately call their veterinarian when they think their pet has been exposed to something potentially toxic (Berny et al., 2010; DeClementi, 2007, 2012; Fitzgerald et al., 2006; Grave & Boag, 2010; Mahdi & Van der Merwe, 2013).

The prognosis will depend on the dose ingested and the time that has passed since exposure until treatment is initiated. If ibuprofen, such as any other potentially harmful substance, is ingested, the pet caretaker should bring the animal to the hospital/clinic as soon as possible and prompt and adequate supportive care and symptomatic treatment should be provided. If so, most animals will fully recover. On the other hand, as the time elapses without intervention and the clinical signs become more severe, the prognosis becomes guarded, especially if irreversible lesions, such as kidney papillary necrosis have already occurred (Richardson, 2000; Dunayer, 2004; King & Boag, 2007; Grave & Boag, 2010). In the clinical case presented (Mimi), the outcome was devastating and probably related to the

late intervention and lack of ongoing close monitoring during the first days after exposure, which are crucial for the treatment success. This reinforces the importance of providing essential information about products that must be properly stored (away from the pets) and the relevance of prompt treatment and ongoing monitoring. In other words, it shows the importance of an adequate pet owner education and cooperation.

# Metaldehyde poisoning

## Clinical case:

### Animal identification

| | | |
|---|---|---|
| **Pet name:** Mack<br>**Species:** Canine<br>**Breed:** Mongrel | **Colour:** White<br>**Gender:** Male - Neutered<br>**Age:** 3 y old | **Weight:** 40 kg/88.2 lbs<br>**Insured status:** Not insured |

**Clinical history:**

No relevant information prior to October 2014.

*28th October 2014*

Mack's owner brought Mack to the HVC- Charneca da Caparica for an emergency consultation in the evening. Mack presented with generalized muscle tremors, intense agitation, hypersalivation and panting. The owner reported that Mack vomited 30 min before coming to the hospital and just after the clinical signs appeared. Mack had been exposed to slug baits that were placed on the garden 1 hour ago. The owner brought with him the slug and snail bait container. Its active constituent was metaldehyde in the form of a granular bait.

The risks of this intoxication and the need for stabilization and ongoing supportive care and monitoring were explained to the owner. During Mack's hospitalization, the owner visited him and was often updated about the progress he made.

Based on the history reported and Mack's clinical signs, he was treated as a poisoned patient. The established priority was to evaluate the major body systems (cardiovascular, respiratory and CNS) according to the ABC approach and stabilize Mack. The abnormalities detected with this evaluation were: dyspnea, tachypnea, tachycardia, hyperthermia (39.9 ºC) and mydriasis (with symmetric pupils). Hyperexcitability and generalized tremors were also evident. The upper airway was patent with no fluids/secretions and the chest was clear. The mucous membranes were pink and moist, the CRT was less than two seconds, pulse was strong and the arterial blood pressures were within normal range.

Firstly, an endovenous catheter was placed and fluid therapy initiated with Ringer's Lactate solution at a maintenance rate (2.5 ml/kg/h) since Mack didn't show any signs of dehydration or hypovolemia. Some wet towels were used to decrease Mack's high temperature and later removed as soon as the temperature dropped back to normal. To do so, regular temperature measurements were performed.

In order to induce emesis, morphine sulphate (0.8 mg/Kg IV) was given. Since no response was seen, 3% hydrogen peroxide (3 ml/kg PO) was administered, but again with no success.

In an attempt to reduce restlessness and muscular spasms, acepromazine (0.05 mg/kg IM) and diazepam (0.25 mg/kg IV) were administered. Despite medication, Mack remained panting and with tremors. He was sedated by adding propofol (4 mg/kg IV). To avoid aspiration pneumonia and assure adequate ventilation, a 12.0 mm endotracheal tube was placed and cuffed and 3 L/min of 100% O2 was provided. Mack was maintained under anesthesia with a CRI of propofol (0.1 mg/kg body weight per minute). Vital parameters, including heart rate, blood pressure, ECG, respiratory rate and pulse oximetry, were continuously monitored. ECG abnormalities were not found at any time during Mack's stay in the hospital (See Figure 38).

Figure 38: Mack under anesthesia and continuous monitoring.

After sedation was initiated, arterial blood pressures fell below normal limits, so a crystalloid bolus (20 ml/Kg IV) was given and it solved the problem. The following blood pressure results were normal.

As the attempt to induce emesis failed and the animal was already intubated and in lateral recumbency, a pre-measured (from nose-tip to the last rib), marked and lubricated nasogastric tube was carefully advanced until the stomach in order to empty gastric contents.

Afterwards, 3 g/kg of activated charcoal diluted in water was introduced through the same tube.

A blood sample was obtained and PCV, TS and glucose levels were measured. PCV and TS were WNL but the blood glucose was 27 mg/dl (80 - 120 mg/dl), so Mack was hypoglycemic. Thus, a 30% glucose bolus (1 ml/kg or 300 mg/kg IV) was administered. After this bolus, the blood glucose returned to the normal range (89 mg/dl). However, it dropped again after a while. A second glucose bolus was given (300 mg/kg IV) and followed by a CRI of 5% glucose solution at maintenance rate (2.5 ml/kg/h), replacing the Ringer's Lactate solution. This aimed to maintain blood glucose levels within normal range. However, after 3 hours of infusion, Mack revealed 7% dehydration (decreased skin turgor and dry mucous membranes). Thus, fluid therapy with Ringer's Lactate solution was restarted, associated with glucose, but now at maintenance rate plus replacement of fluid caused by dehydration. Later on, the glucose measurements showed normal blood glucose levels.

Blood glucose levels were often checked but no more abnormal results were detected during Mack's hospitalization.

When the propofol infusion was ended and the animal extubated, the muscular spasms were still present but now milder and limited to the pelvic limbs.

*29th October 2014*

During the following hours, Mack was ataxic and had a few greenish diarrhea episodes. However, his respiratory rate and rhythm were back to normal. Also the cardiovascular parameters remained WNL. Fluid therapy was continued at a maintenance rate during the 29th of October since Mack had no more signs of dehydration.

Regular physical examinations were performed during the day and Mack stayed hydrated, bright, alert and responsive, without pain, HR and RR within normal range, pulse

strong, symmetric and synchronic, normothermic, mucous membranes pink and moist and CRT less than 2 seconds. His arterial blood pressures and blood glucose levels remained normal as well. The results of hematologic profile revealed leukopenia consisting of neutropenia and lymphopenia, but all the other cell counts were WNL. In turn, the biochemical parameters were all within normal range, including hepatic and renal parameters.

*30th October 2014*

On the 30th of October, once again regular physical examinations were performed and a blood sample taken to analyze the hematologic and biochemical profiles.

During the entire day, Mack was happy and with no more muscle tremors, ataxia or diarrhea (see Figure 39). On physical examinations and laboratory analysis no abnormalities were found. The case was again discussed with the owner. The owner was advised to monitor Mack in the next few days and call the hospital or bring him back as soon as any concern arise. Mack went home with the owner.

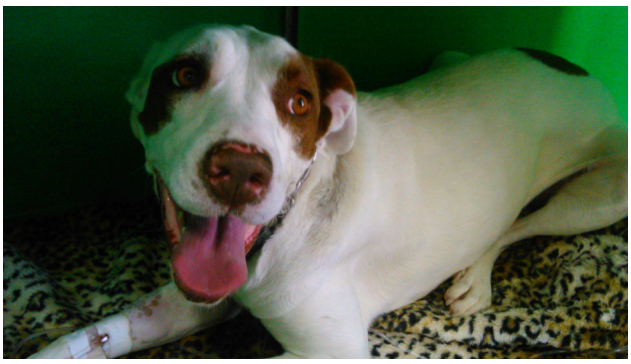

Figure 39: Mack was happy and with no more muscle tremors.

Literature Review

# Metaldehyde Poisoning- dogs

## Introduction

Metaldehyde is a cyclic tetramer of acetaldehyde commonly used as a pesticide. It's the main active constituent of many commercial molluscicide products used in gardens and several agricultural and horticultural crops, including greenhouses, in order to control slugs and snails. It has also been used for control of fish, frogs and leeches and even as a solid camping fuel for small stoves and heaters. It is commercially available in many different formulations, including liquid, spray, powder, granules and pellets. Commonly metaldehyde concentration in the product is less than 4%, however some European formulations can have up to 50% metaldehyde. Some formulations combine it with other substances, such as carbamates, arsenates and organophosphates. Most of this pesticide products have bran and/or molasses in its constitution to make it more attractive for snails and slugs. The problem is that it also becomes attractive for pets and other wild and domestic animals. This compound is toxic for animals and humans and affects their major body systems. Small doses of metaldehyde are enough to cause poisoning and death. Although ingestion of these products is the most common way of exposure, dermal or respiratory exposures can also occur (Dolder, 2003; Puschner, 2006; Gupta, 2007).

There are many reported cases of metaldehyde poisoning that result from accidental or intentional exposure to this compound. Metaldehyde is known to be toxic to all animal species that have been exposed to it, including dogs, cats, horses, cattle, sheep, birds and humans. However, most reported cases involve dogs and cats, probably due to their curious nature. There are also several records regarding human poisonings involving metaldehyde, either through accidental ingestion or suicide attempt. According to the records of poison control centers from many different countries, metaldehyde is between the toxic agents that are most commonly involved in poisoning episodes (Longstreth & Pierson, 1982; Shih et al., 2004; Gupta, 2007; Berny et al., 2010). However, the number of cases concerning metaldehyde toxicosis has been decreasing over the years (Buhl, Berman, & Stone, 2013).

## Pathogenesis

Metaldehyde is known to kill snails and slugs by causing severe dehydration on these mollusks. On animals, its exact mechanism of action remains unknown. It has been reported that, under the low stomach pH, metaldehyde can be hydrolyzed to acetaldehyde. Regarding its mechanism of action, it has been suggested that acetaldehyde is the metabolite responsible for the toxic effects on animals. However, the compound found in the plasma and urine of dogs, in the serum of rats and in the brain tissues of mice exposed to metaldehyde was metaldehyde and not acetaldehyde. Besides, the $LD_{50}$ of this two compounds in rats have been reported and are very different, with 227 to 690 mg/kg for metaldehyde and 1930 mg/kg for acetaldehyde. Thus, this theory seems unlikely. On the other hand, it has been demonstrated that the gamma-aminobutyric acid (GABA) concentration in the brain is decreased after acute oral exposure to metaldehyde. When brain tissue of mice exposed to 1000 mg/kg PO of metaldehyde was analyzed, a reduction of about 50% of GABA content was detected. GABA is the main inhibitory neurotransmitter of mammalian CNS and so it reduces neuronal excitability. Therefore, the increased neuronal excitability manifested by the neurological signs of poisoned animals and the fact that diazepam ($GABA_A$ receptor modulator) attenuates those signs are also suggestive of GABAergic system involvement. Decreased brain levels of norepinephrine and serotonin (5-hydroxytryptamine) and increased monoamine oxidase (MAO) activity have also been associated with metaldehyde toxicity. MAO is the enzyme responsible for the catabolism of norepinephrine and serotonin, hence its increased activity leads to even lower levels of those monoamines. This has been related with lower seizure threshold. However, for a better understanding of the mechanism involved, further investigation is required (Booze & Oehme, 1986; Sparks, Quistad, Cole, & Casida, 1996; Shintani, Goto, Endo, Iwamoto, & Ohata, 1999; Dolder, 2003; Puschner, 2006; Gupta, 2007).

Metaldehyde is rapidly absorbed from the gastrointestinal tract and reaches its maximal plasma concentration within 1 to 3 hours after ingestion, which can be affected by the type of formulation (liquid or solid). It can also be absorbed through the lungs if inhaled or through the skin after dermal exposure. These are less common routes of exposure, however not less important (Gupta, 2007). Its plasma half-life has been reported to be about 27 hours in humans, but in pets it hasn't been reported (Richardson, Welch, Gwaltney-Brant, Huffman, & Rosendale, 2003; Olson & California Poison Control System, 2004). After absorption, metaldehyde is widely distributed through the bloodstream and it has been

detected in many animal tissues. Its residues were found in liver and brain tissues of mice exposed to it (Sparks et al., 1996), and also in kidney tissues of rats (Griffiths, 1984). Metaldehyde and its metabolites can readily cross the blood-brain barrier, hence it can cause CNS signs by interfering with brain neurotransmitters. It is thought to be efficiently metabolized through a process involving the mixed function oxidase system, particularly cytochrome P450. It is also presumably depolymerized into acetaldehyde which is then oxidized to carbon dioxide and excreted through the lungs (Richardson et al., 2003; Puschner, 2006). The extent of unmetabolized metaldehyde excreted through the urine has been reported to be less than 1% in dogs (Booze & Oehme, 1986). In mice about 6% (± 3) of the dose of metaldehyde ingested is excreted through the urine and 1.3% (± 0.6) through the faeces according to reported data (Sparks et al., 1996).

The first clinical signs usually appear within a few minutes to 3-5 hours after exposure and can include vocalization, agitation, anxiety, tachycardia, tachypnea, hyperpnea, cyanosis, hypersalivation, dehydration, mydriasis, hyperesthesia, opistothonus, ataxia, muscle tremors and seizures. Gastrointestinal signs, such as vomiting and diarrhea, may also be seen. Hyperthermia is often present and greatly contributes to patient's morbidity. Temperatures as high as 43°C have been documented. It is known that at temperatures greater than 41.6°C, generalized cellular necrosis rapidly take place and multiple organ failure and DIC may arise. This hyperthermia is probably related to the increased muscular activity (muscle tremors, seizures). Nystagmus can also be evident but is more commonly seen in cats than in dogs. Later, depression and coma may also develop. It has also been reported cases of temporary blindness. In severe cases or when adequate patient stabilization doesn't occur, the animal may die within 4 to 24 hours after metaldehyde ingestion, generally due to respiratory failure. However, the animal can also die a few days later (commonly 2 to 3 days) as a result of liver failure (Dolder, 2003; Richardson et al., 2003; Steenbergen, 2004; Puschner, 2006; Yas-Natan, Segev, & Aroch, 2007; Osweiler et al., 2011).

The electrolyte and acid-base homeostasis can also be compromised in metaldehyde intoxications. Metabolic acidosis, involving decreased bicarbonate levels and blood pH, seems to be a very common condition in this toxicosis. It is presumably related to the acidic nature of metaldehyde or its metabolites and increased lactic acid production due to excessive muscular activity. As a consequence, central nervous system depression and hyperpnea can emerge. Other outstanding biochemical findings reported are increased serum activities of

muscle enzymes, including CK, AST, LDH, which is probably related to muscle injury resulting from seizures and tremors. The subsequent hyperthermia can lead to some degree of rhabdomyolysis causing an additional increase in serum muscle enzymes activity. Other abnormalities, such as glucose disturbances, have also been reported. Hyperglycemia most likely results from stress, while hypoglycemia may be related with the increased muscle effort and consequent increase in glucose consumption by muscle cells. In later stages, liver failure can develop and, consequently, serum liver enzymes activities and bilirubin levels increase (Dolder, 2003; Yas-Natan et al., 2007).

The lowest $LD_{50}$ reported is 100 mg/kg body weight in dogs and humans, 200 mg/kg in cats, 60 mg/kg in horses, 175 mg/kg in guinea pigs, 200 mg/kg in mice, 227 mg/kg in rats and 290 mg/kg in rabbits (Sparks et al., 1996; Olson & California Poison Control System, 2004; Gupta, 2007; Mathews, 2007).

**Diagnosis**

As in the case of ibuprofen toxicosis, the diagnosis of metaldehyde poisoning is often based on the pet exposure history and the presence of suggestive clinical signs. The complete history and thorough physical examination together with blood and urine analysis are essential for establishing a diagnosis and treatment plan. Commonly the owner reports to have placed slug baits through its house premises or even witnessed the ingestion, thus making the diagnosis easier. However, if this is not the case, further investigation may be required and differential diagnosis considered (Richardson et al., 2003; Steenbergen, 2004; Yas-Natan et al., 2007).

To confirm the presence of metaldehyde in the animal's organism, some laboratories are available to test for metaldehyde in stomach contents, serum and urine samples. Metaldehyde has been detected in stomach contents, serum/plasma, urine and even in liver, kidney and brain tissues through gas chromatographic assays (Griffiths, 1984; Booze & Oehme, 1985; Sparks et al., 1996; Richardson et al., 2003). Radiographies can be performed to detect the presence and extension of gastric contents. Besides, as some pellets are radiopaque, it may be visualized on x-rays (Osweiler et al., 2011).

If metaldehyde poisoning can't be confirmed, other differential diagnosis should be ruled out. Many other toxic agents can cause similar symptoms, including CNS excitation (anxiety, tremors, seizures), gastrointestinal upset (vomit, diarrhea) and respiratory signs.

Some examples include: rodenticides, such as strychnine, bromethalin and zinc phosphide, insecticides, such as organochlorine compounds, anticholinesterase compounds (organophosphates and carbamates), pyrethrins and pyrethroids, other pesticides, such as the compound 1080 (sodium monofluoroacetate) and 4-Aminopyridine (avicide), and other substances such as methylxanthines, lead, tremorgenic mycotoxins (penitrem A, roquefortine), cyanotoxins (anatoxin-A, saxitoxins) and illicit drugs (amphetamines). In addition, the clinical signs can also result from other nontoxic etiologies, including infectious (bacterial, viral encephalitis), metabolic (hypoglycemia, hypocalcemia, uremia), traumatic (head trauma), neoplastic and congenital (idiopathic epilepsy, hydrocephalus, portosystemic shunt) (Dolder, 2003; Steenbergen, 2004; Puschner, 2006; Osweiler et al., 2011).

The postmortem findings from animals that died or were euthanized due to metaldehyde poisoning are not pathognomonic. Stomach contents with metaldehyde granules and/or acetaldehyde (similar to formaldehyde) odor are the most revealing findings. In the postmortem evaluation of this animals, other nonspecific lesions have been found, such as congestion of the liver, kidneys and lungs, petechiae and hemorrhages in the gastrointestinal mucosa, epicardium and endocardium, and edema and hemorrhages of the lungs (Dolder, 2003; Richardson et al., 2003; Puschner, 2006; Gupta, 2007; Yas-Natan et al., 2007; Osweiler et al., 2011).

**Treatment and Monitoring**

The main goals of metaldehyde toxicosis treatment are to remove the remaining metaldehyde from the stomach and prevent its further absorption, control clinical signs, monitor and correct any hydroelectrolytic and acid-base disturbances, and provide all the supportive care needed. As for any other emergency situation, our priority is to stabilize the patient as was previously described for the ibuprofen intoxication case. Dyspnea is occasionally present in affected animals, hence ventilatory support and oxygen may be required (Richardson et al., 2003; Gupta, 2007). The next step is decontamination and it is advised at any dose equal or higher than 2 mg/kg body weight (Dolder, 2003).

Emesis is recommended if the metaldehyde was ingested in the last 30 min to 2 hours and only if the animal is asymptomatic. Thus, if the animals presents any tremors or other toxicological signs, it is contraindicated to induce emesis as it can trigger seizures. It has often been reported that feeding a small amount of moist food before inducing emesis may increase

emesis efficacy. Apomorphine is the drug of choice to induce emesis in dogs and it can be applied to the conjunctiva or administered IV or IM at a dose of 0.03 mg/kg or 0.04 mg/kg, respectively. The ocular administration has been proved to have similar efficacy and less side effects (excessive vomiting, excitement, sedation, respiratory depression) than the IV administration. Hydrogen peroxide (3%) has also been successfully used to induce vomit in dogs at a dose of 1 to 5 ml/kg body weight (never exceeding 45 ml per dog). If a large amount of metaldehyde-containing product was ingested and/or the emesis induction was unsuccessful or contraindicated, gastric lavage is a procedure to be considered. It requires GA and endotracheal tube placement (with the cuff inflated) to prevent aspiration of gastric contents during lavage (Richardson et al., 2003; Dolder, 2003; Mathews, 2007; Cote, Collins, & Burczynski, 2008; Ramsey, 2011). In the clinical case presented, emesis was attempted through the use of hydrogen peroxide (3%). However, as previously mentioned, emesis should be avoided if the animal already presents clinical signs.

In addition, activated charcoal should be administered orally at a dose of 0.5 to 4 g/kg of body weight. After being diluted with water, it can be given through a large syringe or stomach tube. However, if the animal presents toxicological signs (seizures, depression, coma, vomiting, etc.) or is under sedation, a cuffed endotracheal tube must be placed, again to minimize the risk of aspiration pneumonia. If gastric lavage was previously performed, the gastric tube can also be used to administrate the activated charcoal. It may be helpful to give one-half of the initial dose every 4 to 8 hours (Richardson et al., 2003; Steenbergen, 2004; Ramsey, 2011). The early administration of activated charcoal has proved to reduce up to 45.3% the gastrointestinal absorption of metaldehyde in rats. The greater effect of activated charcoal was achieved when given 30 min after oral exposure to metaldehyde and in higher activated charcoal: metaldehyde ratios (≥5:1). The same is likely to be true in dogs and cats (Shintani et al., 1999). It may also be beneficial to combine the activated charcoal with a cathartic in order to stimulate its evacuation. Sorbitol (70%) is often added to the first administration of activated charcoal at a dose of 3 ml/kg. However, if the animal is dehydrated or has diarrhea, cathartic agents are contraindicated (Richardson et al., 2003; Steenbergen, 2004; Mathews, 2007). Activated charcoal was administered to Mack (the clinical case presented in this report) in accordance with the literature, although no cathartic agent was added to it.

Methocarbamol is a muscle relaxant that has been successfully used to control muscle tremors and seizures in dogs exposed to metaldehyde. The recommended dose of administration is 55 to 220 mg/kg body weight IV. One-half of this dose should be given first at a maximum rate of 2 ml per minute. The second half is administered as required to control tremors. Methocarbamol administration can be repeated if necessary, however, the daily dosage should never exceed 330 mg/kg body weight (Richardson et al., 2003; Gupta, 2007; Mathews, 2007). Administration of diazepam or phenobarbital to mice have been associated with milder clinical signs (Sparks et al., 1996). Additionally, other experimental studies involving oral administrations of metaldehyde to mice revealed that intraperitoneal administration of diazepam or clonidine (20 min after metaldehyde exposure) reduced the mortality rate and it was also associated with a lower reduction of brain levels of GABA, norepinephrine and serotonin (Homeida & Cooke, 1982). The same is perhaps also true for pets. In dogs, diazepam has also been successfully used to control metaldehyde-induced seizures. It is reported to be used within a range of 1 to 5 mg/kg IV, administered until seizures are controlled. It can also be used at 0.1 to 0.5 mg/kg body weight per hour (CRI). According to more recent data, a diazepam bolus of 0.5 to 1 mg/kg IV (or intrarectally if no IV access available) is recommended and it can be repeated every 10 min if needed up to three doses. A CRI can also be useful at a rate of 1 mg/kg/h, which can be slightly increased until the desired effect is achieved. In refractory cases, inhalant anesthetics, barbiturates and propofol have been used to control seizures. Barbiturates should be reserved for cases refractory to the previous options as it has the potential to compete with the enzyme that metabolizes acetaldehyde. If required, sodium phenobarbital at a dose of 12 mg/kg IV can be administered and if the desired effect is not achieved, a dose of 4 to 6 mg/kg IV can be given every 20 min up to a maximum of 18 to 24 mg/kg. In turn, pentobarbital can be used at 3 up to 15 mg/kg IV, but it should be administered slowly and stopped once seizures are under control. Propofol can be used as an intravenous bolus of 6 to 7 mg/kg IV in an unpremedicated dog or 1 to 4 mg/kg IV in a premedicated dog. A forth of the total dose should be given slowly and every 30 seconds until seizures are controlled. Also a CRI of propofol may be of benefit at 0.1 to 0.4 mg/kg/min. It is commonly reported that more than one sedative or anesthetic are required to control the clinical signs (Richardson et al., 2003; Dolder, 2003; Steenbergen, 2004; Gupta, 2007; Yas-Natan et al., 2007; Ramsey, 2011). A study was conducted to investigate if dogs with prolonged status epilepticus caused by metaldehyde (and other toxins) would have recurrent seizures after it and need long-term

therapy with anticonvulsant agents. After a mean follow-up of more than 2 years, the 20 dogs involved had no more seizure episodes. This suggests that affected animals will probably not need prolonged treatment with anticonvulsants (Jull, Risio, Horton, & Volk, 2011). In the clinical case presented, acepromazine and diazepam were administered to reduce restlessness and muscle tremors. As it didn't solve the problem, the clinician opted for a propofol bolus of 4 mg/kg IV (since the animal was already premedicated with the aforementioned drugs), followed by a CRI of propofol.

It is very important to maintain the affected animals under continuous monitoring and provide them all the symptomatic treatment needed. Fluid therapy is recommended to control body temperature, prevent or correct dehydration and restitute electrolytes and acid-base balances. However, fluid diuresis is generally not needed as metaldehyde renal excretion is very low, so it won't accelerate its elimination from the body. The exception occurs when the pet underwent prolonged seizures and the resulting excessive levels of serum myoglobin led to renal damage, thus fluid diuresis may help avoiding further damage (Dolder, 2003; Steenbergen, 2004).

Hyperthermia usually resolves when tremors and seizures are controlled, however fans and wet towels can be used to cool the animal when the temperature becomes cause for concern. Ice water baths and cold water enemas are totally contraindicated measures as it will easily lead to animal's hypothermia (Dolder, 2003; Richardson et al., 2003; Steenbergen, 2004; Mathews, 2007).

Mild metabolic acidosis is usually corrected with fluid therapy, however if severe or refractory, sodium bicarbonate administration should be considered. Some disruptions in the electrolytes balance may also need intervention. Regular physical examinations and blood and urine analysis, including temperature, blood gases, electrolytes, anion gap and urine pH measurements, should be performed. Besides, it should always be kept in mind that liver failure may occur in later stages, thus serum liver enzymes activities should also be regularly monitored, at least at admission and 72 hours after metaldehyde exposure. Further monitoring will vary according to the individual case. During hospital stay, any other clinical manifestation should be controlled and adequate supportive care provided. Antiemetic agents may be required to control vomit in some patients (Dolder, 2003; Richardson et al., 2003; Steenbergen, 2004).

In conclusion, adequate animal stabilization and control of clinical signs (tremors, seizures, hyperthermia, etc.) and laboratorial abnormalities through prompt and ongoing supportive care and symptomatic treatment are essential for a successful management of metaldehyde intoxication. Furthermore, there is no antidote available for this toxicosis (Gupta, 2007; Mathews, 2007; Yas-Natan et al., 2007).

**Prevention and Outcomes of metaldehyde toxicosis**

Once again the problem is closely related to the fact that some owners are not properly informed about how to manage their pets and what is dangerous for them. The preventive measures previously described for the ibuprofen toxicosis case also applies here. Commonly, the pet is exposed to metaldehyde because his owner spread slug baits through the garden and let him have easy access to it. In other cases, metaldehyde containers (either slug baits or camping fuel) are left opened or in some place of easy access. The dogs can also have access to the outside and be exposed to metaldehyde when scavenging through the treated areas. Therefore, proper pet owner education is the key to prevent the occurrence of these incidents. They should be informed about the potential complications of metaldehyde exposure and alerted to the fact that metaldehyde containers, as well as any other potentially hazardous substance, should be properly stored in pet proof premises. Owners should limit their pet access to the treated areas or just find an alternative, nontoxic measure to control snails and slugs, thus warranting their pet safety. Besides, owners should be advised to immediately call their veterinarian when they think their pet has been exposed to something potentially toxic (Yas-Natan et al., 2007; Osweiler et al., 2011).

If the pet caretaker suspects exposure to metaldehyde, the animal should be brought to the hospital as soon as possible. Upon admission, prompt and adequate supportive care and symptomatic treatment should be provided. When this is achieved, most dogs will fully recover. On the other hand, as the time elapses without intervention and the clinical signs become more severe, the prognosis becomes worse and death may occur within hours of exposure. The mortality rate has been reported to vary from 0 to 16.7%. However, the outcome will depend on the metaldehyde concentration of the ingested product, the total amount ingested and especially on the time elapsed since exposure until treatment is initiated (Studdert, 1985; Firth, 1992; Puschner, 2006; Yas-Natan et al., 2007; Osweiler et al., 2011). In contrast to the earlier mentioned case (ibuprofen poisoning), the clinical case of exposure to metaldehyde had a positive outcome, probably as a result of an early intervention and

ongoing treatment and monitoring. As far as we know Mack had his clinical signs resolved and did not have long term sequelae.

# Chapter IV - Conclusion

These six months of internship were a unique experience to me. It was a real pleasure to integrate amazing teams full of experienced veterinarians. It was an eye-opener to how the veterinary tasks are dealt with in the private small animal practice. The exposure to both Portuguese and British veterinary practice was very welcoming and allowed me to extend and strengthen my previous knowledge and to develop management skills, those that are not learned in the class rooms. The rotation through the different services available in the hospitals greatly contributed to it.

The present report does not intend to describe my entire traineeship experience. It would be exhaustive and impractical to detail all the knowledge acquired and cases attended. However, all the different situations contributed to my personal and professional development. My main passion lies within small animal practice, hence I decided to do my internship and develop this report in this area. Despite my wide range of interests within the small animal's medicine and surgery, I chose to describe only four of the really interesting clinical cases attended due to space limitation.

I want to emphasize that the end-of-course traineeship is, in my opinion, the best way to finish the veterinarian master degree, enabling a smooth transition from university student to young professional. The daily contact with animals and their owners gave me the opportunity to train and improve my practical and communication skills.

# Bibliography

Adams, S. S., Bough, R. G., Cliffe, E. E., Lessel, B., & Mills, R. F. N. (1969). Absorption, distribution and toxicity of ibuprofen. *Toxicology and Applied Pharmacology*, *15*(2), 310–330. http://doi.org/10.1016/0041-008X(69)90032-5

Bates, N., Chatterton, J., Robbins, C., Wells, K., Hughes, J., Stone, M., & Campbell, A. (2013). Lipid infusion in the management of poisoning: a report of 6 canine cases. *Veterinary Record*, *172*(13), 339–339. http://doi.org/10.1136/vr.101036

Benitah, N. (2006). Canine Nasal Aspergillosis. *Clinical Techniques in Small Animal Practice*, *21*(2), 82–88. http://doi.org/10.1053/j.ctsap.2005.12.015

Berny, P., Caloni, F., Croubels, S., Sachana, M., Vandenbroucke, V., Davanzo, F., & Guitart, R. (2010). Animal poisoning in Europe. Part 2: Companion animals. *The Veterinary Journal*, *183*(3), 255–259. http://doi.org/10.1016/j.tvjl.2009.03.034

Billen, F., Guieu, L.-V., Bernaerts, F., Mercier, E., Lavoué, R., Tual, C., … Clercx, C. (2010). Efficacy of intrasinusal administration of bifonazole cream alone or in combination with enilconazole irrigation in canine sino-nasal aspergillosis: 17 cases. *The Canadian Veterinary Journal*, *51*(2), 164–168.

Billen, F., Peeters, D., Peters, I. R., Helps, C. R., Huynen, P., De Mol, P., … Clercx, C. (2009). Comparison of the value of measurement of serum galactomannan and Aspergillus-specific antibodies in the diagnosis of canine sino-nasal aspergillosis. *Veterinary Microbiology*, *133*(4), 358–365. http://doi.org/10.1016/j.vetmic.2008.07.018

Bischoff, K. (2007). Chapter 23 - Toxicity of over-the-counter drugs. In R. C. Gupta (Ed.), *Veterinary Toxicology* (pp. 363–390). Oxford: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780123704672501206

Bischoff, K., & Mukai, M. (2012). Chapter 28 - Toxicity of over-the-counter drugs. In R. C. Gupta (Ed.), *Veterinary Toxicology (Second Edition)* (pp. 443–468). Boston: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780123859266000284

Bojrab, M. J., Waldron, D. R., & Toombs, J. P. (2014). *Current Techniques in Small Animal Surgery, Fifth Edition*. CRC Press.

Bolfer, L., McMichael, M., Ngwenyama, T. R., & O'Brien, M. A. (2014). Treatment of Ibuprofen Toxicosis in a Dog with IV Lipid Emulsion. *Journal of the American Animal Hospital Association*, *50*(2), 136–140. http://doi.org/10.5326/JAAHA-MS-5979

Booze, T. F., & Oehme, F. W. (1985). Gas Chromatographic Analysis of Metaldehyde in Urine and Plasma. *Journal of Analytical Toxicology*, *9*(4), 172–173. http://doi.org/10.1093/jat/9.4.172

Booze, T. F., & Oehme, F. W. (1986). An Investigation of Metaldehyde and Acetaldehyde Toxicities in Dogs. *Toxicological Sciences*, *6*(3), 440–446. http://doi.org/10.1093/toxsci/6.3.440

Buhl, K. J., Berman, F. W., & Stone, D. L. (2013). Reports of metaldehyde and iron phosphate exposures in animals and characterization of suspected iron toxicosis in

dogs. *Journal of the American Veterinary Medical Association*, *242*(9), 1244–1248. http://doi.org/10.2460/javma.242.9.1244

Bushra, R., & Aslam, N. (2010). An Overview of Clinical Pharmacology of Ibuprofen. *Oman Medical Journal*, *25*(3), 155–1661. http://doi.org/10.5001/omj.2010.49

Ce, H. (1984). Nasal aspergillosis and penicilliosis in dogs: results of treatment with thiabendazole. *Journal of the American Veterinary Medical Association*, *184*(1), 48–50.

Claeys, S., Lefebvre, J.-B., Schuller, S., Hamaide, A., & Clercx, C. (2006). Surgical treatment of canine nasal aspergillosis by rhinotomy combined with enilconazole infusion and oral itraconazole. *Journal of Small Animal Practice*, *47*(6), 320–324. http://doi.org/10.1111/j.1748-5827.2006.00154.x

Clarke, D. L., Lee, J. A., Murphy, L. A., & Reineke, E. L. (2011). Use of intravenous lipid emulsion to treat ivermectin toxicosis in a Border Collie. *Journal of the American Veterinary Medical Association*, *239*(10), 1328–1333. http://doi.org/10.2460/javma.239.10.1328

Cohn, L. A. (2014). Canine Nasal Disease. *Veterinary Clinics of North America: Small Animal Practice*, *44*(1), 75–89. http://doi.org/10.1016/j.cvsm.2013.08.002

Cortinovis, C., Pizzo, F., & Caloni, F. (2015). Poisoning of dogs and cats by drugs intended for human use. *The Veterinary Journal*, *203*(1), 52–58. http://doi.org/10.1016/j.tvjl.2014.11.004

Cote, D. D., Collins, D. M., & Burczynski, F. J. (2008). Safety and efficacy of an ocular insert for apomorphine-induced emesis in dogs. *American Journal of Veterinary Research*, *69*(10), 1360–1365. http://doi.org/10.2460/ajvr.69.10.1360

Court, M. H. (2013). Feline drug metabolism and disposition: pharmacokinetic evidence for species differences and molecular mechanisms. *The Veterinary Clinics of North America. Small Animal Practice*, *43*(5). http://doi.org/10.1016/j.cvsm.2013.05.002

Crandell, D. E., & Weinberg, G. L. (2009). Moxidectin toxicosis in a puppy successfully treated with intravenous lipids. *Journal of Veterinary Emergency and Critical Care*, *19*(2), 181–186. http://doi.org/10.1111/j.1476-4431.2009.00402.x

Culp, W. T. N., Mayhew, P. D., & Brown, D. C. (2009). The effect of laparoscopic versus open ovariectomy on postsurgical activity in small dogs. *Veterinary Surgery: VS*, *38*(7), 811–817. http://doi.org/10.1111/j.1532-950X.2009.00572.x

Dagenais, T. R. T., & Keller, N. P. (2009). Pathogenesis of Aspergillus fumigatus in Invasive Aspergillosis. *Clinical Microbiology Reviews*, *22*(3), 447–465. http://doi.org/10.1128/CMR.00055-08

Day, M. J. (2009). Canine sino-nasal aspergillosis: parallels with human disease. *Medical Mycology*, *47*(s1), S315–S323. http://doi.org/10.1080/13693780802056038

DeClementi, C. (2007). Chapter 91 - Prevention and treatment of poisoning. In R. C. Gupta (Ed.), *Veterinary Toxicology* (pp. 1139–1158). Oxford: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780123704672501887

DeClementi, C. (2012). Chapter 104 - Prevention and treatment of poisoning. In R. C. Gupta (Ed.), *Veterinary Toxicology (Second Edition)* (pp. 1361–1379). Boston: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B978012385926600096X

De Lorenzi, D., Bonfanti, U., Masserdotti, C., Caldin, M., & Furlanello, T. (2006). Diagnosis of canine nasal aspergillosis by cytological examination: a comparison of four different collection techniques. *Journal of Small Animal Practice*, *47*(6), 316–319. http://doi.org/10.1111/j.1748-5827.2006.00153.x

Devitt, C. M., Cox, R. E., & Hailey, J. J. (2005). Duration, complications, stress, and pain of open ovariohysterectomy versus a simple method of laparoscopic-assisted ovariohysterectomy in dogs. *Journal of the American Veterinary Medical Association*, *227*(6), 921–927.

Dolder, L. K. (2003). Metaldehyde toxicosis. *Veterinary Medicine*, *98*(3), 213–215.

Dunayer, E. K. (2004, January 7). Toxciology Brief: Ibuprofen toxicosis in dogs, cats, and ferrets. Retrieved May 5, 2015, from http://veterinarymedicine.dvm360.com/toxciology-brief-ibuprofen-toxicosis-dogs-cats-and-ferrets

Firth, A. M. (1992). Part 2: Treatment of Snail Bait Toxicity in Dogs: retrospective Study of 56 Cases. *Journal of Veterinary Emergency and Critical Care*, *2*(1), 31–36. http://doi.org/10.1111/j.1476-4431.1992.tb00021.x

Fitzgerald, K. T., Bronstein, A. C., & Flood, A. A. (2006). "Over-The-Counter" Drug Toxicities in Companion Animals. *Clinical Techniques in Small Animal Practice*, *21*(4), 215–226. http://doi.org/10.1053/j.ctsap.2006.10.006

Forrester, M. B., & Stanley, S. K. (2004). Patterns of animal poisonings reported to the Texas Poison Center Network: 1998-2002. *Veterinary and Human Toxicology*, *46*(2), 96–99.

Fröhlich, C. (2008). *Laparoscopic ovariectomy in female dogs: A cooperation between VUW and the University of Samsun*. na. Retrieved from http://www.vetmeduni.ac.at/fileadmin/v/hs/hochschulschriften/diplomarbeiten/AC07121915.pdf

Grave, T. W., & Boag, A. K. (2010). Feline toxicological emergencies: When to suspect and what to do. *Journal of Feline Medicine & Surgery*, *12*(11), 849–860. http://doi.org/10.1016/j.jfms.2010.09.006

Greene, C. E. (2013). *Infectious Diseases of the Dog and Cat*. Elsevier Health Sciences.

Griffiths, C. J. (1984). The determination of metaldehyde in biological material by head-space gas chromatography. *Journal of Chromatography A*, *295*, 240–247. http://doi.org/10.1016/S0021-9673(01)87619-6

Gupta, R. C. (2007). Chapter 47 - Metaldehyde. In R. C. Gupta (Ed.), *Veterinary Toxicology* (pp. 518–521). Oxford: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780123704672501449

Haworth, M. D., & Smart, L. (2012). Use of intravenous lipid therapy in three cases of feline permethrin toxicosis. *Journal of Veterinary Emergency and Critical Care*, *22*(6), 697–702. http://doi.org/10.1111/j.1476-4431.2012.00804.x

Homeida, A. M., & Cooke, R. G. (1982). Anti-convulsant activity of diazepam and clonidine on metaldehyde-induced seizures in mice: effects on brain γ-amino butyric acid concentrations and monoamine oxidase activity. *Journal of Veterinary Pharmacology and Therapeutics*, *5*(3), 187–190. http://doi.org/10.1111/j.1365-2885.1982.tb00429.x

Hornfeldt, C. S., & Murphy, M. J. (1997). Poisonings in animals: the 1993-1994 report of the American Association of Poison Control Centers. *Veterinary and Human Toxicology*, *39*(6), 361–365.

Jackson, T. W., Costin, C., Link, K., Heule, M., & Murphy, M. J. (1991). Correlation of serum ibuprofen concentration with clinical signs of toxicity in three canine exposures. *Veterinary and Human Toxicology*, *33*(5), 486–488.

J Brad Case, S. J. M. (2011). Surgical time and severity of postoperative pain in dogs undergoing laparoscopic ovariectomy with one, two, or three instrument cannulas. *Journal of the American Veterinary Medical Association*, *239*(2), 203–8. http://doi.org/10.2460/javma.239.2.203

Johnson, L. R., Drazenovich, T. L., Herrera, M. A., & Wisner, E. R. (2006). Results of rhinoscopy alone or in conjunction with sinuscopy in dogs with aspergillosis: 46 cases (2001–2004). *Journal of the American Veterinary Medical Association*, *228*(5), 738–742. http://doi.org/10.2460/javma.228.5.738

Jones, R. D., Baynes, R. E., & Nimitz, C. T. (1992). Nonsteroidal anti-inflammatory drug toxicosis in dogs and cats: 240 cases (1989-1990). *Journal of the American Veterinary Medical Association*, *201*(3), 475–477.

Jull, P., Risio, L. D., Horton, C., & Volk, H. A. (2011). Effect of prolonged status epilepticus as a result of intoxication on epileptogenesis in a UK canine population. *Veterinary Record*, *169*(14), 361–361. http://doi.org/10.1136/vr.d4750

Khan, S. A., & McLean, M. K. (2012). Toxicology of frequently encountered nonsteroidal anti-inflammatory drugs in dogs and cats. *The Veterinary Clinics of North America. Small Animal Practice*, *42*(2), 289–306, vi–vii. http://doi.org/10.1016/j.cvsm.2012.01.003

King, L. G., & Boag, A. (2007). *BSAVA Manual of Canine and Feline Emergency and Critical Care*. Wiley.

Kuehn, N. F. (2014). Diagnostic imaging for chronic nasal disease in dogs. *Journal of Small Animal Practice*, *55*(7), 341–342. http://doi.org/10.1111/jsap.12239

Lhermette, P., & Sobel, D. (2008). *BSAVA Manual of Canine and Feline Endoscopy and Endosurgery*. Wiley.

Longstreth, W. T., & Pierson, D. J. (1982). Metaldehyde poisoning from slug bait ingestion. *Western Journal of Medicine*, *137*(2), 134–137.

Magdalou, J., Chajes, V., Lafaurie, C., & Siest, G. (1990). Glucuronidation of 2-arylpropionic acids pirprofen, flurbiprofen, and ibuprofen by liver microsomes. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *18*(5), 692–697.

Mahdi, A., & Van der Merwe, D. (2013). Dog and Cat Exposures to Hazardous Substances Reported to the Kansas State Veterinary Diagnostic Laboratory: 2009–2012. *Journal of Medical Toxicology*, *9*(2), 207–211. http://doi.org/10.1007/s13181-013-0289-8

Mathews, K. (2007). *Veterinary emergency and critical care manual*. Guelph, Ontario: Lifelearn.

Mathews, K., Davidson, A. P., Koblik, P. D., Richardson, E. F., Komtebedde, J., Pappagianis, D., … Kass, P. H. (1998). Comparison of topical administration of clotrimazole through surgically placed versus nonsurgically placed catheters for treatment of nasal aspergillosis in dogs: 60 cases (1990-1996). *Journal of the American Veterinary Medical Association*, *213*(4), 501–506.

Mathews, K., & Sharp, N. (2006). Canine nasal aspergillosis-penicilliosis. *Infectious Diseases of the Dog and Cat. St Louis, MO: Saunders Elsevier*, *613*. Retrieved from http://www.vet.unicen.edu.ar/html/Areas/Documentos/EnfermedadesInfecciosas/2013/Libros/Greene%20-%20Infectious%20diseases%20ot%20the%20Dog%20and%20Cat%203rd%20Ed/64%20Aspergillosis%20and%20Penicilliosis.pdf

Maton, B. L., Simmonds, E. E., Lee, J. A., & Alwood, A. J. (2013). The use of high-dose insulin therapy and intravenous lipid emulsion to treat severe, refractory diltiazem toxicosis in a dog. *Journal of Veterinary Emergency and Critical Care*, *23*(3), 321–327. http://doi.org/10.1111/vec.12053

Matyjasik, H., Adamiak, Z., Pesta, W., & Zhalniarovich, Y. (2011). Laparoscopic procedures in dogs and cats. *Polish Journal of Veterinary Sciences*, *14*(2). http://doi.org/10.2478/v10181-011-0049-0

Medeiros, R. J., Monteiro, F. de O., Silva, G. C. da, & Nascimento Júnior, A. (2009). Poisoning dogs and cats attended in the Veterinarian College of Federal Fluminense University during the period of 2002 to 2008. *Ciência Rural*, *39*(7), 2105–2110. http://doi.org/10.1590/S0103-84782009005000151

Meler, E., Dunn, M., & Lecuyer, M. (2008). A retrospective study of canine persistent nasal disease: 80 cases (1998–2003). *The Canadian Veterinary Journal*, *49*(1), 71–76.

Mills, R. F. N., Adams, S. S., Cliffe, E. E., Dickinson, W., & Nicholson, J. S. (1973). The Metabolism of Ibuprofen. *Xenobiotica*, *3*(9), 589–598. http://doi.org/10.3109/00498257309151547

Moore, A. H., & Ragni, R. A. (Eds.). (2012). Front Matter. In *Clinical Manual of Small Animal Endosurgery* (pp. i–xi). John Wiley & Sons, Inc. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/9781118702826.fmatter/summary

O'Brien, T. Q., Clark-Price, S. C., Evans, E. E., Di Fazio, R., & McMichael, M. A. (2010). Infusion of a lipid emulsion to treat lidocaine intoxication in a cat. *Journal of the*

*American Veterinary Medical Association*, *237*(12), 1455–1458. http://doi.org/10.2460/javma.237.12.1455

Olson, K. R., & California Poison Control System. (2004). *Poisoning & drug overdose*. New York: Lange Medical Books/McGraw-Hill.

Osweiler, G. D., Hovda, L. R., Brutlag, A. G., & Lee, J. A. (2011). *Blackwell's Five-Minute Veterinary Consult Clinical Companion: Small Animal Toxicology*. John Wiley & Sons.

Peeters, D., & Clercx, C. (2007). Update on Canine Sinonasal Aspergillosis. *Veterinary Clinics of North America: Small Animal Practice*, *37*(5), 901–916. http://doi.org/10.1016/j.cvsm.2007.05.005

Peeters, D., Day, M. J., & Clercx, C. (2005). An Immunohistochemical Study of Canine Nasal Aspergillosis. *Journal of Comparative Pathology*, *132*(4), 283–288. http://doi.org/10.1016/j.jcpa.2004.11.002

Plickert, H. D., Tichy, A., & Hirt, R. A. (2014). Characteristics of canine nasal discharge related to intranasal diseases: a retrospective study of 105 cases. *Journal of Small Animal Practice*, *55*(3), 145–152. http://doi.org/10.1111/jsap.12175

Pomrantz, J. S., & Johnson, L. R. (2010). Repeated rhinoscopic and serologic assessment of the effectiveness of intranasally administered clotrimazole for the treatment of nasal aspergillosis in dogs. *Journal of the American Veterinary Medical Association*, *236*(7), 757–762. http://doi.org/10.2460/javma.236.7.757

Pomrantz, J. S., Johnson, L. R., Nelson, R. W., & Wisner, E. R. (2007). Comparison of serologic evaluation via agar gel immunodiffusion and fungal culture of tissue for diagnosis of nasal aspergillosis in dogs. *Journal of the American Veterinary Medical Association*, *230*(9), 1319–1323. http://doi.org/10.2460/javma.230.9.1319

Poortinga, E. W., & Hungerford, L. L. (1998). A case–control study of acute ibuprofen toxicity in dogs. *Preventive Veterinary Medicine*, *35*(2), 115–124. http://doi.org/10.1016/S0167-5877(98)00051-8

Pope, J. F. A., & Knowles, T. G. (2014). Retrospective analysis of the learning curve associated with laparoscopic ovariectomy in dogs and associated perioperative complication rates. *Veterinary Surgery: VS*, *43*(6), 668–677. http://doi.org/10.1111/j.1532-950X.2014.12216.x

Prescott, L. F. (1979). The third Lilly Prize Lecture. University of London, January, 1979. The nephrotoxicity and hepatotoxicity of antipyretic analgesics. *British Journal of Clinical Pharmacology*, *7*(5), 453–462.

Prisco, R. (2002). *Instrumental Laparoscópico* (edição especial) (pp. 11–20). Retrieved from http://www.apurologia.pt/acta/2-2002/Instrum-lap.pdf

Puschner, B. (2006). Chapter 57 - Metaldehyde. In M. E. P. A. Talcott (Ed.), *Small Animal Toxicology (Second Edition)* (pp. 830–839). Saint Louis: W.B. Saunders. Retrieved from http://www.sciencedirect.com/science/article/pii/B0721606393500605

Ramsey, I. (2011). *BSAVA Small Animal Formulary*. Wiley.

Richardson, J. A. (2000). Management of Acetaminophen and Ibuprofen Toxicoses in Dogs and Cats. *Journal of Veterinary Emergency and Critical Care*, *10*(4), 285–291. http://doi.org/10.1111/j.1476-4431.2000.tb00013.x

Richardson, J. A., Welch, S. L., Gwaltney-Brant, S. M., Huffman, J. D., & Rosendale, M. E. (2003). Metaldehyde toxicoses in dogs. *Compendium*, *25*(5), 376–380.

Rothuizen, J. (1985). Laparoscopy in small animal medicine. *Veterinary Quarterly*, *7*(3), 225–228. http://doi.org/10.1080/01652176.1985.9693987

Saunders, J. H., Clercx, C., Snaps, F. R., Sullivan, M., Duchateau, L., van Bree, H. J., & Dondelinger, R. F. (2004). Radiographic, magnetic resonance imaging, computed tomographic, and rhinoscopic features of nasal aspergillosis in dogs. *Journal of the American Veterinary Medical Association*, *225*(11), 1703–1712. http://doi.org/10.2460/javma.2004.225.1703

Saunders, J. H., Duchateau, L., Störk, C., & van Bree, H. (2003). Use of computed tomography to predict the outcome of a noninvasive intranasal infusion in dogs with nasal aspergillosis. *The Canadian Veterinary Journal*, *44*(4), 305–311.

Saunders, J. H., & Van Bree, H. (2003). Comparison of Radiography and Computed Tomography for the Diagnosis of Canine Nasal Aspergillosis. *Veterinary Radiology & Ultrasound*, *44*(4), 414–419. http://doi.org/10.1111/j.1740-8261.2003.tb00478.x

Schochet, R. A., & Lappin, M. R. (2005). Delayed recurrence of nasal aspergillosis in a dog. *The Journal of Small Animal Practice*, *46*(1), 27–30.

Schuller, S., & Clercx, C. (2007). Long-term outcomes in dogs with sinonasal aspergillosis treated with intranasal infusions of enilconazole. *Journal of the American Animal Hospital Association*, *43*(1), 33–8. http://doi.org/10.5326/0430033

Shariati, E., Bakhtiari, J., Khalaj, A., & Niasari-Naslaji, A. (2014). Comparison between two portal laparoscopy and open surgery for ovariectomy in dogs. *Veterinary Research Forum : An International Quarterly Journal*, *5*(3), 219–223.

Sharman, M., Lenard, Z., Hosgood, G., & Mansfield, C. (2012). Clotrimazole and enilconazole distribution within the frontal sinuses and nasal cavity of nine dogs with sinonasal aspergillosis. *Journal of Small Animal Practice*, *53*(3), 161–167. http://doi.org/10.1111/j.1748-5827.2011.01181.x

Sharman, M., & Mansfield, C. (2012). Sinonasal aspergillosis in dogs: a review. *Journal of Small Animal Practice*, *53*(8), 434–444. http://doi.org/10.1111/j.1748-5827.2012.01245.x

Sharp, N., Burrell, M. H., Sullivan, M., & Cervantes-Olivares, R. A. (1984). Canine nasal aspergillosis: serology and treatment with ketoconazole. *Journal of Small Animal Practice*, *25*(3), 149–158. http://doi.org/10.1111/j.1748-5827.1984.tb00462.x

Sharp, N., Harvey, C. E., & O'Brien, J. A. (1991). Treatment of canine nasal aspergillosis/penicilliosis with fluconazole (UK-49,858). *Journal of Small Animal Practice*, *32*(10), 513–516. http://doi.org/10.1111/j.1748-5827.1991.tb00868.x

Sharp, N., & Sullivan, M. (1989). Use of ketoconazole in the treatment of canine nasal aspergillosis. *Journal of the American Veterinary Medical Association*, *194*(6), 782–786.

Sharp, N., Sullivan, M., Harvey, C. E., & Webb, T. (1993). Treatment of Canine Nasal Aspergillosis with Enilconazole. *Journal of Veterinary Internal Medicine*, *7*(1), 40–43. http://doi.org/10.1111/j.1939-1676.1993.tb03167.x

Shih, C.-C., Chang, S.-S., Chan, Y.-L., Chen, J.-C., Chang, M.-W., Tung, M.-S., … Yang, C.-C. (2004). Acute metaldehyde poisoning in Taiwan. *Veterinary and Human Toxicology*, *46*(3), 140–143.

Shintani, S., Goto, K., Endo, Y., Iwamoto, C., & Ohata, K. (1999). Adsorption effects of activated charcoal on metaldehyde toxicity in rats. *Veterinary and Human Toxicology*, *41*(1), 15–18.

Shrestha, B., Reed, J. M., Starks, P. T., Kaufman, G. E., Goldstone, J. V., Roelke, M. E., … Court, M. H. (2011). Evolution of a major drug metabolizing enzyme defect in the domestic cat and other felidae: phylogenetic timing and the role of hypercarnivory. *PloS One*, *6*(3), e18046. http://doi.org/10.1371/journal.pone.0018046

Sissener, T. R., Bacon, N. J., Friend, E., Anderson, D. M., & White, R. a. S. (2006). Combined clotrimazole irrigation and depot therapy for canine nasal aspergillosis. *Journal of Small Animal Practice*, *47*(6), 312–315. http://doi.org/10.1111/j.1748-5827.2006.00017.x

Sparks, S. E., Quistad, G. B., Cole, L. M., & Casida, J. E. (1996). Metaldehyde Molluscicide Action in Mice: Distribution, Metabolism, and Possible Relation to GABAergic System. *Pesticide Biochemistry and Physiology*, *55*(3), 226–236. http://doi.org/10.1006/pest.1996.0052

Steenbergen, V. M. (2004). Toxicology brief: Taking the bait-Metaldehyde toxicosis. *Veterinary Technician*, *25*(4), 259–261.

Studdert, V. P. (1985). Epidemiological features of snail and slug bait poisoning in dogs and cats. - PubMed - NCBI. Retrieved May 27, 2015, from http://www.ncbi.nlm.nih.gov/pubmed/4062739

Sullivan, M. (2008). Rhinoscopy: a diagnostic aid? *Journal of Small Animal Practice*, *28*(9), 839 – 844. http://doi.org/10.1111/j.1748-5827.1987.tb01350.x

Sykes, J. E. (2013). *Canine and Feline Infectious Diseases*. Elsevier Health Sciences.

Talbot, J. J., Johnson, L. R., Martin, P., Beatty, J. A., Sutton, D. A., Billen, F., … Barrs, V. R. (2014). What causes canine sino-nasal aspergillosis? A molecular approach to species identification. *The Veterinary Journal*, *200*(1), 17–21. http://doi.org/10.1016/j.tvjl.2014.01.009

Tasker, S., Knottenbelt, C. M., Munro, E. a. C., Stonehewer, J., Simpson, J. W., & Mackin, A. J. (1999). Aetiology and diagnosis of persistent nasal disease in the dog: a retrospective study of 42 cases. *Journal of Small Animal Practice*, *40*(10), 473–478. http://doi.org/10.1111/j.1748-5827.1999.tb02998.x

Ternamian, A. (2002). The importance of Visual Port Insertion During Laparoscopy. Retrieved from http://www.obgyn.net/gynecological-oncology/importance-visual-port-insertion-during-laparoscopy

Tobias, K. M., & Johnston, S. A. (2013). *Veterinary Surgery: Small Animal: 2-Volume Set*. Elsevier Health Sciences.

Vane, J. R., Bakhle, Y. S., & Botting, R. M. (1998). Cyclooxygenases 1 and 2. *Annual Review of Pharmacology and Toxicology*, *38*(1), 97–120. http://doi.org/10.1146/annurev.pharmtox.38.1.97

Van Goethem, B., Rosenveldt, K. W., & Kirpensteijn, J. (2003). Monopolar versus bipolar electrocoagulation in canine laparoscopic ovariectomy: a nonrandomized, prospective, clinical trial. *Veterinary Surgery: VS*, *32*(5), 464–470. http://doi.org/10.1053/jvet.2003.50052

Van Goethem, B., Schaefers-Okkens, A., & Kirpensteijn, J. (2006). Making a rational choice between ovariectomy and ovariohysterectomy in the dog: a discussion of the benefits of either technique. *Veterinary Surgery: VS*, *35*(2), 136–143. http://doi.org/10.1111/j.1532-950X.2006.00124.x

Vanherberghen, M., Bureau, F., Peters, I. R., Day, M. J., Lynch, A., Fievez, L., … Peeters, D. (2013). Cytokine and transcription factor expression by Aspergillus fumigatus-stimulated peripheral blood mononuclear cells in dogs with sino-nasal aspergillosis. *Veterinary Immunology and Immunopathology*, *154*(3–4), 111–120. http://doi.org/10.1016/j.vetimm.2013.05.009

Villar, D., Buck, W. B., & Gonzalez, J. M. (1998). Ibuprofen, aspirin and acetaminophen toxicosis and treatment in dogs and cats. *Veterinary and Human Toxicology*, *40*(3), 156–162.

Wolf, A. M. (1992). Fungal Diseases of the Nasal Cavity of the Dog and Cat. *Veterinary Clinics of North America: Small Animal Practice*, *22*(5), 1119–1132. http://doi.org/10.1016/S0195-5616(92)50304-7

Xavier, F. G., Kogika, M. M., & de, S. (2002). Common causes of poisoning in dogs and cats in a Brazilian veterinary teaching hospital from 1998 to 2000. *Veterinary and Human Toxicology*, *44*(2), 115–116.

Yas-Natan, E., Segev, G., & Aroch, I. (2007). Clinical, neurological and clinicopathological signs, treatment and outcome of metaldehyde intoxication in 18 dogs. *Journal of Small Animal Practice*, *48*(8), 438–443. http://doi.org/10.1111/j.1748-5827.2007.00360.x

Zonderland, J.-L., Störk, C. K., Saunders, J. H., Hamaide, A. J., Balligand, M. H., & Clercx, C. M. (2002). Intranasal infusion of enilconazole for treatment of sinonasal aspergillosis in dogs. *Journal of the American Veterinary Medical Association*, *221*(10), 1421–1425. http://doi.org/10.2460/javma.2002.221.1421

# Appendix A

Table 1. Surgical cases attended at HVC – Portugal and Medivet Hendon – UK between 01/09/2014 and 15/04/2015, separated according to species, involved system and procedure performed.

| **Surgical Cases** | Dogs | Cats | Total |
|---|---|---|---|
| **Dentistry** | | | 22 |
| -Teeth cleaning/scaling/polishing | 9 | 4 | 13 |
| -Tooth extraction | 5 | 4 | 9 |
| **Dermatological** | | | 21 |
| -Abscess drainage | 2 | 3 | 5 |
| -FB removal | 1 | | 1 |
| -Folds removal (face) | 1 | | 1 |
| -Mass removal | 9 | | 9 |
| -Wound surgical repair | 3 | 2 | 5 |
| **Digestive/Hepatobiliary** | | | 13 |
| -Anal sac (abscess) flush | 3 | 1 | 4 |
| -Cholecystectomy | 1 | 2 | 3 |
| -Enterectomy | 1 | | 1 |
| -Esophagostomy | | 1 | 1 |
| -Gastrotomy | 3 | | 3 |
| -Mass removal (mouth) | | 1 | 1 |
| **Endocrine** | | | 1 |
| -Parathyroidectomy | 1 | | 1 |
| **Genito-Urinary/Reproductive** | | | 70 |
| -Castration | 9 | 14 | 23 |
| -C-section | 1 | 1 | 2 |
| -Cystotomy | 2 | | 2 |
| -Laparoscopic spay | 15 | | 15 |
| -Spay (normal and pyometra) | 6 | 18 | 24 |
| -Unilateral mastectomy | | 1 | 1 |
| -Uretrostomy | | 2 | 2 |
| -Vaginosplasty | 1 | | 1 |
| **Lymphatic** | | | 6 |
| -Popliteal lymph node removal | 1 | | 1 |
| -Splenectomy | 5 | | 5 |
| **Musculoskeletal** | | | 24 |
| -Cruciate rupture/patella luxation repair (TPLO, TTA, Lateral imbrication) | 7 | | 7 |
| -Exploratory laparotomy | 3 | 1 | 4 |
| -Femoral fracture repair | 1 | | 1 |
| -Femoral head ostectomy | | 1 | 1 |
| -Hock fracture repair | 1 | | 1 |
| -JPS | 2 | | 2 |

| | | | |
|---|---|---|---|
| -Mandibular symphysis fractures repair (cerclage wire) | | 5 | 5 |
| -Re-opened spay wound/hernia repair | 2 | 1 | 3 |
| **Neurological/Auditory** | | | 10 |
| -Bulla osteotomy and flush | | 2 | 2 |
| -Hemilaminectomy | 6 | | 6 |
| -Lateral ear canal resection (Bilateral Zepp) | 1 | | 1 |
| -Ventral-slot | 1 | | 1 |
| **Respiratory** | | | 2 |
| -Frontal sinus trephination | 1 | | 1 |
| -Nasal carcinoma removal | | 1 | 1 |
| **Total** | 104 | 65 | 169 |

Source: author

Table 2. Clinical cases attended at HVC – Portugal and Medivet Hendon – UK between 01/09/2014 and 15/04/2015, separated according to species, condition, and affected system.

| **Clinical Cases** | **Canine** | **Feline** | **Total** |
|---|---|---|---|
| **Cardiovascular** | | | 13 |
| -Heartworm | 1 | | 1 |
| -Others (CHF, neoplasia, etc.) | 10 | 2 | 12 |
| **Dermatological/Otologic** | | | 45 |
| -Abscess | 8 | 4 | 12 |
| -Bite wound | 9 | 2 | 11 |
| -Cutaneous myiasis | 1 | | 1 |
| -Other injuries (wounds, lacerations, degloving, etc.) | 3 | 5 | 8 |
| -Others (dermatitis, otitis, otohematoma, neoplasia, etc.) | 11 | 2 | 13 |
| **Digestive/Hepatobiliary** | | | 170 |
| -Anal fistula/traumatic injury | 4 | 1 | 5 |
| -Constipation, Fecaloma | 6 | 3 | 9 |
| -FB (GIT) | 7 | 2 | 9 |
| -Gastroenteritis (different etiologies) | 45 | 22 | 67 |
| -Gingivitis/Stomatitis complex | | 4 | 4 |
| -Hemorrhagic Gastroenteritis (parvovirus, many without definitive diagnosis) | 26 | 3 | 29 |
| -Hepatobiliary affections (hepatitis, cholangiohepatitis, neoplasia, abscess, etc.) | 9 | 6 | 15 |
| -Intestinal volvulus | 1 | | 1 |
| -Oral abscess | 1 | 1 | 2 |
| -Oral or anal injuries | | 2 | 2 |
| -Pancreatitis | 9 | 7 | 16 |
| -Peritonitis | 3 | 1 | 4 |
| -Rectal prolapse | | 2 | 2 |

| | | | |
|---|---|---|---|
| -Others (oral wound, periodontal disease, IBD, etc.) | 4 | 1 | 5 |
| **Endocrine/Metabolic** | | | 26 |
| -Diabetes mellitus/Diabetic Ketoacidosis | 9 | 2 | 11 |
| -Hyperadrenocorticism (Cushing's disease) | 6 | | 6 |
| -Hyperthyroidism | | 7 | 7 |
| -Hypoadrenocorticism (Addison's disease) | 1 | | 1 |
| -Insulinoma | | 1 | 1 |
| **Genitourinary/Reproductive** | | | 70 |
| -Benign prostatic hyperplasia (BPH) | 1 | | 1 |
| -CKD | 8 | 9 | 17 |
| -Cystitis/FLUTD/Blocked urethra | 6 | 29 | 35 |
| -Hydronephrosis | | 2 | 2 |
| -Neoplasia(mammary, prostatic) | 5 | 1 | 6 |
| -Pyelonephritis | | 2 | 2 |
| -Pyometra | 5 | | 5 |
| -Others (post-op castration/spay) | 2 | | 2 |
| **Hemolymphatic** | | | 38 |
| -Ehrlichiosis | 5 | | 5 |
| -Feline infectious anemia(FIA) or feline hemotropic mycoplasmosis (FHM) | | 9 | 9 |
| -Immune-mediated hemolytic anemia (IMHA) | 2 | 1 | 3 |
| -Leukemia (Lymphoid) | 1 | | 1 |
| -Lymphoma (Mediastinal, Multicentric)/other cancer in the lymph nodes | 3 | 2 | 5 |
| -Other Hemolytic Anemias | 2 | 1 | 3 |
| -(Pyo)granulomatous lymphadenitis | 2 | | 2 |
| -Splenic mass/neoplasia/rupture | 8 | 2 | 10 |
| **Intoxication (and Adverse Reactions to drugs)** | | | 32 |
| -Advocate | | 2 | 2 |
| -Alphachloralose | 1 | | 1 |
| -Anticoagulant Rodenticide | 1 | | 1 |
| -Carprofen | 1 | | 1 |
| -Chocolate | 4 | | 4 |
| -Ethylene glycol (antifreeze) | | 2 | 2 |
| -Fentanyl (Recuvyra®) | 1 | | 1 |
| -Ibuprofen | 2 | 1 | 3 |
| -Lilies | | 2 | 2 |
| -Metaldehyde | 1 | | 1 |
| -Paracetamol (acetaminophen) | 3 | | 3 |
| -Pine processionary | 1 | | 1 |
| -Propofol (perivascular) | | 1 | 1 |

| | | | |
|---|---|---|---|
| -Pyrethroid | 1 | | 1 |
| -Raisins | 4 | | 4 |
| -Synulox | | 1 | 1 |
| -Vaccination (adverse reaction to Leishmune®) | 2 | | 2 |
| -Varnish | | 1 | 1 |
| **Musculoskeletal/Auditory** | | | 65 |
| -Amputation | 3 | | 3 |
| -Dysplasia (hip, elbow) | 5 | | 5 |
| -Evisceration | 1 | | 1 |
| -Femoral head ostectomy (FHO) | 1 | 1 | 2 |
| -Fractures (limbs) | 7 | 5 | 12 |
| -Hernia (inguinal, umbilical) | 2 | | 2 |
| -Lateral ear canal resection (Bilateral Zepp) | 1 | | 1 |
| -Osteoarthritis (degenerative joint disease -DJD) | 4 | 2 | 6 |
| -Osteosarcoma | 2 | 1 | 3 |
| -Patella luxation/cruciate rupture | 19 | | 19 |
| -Others (lameness) | 6 | 5 | 11 |
| **Neurological/Neuromuscular** | | | 59 |
| -Brain tumor | 3 | | 3 |
| -Cerebellar ataxia | 1 | 1 | 2 |
| -Cognitive Dysfunction | 1 | | 1 |
| -Intervertebral Disk Disease (IVDD) | 21 | 2 | 23 |
| -Meningitis | 2 | | 2 |
| -Seizures (many etiologies) | 16 | 6 | 22 |
| -Spinal trauma | | 2 | 2 |
| -Vestibular disease | 1 | 3 | 4 |
| **Ophthalmological** | | | 11 |
| -Corneal ulcer | 4 | 2 | 6 |
| -Glaucoma | 2 | | 2 |
| -Hyphema | 1 | | 1 |
| -Retrobulbar neoplasia | 2 | | 2 |
| **Respiratory** | | | 34 |
| -Angiostrongylosis | 1 | | 1 |
| -Aspergillosis (sino-nasal) | 1 | | 1 |
| -Feline asthma/Chronic bronchitis | | 3 | 3 |
| -Flail chest | 2 | | 2 |
| -Nasal discharge (undetermined cause) | 2 | | 2 |
| -Neoplasia/nodular pattern in the lungs | 2 | 2 | 4 |
| -Pleural effusion | 1 | 5 | 6 |
| -Pneumonia | 5 | 2 | 7 |
| -Pulmonary contusion | 1 | 1 | 2 |

| | | | |
|---|---|---|---|
| -Respiratory distress (undetermined cause) | | 4 | 4 |
| -Tracheal collapse | 2 | | 2 |
| **R.T.A.** | 7 | 18 | 25 |
| **Others** (multi-system, medical border, etc.) | 16 | 43 | 64 |
| -Feline infectious peritonitis (FIP) | | 2 | 2 |
| -FHV-1 infection | | 2 | 0 |
| -Leishmaniasis | 3 | | 3 |
| **Total** | 394 | 258 | 652 |

Source: author