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DIAGNOSTIC VALUE OF MRI IN DOGS WITH INFLAMMATORY NASAL DISEASE

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This Master project was carried out in the Department of Diagnostic Imaging, at the Queen's Veterinary School Hospital, Cambridge Veterinary School, University of Cambridge from 21th of September 2009 to the 30th of April 2010.

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Congress Presentation

Part of the results of this research will be presented in the *Southern European Veterinary Conference* (Barcelona from 30 September to 3 October, 2010) in both abstract and poster formats.

DIAGNOSTIC VALUE OF MRI IN DOGS WITH INFLAMMATORY NASAL DISEASE

This study determines the value of low-field magnetic resonance imaging in differentiating nasal aspergillosis from chronic rhinitis in dogs. The Queen's Veterinary School Hospital magnetic resonance imaging database (2002-2009) was searched for dogs that had undergone MRI of the nasal cavity. Forty-one cases were included of which twenty five were classified as Rhinitis and sixteen as Aspergillosis. On MRI, destruction of the turbinates was classified as mild, moderate or severe. The cribriform plate and vomer/nasal septum destruction were classified as present or absent as well as the involvement of the frontal sinus. Images were examined to assess the signal intensity of mucus and turbinates and classified as hypointense, hyperintense and isointense on the brightest area on the same slice. On T_1W images, the intensity was compared with muscle and on T_2W images with periorbital fat and brain.

Turbinate destruction was statistically associated (p=0.005) with aspergillosis. Most of the Rhinitis cases (48%) had no turbinate destruction but a significant number had mild destruction (40%). Six Aspergillosis cases (37.5%) had moderate turbinate destruction and only three cases (18.8%) had severe destruction. Cribriform plate and vomer/nasal septum destruction, although not statistically associated with any pathology, were more frequent in Aspergillosis. There was no statistical association with Rhinitis or Aspergillosis with respect to frontal sinus involvement. On T_1W scans, Aspergillosis was associated with turbinate hyperintensity, whilst Rhinitis was associated with turbinate hypointensity (p=0.007). On T_2W scans, the turbinate intensity was not statistical significant with Rhinitis or Aspergillosis, however the majority (60%) of Rhinitis cases exhibited hypointense turbinates, whereas the majority (56.3%) of Aspergillosis cases had isointense turbinates. On T_1W, mucus intensity was not statistical associated with Rhinitis or Aspergillosis, but it was noticed that mucus hyperintensity was the most significant feature in both groups. On T_2W scans, there was no statistical significance when comparing with fat and brain, with mucus, nonetheless it was noted that the majority of cases showed hyperintense mucus. It was concluded that T_1W scans provided more information since the turbinate hyperintensity seen was associated with Aspergillosis while hypointensity was with Rhinitis. On T_2W images relevant differences were seen but these were not associated with specific pathology.

Keywords: Aspergillosis; Rhinitis; MRI; T_1W; T_2W; Mucus; Turbinates

VALOR DIAGNÓSTICO DA RESSONÂNCIA MAGNÉTICA NUCLEAR EM DOENÇAS INFLAMATÓRIAS NASAIS NO CÃO

Este estudo pretende determinar o valor da ressonância magnética nuclear (RMN) de baixo campo no diagnóstico diferencial entre aspergilose e rinite crónica em cães. Foram pesquisados registos de cães que realizaram RMN da cavidade nasal no Queen's Veterinary School Hospital em Cambridge. A população era constituída por 41 casos dos quais 25 foram classificados como Rinite e 16 como Aspergilose. Nas imagens de RMN, a destruição das conchas nasais foi classificada como ligeira, moderada e grave. A destruição da placa cribiforme e do vómer/septo nasal e o envolvimento do seio frontal foram classificadas como presente ou ausente. Analisaram-se as imagens para avaliar a intensidade do muco e das conchas nasais, que foram classificados como hipointensas, hiperintensas ou isointensas na zona mais brilhante do mesmo corte. Nas imagens de T_1W, a intensidade foi comparada com o músculo e nas imagens em T_2W com a gordura periorbital e cérebro. Verificou-se que a destruição das conchas nasais estava estatisticamente associada com a Aspergilose (p=0.005). A maior parte dos casos de Rinite (48%) não apresentavam destruição das conchas nasais, no entanto um número considerável tinha uma destruição ligeira (40%). Seis casos (37.5%) de Aspergilose tinham uma destruição moderada das conchas nasais e só três casos (18.8%) tinham destruição grave. Apesar de a destruição da placa cribriforme e do vómer/septo nasal se ter observado com mais frequência nos casos de Aspergilose, não se demonstrou qualquer associação estatisticamente significativa. Também não se observou associação estatisticamente significativa entre qualquer das duas patologias e o envolvimento do seio frontal. Em T_1W, a Aspergilose estava estatisticamente associada com a hiperintensidade das conchas nasais, enquanto a Rinite estava associada com a hipointensidade das mesmas (p=0.007). Em T_2W, não se observou nenhuma associação com significado estatístico entre a intensidade das conchas nasais e qualquer das duas patologias, contudo a maioria dos casos de Rinite (60%) apresentava conchas nasais hipointensas, enquanto a maioria dos casos de Aspergilose (56.3%) tinha conchas nasais isointensas. A intensidade do muco em T_1W, não estava estatisticamente associada a nenhuma das patologias, de facto a hiperintensidade do muco foi a característica mais frequentemente encontrada em ambos os grupos. Quando foi comparada a intensidade do muco em T_2W com a gordura e o cérebro, também não foram encontradas quaisquer associações estatisticamente significativas, sendo de realçar que a maioria dos casos apresentava muco hiperintenso. Em conclusão, pode-se afirmar que as imagens em T_1W têm mais valor diagnostico que as de T_2W, uma vez que se descreveram associações estaticamente significativas nesta sequência.

Palavras-chave: Aspergilose; Rinite; RMN; T_1W; T_2W; Muco; Conchas Nasais.

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Abbreviations and symbols

AGDD – Agar-Gel Double ImmunoDiffusion
AR- Allergic Rhinitis
BID – *bis in die*
CD – Cluster of Differentiation
CNS- Central Nervous System
CSF – Cerebrospinal Fluid
CT – Computed tomography
ELISA – Enzyme-Linked Immuno Sorbent Assay
FeLV – Feline Leukaemia Virus
FIP – Feline Infectious Peritonitis
FLAIR – Fluid Attenuation Inversion Recovery
IDST – Intradermal skin test
IFN - Interferon
Ig – Immunoglobulin
Il - Interleukin
Kg – Kilograms
LPR – Lymphoplasmacytic rhinitis
MHC – Major Histocompatibility Complex
Ml - Milliliters
MR – Magnetic Resonance
MRI- Magnetic Resonance Imaging
NSAID- Non Steroidal Anti-Inflammatory Drug
PO – *Per os*
QVSH – Queen's Veterinary School Hospital
RF – Radiofrequency
SE – Spin Echo
SID – *Semel in die*
SP – Species
SPSS - Statistical Package for the Social Sciences
STIR – Short Time Inversion Recovery
TE – Echo time
TGF – Transforming growth factor
TR – Repetition Time

1 Externship Report

The externship was done at the Queen's Veterinary School Hospital (QVSH), Department of Veterinary Medicine of the University of Cambridge, between the 21th of September 2009 and the 30th of April 2010 (41 weeks).

The QVSH is a teaching and referral hospital with a national and international reputation as a centre for clinical excellence. It offers referral and advice in the areas of small animal studies (orthopaedics, soft tissue surgery, internal medicine, ophthalmology, oncology, neurology and anaesthesia and intensive care), farm animal studies, equine studies and diagnostic imaging (radiography, ultrasound, MRI and scintigraphy).

Diagnostic imaging department

The diagnostic imaging department offers the following activities:

- Three dedicated radiography suites for imaging small and large animals
- A digital radiography system
- Digital image intensification for functional studies and interventional radiology, e.g. balloon valvuloplasty and pacemaker implantation
- High resolution ultrasound machines for imaging small and large animals including echocardiography
- Scintigraphy and nuclear medicine used mainly for equine patients, but also occasionally for small animals
- A dedicated magnetic resonance imaging for small animals as well as magnetic imaging for standing horses. Indications for small animal MRI include: neurological and spinal disease, tumour assessment for surgical or radiotherapy planning, evaluation of nasal disease, obscure lameness and discharging sinuses.

Thirty one of the 41 weeks spent at the QVSH, were at the diagnostic imaging department. The clinical staff present during the externship was: Michael Herrtage, Barbara Posh, Valentina Piola, Abby Caine, Nicholas Rousset and Julie Sales.

The following activities were done:

- Assisted at several seminars, including orthopaedics, hip and elbow dysplasia, skull, abdominal and thoracic imaging.
- Participated in morning rounds, in which x-rays and MRI scans from the day before were evaluated and discussed by board-certified specialists.
- Studied different positioning techniques in dogs and cats.

- Was able to see several contrast studies on x-rays and under fluoroscopy.
- Trained ultrasonographic techniques, learnt how to make differentials based on ultrasonographic results, and assisted several ultrasonographic guided biopsies and fine-needle aspirates.
- Several pathologies were seen on MRI like: Chiari-like malformation and Syringomyelia in the Cavalier King Charles Spaniel, spinal disc herniation and brain tumours.
- Other activities assisted included: echocardiography, joints and tendons ultrasonography and myelography.
- All the x-rays and MRI scans from 2000 were available for consult during all the stay at the hospital

Small animal hospital

During the externship I was able to rotate through different areas of small animal medicine. I spent 2 weeks on neurology, 2 weeks on internal medicine, 1 week on oncology, 1 week in soft tissue surgery and 1 week in orthopaedic surgery. Besides this I did 20 night-shift (12 hrs), in which I was able to follow and study medications for intensive and critical care, as well as study pain management in small animals.

Since the hospital is open 24 hours, I was also able to help the interns during emergencies such as intoxications, bites, road-traffic accidents and seizures. The following activities were done, during the weeks at the small animal hospital:

- **Neurology**

During the two weeks in neurology, I assisted morning rounds, consults, imaging, surgeries and treatment of dogs with neurologic deficits. The neurology clinical staff, with whom I was able to work, included Nick Jeffery, An Vanhaesebrouck, John Parker and Tom Harcourt-Brown.

During consults I was able to learn how to do a neurologic exam and what further exams we should do to an animal with neurologic deficits. The main pathologies seen were: *Myastenia Gravis*, Lafora disease, idiopathic epilepsy, chronic seizures, atlanto-axial subluxation, Cavalier King Charles falling syndrome, spinal disc herniation, chiari malformation, brain tumours, hydrocephalus and epilepsy/narcolepsy in horses.

The surgeries seen included ventral slot, dorsal laminectomy and stabilisation of an atlanto-axial subluxation by a transarticular screw fixation.

- **Internal Medicine**

During the 2 weeks in the internal medicine department I was able to do consults, admit patients at the hospital, write referral letters and participate on morning and afternoon rounds. I studied pathophysiology and treatment of cases, such as *diabetes mellitus*, hypoadrenocorticism, hyperadrenocorticism, aspergillosis, renal insufficiency (chronic and acute), pancreatitis, leptospirosis, hypertiroidism, cardiomyopathies, rhinitis, urolithiasis and immune-mediated diseases. I was also able to see 2 rhinoscopy procedures and 1 pace-maker implantation.

I had the opportunity to work with Michael Herrtage, Penny Watson, Barbara Skelly, Mark Reading, Ben Harris and Allison Collings.

- **Oncology**

The oncology department facilities include orthovoltage and megavoltage radiotherapy and radiation treatment planning assisted by MRI, chemo- and photodynamic therapy for animals with very superficial carcinomas. The clinical staff included: Jane Dobson, Malcolm Brearley and Frances Taylor

During the week in the oncology department, I was able to see consults (first or check-ups) and assist and help during radiotherapy and chemotherapy sessions. Some of the pathologies seen were epitheliotropic cutaneous lymphoma, mast cell tumour, nasal tumours, multicentric lymphoma, osteosarcoma, haemagiosarcoma, glioma, transitional cell carcinoma, histiocytic sarcoma and anal sac gland carcinoma.

- **Soft Tissue Surgery**

On soft tissue surgery, I participated on several surgeries such as spays and castrations, epulis removal, dental surgery on a rabbit, removal of a pancreatic limb due to an insulinoma, removal of an infiltrative lipoma and wound closures. Besides morning and afternoon rounds, I learn how to do dressings and how to do pain scores in surgical patients. During the week, I worked with Jane Ladlow, Jackie Demetriou and Graham Hayes.

- **Orthopaedics**

During the week that I spent in orthopaedics, I participated in morning and afternoon rounds, did consults and referral letters and evaluated post-operative x-rays. I was also able to participate on surgeries, such as total hip replacement, TPLO, distal femoral osteotomy and fracture reduction with locking plates. The clinical staff with whom I worked was Sorrel Langley-Hobbs and Ian Nicholson.

The student	The supervisor
	
(Ana Rita Furtado)	(Michael Herrtage)

2 Literature review

2.1 Introduction

Respiratory system diseases are considered to be common and important causes of morbidity and mortality in animals and humans, mainly because the respiratory tract is in direct contact with the physical environment and is exposed to airborne microorganisms such as viruses, bacteria, fungi and parasites. The nose is the first part of the respiratory tract and is more likely to be subjected to these insults. Clinicians of all the veterinary fields are daily contacted to diagnose and treat respiratory tract disorders.

Aspergillosis and lymphoplasmacytic rhinitis are the most common inflammatory nasal diseases, and because of that their correct diagnose is essential. With the increased availability of non-invasive diagnostic techniques, such as magnetic resonance, it has started to be used more often to evaluate animals with nasal disease.

The purpose of this chapter is: i) to provide background information about the most common nasal inflammatory diseases in dogs, ii) to review the salient literature about nasal imaging on dogs, iii) to provide recent knowledge about other ancillary diagnostic methods and treatment and lastly iv) to draw conclusions from the literature review, to set the research aims of the present study.

2.2 Respiratory system general considerations

Respiration is essential for all the animals, including humans, since it enables gas exchange between the external environment and a circulatory system. Animals have adapted differently in order to perform such exchanges, for instance, in amphibians besides lungs, the skin plays a vital role in gas exchange, birds have a unique organ called air sacs which besides gas exchanging also helps balancing during the flight, reptiles do not possess a diaphragm, instead intercostal muscles are the responsible for breathing movements. Insects possessed a series of external openings called spiracles, and the gas exchanges are performed by simple diffusion in a tracheal system and fishes use the oxygen in water, in order to survive, in most of them the gas exchanges are performed through the gills.

For mammals, the respiratory system is essential thus it allows gas exchange through all the parts of the body and it will be discussed in more detail in the following chapters.

Based on anatomical features the respiratory system can be divided into upper respiratory tract (nasal passages, pharynx and larynx) and lower respiratory tract (trachea, bronchi, bronchiole, and lungs). For this study, the author decided to adopt a classification based on physiology and function, which can be divided into conducting, transitional and gas exchange

systems (López, 2007). The conducting system includes the nasal cavity, paranasal sinuses, pharynx, larynx, trachea and extrapulmonary and intrapulmonary bronchi, all of them lined by a pseudostratified ciliated columnar cells plus a variable proportion of secretory goblet and serous cells (López, 2007).

The nasal cavity is the facial portion of the respiratory passage way, and it's composed of bony and cartilaginous parts and extends from the nostril to the choanae (Haagen & Herrtage, 2010) and apart from olfaction it has the important function of modifying the incoming air before presented to the lower respiratory passages (Dyce, Sack, & Wensing, 2010). Dilation of the nostrils changes the pattern of flow of inspired air, so to sample environmental odours, the nostrils are dilated, and forced inspiration occurs, allowing the air to flow around the ethmoidal conchae, where the olfactory receptors are situated (Haagen & Herrtage, 2010).

The canine nasal cavity lacks organized mucosal lymphoid aggregates but does have diffuse lymphoid populations. IgA plasma cells predominate over IgM and IgG plasma cells within the nasal mucosa, and are particularly distributed around glandular tissue (Day, 2009).

Mast cells are also prominent within in the nasal mucosa (beneath mucosal epithelium) and are present in greater number than in the lower respiratory tract. Similarly, there are significant numbers of antigen presenting cells such as CD1 (dendritic cell marker) and MHC. T cells are, also more prominent within the nasal mucosa of adult dogs compared with puppies (Day, 2009). All these observations are consistent with the greater exposure of the nasal mucosa to inhaled antigens.

The paranasal sinuses are diverticula of the nasal cavity that excavate the skull bones, largely after birth (Dyce, et al., 2010). Although the function of the paranasal sinus is still obscure, in the literature they are considered to offer thermal and mechanical protection to the rostral portions of the brain, orbits and nasal cavities (Dyce, et al., 2010; Haagen & Herrtage, 2010).

The larynx forms a cartilaginous connection between the pharynx and the tracheobronchia tree, and was originally developed as a device to protect the lower respiratory passages against inundation, and although this remains as its primary role, phonation is also a role to be considered (Dyce, et al., 2010). Trachea and bronchi (tracheobronchial tree) form a continuous system of tubes conducting the air between the larynx and bronchioles (Dyce, et al., 2010).

The transitional system of the respiratory tract is constitute by the bronchioles (primary, secondary and tertiary), which serve as a transition zone between the ciliated conducting system and the gas exchange system. In this system, goblet cells are replaced by neuroendocrine and Clara cells, which play an important role in detoxification of xenobiotics (López, 2007).

Alveolar ducts and alveoli form the gas exchange system; the last ones are superficially lined by two types of cells: type I pneumocytes and type II pneumocytes (López, 2007).

The portals of entry of pathogens, allergens and toxic substances can be divided into three major groups: aerogenous, haematogenous and direct extension. The aerogenous way is the most common route in transmission of most infections in domestic animals, through it pathogens, toxic gases and foreign particles such as food can gain access to the respiratory system via inspired air (López, 2007). The haematogenous way is commonly seen in septicaemias, sepsis, protozoal infections and viruses that target endothelial cells (López, 2007) and the direct extension is less common but occurs when the pathogens reach the pleura and lungs through penetrating injuries (López, 2007).

Mechanisms of defence are closely related to the mechanisms of mucosa cleaning, so they will be approached together as a single mechanism. The final goal of this mechanism is to keep the lungs sterile, preventing insoluble and soluble particles, allergens and bacteria reaching the lower respiratory tract.

This mechanism can be divided into two phases: the deposition and the clearance (López, 2007). Deposition is the process by which particles are trapped in the pseudostratified mucosa in the nose, which consists of ciliated cells, intermediate cells, basal cells and goblet cells, with the tall ciliated cells with microvilli the most predominant type (Haagen & Herrtage, 2010). Clearance is the process by which those particles are destroyed, neutralized, or removed from the mucosal surfaces (López, 2007), being the principal mechanisms involved the sneezing reflex (stimulation of the sensory receptors in the nasal mucosa) (Haagen & Herrtage, 2010), the coughing reflex, phagocytosis and the propulsive movement of mucociliary cells, that transports the mucus blanket with insoluble particles towards the pharyngeal end of the oesophagus, the soluble particles are caught in the periciliary area and are absorbed (Haagen & Herrtage, 2010).

In addition to this, other cells contribute to the defence mechanism like: M cells (microfold cells), which are modified epithelial cells covering the bronchial associated lymphoid tissue (BALT) and antigen-presenting cells (APC's) like macrophages and dendritic cells (López, 2007).

Immunoglobulin (Ig) A, IgG and IgM, produced by mucosal plasma cells, plays an important role in local immunity of the conducting system, especially in preventing attachment of pathogens to the mucociliary blanket (López, 2007).

Alveoli lack ciliated and mucus-producing cells but instead its defence is provided by the pulmonary alveolar macrophages, which attach bacteria and any other particle that reaches the alveolar region (López, 2007). These particles are then phagocytised and discriminated

between “self” and “foreign” antigens, by the Fc receptors on the surface of macrophages like: complement receptors (C3b, C3a, C5a), tumour necrosis factor (TNF) and CD40 receptors (López, 2007).

2.3 Anatomy of the nasal passages of the dog

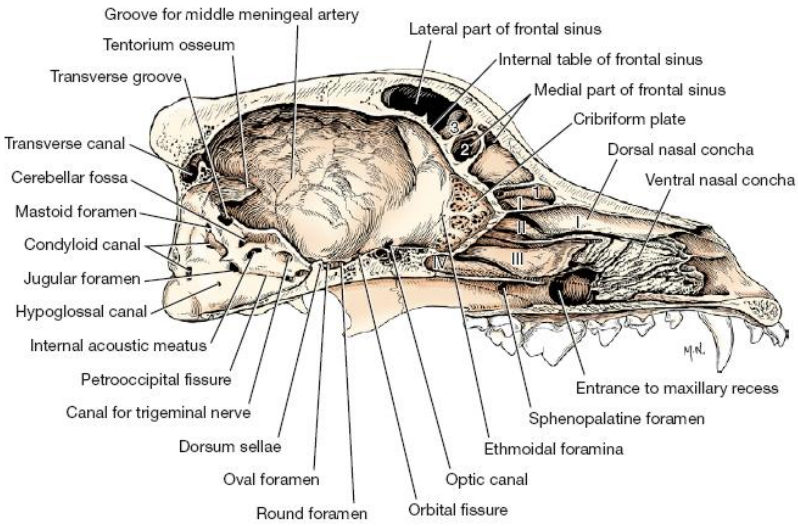
The domestic dog (*Canis familiaris*) has a big variation in body size and skull size, which comes in more shapes than any other mammal (Evans, 1993). Generally, the canine skull is classified according to its shape: dolichocepahic if it is long and narrow, like Border Collies; brachycephalic if it is short and wide, like Boxer, and mesaticephalic if it has medium proportions like Labrador Retriever (Evans, 1993).

The nose in the broad sense comprises the external nose, the paired nasal cavities, and the paranasal sinuses (Dyce, et al., 2010). Within the nasal cavity are three sets of nasal conchae (dorsal, ventral and ethmoidal), which are cartilaginous or slightly ossified scrolls covered with nasal mucosa (Harcourt-Brown, 2006a). The dorsal nasal concha is the first endoturbinate and the longest. It arises from the dorsal part of the cribriform plate caudally as well as from the medial part of the roof plate (Evans, 1993), and is formed by a single curled scroll of bone (Harcourt-Brown, 2006a). The second endoturbinate, is the ventral nasal concha, it arises from its basal lamina near the middle of the lateral lamina (Evans, 1993), and it is divided by a series of tightly folded scrolls (Harcourt-Brown, 2006a). The ethmoidal conchae occupy the caudal part of the nasal cavity and consist of a number of bony scrolls that are into two groups: ectoturbinates and endoturbinates (Harcourt-Brown, 2006a), that viewed from the medial side have the same general form as the second endoturbinates (Evans, 1993). The ethmoidal conchae are attached to the cribriform plate and extend dorsally into the frontal sinus (Harcourt-Brown, 2006a).

The frontal sinus, is divided into rostral, medial and lateral compartments, and is localized dorsocaudal to the nasal meati (dorsal, middle and ventral) (Harcourt-Brown, 2006a).

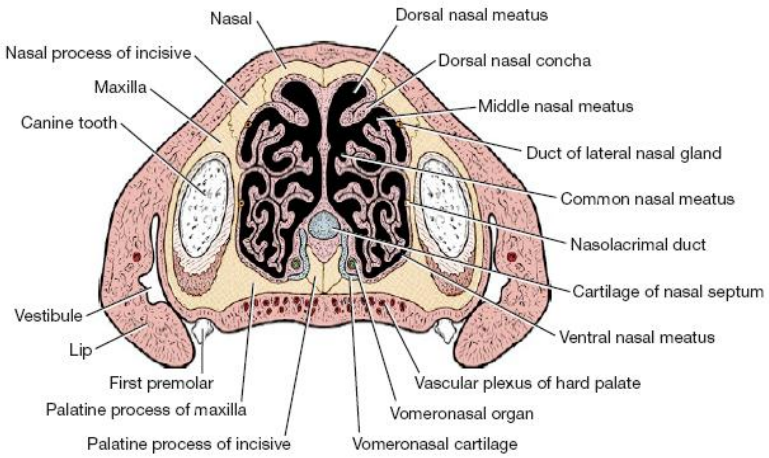
The cribriform plate is part of the ethmoid bone and articulates ventrally with the presphenoid to form the spheno-ethmoid suture and with the vomer bone to form the vomero-ethmoid suture (Evans, 1993).

Figure 1 - Saggital section of the skull, medial view



Roman numerals *I, II, III, IV* indicate endoturbinates in the nasal cavity; Arabic numerals *1, 2, 3* are ectoturbinates located in the frontal sinus (Adapted from Evans & Lahunta, 2010).

Figure 2 – Transverse section of the nasal cavity.



(Adapted from Evans & Lahunta, 2010)

2.4 Importance of clinical history in dogs in nasal disease

The medical history of a dog with a disease of the nasal cavity and paranasal sinuses is very important, since nasal disease signs like depigmentation, inflammation, crust formation, dehydration and hyperkeratosis are usually noted by the owner (Haar, 2006).

The reported presence or absence of epistaxis, sneezing, stertor, coughing, gagging, facial deformity and pain, mouth breathing, systemic signs of illness and in rare instances central nervous system give much useful information (Hawkins, 2009; Sullivan, 1987).

The susceptibility of the breed, sex and age, should also be considered by the clinician. The literature shows that dolicocephalic and mesaticephalic breeds are prone to intranasal disease but brachycephalic breeds are less affected (Knotek, Fichtel, Kohout, & Benák, 2001;

Mathews & Sharp, 2006; J. Saunders, et al., 2004; Sharp, Sullivan, & Harvey, 1992), maybe because the latter is mainly a mouth-breathing breed and has less nasal tissue (Sullivan, 1987). The same study shows that the knowledge of the age range of the most frequent diseases (nasal neoplasia, aspergillosis and chronic hyperplastic rhinitis) can be helpful (Sullivan, 1987). The majority of nasal tumours occur in older dogs, while aspergillosis and chronic hyperplasic rhinitis are found in younger dogs (Sullivan, 1987; Tasker, et al., 1999). Male dogs appeared to be at greater risk than female dogs (J. Saunders, et al., 2004). It is also important to determine the duration of signs, whether clinical signs are static or evolving, if the patient has ever exhibited the presenting signs before, if treatment was initiated in the past and the success or failure of such treatment (Padrid, 2007).

Additional questions should be asked about the animal´s general condition (Haagen & Herrtage, 2010) like exercise intolerance, food habits, drinking and other changes in habits.

Questions about the environment and geographic origin can also be useful. It has been described in the literature that questions concerning the habits of the owners can sometimes bring some important information like if there is any smoker in the house, changed carpeting recently, or any house significant renovation in the last 6 months (Padrid, 2007). Travel history should also be assessed, to determine if the pet has an increased risk of diseases such as mycotic infection or heartworm (Padrid, 2007). It is also reported that aspergillosis affects more frequently farm dogs, with no specific access to heavy concentrations of fungal spores (Sullivan, 1987).

2.5 Clinical signs of nasal disease in the dog

Clinical sings related to the upper respiratory tract, are among the most common presenting complaints encountered in small animal practice and are frequent reasons for referral to specialty practices and veterinary teaching hospitals (Ford, 2005). The following paragraphs are a brief enumeration and explanation of the most common signs of nasal disease.

2.5.1 Nasal discharge

It is most commonly associated with disease localized within the nose, nasal cavity and paranasal sinuses, although it may also develop with disorders of the lower respiratory tract (Ford, 2005), such as bacterial pneumonia and infectious tracheobronchitis, or systemic disorders such as coagulopathies and systemic hypertension. It is reported that discharge occurring only when sneezing indicates a less productive mucosal disease than does continuous discharge (Haagen & Herrtage, 2010).

Based on the nature of the nasal discharge, rhinitis can be classified as serous, which may be seen initially with a variety of nasal diseases (Kuehn, 2009) but can also be normal (Hawkins, 2009), catarrhal which is a slightly more severe sign, and its characterized by an increased of mucus production due to hyperplasia of goblet cells (López, 2007). Purulent or mucopurulent implies inflammation (Hawkins, 2009) and secondary bacterial invasion (Kuehn, 2009) and a neutrophilic exudates occurs (López, 2007), fibrinous when there is an increase of vascular permeability, resulting in exudation of plasma fibrinogen, which coagulates into fibrin and granulomatous when infiltration of numerous active macrophages and lymphocytes occurs, and it is characteristic of chronic allergic inflammation and nasal fungal infection (López, 2007).

The profuse, purulent nature of the discharge in aspergillosis helps to differentiate this condition from nasal neoplasia in which the discharge tends to be more serous and intermittent (Sharp, et al., 1992).

2.5.2 Epistaxis

A previous study (Bissett, Drobatz, McKnight, & Degernes, 2007) describes that owners and veterinarians see epistaxis episodes as an emergency. Epidemiologically it was seen in the same study, that epistaxis was more likely to occur in old dogs (≥ 6 years old), male, and large (≥ 26 Kg.) breeds.

It has been commonly suggested in the veterinary literature that epistaxis is most often a result of local disorders, unilateral, chronic and associated with other nasal tract signs (Bissett, et al., 2007; Dhupa & Littman, 1992). However, a recent study (Mylonakis, et al., 2008), reported systemic disorders to be the most common cause of epistaxis.

When epistaxis is a result of a systemic disorder is said to be bilateral and acute, although it was shown that unilateral epistaxis was not pathognomonic for a local disorder (Bissett, et al., 2007).

Epistaxis can be associated with mucopurulent exudates from any aetiology but when prolonged is usually associated with trauma, local aggressive diseases like neoplasia or mycotic infection, systemic hypertension or bleeding disorders (Hawkins, 2009).

One of the most well-known causes of epistaxis, in dogs, is trauma (Bissett, et al., 2007; Dhupa & Littman, 1992; Gough, 2007), due to the fractures of the maxillae or displaced nasal bones fragments that contribute to the occlusion of the airway (Chandler, Thompson, Sutton, & Price, 1991). However, until the present date, there were no published studies that related trauma with epistaxis in dogs.

Local disease processes have been reported to be a common cause of epistaxis in dogs. Previous studies showed that nasal and paranasal neoplasia (Bissett, et al., 2007; Strasser & Hawkins, 2005) like adenocarcinoma, lymphoma and squamous cell carcinoma (Gough, 2007) and mycotic rhinitis (Bissett, et al., 2007; Mathews, et al., 1998; Strasser & Hawkins, 2005) due to *Aspergillus* spp. (Gough, 2007; Tasker, et al., 1999) have long been regarded as the most important causes.

Other causes of local diseases included idiopathic rhinitis (Bissett, et al., 2007; Strasser & Hawkins, 2005; Windsor, Johnson, Herrgesell, & Cock, 2004), parasitic rhinitis (Strasser & Hawkins, 2005), nasal foreign body (Bissett, et al., 2007), periapical abscesses (Bissett, et al., 2007; Gough, 2007), and arteriovenous malformation. (Bissett, et al., 2007; Hawkins, 2009)

Systemic disorders previously reported to cause epistaxis in dogs include hereditary/congenital diseases like immune-mediated thrombocytopenia (Mylonakis, et al., 2008; Tilley & Smith, 2007) and von Willebrand disease (Hawkins, 2009; Tilley & Smith, 2007), and various systemic infections like canine distemper virus (Gough, 2007), leishmaniasis, monocytic ehrlichiosis (Mylonakis, et al., 2008) and babesiosis (Tilley & Smith, 2007). Other less common causes reported are rodenticide toxicity and systemic arterial hypertension (Hawkins, 2009; Mylonakis, et al., 2008).

2.5.3 Stertor/reverse-sneezing and stridor

The second most common clinical sign associated with upper respiratory disease in dogs is stertor, which can be intermittent, yet persistent or continuous snorting, also called stertorous breathing (Ford, 2005), caused by air passing through a narrowed nasopharynx, pharynx or trachea and meeting resistance because of partial obstruction of these regions (Tilley & Smith, 2007). Reverse-sneezing is characterized by paroxistical stertous, which is believed to be a patient's attempt to displace matter trapped in the nasopharynx and move it into the oropharynx to be swallowed (Ford, 2005).

Stridor is a common sign for obstructive nasal disease (Haagen, 2009) and it is characterized by a higher-pitched sounds that result when rigid tissues are vibrated by the passage of air, usually is a result of nasal or laryngeal partial or complete obstruction (Haagen & Herrtage, 2010; Tilley & Smith, 2007).

These signs are commonly seen in brachycephalic breeds, and the most frequent causes are between others foreign bodies (Gough, 2007), brachycephalic obstructive syndrome (Stanley, 2007), neoplasia most frequently due to squamous cell carcinoma and adenocarcinoma (Haagen, 2009), stenosis, secretions in the airway lumen and upper airway infection and haemorrhage (Tilley & Smith, 2007).

2.5.4 Sneezing

A sneeze is an explosive release of air from the lungs through the nasal cavity and mouth and is a protective reflex to expel irritants from the nasal cavity (Hawkins, 2009). It can due to any mucosal irritation or inflammation (Tilley & Smith, 2007), but usually respiratory disorders cause acute-onset and persistent (Hawkins, 2009).

The most common causes of sneezing are excess of nasal secretions, foreign bodies, neoplasia, parasites (Tilley & Smith, 2007), infections (viral, fungal or bacterial) and inflammatory nasal disease like lymphoplasmacytic rhinitis (Gough, 2007).

Table 1 – Anatomic limits of upper respiratory tract and defining clinical signs adapted from (Ford, 2005)

Anatomic limits	Clinical Signs
Nose, Nasal Cavity and Paranasal Sinuses	Sneezing and/or Nasal Discharge
Nasopharynx, choanae and soft palate	Stertor and Reverse-Sneezing
Larynx	Stridor

2.5.5 Other reported signs

Other reported signs, included nasal depigmentation and nasal pain, which become more obvious when the animal begins to react adversely to the owner´s customary petting (Haagen & Herrtage, 2010). Dyspnoea expresses difficulty in breathing or respiratory distress (Tilley & Smith, 2007). The major causes related to upper airway diseases are obstruction (Haagen & Herrtage, 2010; Tilley & Smith, 2007) due to stenotic nares, infection, inflammation, neoplasia or trauma (Tilley & Smith, 2007).

Coughing is a sudden forceful expiration of air through the glottis, preceded by an exaggerated inspiratory effort and usually accompanied by an audible sound (Tilley & Smith, 2007). It can be caused by lower and upper respiratory tract diseases which include rhinitis, sinusitis, foreign body and neoplasia (Tilley & Smith, 2007).

2.6 Physical Examination

After clinical history has been taken, a thorough clinical examination of the respiratory tract should be followed. Firstly, it is advised to examine the oral cavity, with emphasis on the maxilla, hard palate (trauma and congenital cleft palate on puppies) and canine teeth, which despite appearing normal can occult periodontal disease (Ford, 2005). Soft palate elongation may be also easily appreciated during visual inspection of the posterior pharynx in many patients without sedation (Padrid, 2007).

Palpation of the nasal architecture may reveal structural abnormalities like humps, nasal pain and nasal asymmetry suggestive of bone distortion from neoplasia or mycotic infection (Padrid, 2007).

Unfortunately, even when reported by the owners, the nature of the nasal discharge and side(s) involved can be difficult to assess since in many dogs the discharge is intermittent and therefore absent at the time of the examination (Sullivan, 1987). Checking the patency of the nasal airway is also very important and be easily accessed by using a thread or wisp of cotton wool and suspending it in front of each nostril. In this way an undisclosed bilateral lesion may be detected (Haagen & Herrtage, 2010; Sullivan, 1987).

2.7 Differential Diagnoses

Nasal diseases usually requires ancillary diagnostic techniques in order to reach a final diagnose, however through the history, signs and physical examination, the clinician needs to have in mind some differentials in order to proceed with the investigation.

Nasal disease in dogs can be a result of several conditions like neoplasia, inflammation, infection (primarily fungal), trauma, foreign body or, less commonly, parasitic infestation (Miles, Dhaliwal, Moore, & Reed, 2008). Determine the underlying cause in dogs with nasal disease can be challenging and frustrating, and often necessitating multiple diagnostic procedures at substantial cost to the client (Miles, et al., 2008).

Several studies report neoplasia as the most common disease in middle-aged to older dogs of mesaticephalic and dolicocephalic conformations, with chronic nasal signs (Harcourt-Brown, 2006a; Petite & Dennis, 2006; Sullivan, 1987; Tasker, et al., 1999) being the most common tumour the adenocarcinoma followed by chondrosarcoma (Tasker, et al., 1999).

Two studies reported chronic hyperplastic rhinitis as the second most common disease (Sullivan, 1987; Tasker, et al., 1999), although one reported aspergillosis being the second most frequent nasal disease (Harcourt-Brown, 2006b).

The diagnosis of aspergillosis can be challenging, and the clinician should consider that a correct diagnosis should be confirmed by at least two or three independent methods (Table 2) in order to avoid false positive or negative results (Sharp, et al., 1992).

Table 2 – Diagnostic criteria for nasal aspergillosis

Diagnostic criteria for nasal aspergillosis
• Radiologic evidence of fungal rhinitis
• Rhinoscopical evidence of fungal plaques
• Mycological evidence of *Aspergillus* or *Penicillium* species (as shown by culture, cytology or histopathology)
• Serological evidence of *Aspergillus* or *Penicillium*

Other diseases less commonly reported in the literature as frequent (Harcourt-Brown, 2006b; Sullivan, 1987; Tasker, et al., 1999), were foreign bodies, which should always be considered when a patient presents with a sudden onset of violent and persistent sneezing (Chandler, et al., 1991), dental problems that result in bone necrosis and subsequently communication between oral and nasal cavity (Ford, 2005), oronasal fistula characterised by the presence of food material in the nasal cavity, which has gained entrance via a defect in either the hard or soft palate (Chandler, et al., 1991), destructive rhinitis, polyp and undiagnosed diseases.

Diagnostic tests are obviously required to confirm the presence of these respiratory diseases, however respiratory medicine is considered to be underdeveloped subspecialty in veterinary medicine, and most of the commonly available tests are used to point the clinician in the right direction, and to rule out the presence of other potentially confounding disorders (Padrid, 2007).

2.8 Imaging studies

Nasal imaging is a decisive component of the diagnostic assessment of animals with signs of nasal disease, allowing the study of the bone and soft tissue structures that are not visible by physical examination or rhinoscopy (Hawkins, 2009).

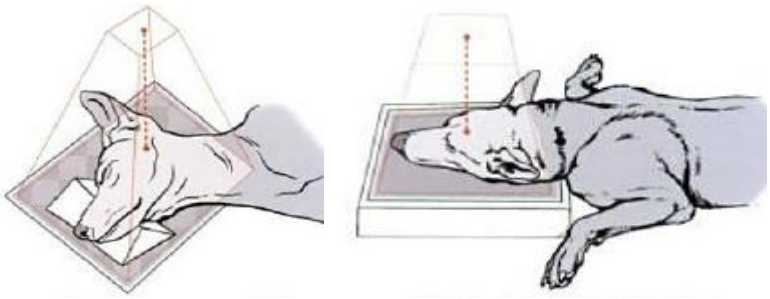
However, it must be taken into consideration that imaging studies should always be performed prior to the rhinoscopy and biopsy procedures (J. Saunders, et al., 2004). The two major reasons for that are: the results of nasal imaging can help directing biopsy instruments to the most abnormal regions and the resultant haemorrhage from rhinoscopy and biopsy may obscure subtle soft tissue lesions and induce imaging abnormalities (Hawkins, 2009; J. Saunders, et al., 2004).

2.8.1 Radiography

The canine head consists of 50 bones and numerous soft-tissue structures, which create challenges when making and interpreting survey radiographs of the nasal cavity and paranasal sinuses (Pownder, Rose, & Crawford, 2006).

Radiographs should be made under general anaesthesia to achieve perfect positioning (Pownder, et al., 2006). The standard radiographic examination for nasal diseases consists of a latero-lateral (LL) and dorso-ventral (DV) projection of the skull (Haagen, 2005) (Figure 3), however the value of the DV is limited by the superimposition of the mandible over much of the nasal chamber (Dennis, Kirberger, Wrighley, & Barr, 2001; Haagen & Herrtage, 2010).

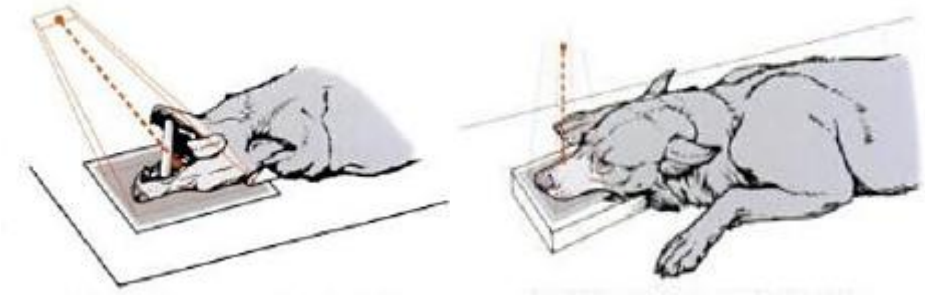
Figure 3 - Positioning of the head. LL and DV projections.



(Adapted from Waibl, Mayrhofer, Matis, Brumberg, & Köstlin, 2005)

In examination of the nose and nasal sinuses, special projections are required (Figure 4), such as the open mouth rostroventral-dorsocaudal (RV-DCd) and intra-oral DV projections (Haagen, 2005), providing the last one minimal superimposition of structures over the area of interest (Haagen & Herrtage, 2010) and the rostrocaudal (skyline) projection, which is useful for the evaluation of frontal sinuses (Haagen & Herrtage, 2010).

Figure 4 – Positioning of the head. Open mouth RV-DCd and intra-oral DV projections.



Adapted from (Waibl, et al., 2005)

Radiographic evaluation of a diseased nasal cavity is difficult because of the superimposition of bony structures and the complexity of the nasal turbinates (J. Saunders, et al., 2004). There are nasal structures that are never visible on radiographs like nasal septum and caudal

recesses, others are only sometimes visible like orbital lamina of the maxillary recesses. The cribriform plate, the naso-orbital wall and the vomer are well visualised, but changes like destruction need to be severe to be seen (Petite & Dennis, 2006; J. Saunders & Bree, 2003; J. Saunders, et al., 2004). This proves that conventional radiography has a low diagnostic value for evaluation of cerebral involvement and aggressive nasal disease, which is a very important prognostic factor (Petite & Dennis, 2006).

The radiographs may provide all the information that is needed or they may serve as a primary examination for prioritizing the differential diagnosis (Haagen, 2005). In fact, if an appropriate technique is used, they are considered useful for identifying the extent and severity of the disease, localizing sites for biopsy within the nasal cavity (Hawkins, 2009) and showing opacifications of the nasal cavity and frontal sinuses (except when lesions are bilateral and subtle) (J. Saunders, et al., 2004).

Nasal radiographs should be evaluated for increased fluid density, loss of turbinates, lysis of facial bones, radiolucency at the tips of tooth roots and the presence of radiodense material (Hawkins, 2009).

In the past, it was believed that radiography was not reliable for diagnosis of chronic nasal disease (Gibbs, Lane, & Denny, 1979). However, a study (Russo, Lamb, & Jakovljevic, 2000) identified some of the features that are most often found in radiographs of dogs with inflammatory nasal disease, and showed that dogs with rhinitis often get lucent foci in the nasal cavity (82%), usually the frontal sinus is not involved, focal or multifocal loss of turbinate detail could be present and localized soft tissue opacities could be seen. The majority of these lesions were frequently found in the rostral part of the nasal cavity (Russo, et al., 2000).

A more recent study showed that radiography like CT, provides a diagnosis mainly on the basis of the cavitated appearance of the nasal cavity and the presence of hyperostotic bone (J. Saunders, et al., 2004). However, it is particularly important to notice that in some cases, lesions identified on CT and MRI that could be significant with regard to prognosis and treatment are not visible on radiographs (Petite & Dennis, 2006).

Russo et al., showed that radiography had a high degree of accuracy for distinguishing between nasal neoplasia and aspergillosis, and a more recent study demonstrated that it has a sensitivity of 72-84% for aspergillosis (J. Saunders & Bree, 2003). Nevertheless, trapped fluid can be misinterpreted as solid tissue on some radiographs, and tumour size may be overestimated in these cases (Petite & Dennis, 2006).

In fact, a very high sensitivity was obtained in dogs with generalized lesions, whereas, the sensitivity was lower in dogs with localized lesions (J. Saunders & Bree, 2003; Jimmy

Saunders, et al., 2002), frontal sinus lesions or bilateral and subtle lesions (Gibbs, et al., 1979; J. Saunders, et al., 2004).

2.8.2 Computed tomography

CT is a reliable, noninvasive technique for use in the diagnosis of nasal disease in dogs (J. Saunders, Bree, Gielen, & Rooster, 2003), and has been used in dogs with a possible diagnosis of fungal rhinitis, nasal tumours, nonspecific rhinitis, and foreign-body rhinitis (Drees, Forrest, & Chappell, 2009; J. Saunders & Bree, 2003; J. Saunders, et al., 2003; Jimmy Saunders, et al., 2002).

Nasal CT is a powerful tool and greatly enhances the ability to establish an accurate and definitive diagnosis of chronic nasal disease in the dog, since cross sectional imaging provides good assessment of the extent of nasal disease, identifies areas of the nose to examine via rhinoscopy (Lefebvre, Kuehn, & Wortinger, 2005), and permits the evaluation of the character of lesions in the nasal cavities (Rycke, Saunders, Gielen, Bree, & Simoens, 2003; J. Saunders, et al., 2003).

CT uses the same basic physical principles as diagnostic x-ray, except it depicts the shades of gray in cross-section (Gavin & Bagley, 2009). The interpretation of CT scans is also very similar in principle to radiographic interpretation, with mineralised and bony material appearing radiopaque, fluid and soft tissue producing intermediate grey shaded and fat being more radiolucent. However, unlike radiography, differentiation between soft tissue and fluid is possible and internal soft tissue architecture is visible (Dennis, 2003).

It was found to be more sensitive than radiography for diagnosis of nasal aspergillosis in dogs, because the axial images permits accurate visualization of all the structures that are not visible on radiography (two-dimensional projections) as well as abnormalities like cribriform plate lysis (dorsal reconstructions), bilaterality and retrobulbar involvement (J. Saunders & Bree, 2003; Jimmy Saunders, et al., 2002).

Furthermore, CT was found to be more sensitive than radiography for diagnosis of nasal aspergillosis in the dog because of a better demonstration of mucosal thickening, frontal sinus lesions and a cavitated–like process (J. Saunders & Bree, 2003). The most common CT findings in dogs with nasal aspergillosis included moderate to severe turbinate destruction with a variable amount of abnormal soft tissue in the nasal passages, non-specific thickening of the mucosa along the bones of the frontal sinus, maxillary recess and nasal cavity; and thickened reactive bone (Jimmy Saunders, et al., 2002).

The diagnosis of rhinitis has also been studied for the past few years; non-specific rhinitis was seen as a non-destructive process affecting both entire nasal cavities of the dog with a

minimal to moderate amount of fluid in the frontal sinus (J. Saunders, et al., 2003). CT also enables the detection of highly attenuating foreign bodies, such as metal or glass. When not visible, the foreign body rhinitis is characterized with features similar to a localized nasal aspergillosis, which can make the differentiation between them difficult (J. Saunders, et al., 2003).

In conclusion, nasal computed tomography is a powerful tool and greatly enhances the ability to establish an accurate definitive diagnosis of nasal disease, since it helps differentiating idiopathic inflammatory disease from fungal rhinitis and neoplasic diseases from non-neoplasic diseases (Kuehn, 2006a).

2.8.3 Magnetic resonance imaging

2.8.3.1 Basic Physics

MRI was utilized in veterinary medicine primarily as a research tool in the 1980´s and early 1990´s (Gavin & Bagley, 2009), and over the past years, has been replacing computed tomography (CT) as a method of diagnostic imaging (Dennis, 1998).

Magnetic resonance imaging allows investigators to make multiplanar images without repositioning the dog (Rycke, et al., 2003). The three common planes are transverse (axial), dorsal (coronal) and the sagittal plane. The images are made from different slices within these planes, which are formed from the three magnetic gradients used (Gavin & Bagley, 2009).

The transverse plane allows better evaluation of the nasal turbinates, which is essential for the diagnosis of diseases that affect the nasal region. Therefore, this is the reference plane for the assessment of nasal pathologic changes (Rycke, et al., 2003).

The dorsal plane is most appropriate for use in determining the integrity of the cribriform plate in dogs with nasal tumours or nasal aspergillosis. Images through the dorsal plane also provide a more general view of the entire nasal cavities allowing easier characterization of disease processes (Rycke, et al., 2003).

The sagittal plane is of more use for spinal studies, because in small animals the whole spine can easily be included, and abnormalities like disc prolapsed, cord compression and nerve root impingement can easily be recognised (Elliot & Skerritt, 2010).

The current clinical applications for MRI rely on visualization of the resonance of the hydrogen atom nucleus (Gavin & Bagley, 2009), because a single hydrogen atom produces a relatively large magnetic moment and resonates very well, and in addition to this is very abundant within the body (Elliot & Skerritt, 2010). The size of the magnetic field is

dependent on the speed of movement (magnetic movement) and the size of the charge, since the hydrogen nucleus has a small electric charge it spins very fast (Gavin & Bagley, 2009).

However, in reality, it is not only hydrogen that can resonate, any atom with an odd mass number such as carbon (13), sodium (23) and phosphorous (31) would be suitable, since they also possess the ability to resonate and produce images (Elliot & Skerritt, 2010).

The hydrogen proton, as all other protons, carries a positive electrical charge and spins permanently on its own axis, which creates a corresponding magnetic field (magnetic moment) around the proton that possesses properties of size and direction (Dennis, 1998; Elliot & Skerritt, 2010; Gavin & Bagley, 2009). The body has many billions of microscopic magnetic moments that are completely randomly orientated, so they cancel each other out in a manner that their macroscopic magnetic field is zero (Elliot & Skerritt, 2010).

When an animal is placed within a large external magnetic field (scanner), the randomly spinning protons will come into alignment with the external field, although some of them will align against the field (anti-parallel state), largely cancelling each other out (Gavin & Bagley, 2009).

If protons are then bombarded with a series of RF pulses at a similar frequency to the rotational movement, they resonate (Dennis, 1998). This is due to the fact that the RF pulse imparts sufficient energy to allow more protons to adopt the anti-parallel state and it brings all the hydrogen spins into phase with each other, this means that the spins are no longer cancelling each other out, but instead each microscopic magnetic field is in unison with its neighbours (Elliot & Skerritt, 2010). After each brief RF pulse, the protons try to realign with the magnetic field, but are quickly unbalanced again by the next RF pulse, this produces cross-sectional images of tissues using a combination of magnetic fields and RF signals (Dennis, 1998).

This process continues for several minutes during which time the protons themselves emit a detectable RF signal which is related in strength and frequency to their chemical environment and position within the tissues (Dennis, 1998).

When RF transmission is turned off, three things happen simultaneously but independently of each other. Firstly, the absorbed RF energy is retransmitted as the useable MR signal, which depends on how much hydrogen there is in a particular tissue (proton density). Secondly, the spins that were in phase begins to slow down relative to others (dephase), this event is referred to as T_2, transverse or spin spin relaxation. The other event, occurs when the extra excited protons that were using the RF energy to adopt the anti-parallel state begin to return to their usual state, this is called T_1, longitudinal recovery or spin lattice relaxation (Elliot & Skerritt, 2010; Gavin & Bagley, 2009).

The emitted RF signal is detected by an aerial and rapidly converted by a computer into a series of cross-sectional images which can be viewed within a few seconds of the end of the study, the images are 'maps' of the locations of hydrogen nuclei (protons) within the tissues, based on their different chemical environments (Dennis, 1998) as well as their T_1 and T_2 relaxation (Elliot & Skerritt, 2010).

Thus, MRI is able to make high-quality images, not only because of the energy of the spinning protons, but also due to the abundance of hydrogen protons present in the body (Gavin & Bagley, 2009). Tissues containing a high concentration of hydrogen protons such as fat and CSF will generate more signal than others, containing little or no hydrogen protons, like cortical bone and lung (Elliot & Skerritt, 2010).

The spin echo sequences, uses an additional 180º RF pulse, which is divided into two 90º pulses. The first one will bring spins back into phase whilst the second will continue to push spins out of phase but in the opposite direction, this makes fast moving spins proceeding in the same direction but behind slow moving ones. Once the RF pulse has been turned off, they gradually catch up, and are said to rephrase or refocus, the corresponding regrowth of signal is detected in the receiver coil, referred to as an echo (Elliot & Skerritt, 2010).

The refocusing process forms the basis of spin echo (SE) pulse sequences and manipulation of the timing of these 90º and 180º RF pulses determines image contrast (Elliot & Skerritt, 2010). However a single echo is not sufficient to give an image, so this process is repeated hundreds of times (Elliot & Skerritt, 2010).

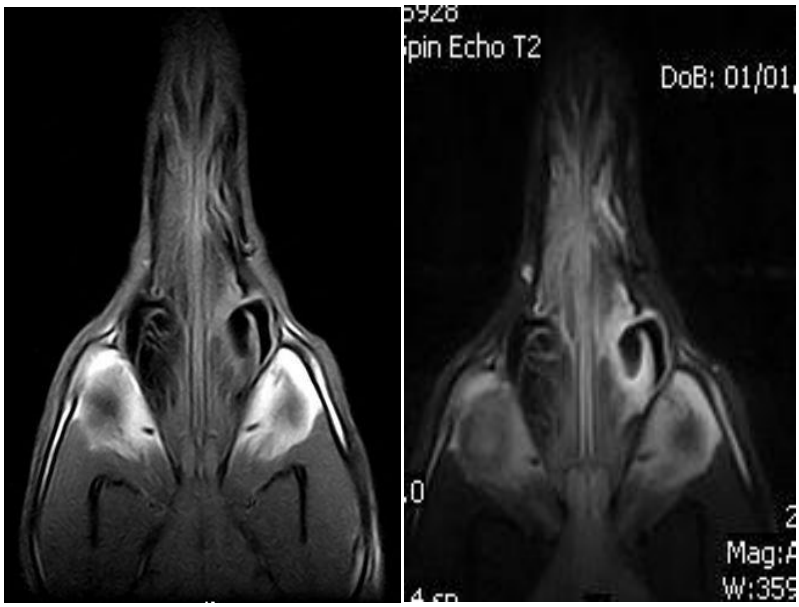
The two relaxation processes, T_1 and T_2, occurred simultaneously but are completely independent of each other, so by choosing the right echo time and repetition time (time between one 90º RF pulse and the next) the sequence can be optimized in favor of the desired contrast. Thus images are said to be T_1/T_2 weighted or T_1W/T_2W (Elliot & Skerritt, 2010). The proton density weighted minimizes both T_1 and T_2 effects, so any contrast in this image is down to absolute numbers of hydrogen atoms rather than either of the relaxation processes (Elliot & Skerritt, 2010).

Table 3 – Relaxation sequences formation

TR \ TE	Short	Long
Short	T_1W	Optimize both T_1 and T_2 (very poor quality images)
Long	Proton density weighted (PDW)	T_2W

Since fluid contains a lot of hydrogen protons, it will required long TR values to allow a full T_1 relaxation, so it appears bright or hyperintense on T_2-weighted and PDW images and dark or hypointense on T_1-weighted images (Dennis, 2003; Gavin & Bagley, 2009). On the contrary, fat is hyperintense on PDW and T_2 and T_1-weighted images (Gavin & Bagley, 2009).

Figure 5 – T_1W (left) and T_2W (right) sequences of the same dog



Pulse sequences, describe the sequence and timing of RF pulses and gradient applications required to produce an image (Elliot & Skerritt, 2010). The STIR sequence allows for a T_2-weighted type of image with uniform loss of the fat signal, which would normally be bright on conventional SE sequences (Elliot & Skerritt, 2010; Gavin & Bagley, 2009). It is used to demonstrate lesions in parts of the body such as the abdomen or orbits, which contain lots of fat (Elliot & Skerritt, 2010).

The FLAIR sequence is similar to the previous one, but it coincides with null point of water, so they show low signal intensity in areas of free fluid (Elliot & Skerritt, 2010; Gavin & Bagley, 2009), bound fluid, on the other hand, has a quicker relaxation time because it is able to impart energy to the molecules with it is bound (Elliot & Skerritt, 2010). In this sequence, oedema, tumour, necrosis or other pathology will retain a high signal, whilst areas of free fluid, such as CSF will appear dark (Elliot & Skerritt, 2010). This is particularly useful in brain studies, since it demonstrates periventricular lesions (Elliot & Skerritt, 2010) and gets rid of the usually hyperintese fluid signal from the vertebral spinal fluid (Gavin & Bagley, 2009).

Contrast is the mechanism in image quality, which allows differentiation of the various tissues according to their signal intensity (Elliot & Skerritt, 2010). Paramagnetic contrast media such

as dimeglumine gadopentetate (gadolium) can be injected intravenously and used to demonstrate areas of blood-CNS barrier breakdown or increased tissue vascularity (Dennis, 2003).

The contrast agent leaks into the tissue and changes its relaxation, leading to increased signal intensity on T_1-weighted (Gavin & Bagley, 2009). Nonetheless, gadolinium is not seen on T_1-weighted, it affects the relaxation of the hydrogen proton in the molecules, so the amount of contrast required for this effect is not concentration dependent (Gavin & Bagley, 2009).

2.8.3.2 Practical aspects

It is known that MRI uses a combination of a magnetic field and RF energy to create cross-sectional images of the area under examination (Dennis, 2003). Since the scanner generates a very powerful magnetic field, care must be taken not to introduce any ferrous metallic objects into the surrounding as they can become potentially dangerous missiles (Dennis, 1998).

Collars should be removed before scanning and because microchips cannot be removed, they commonly produce small areas of signal void (black areas on the image) in the neck. They are usually distant from the tissues being examined and are themselves unaffected by the magnetic field (Dennis, 1998). People and animals with pacemakers should not enter the scanning area, and watches, credit cards and computer discs should also be left outside the room (Dennis, 1998).

No painful stimuli result from MRI investigations, so in terms of anaesthesia or sedation, the only thing needed is that the animal remains still throughout the procedure, being the combination of medetomidine (0.025ml/kg) and butorphanol (0.01 ml/kg) the ideal for short studies in dogs (Elliot & Skerritt, 2010).

Any anaesthetic and handling materials must be composed of non-ferrous material such as plastic or aluminium and, if patient monitoring equipment is used, it should be MRI-compatible (Dennis, 1998).

The animal should be positioned as straight as possible on the scan table and immobilised so as to prevent movement artefact, even anaesthetised animals can roll to one side if not adequately secure. It seems easier to position dogs in dorsal recumbency, however ventral recumbency can also be used (Elliot & Skerritt, 2010).

The amount of energy absorbed during an examination is of concern, however for the time utilized in veterinary imaging due to the anaesthesia concerns, the amount of energy for MR studies has not been a problem to date (Gavin & Bagley, 2009).

To sum up, one of the major limiting facts with many MRI scanners is the need for immobility of the area being scanned, usually for 30 to 60 minutes at a time (Dennis, 1998).

Nevertheless, the major advantages of MRI over other imaging techniques is its ability to image in any plane without using ionising radiation (Elliot & Skerritt, 2010), excellent quality images with high contrast and good spatial resolution (A. Avner, Dobson, Sales, & Herrtage, 2008)

2.8.3.3 Current applications in small animal veterinary medicine

MRI is now being used for the diagnosis and staging of conditions of the intracranial nervous system such as cerebral oedema, brain tumours such as meningioma and glioma, idiopathic epilepsy and congenital disorders such as hydrocephalus or cerebellar hyperplasia (Gavin & Bagley, 2009).

Inflammatory diseases like granulomatous meningitis or encephalitis can also be accessed through a MRI scan. Furthermore, MRI can also be a useful tool in cases of raised intracranial pressure or haemorrhage due to a cranial trauma (Gavin & Bagley, 2009). Extra-cranial structures that are also frequently studied in MRI scans are the frontal sinuses, nose, orbit, nasal chambers, maxillae, mandibles and tympanic bullae (Dennis, 1998; Gavin & Bagley, 2009).

MRI is considered an excellent imaging modality for documenting the extent of nasal diseases such as tumours, giving several important prognostic features, such as breaching of the nasal septum, intracranial extension, occlusion of the major airway passage and fluid trapped in the frontal sinuses or caudal nasal cavity, that can be differentiated from tumour tissue (Petite & Dennis, 2006).

Similar to intracranial diseases processes, spinal diseases may result in anatomical alterations of spinal tissues or affect spinal cord elements at a microscopic, physiologic, or functional level. MRI is currently the gold standard for evaluation of the spine and spinal cord (Gavin & Bagley, 2009). Some of the diseases more frequently diagnosed are prolapsed of intervertebral discs, detection of cauda equina, Wobbler's syndrome, atlantoaxial subluxation, syringomyelia/hydromyelia and neoplasia (Dennis, 1998; Gavin & Bagley, 2009).

Although MRI is not used as commonly on orthopaedic cases as on neurological cases, it has been reported to be useful for shoulder lameness. This pathology is difficult to diagnose based on conventional examination, including palpation and radiography (Gavin & Bagley, 2009). MRI images can be an alternative to more invasive procedures such as arthroscopy, since they show all soft tissue components of long bones and joints, including articular cartilage, synovial fluid, ligaments and menisci (Dennis, 1998).

Abdominal and thoracic diseases can provide exquisite anatomical detail of organ systems. Abdominal studies are aided by general anaesthesia, which reduces motion from the

gastrointestinal tract. It is used for detecting abnormalities of the liver (portosystemic shunts) and associated structures, kidneys and adrenal glands (Gavin & Bagley, 2009). Although initially thoracic scans were not useful due to the motion that occurs with respiratory and the cardiac cycles, nowadays there are breath-hold techniques and respiratory and cardiac gating that allow the diagnosis of diseases such as pulmonary metastatic lesions and thymomas (Gavin & Bagley, 2009).

2.8.3.4 Magnetic resonance nasal anatomy

Figure 6 – Transverse sections through the nasal cavity, tongue and lower jaw

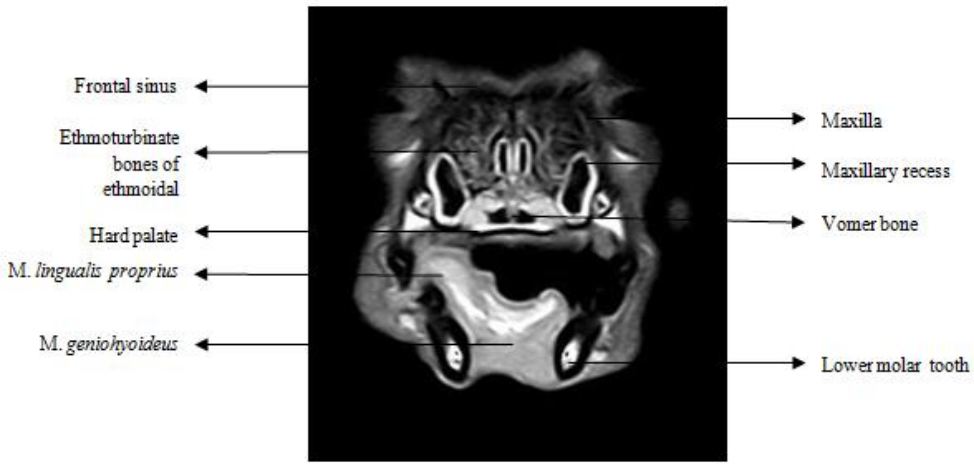


Figure 7 – Dorsal sections through the nasal cavity and cranium

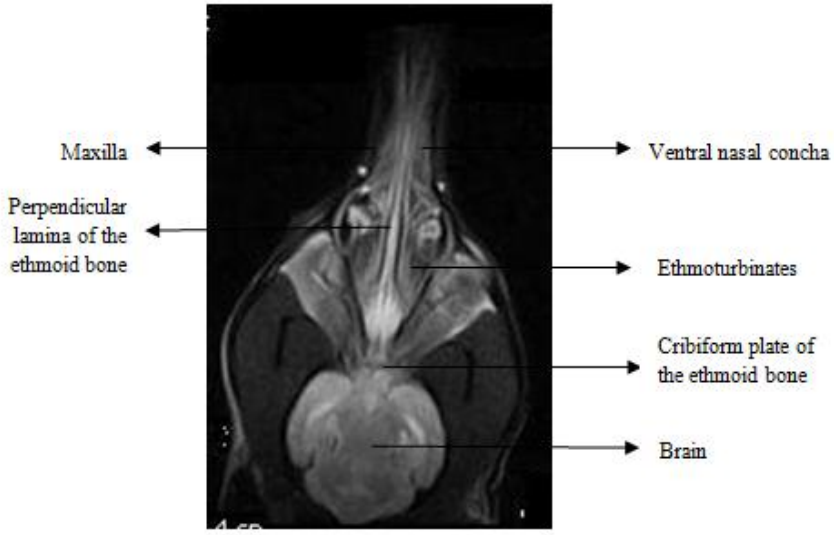
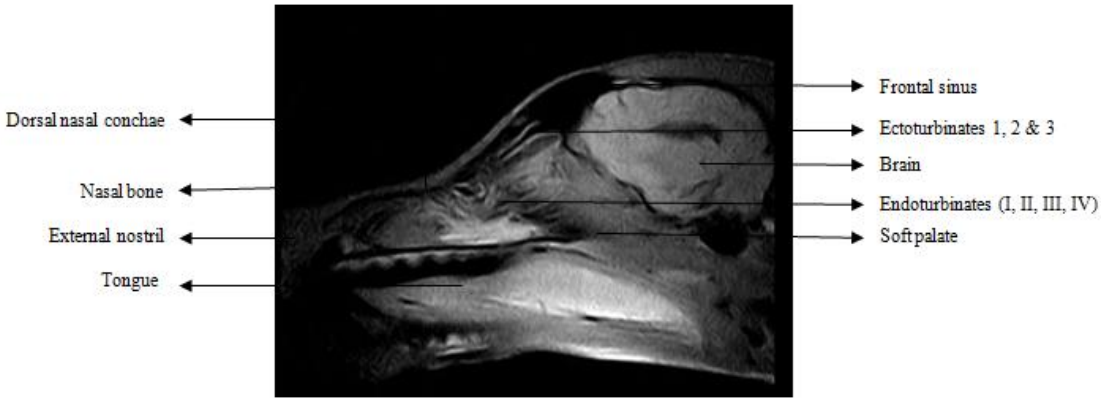


Figure 8 - Saggital section through the nasal cavity and cranium



2.8.3.5 MRI appearance of nasal diseases

With the use of different sequences, MRI has improved relative contrast between normal and abnormal soft tissue in the nasal cavity, and so it may aid in the identification of diseases of the nasal cavity and frontal sinuses (J. Saunders, et al., 2004).

The normal sinonasal secretions are aqueous (hypointense on T_1-weighted and hyperintense on T_2-weighted), but if the disease starts to progress they become thickened (hypointense on T_1-weighted and hypointense on T_2-weighted) (J. Saunders, et al., 2004).

When chronically retained secretions, exudates, fungal colonies or blood (acute haemorrhage) is present, on MRI scans, the soft tissue appears hypo to iso-intense on T_1-weighted images, and is hypointense on T_2-weighted images (J. Saunders, et al., 2004).

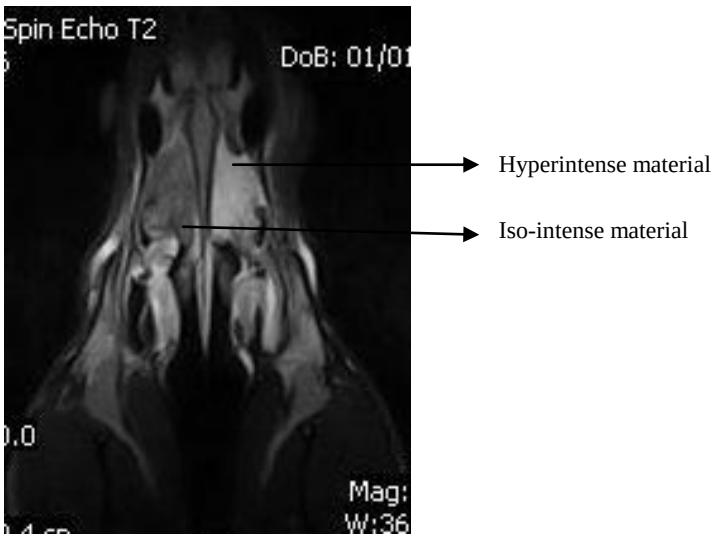
The variety of signalment of secretions is due to the protein content of sinonasal secretions, which can affect the signal intensity on both T_1- and T_2- weighted images, causing various combinations of hypointense or hyperintense signals (Miles, et al., 2008). The signal intensity on T_1-weighted changes from hypointense to hyperintense as the protein concentration of the secretions increases, and conversely the signal intensity on T_2-weighted changes from hyperintense to hypointense as the protein concentration increases. Once the protein concentration exceeds 28%, the secretions become inspissated and hypointense on both T_1- and T_2-weighted images (Miles, et al., 2008).

Owing to possible signal overlap between secretions and fungal colonies, it is often impossible to differentiate the two (J. Saunders, et al., 2004). Therefore, when visible, fungal colonies when present, appear as a hypointense mass on T_1-weighted adjacent to the wall of the nasal cavity, have no contrast enhancement of intensity, and are hypointense on T_2-weighted images. This is believed to be the result of the combined presence of metal irons (iron and manganese) that are essential elements in fungal amino acid metabolism and

calcium salt deposits (endogenous fungal products) in necrotic areas of the mycelium (J. Saunders, et al., 2004).

Differentiation between soft tissue and secretions may be affected by image quality and technique (Miles, et al., 2008). Thus, hyperintense material in the nasal cavity, on T_2-weighted images, is compatible with secretions with a high water or protein content, fungal colonies, retention cysts or polyposis (J. Saunders, et al., 2004).

Figure 9 – Hyperintense and iso-intense material in the nasal cavity on a T_2W image



Acquisition of FLAIR images may have helped differentiate soft tissue structures in the sinuses and evaluate cribriform plate erosion (Miles, et al., 2008). Fat suppression techniques are often used in human sinonasal imaging to help delineate soft tissue structures in the sinuses and periorbital regions. However, the uncertainty of the usefulness of these techniques, is still unknown, since dogs lack of fat tissue in the sinuses (Miles, et al., 2008).

If gadolinium contrast is used in dogs with nasal disease, post-contrast T1-weighted images tend to enhance the nasal mucosa in dogs with inflamed mucosa (J. Saunders, et al., 2004).

A study (M. Saunders, Jones, & Kabala, 1999) was made to evaluate the parameters of nasal airway anatomy on MRI when correlated with signs of nasal patency. It was concluded that MRI does not correlate with subjective nasal patency, and in fact the measurement of nasal anatomy was shown to be inadequate to define the subtle changes responsible for the variations in nasal airways or other parameters which affect the sensation of nasal airflow and resistance such as moisture or receptors or the rate of inspiration.

It was also proven that the lack of mass effect on MRI images was significantly associated with inflammatory disease and animals with vomer bone lysis, cribriform plate erosion, paranasal bone destruction, and sphenoid sinus and nasopharyngeal invasion by a mass, were

significantly associated with a diagnosis of neoplasia (Miles, et al., 2008). Nevertheless, a mass effect is not synonym of neoplasia, because dogs with non-neoplasic disease can have a mass effect present on MRI images, such as granulomas secondary to tooth root abscesses (Miles, et al., 2008).

In conclusion, because of the possible signal overlap between secretions and fungal colonies and because there is no way to predict prior to cross-sectional imaging how desiccated secretion will appear and differentiation between secretions and fungal colonies is, in the majority of cases, not possible (J. Saunders, et al., 2004). In addition to this, the absence of a mass on MRI could be used to exclude neoplasia as the underlying cause, unless it is an infiltrative rather than proliferative process (Miles, et al., 2008).

2.8.3.6 MRI versus CT

CT and MRI are superior to radiography for defining the extent and character of lesions in the nasal cavities (Petite & Dennis, 2006; Rycke, et al., 2003; J. Saunders & Bree, 2003; J. Saunders, et al., 2004). In addition, they are also useful for staging of tumours and planning for surgery and radiation treatments (Rycke, et al., 2003); they also have prognostic value in dogs with nasal aspergillosis (Mathews, et al., 1998; Rycke, et al., 2003).

MRI is generally accepted as the modality of choice for many applications of cross-sectional soft tissue imaging, such as intracranial invasion of nasal neoplasia, anatomic features, and secondary pathologic lesions attributed to brain neoplasm, whereas CT is not only preferred for cross-sectional bone imaging (permitting the visualization of minimal lytic and hyperostotic lesions) but also enables soft tissue visualization (Drees, et al., 2009; J. Saunders, et al., 2004).

Taking in consideration the evaluation of the nasal cavity, MRI produces highly detailed images of soft tissue, far in excess of the detail provided by CT (Dennis, 2003; Rycke, et al., 2003). Nonetheless, MRI is insensitive for recognition of cortical bone in which hydrogen protons are immobile and produce a signal void that cannot be differentiated from that of air, desiccated secretions, mycetomas, acute haemorrhage and calcium (J. Saunders, et al., 2004).

CT has been said to be superior for detection of cribriform plate involvement (intracranial extent of disease) and to provide better anatomic detail of tumours and secondary changes. Turbinate lysis and lysis of the tiny bony part of the nasal septum were reported to be easier to recognise on CT images than on MR images (Drees, et al., 2009).

However, the same study (Drees, et al., 2009) recognised a significant difference between the modalities for the presence of fluid and thickened mucosa in the nasal cavity. They were identified more frequently in MRI scans than on CT scans, most likely because of the

increased conspicuity of small amounts of fluid on the MRI T_2-weighted images, whereas small amounts of fluid might not be differentiated from the mucosal lining in all CT images.

In conclusion, advantages of CT over MRI are lower cost and a reduced amount of time to conduct an examination. Advantages of MRI over CT include the use of multiplanar capabilities (Rycke, et al., 2003), good observation of small quantities of fluid in the nasal cavity (Drees, et al., 2009). However, MRI is less sensitive than CT for subtle bony lesions, such as minor osteolysis, or for areas of calcification (Dennis, 2003). Both were reported to equally identify soft tissue abnormalities in the frontal sinuses (Drees, et al., 2009).

2.9 Other ancillary diagnostic tests

2.9.1 Rhinoscopy

Rhinoscopy is one of the most helpful studies when it comes to nasal disease, since it is the only technique that allows direct visualization and identification of abnormal soft tissue as well as fungal plaques (J. Saunders, et al., 2004). However, rhinoscopy does not allow evaluation of bony structures, and extensive turbinate destruction must be present before the frontal sinuses and cribriform plate can be seen (J. Saunders, et al., 2004).

Rhinoscopy defines the use of an otoscope or endoscope, flexible or rigid, to explore and visualize the nasal cavities (Elie & Sabo, 2006). It is a demanding, and yet diagnostically valuable, method of visualization, whereas it makes possible to directly inspect the status of the mucosa, nature of the content of the nasal cavities and sinuses (mucoid, purulent or blood), appearance of the turbinates (absence, swelling, change in colour and atrophy), presence of etiologic agents like fungal colonies or parasites, and masses like polyps, neoplasms, cysts and granulomas (J. Saunders, et al., 2004).

It is also excellent for finding foreign bodies, oronasal fistulas, obstructive disease indicating tumour with no conclusive radiographic findings and severe rhinitis with suspicion of aspergillosis (Haagen, 2005; Knotek, et al., 2001), which appears as greenish-white plaques growing on turbinate mucosa (Sharp, et al., 1992).

The main indications for rhinoscopy are a history of unilateral nasal discharge, probable entrance of a foreign body, obstructive disease such as neoplasia and severe rhinitis with suspicion of aspergillosis, in which loss of nasal conchae is considered pathognomic for this condition (Haagen, 2005; Haagen & Herrtage, 2010; Harcourt-Brown, 2006b).

Rhinoscopy is generally a safe procedure since it is considered minimally-invasive, and the most common finding is bleeding that may persist for a short while after the procedure but it should stop on its own (Harcourt-Brown, 2006a).

Before rhinoscopy it is recommended to perform a full clinical examination, blood count, serum chemistries, coagulation profile (including von Willebrand's factor level) urinalysis and thoracic radiographs (Degner, 2006; Elie & Sabo, 2006). Furthermore, in advance of rhinoscopy, patients suffering from epistaxis, should be screened for coagulation abnormalities, systemic hypertension, and diseases of vascular fragility such as systemic lupus erythematosus and hypothyroidism (Elie & Sabo, 2006).

The dog should then be anaesthetised (topical anaesthesia of the nostril and vestibule may be helpful), intubated and placed in lateral or ventral dorsal recumbency, if possible with the body raised slightly higher than the head (Haagen, 2005; Harcourt-Brown, 2006a). A cuffed endotracheal tube is used to create an air/watertight seal, and the mouth is held open with a gag. The dog's head should be placed on a grid laid over a stainless steel tray, in order to collect the fluid flushed through the nasal passages for further examination (Harcourt-Brown, 2006a).

The use of an otoscope is often preferable for diagnosis and removal of foreign bodies which have entered the nasal cavity via nostril (Haagen, 2005). Rigid endoscopes can be more useful for examining the nasal passages than otoscopes and flexible endoscopes, as they have been designed to be used within a sheath, which allows insuflation or irrigation of cavities, and enable examination of almost the entire upper respiratory tract (Harcourt-Brown, 2006a). Notwithstanding, despite the increased visibility afforded by a rigid endoscope relative to the otoscope, nearly 50% of the caudal portion of the nasal cavity still remains inaccessible (Elie & Sabo, 2006).

There are some limitations to the use of a rigid rhinoscope, particularly the size of the patient and the status of the mucosa nasal cavity, since in some dogs rhinoscopy may be relatively difficult due to narrow nasal airways and frequent bleeding (Knotek, et al., 2001). So, some authors prefer to use the flexible endoscopes which are passed up to the choanae retroflexed through the soft palate from the mouth (Knotek, et al., 2001).

The flexible endoscope provides the most extensive view of the nasal cavity (Elie & Sabo, 2006) including the examination of the frontal sinus, since it is not possible to enter the frontal sinus from the nasal cavity, (its entrance lies dorsocaudal to the dorsal meatus) with a rigid endoscope. However, trephining a hole into the sinus allows the introduction of a rigid endoscope into the frontal sinus (Harcourt-Brown, 2006a).

When fungal colonies are seen on the nasal passages and on frontal sinus, they should be biopsied for cultural or cytological examination (Sharp, et al., 1992). In fact, fungal plaques are commonly seen in the frontal sinus, suggesting that the term sinonasal aspergillosis or

fungal rhinosinusitis might be more appropriate for the condition than nasal aspergillosis (Lynelle Johnson, Drazenovich, Herrera, & Wisner, 2006).

At the end of rhinoscopy the dog's head should be tipped forward and downwards to allow blood and fluids to drain out of the nasal passages, and frequently the dogs are hospitalised overnight as further bleeding is more likely in those sent home the same evening (Harcourt-Brown, 2006a).

2.9.2 Nasal biopsy

Indications for nasal biopsy include gross facial deformity or radiographic evidence of destruction or deviations of the nasal structures, or loss of trabecular detail (Padrid, 2007).

Biopsy collection via direct visualization is preferred whenever feasible; however, this approach may be thwarted by the anatomic limits of the patient's nasal cavity, as well as the comparative dimensions of the indwelling instrument in use (Elie & Sabo, 2006). Nonetheless, it is frequently necessary to consider blind nasal biopsies, and care must be taken to insure that the instrument does not penetrate the cribriform plate, since inadvertent penetration of this nasal anatomic barrier to the brain has fatal consequences (Elie & Sabo, 2006).

There are numerous methods for tissue sampling from the nasal cavity such as nasal flush or lavage, nasal swab, scraping, fine-needle aspiration biopsy, impression smears, and turbinectomy and pinch biopsy (Hawkins, 2009; Knotek, et al., 2001).

The least traumatic techniques are nasal flush and nasal swab, and unlike other techniques nasal swabs can be collected from an awake animal and are useful to indentify cryptococcal organisms cytologically (Hawkins, 2009). Nasal flush is performed by positioning a soft catheter in the caudal region of the nasal cavity via the oral cavity and internal nares, and then approximately 100 ml of sterile saline solution is injected in pulses by syringe. The fluid exiting is collected and examined cytologically (Hawkins, 2009).

Impression smears and fine needle aspiration biopsy are techniques that provide very consistent results in cases of advanced neoplasic processes (Knotek, et al., 2001).

A representative biopsy should be used to make impression smears, which are stained using a rapid cytology stain, this will here confirm it. Although many conditions can be diagnosed reliably from cytology, histopathology should be used to confirm the diagnosis in all cases (Benitah, 2006; Harcourt-Brown, 2006a).

Nowadays, pinch biopsy is the preferred technique; it uses an alligator cup biopsy forceps to obtain pieces of nasal mucosa for histologic evaluation (Hawkins, 2009). It must be taken into consideration that after the first samples are taken, bleeding will prevent further visual

guidance, therefore the forceps are passed blindly to the position identified during rhinoscopy evaluation (Hawkins, 2009).

Turbinectomy is a more invasive technique that provides the best tissue specimens for histologic examination and allows the removal of abnormal or poorly vascularised tissue, fungal granulomas and permits placing drains for subsequent topical nasal therapy (Hawkins, 2009).

Masses or areas of deformity should be biopsied, as should areas of inflammation since some diagnoses will only be made from biopsies of inflamed areas (Harcourt-Brown, 2006a). It is also recommended to obtain multiple samples of abnormal nasal tissue (six or more) for histopathology. If no localized lesions are identified radiographically or rhinoscopically, multiple biopsies (six to ten) should be obtained randomly from both sides of the nasal cavity (Hawkins, 2009).

Additional samples should be housed in a sterile agar-impregnated culture, and kept refrigerated. In the event bacterial or fungal cultures are indicated, based on preliminary histopathology findings, the preserved culture specimen may be submitted within 72 hours of its collection (Elie & Sabo, 2006).

Although clinicians often try to get as many samples as possible from abnormal tissue, it is sometimes difficult to decide if the tissue is abnormal so, on occasion, apparently normal tissues will yield a diagnosis (Harcourt-Brown, 2006a). When rhinoscopy is not available, high quality samples for cytology and histology may be obtained during operative procedures in the nasal cavity such as rhinotomy or turbinectomy (Harcourt-Brown, 2006a).

The complications most commonly seen are haemorrhage, brain trauma, and aspiration of blood, saline solution or exudates (Hawkins, 2009). Despite all these complication, nasal biopsy continue to be an essential diagnostic procedure and, as described before, is sometimes the only way to confirm a diagnosis (Sullivan, 1987).

2.9.3 Histopathology

The histopathologic diagnosis is done from samples fixed on 10% formaldehyde and stained, routinely, using haematoxylin and eosin (HE), which is able to show some types of fungi as dermatophytes (Mackin, 2004; Pérez & Carrasco, 2000).

However, most of the potentially pathogenic species of fungi are poorly stained with this technique, so special staining techniques are needed, such as periodic acid-Schiff (PAS), Gridley technique or the silver methenamine techniques (Pérez & Carrasco, 2000).

Neoplastic disease is frequently associated with inflammation, so histologic examination of small or superficial biopsy specimens obtained by means of rhinoscopy may lead to

misdiagnosis of inflammatory disease. In instances, when neoplasia is suspected because of mass effect on MRI but histologic examination of a biopsy specimen revealed only inflammation, is recommended to repeat rhinoscopy or rhinotomy (Miles, et al., 2008).

Histologic identification of fungal hyphae in biopsy specimens has traditionally been used to confirm the diagnosis of aspergillosis (Blanco & García, 2000; Lynelle Johnson, et al., 2006; Mathews, et al., 1998). Nevertheless, it does not identify the precise fungal agent present (Blanco & García, 2000).

A study (Peeters, Day, & Clercx, 2005) suggested that nasal aspergillosis in dogs is not associated with mucosal invasion, thus histologic evidence of disease may not be obtained in all cases, and detection of plaques, along with histologic examination of plaque material, is of critical importance in confirming the diagnosis (Lynelle Johnson, et al., 2006).

Another point that should be taken into consideration is that the presence of fungal elements does not rule out other underlying problems, such as nasal foreign bodies or neoplasia, so an complete anterograde as well as retrograde rhinoscopy should always be performed (Lorenzi, Bonfanti, Masserdotti, Caldin, & Furlanello, 2006).

As the inflammatory cellular response to *Aspergillus* spp. is primarily neutrophilic, a large number of moderately lytic neutrophils is usually seen and many samples show bacterial phagocytosis due to secondary bacterial infection (Lorenzi, et al., 2006).

2.9.4 Nasal cultures

Microbiologic cultures specimens are recommended but can be difficult to interpret, since small animals have a wide range of normal intranasal commensal bacterial flora, most notably *Staphylococcus* sp., *Streptococcus* sp., *Escherichia coli*, *Pseudomonas* sp., *Pasteurella* sp., *Bordetella*, and also a variety of other aerobic and anaerobic bacteria as well as fungi such as *Aspergillus* sp. (Harvey, 1984; Padrid, 2007) .

Therefore, bacterial culture from nasal swabs is of little value as the bacteria recovered are usually normal commensals or opportunistic invaders (Sullivan, 1987), in fact, culture of the nasal cavity is sometimes not indicated for chronic sneezing, epistaxis, and/or nasal discharge (Padrid, 2007).

Regardless of the method used, the growth of many colonies of one or two types of bacteria, more likely reflects infection than the growth of many different organisms (Hawkins, 2009).

Previous studies have suggested that results of fungal culture are typically positive in dogs with nasal aspergillosis and are of significant diagnostic value (Mathews, et al., 1998; Tasker, et al., 1999). However, false-positive culture results have been reported with dogs with

neoplasia (Lynelle Johnson, et al., 2006). Thus fungal culture is not always reliable in confirming the diagnosis.

Recent studies (Billen, Clercx, et al., 2009; Pomrantz, Johnson, Nelson, & Wisner, 2007) contradicted older studies by saying that fungal culture of nasal specimens was very specific and sensitive for the diagnosis of nasal aspergillosis in dogs. It is recommended to culture fungal plaque material at 37º C, since it results in the highest positive fungal culture rate in dogs with nasal aspergillosis confirmed, with a sensitivity of 88% and a specificity of 100%. (Billen, Clercx, et al., 2009)

Growth varies with the temperature but also with the humidity of the air, so high humidity is preferred during incubation since it promotes growth, while low humidity slows it down or stops it altogether (Cutsem & Rochette, 1991).

However, it must also be taken into consideration that the type of nasal sample is of major importance for the success of fungal culture, so that blind sampling of nasal secretions with cotton swabs is very unreliable and in contrast, direct sampling of fungal plaques is highly specific and sensitive (Billen, Clercx, et al., 2009).

2.9.5 Serology

A 5 to 10 ml clotted blood sample, preferable spun to remove serum, is all that is required by competent laboratories to test for antibodies to particular fungi, specifically *Aspergillus fumigatus* (Sullivan, 1987).

Serological examination, by a reputable laboratory, is another very helpful technique and is the most accurate way of confirming infection (Sullivan, 1987), however it can show false positives (about 15%) and so must be interpreted in the light of the imaging and rhinoscopical results (Sharp, et al., 1992).

In human medicine, the antibody response to *Aspergillus* spp. infection has been well characterised and over 95% patients with aspergilloma have detectable precipitating IgG antibodies and some IgM antibodies as well (Denning, 1996).

Recent veterinary studies (Billen, Peeters, et al., 2009; Pomrantz & Johnson, 2010; Pomrantz, et al., 2007) showed that serology results are highly specific and with good sensitivity, if the detection of serum *Aspergillus*-specific antibodies is done with AGDD (Agar Gel Double Diffusion) or ELISA (Enzyme Linked Immuno Sorbent Assay).

The AGDD technique is based on the observation of the precipitation in agarose gel of the antibodies directed against *Aspergillus* spp., when they meet in an amount equivalent to *Aspergillus* antigens (Billen, Clercx, & Peeters, 2008).

ELISA can be used to study the evolution of the disease and its response to treatment, since it can analyse the humoral response of the host. In this technique an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal (Billen, et al., 2008).

Billen et al (2009), showed that the specificity was higher for AGDD (100%) than for ELISA (96,8%) (Billen, Peeters, et al., 2009), while sensitivity was higher for ELISA (88,2%) than for AGDD (76,5%). The AGDD specificity was similar to a previous study wherein the specificity was 98% (Pomrantz, et al., 2007).

Sensitivity of the AGDD serologic test in the same study was only 67%, which may suggest that aspergillosis, as the cause of nasal discharge, cannot be ruled out on the basis of negative AGDD test results only (Pomrantz, et al., 2007).

AGDD has the advantage of being inexpensive and easy to perform but does not allow quantification of serum immunoglobulins, whereas ELISA is a rapid quantitative method that allows the detection of very low concentrations of antibodies (Billen, Peeters, et al., 2009).

Serology was shown, by a recent study, not to be a useful indicator of disease status, as results did not reflect response to treatment (Pomrantz & Johnson, 2010). Results from the same study indicated that serial serologic testing for aspergillosis were unpredictable and did not appear clinically useful.

In conclusion, negative results of serologic evaluation cannot be used to rule out nasal aspergillosis, but positive results should be considered highly suggestive of the infection (Pomrantz, et al., 2007).

2.9.6 Leukogram

Haematology and biochemical profiles are only of value in specific diseases, in which the common causes have been ruled out (Sullivan, 1987). In dogs with nasal aspergillosis, we may see neutrophilia, leukocytosis and monocytosis, which reflects chronic inflammation (Tilley & Smith, 2007). When the aspergillosis is systemic in dogs, the findings are very non-specific but often have mature neutrophilia, leukocytosis and lymphopenia (Tilley & Smith, 2007).

Humans, in the cases of neutropenia, prolonged neutropenia, neutrophil function deficits or corticosteroid therapy (which reduces pulmonary macrophage), have proved highly susceptible to systemic *Aspergillus spp.* infections (Denning, 1996).

2.10 Respiratory diseases

Respiratory disease is a term used to describe abnormalities within the respiratory tract, including nose, pharynx, larynx, trachea, bronchi, lungs, and chest cavity (Padrid, 2006). The most common signs of respiratory disease are sneezing, reverse-sneezing, snorting, wheezing, noisy breathing, nasal discharge, cough, and changes in respiratory rate, depth and effort (Padrid, 2006). The causes of these signs range from simple nasal allergies accompanied by sneezing and a clear nasal discharge, to life-threatening asthmatic bronchoconstriction. (Padrid, 2006)

Inflammation of the nasal mucosa is called rhinitis, and it must borne in mind that all dogs with chronic nasal discharge will have a rhinitis, this will be seen on endoscopy (increased mucus, pus and inflammation and possibly ulceration, which is probably caused by fungal toxins in the nasal discharge) and mirrored in the cytology and histology (Harcourt-Brown, 2006b; Sharp, et al., 1992).

Rhinitis can be classified based on the nature of exudates, age of lesions, severity of the insult and aetiological agent (López, 2007). The nature of exudates is highly associated with the nature of nasal discharge and can be classified as serous, catarrhal, purulent, fibrinous or ganulomatous (López, 2007). The age of lesions can be classified as acute, subacute or chronic (López, 2007) and the severity of the insult can be classified as mil, moderate and severe (López, 2007).

As far as the aetiology is concerned, the inflammatory nasal diseases can be divided into viral, bacterial, neurogenic, mycotic, and non-specific (including allergic rhinitis), since the last two types of rhinitis are the most common and the object of this study, they will be discussed on other chapters.

Viral rhinitis in dogs is a prominent sign of canine distemper, kennel cough (Knotek, et al., 2001) and herpes virus infection in newborn puppies, which is characterized by profuse mucopurulent nasal discharge (Haagen & Herrtage, 2010).

Although primary bacterial rhinitis is not common (Haagen & Herrtage, 2010; Hawkins, 2009; Tasker, et al., 1999), it is possible that some bacteria may be responsible for actually initiating an acute rhinitis and this is probably the case with *Bordetella bronchiseptica* and possibly *Pasteurella multocida* (Chandler, et al., 1991; Haagen & Herrtage, 2010). Broad spectrum antibiotic therapy leads to clinical improvement, but the response is usually temporary (Hawkins, 2009).

Neurogenic rhinitis is reported in the literature, to be caused by a lesion in the parasympathetic nerves caused primarily by otitis media (Haagen & Herrtage, 2010).

The most effective treatments for non-infectious airway inflammation have been injectable and oral corticosteroids such as prednisone and prednisolone (Padrid, 2006). Nevertheless, not all diseases respond to the steroid therapy and in addition potential systemic effects can occur from the chronic use of corticosteroids, including behavioural changes, polydipsia, polyuria, increased appetite, skin and urinary tract infections, pancreatitis and *diabetes mellitus* (Plumb, 2005). Fortunately, corticosteroids can now be given by inhalation and this route minimizes their systemic absorption (Padrid, 2006).

2.10.1 Lymphoplasmacytic Rhinitis

First described in 1987 (Burgener & Slocombe, 1987), idiopathic lymphoplasmacytic rhinitis is characterized by the histopathological appearance of inflammation of the nasal mucosa dominated by lymphocytes and plasma cells (Day, 2009). It is a common cause of nasal discharge in the dog, but in disease the clinical signs tend to be less severe than in sino-nasal aspergillosis (Windsor, et al., 2004).

Because dogs with lymphoplasmacytic rhinitis may have neutrophilic inflammation of the nasal mucosa, in addition to the characteristic lymphoplasmacytic infiltration, less specific terms such as chronic rhinitis, chronic inflammatory rhinitis, and idiopathic chronic rhinitis, have also been used to identify this condition (Hawkins, et al., 2008; Mackin, 2004).

Epidemiology and clinical signs

Idiopathic LPR was reported to be a middle age to older dogs disease and is most often bilateral, even among dogs with unilateral nasal discharge (Windsor, et al., 2004). It is also characterized to be a disease refractory to treatment with antimicrobials, antihistamines and glucocorticoids (Windsor, et al., 2004). A breed susceptibility to LPR was proven in a study, which found Whippets and Dachshunds to be particularly susceptible to this disease (Sullivan, 1987).

LPR is typically not a destructive disease, but occasionally turbinate destruction is seen on MRI, mimicking a fungal rhinitis (Kuehn, 2009). Clinical signs of this disease are often typical of other chronic nasal disorders like nasal discharge, sneezing and occasionally epistaxis or cough (Windsor, et al., 2004). And in contrast to chronic rhinitis in people, in dogs typically occurs without concurrent conjunctivitis or allergic lower airway disease (Mackin, 2004).

Aetiology

The aetiology of this condition in dogs is unknown, so the diagnosis is made by the elimination of other common diseases (Harcourt-Brown, 2006b; Padrid, 2006; Sullivan, 1987). Yet, a number of cases are presented in which the signs are clinically and radiologically indistinguishable from dogs with aspergillosis, however serology from these dogs give negative results when tested for antibodies to *Aspergillus* and *Penicillium* species, and no fungus are detected endoscopically (Sullivan, 1987).

In human medicine, airborne fungi have been shown to play a key role in the pathogenesis of disease in some patients with chronic rhinosinusitis (Peeters, et al., 2008). However in dogs, this type of rhinitis is often classified as idiopathic destructive rhinitis, and it is thought to be due to penetration of a foreign body, resulting in a severe turbinate necrosis, although the foreign body is no longer present (Sullivan, 1987).

In two veterinary medicine studies (Peeters, et al., 2008; Windsor, et al., 2006), molecular analysis has shown higher loads of fungal DNA in nasal biopsies from dogs with LPR than in nasal biopsies from healthy dogs, suggesting a possible role for fungal organisms in LPR of dogs.

Another possible, explanation for this, still, idiopathic disease is *Bartonella* infection (Hawkins, et al., 2008). However, two recent studies failure to prove an association between *Bartonella* infection and idiopathic rhinitis (Hawkins, et al., 2008; Windsor, et al., 2006). Nevertheless, *Bartonella vinsonii* has been proved to be linked with canine granulomatous rhinitis (Pappalardo, Brown, Gookin, Morrill, & Breitschwerdt, 2000) and *Bartonella* sp. may be associated with epistaxis in dogs (Henn, et al., 2005).

In addition to this, *Pseudomonas aeruginosa*, *Pasteurella multocida* and *Staphylococcus* spp., present on the mucosa of respiratory tract of animals without clinical disease are mentioned most frequently from the possible bacterial agents causing rhinitis in the dog (Knotek, et al., 2001).

Pathogenic *Mycoplasma* spp., *Chlamydophila* spp. have been reported as possible LPR agents, however in a study, molecular evidence of current infection or invasion of nasal tissue by those agents could not be established (Windsor, et al., 2006).

An allergic aetiology has also been suggested, since a study showed that histopathological examination of biopsy samples of dogs with LPR, revealed an increased gene expression for IL-5, which is consistent with a partial Th2 cell regulated immunity, which is consistent with an allergic pathogenesis (Day, 2009). Nevertheless, evidence of allergy in the canine upper respiratory tract is not well described, and dogs with LPR have demonstrated poor response to antihistamines and glucocorticoids (Windsor, et al., 2004).

Pathophysiology and inflammatory response

The inflammation of the nasal mucosa leads to vasodilatation and increased vascular permeability with associated congestion and oedema of the nasal tissues. Exudation of fluid from leaky vessels walls leads to both mucosal oedema and accumulation of serous fluid in the nasal lumen (Mackin, 2004). With chronic rhinitis, nasal secretions become progressively more mucoid as mucus-secreting glands proliferate and become hypersecretory in response to chronic inflammation (Mackin, 2004). At the end, this will lead to destruction of cilia and squamous metaplasia of the respiratory epithelium, causing disruption of mucociliary clearance and accumulation of secretions (Mackin, 2004).

The reduced mucociliar clearance creates a perfect microenvironment for commensal bacteria proliferation (e.g. *Staphylococcus intermedius*), with a resultant increased susceptibility to secondary infections. Infections will lead to a recruitment of neutrophils and subsequently the development of purulent exudates (Mackin, 2004).

Histopathology usually reveals a mixed inflammatory cell infiltration of mature lymphocytes and plasma cells within the nasal mucosa and submucosa. Notwithstanding other inflammatory cells may be present, such as eosinophils and neutrophils. The respiratory epithelium may reveal diffuse changes like hyperplasia, squamous metaplasia, or ulceration. Beneath the epithelial cells there can be submucosal fibrosis or glandular hyperplasia (Mackin, 2004).

Treatment

Idiopathic lymphoplasmacytic rhinitis was initially thought to be a steroid-responsive disease (Burgener & Slocombe, 1987), however a study showed that administration of corticosteroids were often ineffective (Windsor, et al., 2004). This continues to be the traditional primary treatment of choice of LPR. Firstly with immunosuppressive doses, oral prednisone or prednisolone (2mg/kg) for at least 1 to 2 weeks and then tapered over time to anti-inflammatory doses (Mackin, 2004).

Antimicrobial treatment is likely to reduce secondary bacterial without diminishing the discharge caused by the idiopathic LPR, however it was reported to help reducing or change the nature of nasal discharge in some dogs (Windsor, et al., 2004). Empiric antimicrobial treatment is not advised, unless chronic *Bordetella bronchiseptica* or *Pasteurella multocida* (very rare) rhinitis are suspected (Kuehn, 2009).

Anti-histamine medication is rarely effective, but they can occasionally slightly reduce the severity of nasal discharge (Kuehn, 2009). Itraconazole was also reported to have a dramatic improvement in clinical signs in some dogs (Kuehn, 2006b).

LPR exhibit amelioration of clinical signs during treatment with doxycycline or macrolides, suggesting that specific microbes might be involved in the pathogenesis (Windsor, et al., 2006).

Nowadays, it is considered very helpful a long-term administration of antibiotics with immunomodulatory effects combined with non-steroidal inflammatory agents, like doxycycline (3-5 mg/kg, BID, PO) or azithromycin (5mg/kg, SID, PO) in combination with piroxicam (0.3mg/kg, SID, PO). Therapy is likely to be required for a minimum of 6 months if not indefinitely (Kuehn, 2009).

2.10.2 Allergic rhinitis

Allergic rhinitis has not been well characterized in dogs. The nasal mucosal inflammation seems to result from immune-mediated (IgE) responses to allergens, most of them seasonal, like pollens, spores or dust mites (Rosenwasser, 2002).

Although dog cases suspected to have allergic rhinitis are sometimes observed in small animal practice, immunological characteristics of canine AR are not, yet, well understood (Kurata, et al., 2004). It is also possible that food allergens and parasites play a role in some patients (Hawkins, 2009).

A study (McDougal, 1977) reported a case of a dog with upper respiratory symptoms that showed positive reactions to antigens of house dust, grasses and trees in IDST, and the symptoms were improved by immunotherapy with alum-precipitated extracts of these antigens.

In fact, environmental allergens have been identified with IDST and IgE test in dogs with allergic rhinitis, and in addition peripheral blood mononuclear cells obtained from these dogs showed to proliferate under stimulation with extracts of allergens, suggesting that allergic reactions directed to environmental allergens can occur in dogs (Kurata, et al., 2004).

Another study (Meler, Dunn, & Lecuyer, 2008), showed that few dogs with eosinophilic rhinitis, were suspected of having allergic rhinitis, secondary to tobacco smoke, since they improved during hospitalization.

The signs of allergic rhinitis are similar to those of LPR but can be concomitant with allergic dermatitis and conjunctivitis, however further research is required to identify the cause of this type of rhinitis (Windsor, et al., 2004). A good history can reveal a relationship between signs and potential allergens, like cigarette smoke, new perfumes, cleaning agents, furniture or fabric in the house (Hawkins, 2009).

The treatment of this type of rhinitis should involve allergen avoidance, pharmacotherapy (H_1 anti-histamines and corticosteroids), and in severe cases, immunotherapy may be an option

(Rosenwasser, 2002). If treatment is effective, signs will generally resolve within a few days (Hawkins, 2009).

2.10.3 Mycotic Rhinitis

In veterinary medicine, infection of the upper respiratory tract by *Aspergillus* spp., is of greatest clinical significance in the dog. *Aspergillus* causes a spectrum of diseases ranging from colonization, hypersensitivity reactions, through chronic necrotizing infections to rapidly progressive angioinvasion, resulting even in death (Siemieniuch, Skarzynski, & Kozdrowski, 2009).

The term sino-nasal aspergillosis seems more appropriate than the most common used in the literature (nasal aspergillosis) because in most cases, the lesions are undertaking the nasal cavities and frontal sinuses (Peeters & Clercx, 2004).

It is a common cause of nasal discharge in the dogs and is most often caused by *Aspergillus fumigatus* (Billen, et al., 2008; Sharp, Harvey, & Sullivan, 1991). As the fungus does not invade the mucosa or disseminate through the body in canine sino-nasal aspergillosis (Peeters, et al., 2005), this disease resembles a form of human chronic erosive non-invasive fungal sinusitis that occurs in immune competent patients (Day, 2008; Peeters, et al., 2008). The frontal sinus is the predilection site for the organism to become established before its metabolites begin the rapidly destructive process in the turbinates and their bony confines (White, 2006).

In humans, non-invasive fungal sinusitis encompasses three distinct clinical entities: fungal ball, allergic fungal sinusitis and chronic erosive non-invasive fungal sinusitis. The last one, more similar to the form present in dogs, is characterized by destruction of bone in absence of tissue invasion by the fungus and requires both tissue debridement and adjunct medical therapy (Day, 2008).

Epidemiology

Nasal aspergillosis is a common disease in dogs, it affects primarily young to middle aged males, mesaticephalic and dolicocephalic breeds and generally systemically healthy animals without clear evidence of reduced immunocompetence (Day, 2008; Mathews & Sharp, 2006). It, also, can occur concomitantly with, and probably secondary to nasal tumours and nasal foreign bodies (Mathews & Sharp, 2006).

However, it is still not clear why some dogs develop aspergillosis, since spores are present in the nasal cavity of most dogs, yet infection is uncommon (Billen, et al., 2008; Day, 2008;

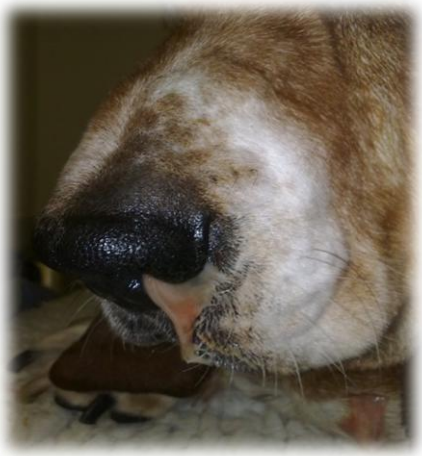
Sullivan, 1987). It is possible that there is a genetic susceptibility, perhaps MHC associated, or a subtle defect in innate immune mechanisms (Day, 2008).

Clinical signs

Typically, aspergillosis has a slow onset, although the history may be confuse by sudden dramatic epistaxis, other signs may have a chronic development like loss of pigmentation in the nasal planum around the nostril, sometimes accompanied by ulceration; facial pain, nasal hyperkeratosis and sensitivity especially over the frontal sinus and nasal discharge, which often fails to respond to antibiotic therapy. Severely affected dogs may also show signs of systemic illness, such as depression and anorexia (Harcourt-Brown, 2006b; Mathews, 2004; Schuller & Clercx, 2007).

When clinical signs of unilateral intranasal disease with accompanying turbinate destruction, the main diagnostic differentials should be neoplasia, fungal rhinitis, lymphoplasmacytic rhinitis or tooth root abcessation (Schochet & Lappin, 2005). Nevertheless, lymphoplasmacytic rhinitis as a primary aetiology for nasal disease is rare in the dog, whereas primary nasal neoplasia and fungal rhinitis are common causes o canine nasal disease (Schochet & Lappin, 2005).

Figure 10 – Nasal discharge on a dog with aspergillosis (19/04/2010)



Aetiology

Mycotic rhinitis cases in the dog are usually caused by *Aspergillus fumigatus* and rarely by *A. niger*, *A. nidulans*, *A. flavus* and *Penicillium sp*. The *Aspergillus* species can produce at least nine toxins (Ferreira, et al., 2007; Harcourt-Brown, 2006b). Aspergillosis and penicilliosis are in fact indistinguishable, other than by the microscopic appearance of their conidiophores,

however the species of *Penicillum* causing nasal penicilliosis have not been defined yet (Mathews & Sharp, 2006).

Aspergillus fumigatus is a worldwide saprotrophic species that plays an essential role in recycling carbon and nitrogen (Latgé, 2001, 2003). It belongs to *Ascomycota* phylum and *Trichomaceae* family, species are differentiated by morphological characteristics and colour of the colonies (Abarca, 2000; Krishnan, Manavathu, & Chandrasekar, 2009). *Aspergillus fumigatus*, is a thermotolerant species, since it grows at temperatures ranging from 20ºC to 50ºC (Quinn, Markey, Carter, Donnelly, & Leonard, 2002).

Although *Aspergillus* species are universally found in air, soil, dust and water, the primary mode of transmission to humans and animals is by inhalation of conidia, however there has been an increasing concern about contaminated food, environmental and occupational exposure to fungal spores of different species (Krishnan, et al., 2009; Quinn, et al., 2002).

It has a simple biological cycle, with a high sporulating capacity, which results in the ubiquitous presence of high concentrations of conidia in the atmosphere indoors and outdoors (Latgé, 2001, 2003). It also does not have a very elaborate mechanism for releasing its conidia into the air, dissemination simply relies on disturbances of the environment and strong air current (Latgé, 1999).

Putative virulence factors

Conidia of *Aspergillus* sp. are inhaled by humans and animals and rarely have any adverse effects since they are eliminated efficiently by innate immune mechanisms that endeavour to prevent mucosal colonization (Latgé, 2001, 2003).

A. fumigatus is known to produce adhesion, pigments, toxic molecules, enzymes polypeptide and allergens responsible for asthma and rhinitis, mycotoxins and β1,3 glucans that are known modulators of the immune system (Latgé, 1999, 2003).

It is thought that *Aspergillus sp.* virulence is directly correlated with its specific biological characteristics such as the release into the air of high concentration of conidia of small size, which are easily inhaled and able to germinate; and rapid mycelia growth at temperatures higher than 37ºC without any specific nutritional requirements (Debeaupuis, Sarfati, Chazalet, & Latgé, 1997; Latgé, 2001). It is also known that they adhere to and penetrate the respiratory epithelial, kill surrounding cells and resist phagocytosis (Mathews & Sharp, 2006).

The virulence of *Aspergillus fumigatus* seems to be more related to the lack of resistance of the host together with the microorganism's ability to adapt to surviving in harsh environments, rather than the expression of specific disease related-protein (Debeaupuis, et al., 1997; Park & Mehrad, 2009).

Different studies showed different results on *Aspergillus fumigatus* genotypes virulence. The first one published (Debeaupuis, et al., 1997), showed that with either clinical or environmental origin fungus were putative infectious strains, and the ability of any stain to become pathogenic in contact with an appropriate host could be linked to the absence of host specificity. However, a study (Aufauvre-Brown, Brown, & Holden, 1998) proved that the differences in virulence between strains of *Aspergillus fumigatus* appeared to exist, suggesting that differences occur, being clinical samples more virulent than environmental ones.

Nevertheless, no true virulence factors have been identified in *A.fumigatus*. In fact, the catalase (breaks down H_2O_2 to water and oxygen), proteases (which allow tissue invasion) and toxin secreted by this fungus do not play a role in the pathogenesis of *A. fumigatus* in experimentally-induced infections (Latgé, 2003; Mathews & Sharp, 2006).

The best documented toxins are fumagillin, gliotoxin and galactomannin. As fumagillin has been proven to inhibit the development of blood vessels, it probably contributes to the tissue loss that accompanies this disease (Harcourt-Brown, 2006b). Gliotoxin inhibits phagocytosis and induces apoptosis in macrophage, and has also a broad range of immunosuppressive actions (Latgé, 1999; Mathews & Sharp, 2006).

The effect of these toxic secondary metabolites could favour invasion by *A. fumigatus*, however their role remains questionable since species of Aspergillus which do not produce them can be pathogenic (Latgé, 1999).

Host immunity

Nonspecific or natural immunity plays a major role in the defence against *A. fumigatus* by recognition and clearance of the organism in immunocompetent hosts (Latgé, 1999).

The defence against inhaled conidia begins in the physical barriers of the respiratory tract, including nasal turbinates and the branching pattern of the bronchial tree. This results in a highly turbulent airflow that deposits most inhaled particles against airway surface fluid (Park & Mehrad, 2009).

The recognition of *Aspergillus* conidia and hyphae occurs via a number of soluble and cell-associated microbial pattern recognition receptors (Park & Mehrad, 2009). Conidial maturation induces a morphological change that involves the loss of the surface proteins (β-glucan, mannan, chitin and galactomannan) and the exposure of the inner cell wall of the fungus (Park & Mehrad, 2009) .

In dogs, the extensive tissue and bone destruction that occurs in nasal aspergillosis appears to be largely mediated by the inflammatory response, which includes a dominance of IgG

plasma cells over IgM and IgA cells (in contrast to the normal balance of nasal immunity) (Blanco & García, 2000; Day, 2009).

Colonization by fungus in the nasal mucosa of dogs, appears to induce a protective, cell-mediated immune response involving macrophages that are regulated by Th1 cells, perhaps via the production of key cytokines such as IL-12, Il-18 and IFN-γ (Day, 2008).

This protective immune response might not be enough to allow complete elimination of the causative organism. If IL-10 and TGF-β are activated, the probability of secondary immunopathological sequelae to infection is reduced but the persistence of the infectious agent is more likely (Day, 2008).

The functional nature of this immune response is distinct from that of LPR, but has clear benefit for the fungus, which establishes a commensal infection in a host that survives the initial pathogenic insult, in addition to this it has been suggested that fungal agents, themselves might be capable of manipulating host immunity by inducing T regulatory cells (Day, 2008; Mathews & Sharp, 2006).

Vaccines are now considered the future in fungal nasal prophylaxis, however a clearer understanding of the mechanisms involved in the immune response to fungal infection is needed to aid the development of effective vaccines (Blanco & Garcia, 2008). For fungal infections the only vaccine proved to results is for *Pythia insidiosum*, which causes a dermatophytosis in horses (Blanco & Garcia, 2008).

2.11 Systemic aspergillosis

In humans, *A. fumigatus* is the most frequent airborne fungal pathogen, which causes severe and usually fatal invasive infections in immune-compromised hosts in developed countries (Latgé, 2003). The airborne conidia have a diameter small enough the reach the alveoli in the lung, and if they overcome the immune defence mechanisms in the lung, they germinate and produce a branched, septate vegetative mycelium that invades the lung tissues (Latgé, 2001).

Cats are rarely seen with nasal aspergillosis, being systemic aspergillosis the most common one (Furrow & Groman, 2009). In fact, 70% of cats with systemic aspergillosis have underlying causes such as feline panleukopenia, FeLV, PIF and endoparasites (Furrow & Groman, 2009).

On the contrary, in dogs, systemic aspergillosis is not very common since the long nasal passages of dogs may more effectively trap *Aspergillus* spores before they reach the lower airways (Mathews & Sharp, 2006).

Predisposing factors for canine systemic aspergillosis may include a combination of optimal climatic conditions, an access to particular strains of *Aspergillus*, and a subtle defect in mucosal immunity that may have a genetic basis (Mathews & Sharp, 2006).

It is most often reported in German Shepherd Dogs that have been suggested to have a hereditary immune deficiency (Pérez, Mozos, Lara, Paniagüa, & Day, 1996; Schultz, et al., 2008).

Contrarily to human systemic aspergillosis, in dogs is mainly caused by *Aspergillus deflectus* and *Aspergillus terreus*, less commonly *Aspergillus niger* and rarely *A. fumigatus* and *A. flavipes* (Bruchim, Elad, & Klainbart, 2006; Clercx, McEntee, Snaps, E.Jacquinet, & Coignoul, 1996; Kim, et al., 2003; Robinson, Connole, King, Pitt, & Moss, 2000; Schultz, et al., 2008).

Dogs with systemic aspergillosis do not have any clinically overt nasal involvement (Mathews & Sharp, 2006). Usually instead, they have nonspecific clinical signs or those related to musculoskeletal, neurological, respiratory and gastrointestinal systems, and also signs of renal involvement like anorexia, weight loss, weakness, lethargy and vomiting (Schultz, et al., 2008). Rarely described, fungal infection of the reproductive organs can occur (Siemieniuch, et al., 2009).

A recent study (Svirshchevskaya, et al., 2009), showed that in mice invasive aspergillosis is related to the fungal germination in the lungs due to a delayed neutrophilic recruitment into the lungs and an abnormal functioning of complement system. Whether this actually happens in humans and dogs has not yet been proven.

Laboratory abnormalities include neutrophilia, azotemia, increased total serum protein concentrations (hyperglobulinemia), hypercalcemia (granulomatous inflammation or renal failure) and isosthenuria (Schultz, et al., 2008).

Radiographic changes consistent with diskospondylitis, osteomyelitis and intrathoracic lymphadenomegaly have also been described (Schultz, et al., 2008). MRI features are reported to be similar to other infectious and non-infectious inflammatory brain diseases (Schultz, et al., 2008).

Treatment with antifungal drugs should be considered in dogs with less severe presentations since long-term remission might be possible (Schultz, et al., 2008).

To sum up, in both dogs and cats the most commonly affected organs in systemic aspergillosis are lungs, kidneys, intestines, liver, lymph nodes and CNS (Schultz, et al., 2008). In contrast to systemic aspergillosis, nasal aspergillosis is known to be a local disease that can affect immunocompetent animals (Mathews & Sharp, 2006).

2.12 Treatment

2.12.1 Anti-fungal treatment

Polyene Macrolide Antibiotics

A number of polyene antifungal antibiotics has been isolated from various strains of *Actinomyces* , but only amphotericin B, nystatin, and pimaricin (natamycin) are used in veterinary medicine (Kahn & Line, 2005).

Amphothericin B (Albecet, AmBisome, Amphocil, Fungizone) has a great affinity to ergosterol (major sterol component of the phospholipid-sterol membranes of fungal cells) rather than to the cholesterol in host cells (Kahn & Line, 2005). As other polyene antifungal drugs, it forms complexes with the sterol components and induces the formation of pores in the fungal membrane, allowing leakage of contents (Kahn & Line, 2005; Ramsey, 2008).

Its main role is as a fungistatic drug, although high drug concentrations and pH values between 6.0 and 7.3 in the surrounding medium may lead to fungicidal effects (Kahn & Line, 2005).

Amphotericin B has been considered, in human medicine, the mainstay of antifungal therapy for invasive and serious mycosis, but nowadays other antifungals such as voriconazole and posaconazole are considered first-line drugs for many of these infections (Porter & Kaplan, 2010). In veterinary medicine, it has been used for the treatment of systemic fungal infections and leishmaniasis (Ramsey, 2008) and it appears to have direct effect on yeasts and fungi and may also act as an immunopotentiator, thus enhancing the host's ability to overcome mycotic infections (Kahn & Line, 2005). Nevertheless, it is contraindicated in animals with hepatic or renal failure (Ramsey, 2008).

The polyenes are not effective against dermatophytes. Nystatin is mainly used for the treatment of mucocutaneous candidiasis, but it is effective against other yeasts and fungi. The antimicrobial activity of pimaricin is similar to that of nystatin, though it is mainly used for the local treatment of candidiasis, trichomoniasis, and mycotic keratitis (Kahn & Line, 2005).

Imidazoles

The discovery of antifungal activity of azole compounds, including imidazoles and triazoles, represented an important advance in the management of superficial and systemic fungal infections (Bodey, 1992). In fact, the azole compounds have a relatively broad spectrum of activity against pathogenic and opportunistic fungi excelled only by amphotericin B (Bodey, 1992).

There are two principal mechanisms of action of the azole compounds. Firstly, they alter the cell membrane permeability of susceptible yeasts and fungi by blocking the synthesis of ergosterol (demethylation of lanosterol is inhibited), the primary cell sterol of fungi (Kahn & Line, 2005). Secondly, they inhibit cytochrome P-450 enzymes (Bodey, 1992), which are required for fatty acid synthesis.

These drugs induce changes on oxidative and peroxidative enzyme activities (Kahn & Line, 2005). Due to the toxic concentrations of hydrogen peroxide that develop intracellularly, the cell membrane alters its permeability leading to leakage of proteins and aminoacids, it also interferes with the uptake of essential nutrients, inhibits catalase systems, decreases fungal adherence, and inhibits mycelia formation (Bodey, 1992; Kahn & Line, 2005). All this will lead to cell membrane and organelle disruption as well as cell death (Kahn & Line, 2005).

Imidazoles may have anti-bacterial, anti-fungal, anti-protozoal, and anti-helmintic activity. Several distinct phenylimidazoles are therapeutically useful antifungal agents with wide spectra against yeasts and filamentous fungi responsible for either superficial or systemic infections. The anti-helmintic thiabendazole is also an imidazole with antifungal properties against *Aspergillus* and *Penicillium* sp., however its use has largely been replaced by the more effective imidazoles (Kahn & Line, 2005).

Clotrimazole, miconazole, enilconazole, ketoconazole, itraconazole, and fluconazole are the most clinically important members of this group (Kahn & Line, 2005), they as all the imidazole derivatives are fungistatic at low concentrations and fungicidal at higher concentrations (Claeys, Lefebvre, Schuller, Hamaide, & Clercx, 2006; Zonderland, et al., 2002).

Miconazole (Daktarin, Malaseb, Surolan) was the first azole available for parenteral administration and has a wide antifungal spectrum against most fungi and yeasts of veterinary interest. Sensitive organisms include *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Candida spp*, *Coccidioides immitis*, *Cryptococcus neoformans* and *Aspergillus fumigatus* (Bodey, 1992; Kahn & Line, 2005). However some *Aspergillus* and *Madurella sp*. are only marginally sensitive (Kahn & Line, 2005).

According to BSAVA formulary (Ramsey, 2008), it is used in dogs to treat fungal otitis (Surolan), dermatophytosis and *Malassezia* dermatitis (Malaseb).

Ketoconazole (Nizoral) has an antifungal spectrum similar to that of miconazole, although is more effective against *Coccidioides immitis* and some other yeasts infections of the skin and mucous membranes (Kahn & Line, 2005). It is also used in veterinary medicine, for the medical management of adrenal tumours, in fact it is used as a primary therapy in dogs that do not tolerate mitotane or trilostane, however as a short-term therapy it is used to provide

evidence for or against diagnose of hyperadrenocorticism (Ramsey, 2008). Hepatotoxicity appears to be most important adverse reaction, although in dogs has not been recognized, unless high doses are used (Ramsey, 2008).

Itraconazole (Intrafungol, Sporanox), it´s a triazole antifungal agent and is one of the most active antifungal, preferred to other imidazoles for the treatment of systemic fungal infections, including aspergillosis and sporotrichosis (Kahn & Line, 2005). It is widely distributed in the body, and because of that it is known to clear some types of fungal meningitis in humans, although is not the drug of choice (Porter & Kaplan, 2010). Because of its high lipid solubility and protein binding, the level of itraconazole tends to be low in blood, urine, CSF and tissues with low protein contents (Porter & Kaplan, 2010; Ramsey, 2008).

The reported side effects of itraconazole included anorexia, vomiting, diarrhoea, lethargy, increased blood urea nitrogen (BUN) and skin ulcerations (Legendre, et al., 1996), which are now believed to occur with itraconazole toxicity due to an immune reaction (Schochet & Lappin, 2005). A study (Schochet & Lappin, 2005) reported itraconazole to be able to induce fever, in dogs, when taking the recommended dose.

In humans, the use of itraconazole is likely to decline due to resistance (Denning, 1996) and to the use of new anti-fungal drugs such as voriconazole and posaconazole (Porter & Kaplan, 2010).

Fluconazole (Diflucan, Fluconazole) is a water-soluble drug, absorbed almost completely after an oral dose; it is used against *Blastomyces*, *Cryptococcus*, *Coccidioides*, *Candida sp*., *Histoplasma* and *Microsporium*. It has variable effectiveness against *Aspergillus sp.* and *Penicillium sp.* infections (Porter & Kaplan, 2010; Ramsey, 2008). It attains therapeutic concentrations in the respiratory tract and in the CSF, actually it is used, in human medicine, for the treatment of cryptococcal and coccidioidal meningitis (Porter & Kaplan, 2010). Due to fact that fluconazole is excreted largely unchanged in urine, adverse reactions (nausea and diarrhoea) can occur in dogs with kidney disease, however drug interactions are less likely to occur with fluconazole than with any other imidazole (Ramsey, 2008).

Enilconazole (Imaverol) is an imidazole that can be applied topically for the treatment of fungal skin infections such as dermatophytosis, in which it inhibits fungal growth in 2 rather than 4-8 treatments SID, as is necessary with other antifungal agents. In addition to this, enilconazole can also be used successfully as infusion into the nasal turbinates in dogs for the treatment of nasal aspergillosis (Kahn & Line, 2005; Lanthier & Chalifoux, 1991; Ramsey, 2008; Sharp, et al., 1991; Zonderland, et al., 2002). It is known not to have any significant side effects during the course of treatment (Lanthier & Chalifoux, 1991), however it was

reported to cause hepatotoxicity in dogs when swallowed (Ramsey, 2008). This drug has the advantage of being inexpensive, short-term and topical therapy.

Clotrimazole (Canesten, Clotrimazole, Lotriderm) was the first imidazole that exhibit potential systemic effects, unfortunately the high doses required for adequate serum concentrations were poorly tolerated (Bodey, 1992).

It is commonly used in superficial fungal infections as well as nasal aspergillosis (Ramsey, 2008), and in human medicine is used for the treatment of a variety of conditions, including the topical treatment of oropharyngeal or oesophageal candidiasis (Mathews, Linder, Davidson, Goldman, & Papich, 2009).

Voriconazole is second generation broad-spectrum triazole that is derived from fluconazole and that has an enhanced antifungal spectrum, compared with older triazoles (L. Johnson & Kauffman, 2003). It is successful against *Candida sp*., *Aspergillus sp*., *Cryptococcus sp.* and other species (Nakaya, et al., 2010). It's used, in human medicine, as a first-line therapy for serious *Aspergillus* spp. infections (systemic), refractory infections with *Pseudallescheria/ Scedosporium* and *Fusarium sp.* (L. Johnson & Kauffman, 2003), or in immunocompetent and immunocompromised hosts (Porter & Kaplan, 2010; Rogers & Frost, 2009).

It is known to have superior effectiveness compared to amphotericin B (Nakaya, et al., 2010). However, it is not effective against *Candida sp*. and it is proven to have more side effects and drug interactions than fluconazole (L. Johnson & Kauffman, 2003).

Nakya et al. (2010) described the effectiveness of a treatment of invasive fungal sinusitis with voriconazole. The three cases reported were treated with successfully with this new agent, which may create new options for the treatment of invasive mycotic sinusitis, changing the conventional treatment with amphotericin B.

In veterinary medicine, a study (Beernaert, et al., 2009) was published reporting the efficacy of voriconazole in pigeons. It was proven to reduce clinical signs and eliminate *A. fumigatus* in racing pigeons experimentally infected with *A. fumigatus*.

At conventional doses the azole compounds have been very well tolerated, even when administered for prolonged periods of time. However, the potential toxicities are gastrointestinal (nausea, vomit and diarrhoea), hepatic (transient increased liver enzymes), endocrinologic (reduced serum testosterone and hypoadrenocorticism), metabolic, haematologic (hyperlipidemia, hypertriglycemia and non-regenerative anaemia), carcinogenic and also occur as the result of drug-drug interactions, for instance with cyclosporine (Bodey, 1992).

Other antifungal agents

Delayed closure of the nasal cavity and frontal sinus combined with topical administration of povidone-iodine applied either as a solution or as dressings has also been described (Moore, 2003). Despite its effectiveness, this technique is very invasive, requires the owner's and patient's compliance and long hospitalization time, so it should only be used in severe cases unresponsive to other treatments.

2.12.2 Current treatment for canine nasal aspergillosis

Nasal aspergillosis is a common condition in dogs that results in massive turbinate destruction and pain. If untreated, lysis of the surrounding skeletal structures, such as the cribriform plate, will occur, and death or euthanasia would be the only options available (Mathews, et al., 1998; Mathews & Sharp, 2006).

The treatment of canine aspergillosis can be changeling, and the existing options are either systemic or topical antifungal therapy. Systemic treatment with oral antimycotic agents such as thiabendazole, ketoconazole, itraconazole, or fluconazole is known to be costly, requires prolonged administration (Mathews, 2004; Schuller & Clercx, 2007) and in addition are not highly active against *Aspergillus* spp. or *Penicillium* spp. (Claeys, et al., 2006; Sharp, et al., 1992).

In fact, different studies showed that that clinical improvement with systemic treatment was only seen in about 50% of dogs treated with thiabendazole and ketoconazole and in 70% of dogs treated with intraconazole or fluconazole (Sharp, et al., 1991; Sharp & Sullivan, 1989).

By comparison, topical treatment for nasal aspergillosis has been shown to have considerably better efficacy (Bray, White, & Lascelles, 1998; Mathews, et al., 1998; Sharp, Sullivan, Harvey, & Webb, 1993; Smith, Andrews, & Biller, 1998). Instillation of antifungal agent into the nasal and paranasal sinuses is performed following either surgical placement catheters or by non-surgical placement of catheters into the nasal sinuses via the nostrils (Sissener, Bacon, Friend, Anderson, & White, 2006).

Topical treatment is performed with clotrimazole or enilconazole, since these drugs have poor solubility and show limited intestinal absorption (Sharp, et al., 1993; Zonderland, et al., 2002). Some studies showed that this treatment is associated with higher success rates and has improved the management of nasal aspergillosis (Claeys, et al., 2006; Mathews, et al., 1998; Schuller & Clercx, 2007).

Many techniques have been investigated to administer medication locally. For many years, the standard treatment consisted of surgical implantation of infusion tubes and irrigation of the nasal cavities and frontal sinuses, twice daily for 7 to 14 days, with topical antifungal

agents (Mathews, et al., 1998; Sharp, et al., 1991; Sharp & Sullivan, 1989; Sharp, et al., 1992). In fact, enilconazole irrigation showed to be successful in 90% of cases (Sharp, et al., 1993), however, as explained above, this requires multiple treatments, is poorly tolerated (Sissener, et al., 2006) and animals must often be hospitalised for up to two weeks (Bray, et al., 1998; Sharp, et al., 1991).

If treatment can be performed at home, most owners find it distressing and few are willing to repeat it (Bray, et al., 1998). In addition to this, the tubes can become prematurely dislodged and an additional surgical procedure is require to replace them (Bray, et al., 1998).

Nowadays in medicine veterinary, the tendency is to use minimally invasive techniques with infusion of enilconazole (1% or 2%) or clotrimazole (1%), by non-surgically placed catheters into the dorsal nasal meatus via general anaesthesia. This technique is known to provide better distribution of the drugs into the sinuses, to be equally effective and to be associated with few complications (Bray, et al., 1998; Mathews, et al., 1998; Richardson & Mathews, 1995; Schuller & Clercx, 2007; Smith, et al., 1998).

Enilconazole is thought to be less toxic and irritating than clotrimazole, especially at low concentrations (Claeys, et al., 2006; Sharp, et al., 1993; Zonderland, et al., 2002), because european preparations of clotrimazole contain isopropanol and propyleneglycol which can be irritating to the mucous membranes (Claeys, et al., 2006; Sharp, et al., 1993; Zonderland, et al., 2002). It was proven, that clotrimazole was not viscous enough to be retained in sinuses after anaesthesia, which was likely to drain into the nasal cavity or swallowed (Mathews, et al., 2009).

Nevertheless, clotrimazole has been used as a topical agent both invasively and non-invasively with similar success rates to enilconazole (Mathews, et al., 1998; Richardson & Mathews, 1995; Smith, et al., 1998). The clinician should always have present that clotrimazole topical infusion is generally contraindicated in animals in which the cribiform plate is damaged (Mathews & Sharp, 2006), and may not be successful in animals with aspergillosis that involves the orbit (Furrow & Groman, 2009).

The instillation of a clotrimazole-containing gel into the frontal sinuses of dogs with nasal aspergillosis was proven to be perhaps beneficial since it may reduce the anaesthetic time associated with current topical drug protocols and has the potential to increase the efficacy of topical treatment by prolonging the drug contact with the fungus. Yet, the ideal retention time for treating nasal mycosis in dogs is not known (Mathews, et al., 2009; Sissener, et al., 2006).

The volume of nasal cavity and frontal sinuses depends on the size of the dogs, extent of turbinate destruction, and volume of accumulated exudates, but on the basis of other studies,

the average volume considered for the frontal sinuses in breed predisposed to fungal rhinitis is 25 ml per side (Benitah, 2006).

Treatment protocols (Mathews, et al., 1998; Smith, et al., 1998; Zonderland, et al., 2002) with variations of this last technique are still under investigation in order to improve treatment success, tolerance by the patient and compliance by the owners.

Endoscopic placement catheters into the frontal sinuses was also described (Claeys, et al., 2006; Zonderland, et al., 2002). The studies concluded that this technique with the intrasinusal administration of enilconazole was 92% effective and fewer infusions (one to three) were required for success when compared with blindly placed intranasal catheters. In addition to this, it seems that extensive rhinoscopic debridement before infusion is critical for the success of the treatment, because it reduces the number of infusion procedures.

In cats, a study (Furrow & Groman, 2009) showed that intranasal infusion of clotrimazole is also an effective treatment without concurrent or subsequent oral administration of antifungal agents. However, further prospective studies are needed to recommend this treatment as the gold standard for nasal aspergillosis in cats.

Glucorticoids should never be administered to dogs with aspergillosis, since their inadvertent use can precipitate the dissemination of the infection in some cases (Mathews & Sharp, 2006).

2.12.3 Surgical considerations

Rhinotomy and turbinectomy besides its diagnostic role are commonly used as part of aspergillosis treatment. Osteotomy of the nasal bones after a dorsal midline incision has been the standard approach to the nasal cavity of dogs (Holmberg, 1996). The dorsal approach is useful to access the entire nasal cavity and the frontal sinus and can be used for most diseases of nasal cavity except for lesions located within the nasopharynx (Degner, 2006). Commonly it is performed by removing a window of bone over the dorsal bridge of the nose and frontal sinuses using a pneumatic drill or alternatively a large intramedullary pin (Degner, 2006).

Ventral approaches to the nasal cavity have also been described; clinicians tend to be reluctant to use it due to concerns with contamination, limited exposure and potential oronasal fistula formation. (Holmberg, 1996) It is useful to access the nasal cavity and nasopharyngeal region, however the frontal sinuses cannot be entered using this approach (Degner, 2006).

Ventral rhinotomy is reported to cause much less postoperative morbidity than does the dorsal approach, for instance the complication of subcutaneous emphysema does not occur (Degner, 2006) and long-term sequelae are reported to be similar but less common to those of dorsal rhinotomies such as persistent oronasal fistula, which may result in chronic infection of the nasal cavity and subsequent nasal discharge (Degner, 2006; Holmberg, 1996).

Complications of operative examination procedures include dehiscence of the macerated wound, subcutaneous emphysema and injury to the brain such as ischemia and penetration of the ethmoidal turbinates that can induce neurological signs (Degner, 2006; Knotek, et al., 2001). Other complications include intra-operative bleeding from the conchae, excessive haemorrhage, anaemia (may necessitate blood transfusions), blood and exudates aspiration, respiratory difficulties, oronasal fistulae, persistent nasal discharge and in exceptional cases even death of the patient (Degner, 2006; Fletcher, Snyder, Messinger, Chiu, & Vite, 2006; Knotek, et al., 2001).

Hazards include profuse bleeding in patients suffering from coagulopathies (Knotek, et al., 2001), pneumocephalus or septic meningoencephalitis secondary to dorsal rhinotomy (Fletcher, et al., 2006). Furthermore, secondary fungal infections or fungal hypersensitivity may develop due to a breakdown of normal defence mechanisms, which may require chronic treatment with antifungal medication (Degner, 2006). Complications associated with anaesthesia in dogs with functional disorders of the circulatory and respiratory apparatus and liver have also been referred (Knotek, et al., 2001).

Administration of intravenous fluids may be necessary for a day or two to maintain normal hydration and replace fluid losses associated with intra-operative bleeding. Serosanguinous discharge from the external nares is present during the first week after surgery. A broad spectrum antibiotic effective against bacteria that are found in nasal cavity should be administered for a period of two weeks to prevent a future infection (Degner, 2006).

2.13 Prognosis

The prognosis of the anti-fungal treatment when well chosen and performed has been reported as excellent. With enilconazole administered, BID, for 7 to 14 days as an invasive topic treatment was successful in 90% of cases (Sharp, et al., 1993), however it was ineffective when used as a non-invasively topical treatment (Bray, et al., 1998).

Clotrimazole has been used topically, both invasively and non invasively (Mathews, et al., 1998; Richardson & Mathews, 1995; Smith, et al., 1998) with 87% success in one study (Mathews, et al., 1998; Pomrantz & Johnson, 2010) and 67% in other (Pomrantz & Johnson, 2010).

Once nasal discharge ceases after clotrimazole treatment, it appears that a recurrence of rhinitis attributable to fungal infection is uncommon (Mathews, et al., 1998; Sharp, et al., 1992). However recurrence of nasal discharge which responds to antibiotics has been reported, and is presumed to be bacterial infection secondary to permanent damage to the nasal mucosa (Mathews, et al., 1998; Sharp, et al., 1993). If it does not resolve in the first two

weeks after treatment, a re-treatment should be performed, since the clinician should consider the initial infection not to be resolved (Schochet & Lappin, 2005).

Dogs with fungal granulomas within the frontal sinuses are often refractory to treatment and may require severe debridement treatments followed by topical application of clotrimazole during the same anaesthetic episode (Mathews & Sharp, 2006).

The re-examination should be done after three to four weeks after the first treatment with clotrimazole, and if there is resolution of the clinical signs after the first treatment, it was shown that there is no benefit to be derived from a routine second treatment (Friend, Williams, & White, 2002; Pomrantz & Johnson, 2010).

Repeated rhinoscopy and treatment at monthly intervals are advised, since it helps following resolution or progression of disease (Pomrantz & Johnson, 2010).

Glucocorticoids are frequently used in veterinary medicine for their anti-inflammatory properties, yet it is known that they can increase the susceptibility to infection and may enhance the fungal infection by inducing immunosuppresion (Schochet & Lappin, 2005).

A study (Friend, et al., 2002) reported a seasonal variation in some of the dogs affected by aspergillosis, and commonly the signs after treatment would cease within 12 months after treatment.

In the case of LPR, clinical signs can take many weeks or months to completely resolve and relapses are common if treatment is tapered or discontinued (Mackin, 2004). So, the prognosis is generally good with respect to management of sign and quality of life. Nevertheless, some degree of clinical signs persists in many dogs (Hawkins, 2009).

The prognosis of dogs with allergic rhinitis is excellent if the allergen can be eliminated, otherwise the prognosis for control is good, but a cure is unlikely (Hawkins, 2009).

2.14 Conclusions from the literature review and research aims

The literature review has shown that inflammatory nasal diseases such as lymphoplasmacytic rhinitis and aspergillosis are important and common diseases that affect healthy and middle-aged dogs.

The correct diagnose of both diseases is still very changeling and no single test result is diagnostic. Aspergillosis diagnosis is based on two or three cumulative positive findings of a comprehensive evaluation tests with appropriate clinical signs. On the contrary, lymphoplasmacytic rhinitis diagnosis is made by exclusion of other possible diagnose differentials, since its aetiology is still very controversial.

Diagnostic imaging studies, such as MRI, are considered to have good resolution to identify or localized early nasal disease, since they provide a thorough assessment of the nasal cavity and sinuses and provide superior insight to the nature and extent of the disease.

Therefore, to test the hypothesis that MRI is a good and reliable imaging study for inflammatory nasal disease, the following aims were set:

1. Determine the statistical association of MRI abnormalities in dogs with aspergillosis or rhinitis.
2. Assess the changes seen on MRI and compare them with rhinoscopy findings.
3. Determine whether MRI features correlate with the histopathologic findings.
4. Propose a new histologic inflammatory score in dogs with inflammatory nasal disease, and determine the statistical association between it and the MRI abnormalities.
5. Assess the association between serology and culture, and MRI findings and final diagnosis.
6. Appraise the findings from the present study and compare them with previous investigations, in order to draw final conclusions.

3 Materials and Methods

3.1 Case Selection criteria

Medical records of dogs examined at the Queen's Veterinary School Hospital of the University of Cambridge, between February 2002 and December 2009, that underwent MRI because of nasal disease, were reviewed. All available MR images were reviewed by the author and one board-certified radiologist, and qualitatively characterized in regard to the level of destruction and intensity.

Dogs were included in the study if results of nasal MRI were available as well as dogs diagnosed with inflammatory or fungal disease. Dogs were excluded from the study if results of MRI were not available, the final diagnose was foreign body, parasitic infection, trauma, neoplasia or if nasal disease was not present.

3.2 Diagnostic Criteria

All the 46 medical records of dogs with inflammatory nasal disease were analyzed and distributed according to three groups (Aspergillosis, Rhinitis and Likely Aspergillosis): 16 were included in the Aspergillosis group, 25 on the Rhinitis group and 5 on the Likely Aspergillosis group.

Aspergillus fumigatus is a commensal fungal infection of the nasal chambers, so at least three positive ancillary diagnostic tests (radiography, MRI, visualization of fungal colonies via rhinoscopy, serology, culture and histopathology) were needed to diagnose pathological fungal nasal disease (J. Saunders, et al., 2004; Sharp, et al., 1992).

Rhinitis was diagnosed on the basis of MRI and rhinoscopy findings. Allergic rhinitis and non-specific rhinitis (LPR) were included on the Rhinitis group, so no distinction was made between these pathologies.

The Likely aspergillosis group was defined as those cases for which a nasal disease was likely due to aspergillosis, but did not have three positive ancillary diagnostic tests. This group was only included for a descriptive and MRI statistics purpose, it was not included in the main statistic evaluation.

3.3 Procedures

The medical records of the dogs with inflammatory nasal disease included in the study were reviewed. For all animals, historical information was reviewed including breed, gender (male, male neutered, female and female neutered), age (in months, although in the results it was converted to years for a better understanding), weight (in kilograms), duration of clinical signs

(onset of symptoms less than 1 month, between 1 and 3 months, between 4 and 6 months, more than 6 months and unknown, when the medical records did not specify a period of time), and response to antibiotics (responsive, non responsive and unknown, when no information was found).

Clinical signs, bacteriology and mycology results, radiology reports, rhinoscopy findings, histopathology results and MRI reports, were also reviewed. The treatment and follow-up information regarding clinical outcome were also obtained for descriptive purposes.

3.3.1 Clinical signs and Leukogram

Clinical examination of these dogs revealed a range of signs including nasal discharge (right side, left side, bilateral or unknown, when information about was not present; mucoid, serous, purulent, muco-purulent and non-specific), epistaxis/haemorrhagic nasal discharge (present or absent), sneezing (present or absent), reverse-sneezing (present or absent), exophthalmus (present or absent), changes in the *planun nasale* like depigmentation and hyperkeratosis (present or absent for both) and other signs present like cough, nose pain and lymphadenopathy.

Leukogram was performed in 38 dogs and classified by the author as lymphopenia, lymphopenia with neutrophilia, lymphopenia with eosinophilia, leucocytosis with neutrophilia, neutrophilia only, eosinophilia only, neutrophilia and eosinophilia, leucocytosis with neutrophilia and eosinophilia, normal (when no changes were observed on the leukogram) and unknown (when no leukogram was performed or no results were found).

3.3.2 Radiology

The radiographic examination was performed on 24 dogs under general anaesthesia or sedation, and included the skull (nasal cavity and frontal sinus, ventro-dorsal open mouth view, lateral or skyline views) and thoracic radiographs were performed, under sedation, in 24 cases each, prior to rhinoscopy.

The radiographs were reported by two board-certified radiologists, who gave a radiological diagnosis when possible. Retrospectively, it was seen when turbinate destruction and soft tissue opacities were present in these radiographs, and they were classified by the author as normal (when there were no evidence of nasal disease), abnormal (when the final diagnosis was consistent with inflammatory nasal disease) and unknown (when there no reports or radiographs available).

Figure 11 – Horizontal beam frontal sinus skyline view x-ray at QVSH, Radiology Department (10/11/2009)

3.3.3 Magnetic Resonance Imaging (MRI)

MRI was performed in each dog under general anaesthesia using a veterinary MRI unit (Vet-MR; Esaote) incorporating an open 0-2 Tesla permanent magnet. The patient head, as described in another study made in the Queen´s Veterinary School Hospital (A. Avner, Dobson, J.M., Sales, J.I. & Herrtage, M.E., 2008)[1], was placed in a dual-phase-array coil (143-158 mm). T_1-weighted (T_1W), T_2- weighted (T_2W) and proton density-weighted (PDW) images of the nasal cavities and paranasal sinuses were acquired in the dorsal, transverse and sagittal planes. T_1W images were also acquired after intravenous administration of 0.1 mmol/kg gadobenate dimeglumine (Multihance; Bracco).

Figure 12 - Nasal MRI scan on a German Shepherd dog at the Veterinary MRI Unit (10/11/2009)

All scans were reported by two board-certified radiologists, who gave a final diagnosis when possible. Subsequently, the scans were reviewed and classified by the author and by a board-

[1] Avner, A., Dobson, J.M., Sales, J.I. & Herrtage, M.E. (2008). Retrospective review of 50 canine nasal tumours evaluated by low-field magnetic resonance imaging. *Journal of Small Animal Practice*, *49*, 233-239.

certified radiologist, over a period of one week in several batches prepared by the author with patients in roughly chronologic order. If a patient had more than one scan, the one used for the study was always the earliest one.

They were firstly classified as normal (when there was no evidence of nasal disease) or abnormal (when there was evidence of destruction, inflammation or mucus). Secondly, the destruction of the turbinates, cribriform plate and vomer/nasal septum was examined and classified as present or absent. The turbinate destruction, when present, was also classified as mild (less than 25% destruction), moderate (between 25% and 50%) and severe (more than 50% destruction). This classification was done according to the transverse slice that appeared to show the greatest degree of destruction on T_1W pre and post-contrast.

The intensity on dorsal T_1W (comparing with muscle) and dorsal T_2W (comparing with periorbital fat and brain) was examined for mucus and turbinates and classified as unknown (when the brain, fat or muscle where not visible), hypointense, hyperintense and isointense (when had the same intensity) on the brightest area on the same slice.

The involvement of the frontal sinus (mucus and granulomas) was classified as present or absent, and the localization of the lesions (right side, left side, bilateral or normal) was also documented.

Table 4 - The magnetic resonance criteria evaluated

The magnetic resonance imaging criteria evaluated
• Presence and localization of the lesions
• Destruction of turbinates (extent on the most destructive slice)
• Integrity of the cribiform plate
• Destruction of the nasal septum/vomer bone
• Appearance of the mucus (intensity on the various sequences)
• Appearance of the turbinates (intensity on the various sequences)
• Involvement of the frontal sinus (enhancement)

3.3.4 Rhinoscopy and Nasal Biopsies

Rhinoscopy was performed in 43 dogs by different hospital clinicians, immediately after MRI. For anterior rhinoscopy, was used a rigid rhinoscope of variable diameter to accommodate for patient size. Posterior rhinoscopy was performed with a flexible rhinoscope using a videoendoscope.

Since September 2009, the dogs were anaesthetized after premedication with sedatives as acepromazine (0.01–0.05 mg/kg) and medetomidine (1-5 µg/kg) and with analgesics as buprenorphine (20µg/kg). Anaesthesia was induced and maintained with intravenous

administration of propofol (1-4 mg/kg) and gas inhalation (isoflurane or sevoflurane); all dogs were intubated and swabs or rolled cotton gauze, were placed behind the soft palate to prevent aspiration of any fluid debris. Rhinoscopy was performed with the dogs in sternal recumbency and with the head tilted downwards to allow fluid from the nasal sinuses to drain rostrally.

Figure 13 - Rhinoscopy on a German Shepherd dog at the QVSH, Surgery Department (10/11/2009)

The oral cavity, nasopharynx and larynx were examined and nasal flushes, swabs and biopsy specimens when collected, from the nasal cavity, were submitted for histopathologic, bacteriologic (aerobic bacterial culture and antibiotic sensitivity testing on each isolate) and mycologic (fungal culture) examination in the hospital pathology laboratory by board-certified veterinary pathologists.

The rhinoscopy reports were reviewed by the author and classified according to the lesions found as normal, inflammation only, inflammation and fungal plaques seen, turbinate destruction only, turbinate destruction and fungal plaques seen, fungal plaques only and unknown when no rhinoscopy was performed.

Methods for obtaining nasal biopsy specimens, listed in the medical record included rhinoscope-guided biopsy, rhinotomy biopsy and blind grab biopsy. The samples, were taken where the tissue appeared to be macroscopically abnormal, and when several areas presented abnormal tissue, a biopsy was taken from each abnormal area. Fine needle aspiration biopsies from lymph nodes were taken, when they were palpably enlarge.

Figure 14 - Biopsy taken during rhinoscopy (10/11/2009)

3.3.5 Histopathology

Histopathology tissue samples were reviewed and classified for 38 dogs according to the inflammation degree, as normal, mild, moderate and severe, by a single board-certified veterinary pathologist. When the samples were not available, they were classified as unknown.

The final degree of inflammation was described according to the number of neutrophils, lymphocytes, plasma cells and eosinophils, as not present, mild, moderate and severe presence of cells, the numbers were then sum up to give an inflammation score from 0 to 12. This score was then converted into the final inflammation score, from 0 to 3, the inflammation was classified as mild; from 4 to 6 was moderate and more than 7 was severe inflammation. The cell type classification was based on the most frequent cell present on the samples given; therefore the final histopathology classification included firstly the name of the most frequent cell, then the second most frequent one and so on.

The epithelial hyperplasia, goblet cell hyperplasia, oedema and fungal presence were also classified as normal, mild, moderate and severe presence.

The histopathology classification was done according to the cell type most found in the samples; inflammation score; acute, subacute or chronic changes; diffuse or localized changes, most frequent cell type hyperplasia found and oedema and fungal presence (Attachment I).

3.3.6 Bacteriology and Mycology

Nasal culture results were classified for 36 dogs as negative, presence of bacteria, presence of fungus, presence of bacteria and fungus and unknown when the author did not find information about it on the medical records. Serology testing for antifungal (*Aspergillus sp.*)

antibodies was performed by means of agar gel immunodiffusion in 34 dogs and classified as positive, negative or unknown (when no information was found).

3.3.7 Final Diagnosis

The final diagnosis in the 46 cases was based on the review of history, radiography, MRI and rhinoscopy findings, culture of the nasal discharge, serology and histopathology results. Despite the groups that were done for this study, the final diagnosis for each case was based on the diagnosis that the clinician did when the patient presented at the hospital. The three main diagnoses were chronic non-specific rhinitis/LPR, allergic rhinitis or aspergillosis. Dogs in which the final diagnosis nasal neoplasia or foreign body were excluded from this study.

3.3.8 Statistics analysis

Statistical analysis of the data was performed using SPSS® 17.0. A database was created with thirty six variables regarding classification of the disease, age, breed, gender, weight, onset of the disease, clinical signs, leukogram, serology, culture, x-ray results, MRI results, rhinoscopy, and clinician final diagnosis of each one of the 46 dogs selected (Appendix I).

On the first phase of the analysis were used descriptive measures such as means, standard deviation and percentages. On the second phase of analysis, statistical testing was done with cross-tabulation (contingency tables) between the categorical variable, Classification (with two categories: Rhinitis and Aspergillosis, being the category defined as Likely aspergillosis coded as missing value) and all the others categorical variables.

In the third phase of the analysis was found useful to cross-tabulate a re-coded Classification (with two categories: Likely aspergillosis and Aspergillosis, being Rhinitis coded as missing values) and the variables related with MRI (affected side, turbinate destruction, vomer/septum destruction, cribriform plate destruction, frontal sinus involvement, mucus and turbinate intensity on T_1W comparing with muscle, mucus and turbinate intensity on T_2W comparing with fat and brain)

For each cross-tabulation non parametric tests were used. The Pearson's chi square test was used in order to test for row and column independence. Null and alternative hypothesis were formulated for these data. The null hypothesis (H_0) established that the Classification and Variable *x* were independent and the alternative hypothesis that the Classification and Variable *x* were not independent. The level of significance accepted was for all cross-tabulations a p value < 0.05.

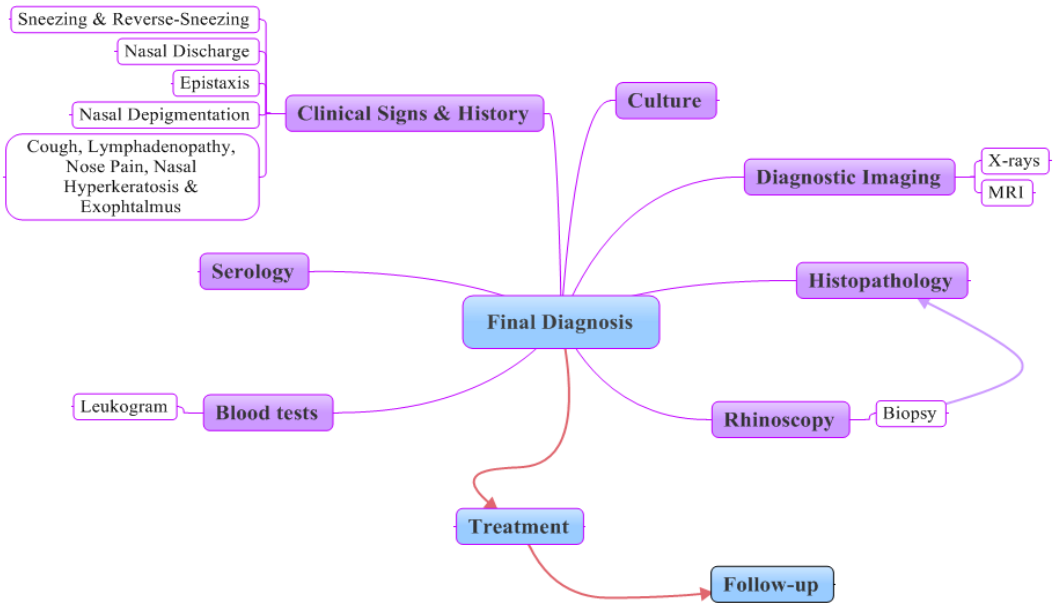
In the analysis some variables were re-coded (Classification, Responsiveness to Antibiotics, X-ray, Nasal Discharge, Serology, Rhinoscopy, Mucus Intensity on T_1W, Affected side, Turbinate Intensity on T_2W Brain and Fat) in order to obtain contingency tables suitable to

Pearson's chi square test. In some of them was not possible to apply this test, because there was not enough criteria (Mehta, 1996) namely, the minimum expected cell count for all cells should be at least 5; for tables larger than 2x2, a minimum expected count of 1 is permissible as long as no more than about 20% of the cells have expected values below 5.

When SPSS reported that the minimum expected frequency was inferior to that level, the Fisher's Exact Test of independence was used to obtain the exact *p* value for a single 2x2 contingency table (cross-tabulation of Classification with Nasal Discharge, Epistaxis, Nasal Hyperkeratosis, Reverse Sneezing, Nasal Depigmentation, Responsiveness to Antibiotics, Affected Side on MRI, X-ray results, MRI results, Cribriform Plate Destruction, Mucus Intensity on T_1W). For the Fisher's exact test were also formulated Null and Alternative hypothesis of independence; the level of significance accepted was also a *p* value < 0.05.

The cases, in which was not possible to calculate an exact *p* value with Pearson's chi square or Fisher's exact test (cross-tabulation between Classification and Turbinate Destruction, Mucus Intensity on T_2W when compared with fat and brain, Turbinate Intensity on T_2W when compared with brain) the Monte Carlo method was used (Maroco, 2003). This method provides a unbiased estimate of the exact *p* value of Pearson's chi Square without the requirements of the asymptotic method (Mehta, 1996). The Monte Carlo method is a repeated sampling method in order to obtain an unbiased estimate of the true *p* value. The level of significance accepted was *p* value <0.05.

Figure 15 – How to reach a final diagnosis in inflammatory nasal diseases



4 Results

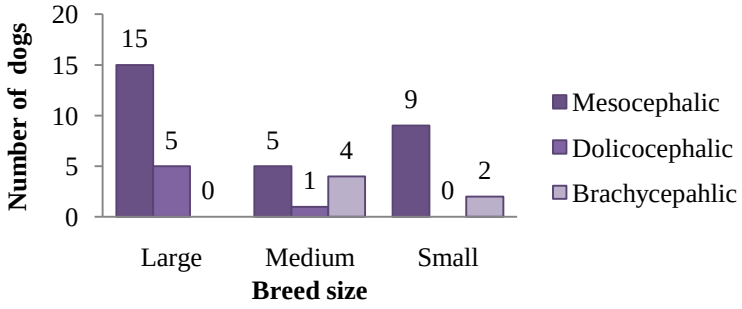
4.1 Patient Characteristics

Initially, records of 52 dogs in which a final diagnosis of inflammatory nasal disease had been made were identified. However, six of these dogs were excluded from the study because the MRI scans were not available for review by the author.

Five (10.8%) of the 46 dogs were cross-breed dogs and were not classified due to the fact that there were no information about the animal size. Forty one (89.2%) of the 46 dogs were purebred, including five Border Collies (10.9%), five Jack Russell Terriers (10.9%), four Labrador Retrievers (8.7%) and three Dashchunds (6,5%).

Twenty (43.5 %) of the 46 dogs were classified as large-breed dogs, ten (21.7%) as medium-breed dogs and eleven (23%) as small-breed dogs. Twenty nine (63%) of the 46 dogs had a mesocephalic skull, six (13%) had a dolicocephalic skull and six (13%) had a brachycephalic skull (Appendix II).

Chart 1-Population skull Size

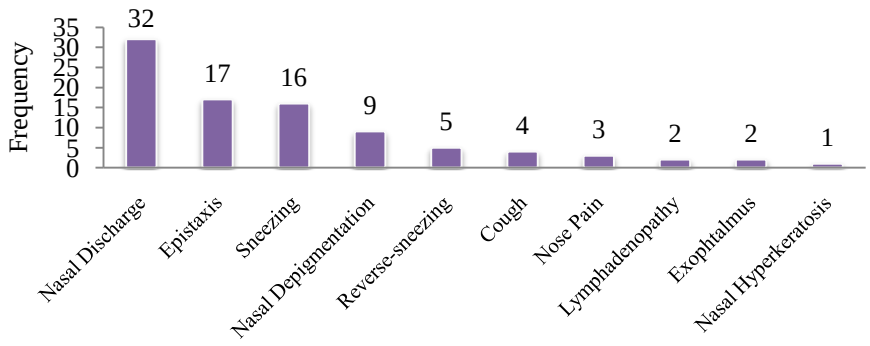


The 46 dogs included in the study ranged from 18 months to 14 years old (mean, 6.7 years; standard deviation, 3.68 years). There were 29 male dogs (63%; 18 castrated and 11 sexually intact) and 17 female dogs (37%; 12 spayed females and 5 sexually intact). Body weight ranged from 2.8 to 46 kg (mean, 23.4 kg; standard deviation, 11.45 kg).

4.2 Clinical Signs

Duration of the clinical signs prior to examination at the Queen's Veterinary School Hospital ranged from two weeks to ten months. Eight (17.4%) of the 46 dogs had signs for less than one month, thirteen (28.3%) had signs for 1 to 3 months, five (10.9%) for 4 to 6 months and three dogs (6.5%) for more than 6 months. The remaining seventeen (37%) dogs did not have any information concerning the duration of clinical signs on the medical records.

Chart 2 - Clinical Signs



Nasal discharge (Chart 2) was the most common clinical complaint and was reported in 32 (70%) of the 46 dogs. The cases that were likely to be aspergillosis were excluded of the statistical analysis. This sign was cross-tabulated with Rhinitis and Aspergillosis (Appendix III) but no statistical significance was found (p=0.08). However, it was seen on fourteen (87.5%) dogs with aspergillosis and on fifteen (60%) dogs with rhinitis.

The discharge was described as mucoid in nine (28%) of the 32 dogs, purulent in three dogs (9.5%), mucopurulent in eleven dogs (34.5%), serous in two dogs (6%) and non-specific in seven dogs (22%). Whether the nasal discharge was unilateral or bilateral was not recorded in one of the 32 dogs. Eleven (35.5%) of the remaining 31 dogs were reported to have bilateral nasal discharge, and twenty (62.5%) dogs were reported to have unilateral nasal discharge (ten from the right nostril and other ten dogs discharge from the left nostril).

The second most common sign seen on dogs with inflammatory nasal disease was epistaxis (37%). The cases that were likely to be aspergillosis were excluded of the statistical analysis. This sign was cross-tabulated with Rhinitis and Aspergillosis (Table 5), and the association was shown to be statistically significant (p=0.002). The table 5 shows that twenty one (84.0%) of the rhinitis patients did not have epistaxis or haemorrhagic nasal discharge, while ten (62.5%) aspergillosis cases were reported to have it.

Table 5 - Classification * Epistaxis Cross-tabulation

			Epistaxis		Total
			Present	Absent	
Classification	Rhinitis	Count	4	21	25
		% within Classification	16.0%	84.0%	100%
	Aspergillosis	Count	10	6	16
		% within Classification	62.5%	37.5%	100%
	Total	Count	14	27	41
		% within Classification	34.1%	65.9%	100%

Sneezing was also a common sign, sixteen (34.8%) dogs had this sign reported. The cases that were likely to be aspergillosis were excluded of the statistical analysis. This sign was cross-tabulated with Rhinitis and Aspergillosis, but the association was shown not to be statistically significant (p=0.72). Analyzing this cross-tabulation, it was seen that most of the Rhinitis (68%) and Aspergillosis (62.5%) cases did not have signs of sneezing reported.(Appendix III).

Nasal depigmentation was seen on nine (19.6%) out of 46 dogs. The cases that were likely to be aspergillosis were excluded of the statistical analysis. This sign was cross-tabulated with Rhinitis and Aspergillosis (Table 6), and the result was shown to be statistically significant (p<0.001). Nasal depigmentation was not observed in any patient with rhinitis, while nine (56.3%) patients with aspergillosis showed it.

Table 6 - Classification * Nasal Depigmentation Cross-tabulation

			Nasal Depigmentation		Total
			Present	Absent	
Classification	Rhinitis	Count	0	25	25
		% within Classification	0%	100%	100%
	Aspergillosis	Count	9	7	16
		% within Classification	56.3%	43.8%	100%
	Total	Count	9	32	41
		% within Classification	22.0%	78.0%	100%

Nasal pain was, only seen in three cases out of 46 cases (6.5%) seen, and all of them belonged to the Aspergillosis group. Nasal hyperkeratosis was seen in only one dog (2.2%). The cases that were likely to be aspergillosis were excluded of the statistical analysis. This result was cross-tabulated with Rhinitis and Aspergillosis (Appendix III), and although no statistical association was found (p=0.39), the only case seen belonged to the Aspergillosis group.

Reverse-sneezing was reported in five dogs (10.9%) and all of them belonged to the Rhinitis group. Of the four cases (8.7%) seen with cough, two of them were classified as Rhinitis and the other two as Aspergillosis. The two cases (4.3%) with lymphadenopathy reported, corresponded one to Rhinitis and the other to Aspergillosis groups. Regarding the two exophthalmus patients (4.3%), one belonged to the rhinitis and the other to the likely aspergillosis group.

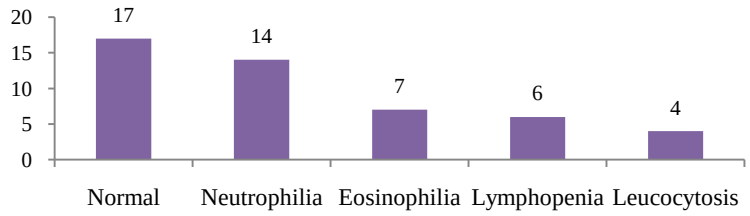
Information about previous treatment response was available in some cases (47%), however twenty nine (63%) of the 46 dogs did not have any data on the medical records concerning previous treatments. Eight dogs (17.4%) improved the symptoms with the administration of antibiotics and nine (19.6%) dogs did not have any improvement with the antibiotic therapy.

The cases that were likely to be aspergillosis were excluded of the statistical analysis. The variable Classification (Rhinitis and Aspergillosis) was cross-tabled with Responsiveness to antibiotics (Appendix IV), but it was not possible to demonstrate any association with statistical significance (p=0.20). However, it was noticed that all the three Aspergillosis cases that were treated with antibiotics did not respond to treatment and seven (58.3%) Rhinitis cases seem to have improved with the antibiotic treatment.

4.3 Leukogram

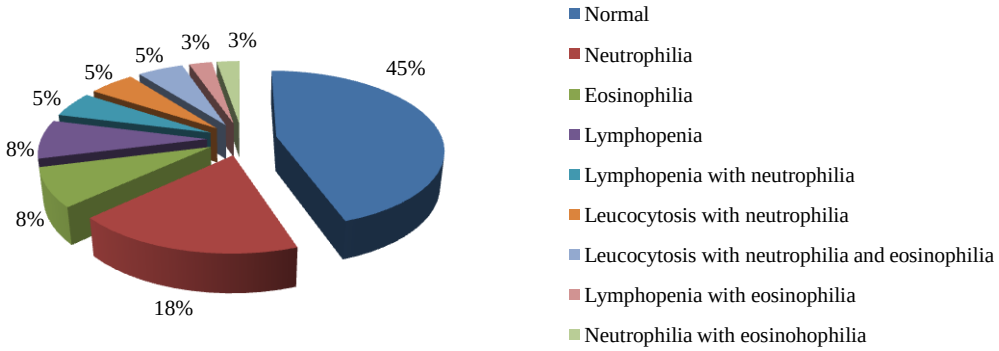
A leukogram was performed on thirty seven (80.4%) of the 46 dogs included in the study. Seventeen (45.9%) were normal and twenty (54.1%) dogs had some abnormalities in the analysis. The abnormalities were studied in detail, and the following results found: fourteen cases (70%) had neutrophilia, seven cases (35%) had eosinophilia, six (30%) had lymphopenia and four (20%) had leucocytosis (Chart 3).

Chart 3 - Leukogram results



Despite the fact that most of the cases had only one major change seen on leukogram, there were some cases in which two or more changes were detected: two (5%) cases of lymphopenia and neutrophilia, two (5%) cases of leucocytosis with neutrophilia, two (5%) cases of leucocytosis with neutrophilia and eosinophilia, one (3%) case of lymphopenia with eosinophilia and another one (3%) of lymphopenia with eosinophilia. The following chart (Chart 4) shows how the data was classified as well as the results in percentage.

Chart 4 – Most prevalent abnormalities found in the leukogram



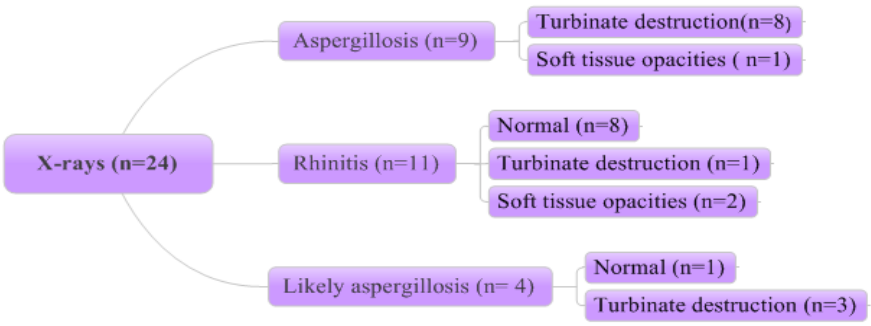
The Leukogram results were cross-tabulated with the Classification groups (Appendix V). The association was shown not to be statistically significant (p=0.36) but it was noticed that only three (27.3%) cases of aspergillosis had an unremarkable leukogram comparing with the rhinitis, in which twelve (50%) were normal. It was also seen that eosinophilia was a rare finding in the aspergillosis cases, in fact only two (18.2%) cases were detected. The cases that were likely to be aspergillosis were excluded of this statistical analysis.

4.4 Ancillary diagnostic tests

4.4.1 Radiography

Radiographs were taken on twenty four of the 46 dogs. Nine (37.5%) cases were classified as normal and radiographic lesions were detected in fifteen (62.5%) of 24 dogs, from which three of them were diagnosed as rhinitis, nine as aspergillosis and three cases as likely aspergillosis. Most of the Aspergillosis cases (88.9%) showed turbinate destruction on x-ray, while most of the Rhinitis cases (72.3%) had x-rays classified as normal. The Likely aspergillosis group showed, like the Aspergillosis group, that turbinate destruction was the most common (75%) lesion found. (Figure 16).

Figure 16 – Radiography descriptive results



The x-ray results were classified as normal and abnormal and were cross-tabulated with Rhinitis and Aspergillosis cases. The statistics results showed that the changes seen on x-rays were significantly associated with Aspergillosis (p=0.001). It was shown that the majority (72.7%) of dogs with Rhinitis did have unremarkable x-ray results and that all (100%) the Aspergillosis cases that underwent an x-ray study had abnormalities reported. The cases that were Likely to be aspergillosis were excluded of this statistical analysis (Table 7).

Table 7 - Classification * X-ray Cross-tabulation

			X-ray		Total
			Normal	Abnormal	
Classification	Rhinitis	Count	8	3	11
		% within Classification	72.7%	27.3%	100%
	Aspergillosis	Count	0	9	9
		% within Classification	0%	100.0%	100%
	Total	Count	8	12	20
		% within Classification	40.0%	60.0%	100%

4.4.2 Magnetic Resonance Imaging (MRI)

All of the 46 dogs included in this study were submitted for a nose MRI scan. All the cases were firstly, divided into two groups: normal and abnormal MRI scans. Forty cases (87%) were classified as abnormal and six (13%) as normal.

This MRI classification (normal and abnormal) was cross-tabulated with the X-ray classification (normal and abnormal), but the association was proven not to be statistically significant (p=0.09). Nevertheless, it was seen that five cases (25%) that had an abnormal MRI scan, were classified as normal on x-ray, and that three (75%) classified as normal on MRI were as well classified as normal on x-ray (Appendix VI).

4.4.2.1 Rhinitis and aspergillosis

The MRI classification (normal and abnormal) was cross-tabulated with Rhinitis and Aspergillosis (Appendix VI), but the association was proved not to be statistically significant (p=0.14). Nevertheless, only five (20%) Rhinitis cases had an MRI considered to be normal and all (100%) the sixteen cases of Aspergillosis had an abnormal MRI reported.

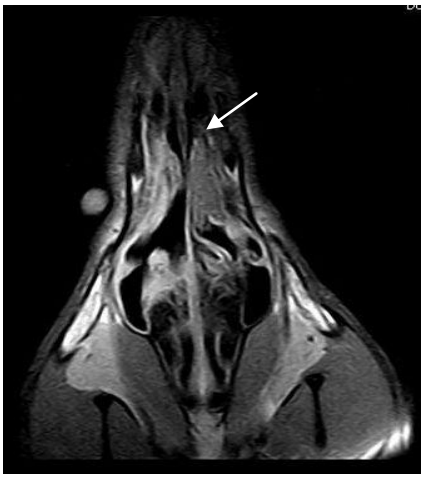
The affected side on MRI was also assorted and it was seen that thirty dogs (65.2%) had bilateral changes, five (10.8%) had unilateral changes and eleven were normal (24%). This feature was cross-tabulated with Rhinitis and Aspergillosis (Appendix VI), but the association was not statistically significant (p=0.64). Most of the Rhinitis (78.6%) and Aspergillosis (87.5%) cases had bilateral nose changes seen on MRI.

The affected side (nasal discharge) was cross-tabulated with the affected side changes seen on MRI (Appendix VI). Twenty seven cases were included, but the association was shown not to be significant statistically (p=0.28). Although not dependable, the results demonstrate that all the eight cases (100%) with bilateral nasal discharge had bilateral changes on MRI scans and that fourteen (73.7%) of the cases with unilateral nasal discharge had bilateral changes seen on MRI scans.

4.4.2.2 Destruction Evaluation

The nasal septum/vomer was destroyed in five (10.9%) out of 46 dogs (Figure 17). This type of destruction was cross-tabulated with Rhinitis and Aspergillosis (Appendix VI), but it was not found any significant statistical association (p=0.07). However, it was noticed that four cases (80%) were diagnosed as Aspergillosis and the other one (20%) as Rhinitis. The cases that were Likely to be aspergillosis were excluded of this statistical analysis.

Figure 17 – Dorsal section. Destruction of the septum/vomer on T1W**.**

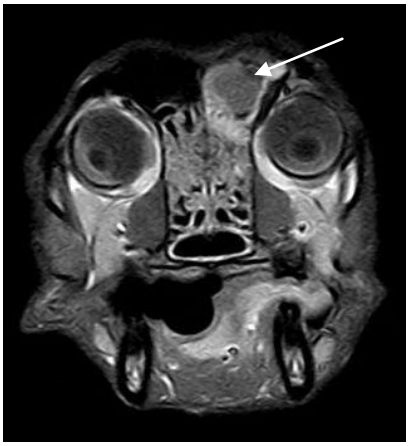


Destruction of the cribriform plate was evident in two (4.3%) out of the 46 dogs. This finding was cross-tabulated with Rhinitis and Aspergillosis (Appendix VI), but the association was not statistically significant (p=0.15); however both dogs were included in the Aspergillosis group.

In sixteen dogs (34.8%), it was also noticed involvement of the frontal sinus, characterized by sinus hyperintensity. A cross-tabulation was done between Classification and this variable (Appendix VI), but the association was not statistically significant (p=0.07). Nevertheless eighteen Rhinitis cases (72%) did not show frontal sinus involvement whereas nine Aspergillosis cases (56.3%) exhibit it on MRI.

Frontal sinus involvement was also cross-tabulated with turbinate destruction seen on MRI, but the association was shown not be statistically significant (p=0.21). It was noticed that most of the cases with (81.3%) or without (63.3%) frontal sinus involvement had destruction seen on MRI (Appendix VI).

Figure 18 – Transverse section. Frontal sinus involvement seen on T1W.



The nasal turbinates were destroyed in thirty two cases (69.6%) out of the 46 initial cases. All the five cases, of the likely aspergillosis group, had destruction of the turbinates present, but they were excluded from the statistical analysis

The turbinate destruction was cross-tabulated with Rhinitis and Aspergillosis (Table 8), and the result was statistically significant (p=0.005). Twelve (48%) of the dogs with Rhinitis did not have turbinate destruction seen on MRI and fourteen (87.5%) of the Aspergillosis cases had turbinate destruction seen.

Table 8 – Classification * Turbinate Destruction Cross-tabulation

			Turbinate Destruction		Total
			Absent	Present	
Classification	Rhinitis	Count	12	13	25
		% within Classification	48.0%	52.0%	100%
	Aspergillosis	Count	2	14	16
		% within Classification	12.5%	87.5%	100%
	Total	Count	14	27	41
		% within Classification	34.1%	65.9%	100%

Whether the turbinate destruction seen on MRI was associated or not with the final inflammation classification done by the histopathologist was also studied in two different perspectives. On the first cross-tabulation, the final inflammation grade was shown to be statistically associated with the presence or absence of turbinate destruction (p=0.01). It was seen that all the cases (100%) that were classified as severe inflammation had turbinate destruction seen on MRI, and that most of the cases without destruction (80%), had moderate inflammation present (Table 9).

Table 9 -Turbinate Destruction * Final Inflammation Grade Cross-tabulation

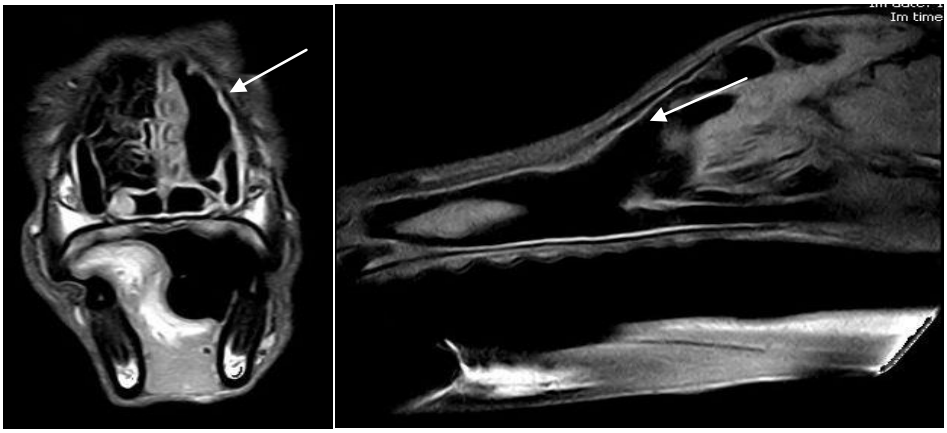
			Final Inflammation Grade			Total
			Mild	Moderate	Severe	
Turbinate Destruction	Absent	Count	2	8	0	10
		% within Turbinate Destruction	20.0%	80.0%	0%	100%
	Present	Count	4	9	15	28
		% within Turbinate Destruction	14.3%	32.1%	53.6%	100%
	Total	Count	6	17	15	38
		% within Turbinate Destruction	15.8%	44.7%	39.5%	100%

The turbinate destruction (present or absent) seen on MRI was cross-tabulated, as well, with the X-ray results (Appendix VI), but the association was shown not to be statistically significant (p=0.58). It was seen that the majority of cases (75%) with a normal x-ray had in fact turbinate destruction seen on MRI, and that the majority of cases (87.5%) with an abnormal x-ray also had turbinate destruction seen.

Serology was also cross-tabulated with turbinate destruction (Appendix VI), this association was shown not to be statistically significant (p=0.45). Thirteen (68.4%) out of the 19 that had a negative fungal serology result had turbinate destruction seen on MRI and twelve (80%) cases were found to have a positive fungal serology with turbinate destruction present.

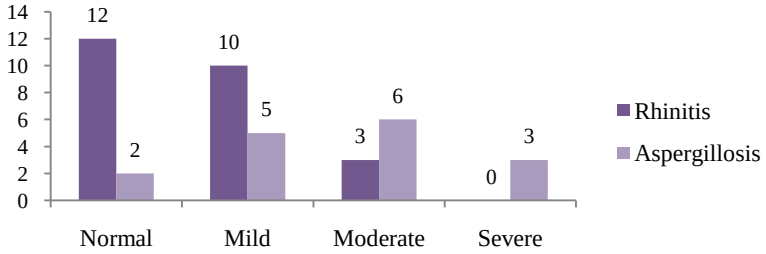
A more detailed study about turbinate destruction seen on MRI was made. It was seen that the majority of cases had mild turbinate destruction (41.3%), followed by moderate (19.6%) and severe destruction (8.7%). Normal turbinates without destruction were also commonly seen (30.4%).

Figure 19 – Turbinate destruction seen on transverse and saggital sections.



The results of this turbinate destruction study, were distributed according to Rhinitis and Aspergillosis (Chart 5). It was noticed that most Rhinitis cases (48%) had no turbinate destruction, a significant number (40%) had mild destruction (40%) and no cases were classified, by the author, as severe. The Aspergillosis cases on the other hand, showed that six cases (37.5%) had moderate turbinate destruction, five (31.3%) had mild destruction and although severe destruction was only see on three cases (18.8%) on MRI, they belonged to dogs with aspergillosis.

Chart 5 – Distribution of Turbinate Destruction (2) according to Rhinitis and Aspergillosis



This type of turbinate destruction classification (2) was also cross-tabulated with the rhinoscopy results (Table 10), and it was shown that this association was statistically significant (p=0.002). Most of the cases (45.8%) that presented with no turbinate destruction on MRI were reported as normal/inflammation after rhinoscopy, likewise most of the cases (96.8%) that exhibited some kind of destruction on MRI were reported to have turbinate destruction or plaques seen during rhinoscopy. Thirteen (54.2%) cases that had turbinate destruction (ten mild and three moderate destruction) were reported as normal/inflammation after rhinoscopy and only one (5.3%) that did not show any turbinate destruction on MRI had, in fact, turbinate destruction or plaques seen during rhinoscopy.

Table 10 - Rhinoscopy * Turbinate Destruction (2) on MRI cross-tabulation

			Turbinate Destruction (2)				Total
			No Destruction	Mild	Moderate	Severe	
Rhinoscopy	Normal/inflammation	Count	11	10	3	0	24
		% within Rhinoscopy	45.8%	41.7%	12.5%	0%	100%
	Turbinate Destruction/ fungal plaques	Count	1	8	6	4	19
		% within Rhinoscopy	5.3%	42.1%	31.6%	21.1%	100%
	Total	Count	12	18	9	4	43
		% within Rhinoscopy	27.9%	41.9%	20.9%	9.3%	100%

This turbinate destruction classification (2) was cross-tabulated with the final inflammation grade (Table 11). This association was shown to be statistically significant (p=0.02) as the

other one, where only two groups of turbinate destruction were considered. It was seen that the majority of the cases (64.7%) with severe inflammation showed mild turbinate destruction and that most of cases with no turbinate destruction seen (80%), had moderate inflammation present.

Table 11 - Turbinate Destruction (2) * Final Inflammation Grade Cross-tabulation

			Final Inflammation Grade			Total
			Mild	Moderate	Severe	
Turbinate Destruction	No Destruction	Count	2	8	0	10
		% within Turbinate Destruction	20.0%	80.0%	0%	100%
	Mild	Count	3	3	11	17
		% within Turbinate Destruction	17.6%	17.6%	64.7%	100%
	Moderate	Count	0	5	3	8
		% within Turbinate Destruction	0%	62.5%	37.5%	100%
	Severe	Count	1	1	1	3
		% within Turbinate Destruction	33.3%	33.3%	33.3%	100%
	Total	Count	6	17	15	38
		% within Turbinate Destruction	15.8%	44.7%	39.5%	100%

4.4.2.2.1 Turbinate Intensity evaluation on T_1W and T_2W

Turbinate intensity on T_1W was compared with muscle intensity. The results showed that seventeen cases (37%) had hypointense turbinates, sixteen (34.8%) were hyperintense and thirteen (28.2%) were isointense to the muscle intensity. The cases that were Likely aspergillosis were excluded of the statistical analysis.

This feature was cross-tabulated with Rhinitis and Aspergillosis (Table 12), and the association was shown to be statistically significant (p=0.007). It was noticed that most of the Aspergillosis cases (68.8%) had turbinate hyperintensity comparing with muscle, and that turbinates were more frequently seen as hypo- (40%) or isointense (40%) to muscle intensity on Rhinitis cases.

Table 12 - Classification * Turbinate Intensity on T_1W Cross-tabulation

			Turbinate Intensity on T_1			Total
			Hyper	Hypo	Iso	
Classification	Rhinitis	Count	5	10	10	25
		% within Classification	20.0%	40.0%	40.0%	100%
	Aspergillosis	Count	11	3	2	16
		% within Classification	68.8%	18.8%	12.5%	100%
	Total	Count	16	13	12	41
		% within Classification	39.0%	31.7%	29.3%	100%

Turbinate intensity on T_1W was also cross-tabulated with Epistaxis (Table 13). This association was shown to be statistically significant (p=0.03), and it was found that twelve (41.4%) out of the 29 cases without epistaxis had an iso turbinate intensity and that nine (53%) out of 17 cases with epistaxis had turbinate hyperintensity.

Table 13 - Epistaxis * Turbinate Intensity on T_1W cross-tabulation

			Turbinate Intensity on T1W			Total
			Hyper	Hypo	Iso	
Epistaxis	Present	Count	9	7	1	17
		% within Epistaxis	52.9%	41.2%	5.9%	100%
	Absent	Count	7	10	12	29
		% within Epistaxis	24.1%	34.5%	41.4%	100%
	Total	Count	16	17	13	46
		% within Epistaxis	34.8%	37.0%	28.3%	100%

Turbinate intensity was also table-crossed with the inflammation final classification (Appendix VI), but the association was shown not to be statistically significant (p=0.38). Nevertheless eight (47.1%) out of the 17 cases with moderate inflammation had turbinate hyperintensity and eight (53.3%) out of the 15 with severe inflammation had hypointense turbinates.

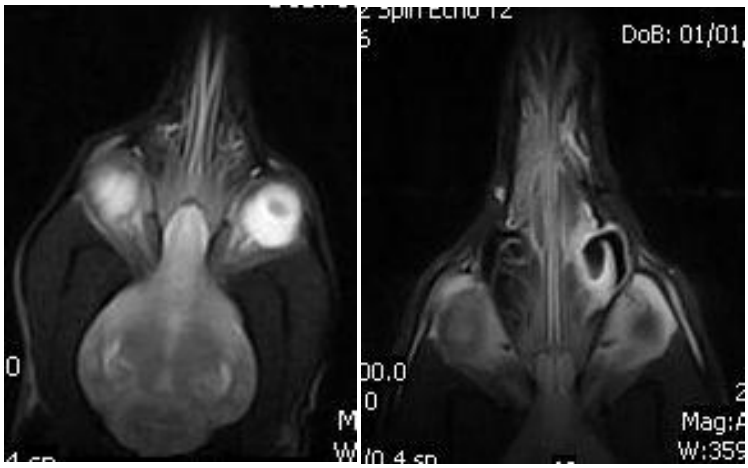
Other cross-tabulation studies with turbinate intensity on T_1W were made (Appendix VI), but all of them were shown not to be statistically significant: Oedema (p=0.80), Epithelial cell hyperplasia (p=0.58) and Nasal discharge (p=0.16). Peculiarly this last association showed that the majority (37.5%) of the cases without discharge had a hyperintense turbinates and that the majority of cases (57.1%) with discharge had hypointense turbinates on T_1W.

On T_2W, only forty five cases were analyzed for turbinate intensity. When compared with fat, twenty three (51.1%) dogs exhibit turbinate hypointensity, four (8.9%) turbinate hyperintensity and eighteen (40%) had turbinate iso-intensity.

When cross-tabulated turbinate intensity (fat) with Rhinitis and Aspergillosis (Appendix VI), it was seen that the association was not statistically significant (p=0.13). The majority (62.5%) of Rhinitis cases exhibit hypointensive turbinates and the majority (56.3%) of Aspergillosis cases had iso-intense turbinates.

Figure 20 – Dorsal sections on T_2W sequence.

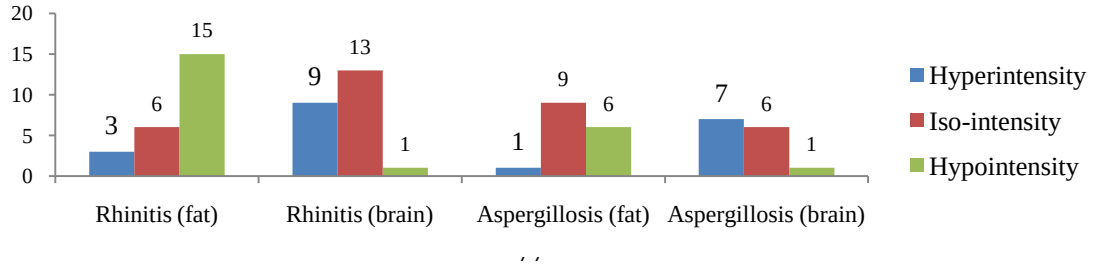
On the left picture, the turbinates are iso-intense to the fat and hypointese to the brain. However on the right picture, the turbinates are hypointense to the fat and hyperintense to the brain.



When comparing with brain, four cases (two rhinitis and two aspergillosis cases) had to be excluded due to the fact that the brain was not present on the MRI slices available. Nevertheless, twenty one cases (50%) had iso-intense turbinates, nineteen (45.2%) had hyperintense turbinates and two (4.8%) cases were hypointense to the brain intensity.

The cross-tabulation between turbinate intensity (brain) and Aspergillosis and Rhinitis (Appendix VI) was shown not to be statistically significant (p=0.76). Most of the Rhinitis cases (56.5%) had iso-intensive turbinates comparing with fat and most of Aspergillosis cases (50.0%) had turbinates more hyperintense than fat. Nevertheless, a high number (42.9%) of Aspergillosis cases had iso-intense turbinates when comparing with fat. The following chart (Chart 6) shows how the cases were distributed according to the turbinate intensity and its comparison with fat and brain intensity on T_2W.

Chart 6 - Turbinate Intensity on T_2W



Other associations were studied for both T_2W comparisons (fat and brain), but none of them were shown to be statistically significant (Appendix VI), were included: Inflammation final classification (fat p=0.52; brain p=0.59), Oedema (fat p=0.38; brain p=0.15), Epistaxis (fat p=1.0; brain p=0.58), Epithelial cell hyperplasia (fat p=0.97; brain p=0.71) and Nasal discharge (fat p=0.51; brain p=0.78).

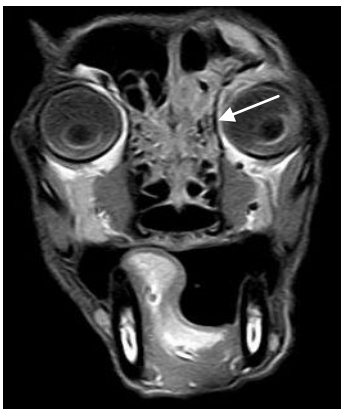
4.4.2.2.2 Mucus Intensity evaluation on T_1W and T_2W

Mucus intensity on T_1W was only studied on forty five dogs, since one of the rhinitis cases did not have any mucus present. Thirty seven cases (82.2%) showed hyperintensity of the mucus when compared with muscle intensity, and only eight (17.8%) were isointense to muscle. There were no cases in which mucus was hypointense to the muscle. The cases that were Likely aspergillosis were excluded of the statistical analysis.

Mucus intensity on T_1W was cross-tabulated with Rhinitis and Aspergillosis (Appendix VI), but the association was shown not to be statistically significant (p=0.31). Mucus hyperintensity was the most frequent feature in Rhinitis (87.5%) as well as in Aspergillosis (75%) cases.

Other statistically associations, with Mucus intensity on T_1W, were made but they were all shown not to be statistically significant (Appendix VI). The associations made included the following variables: Inflammation final classification (p=0.85), Oedema (p=0.33), Epistaxis (p=0.43), Epithelial cell hyperplasia (p=0.89) and Nasal discharge (p=0.68).

Figure 21 – Mucus Hyperintensity on T_1W



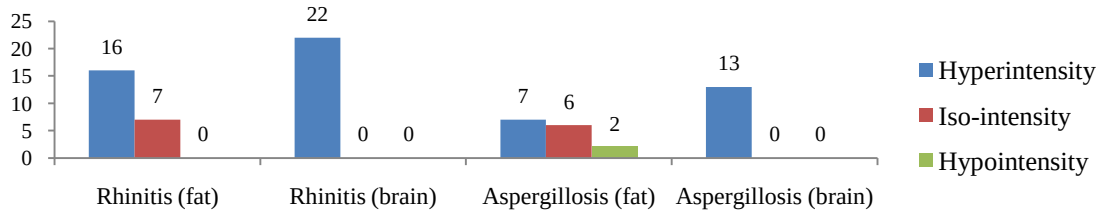
On T_2W, three cases were excluded because fat could not be accessed (Chart 7). Mucus intensity, when compared with fat intensity, showed that twenty eight dogs (60.9%) had mucus hyperintensity, thirteen (28.3%) had mucus isointensity and only two cases (4.3%) had hypointense mucus. The association between Mucus intensity on T_2W (fat) and Classification

was shown not to be statistically significant (p=0.16). It was seen that the majority of the Rhinitis cases (69.6%) had hyperintense mucus; on Aspergillosis cases, even though the mucus was in most cases (46.7%) hyperintense to fat, a considerable number of cases (40%) had isointense mucus to fat (Appendix VI).

When comparing mucus with brain intensity on T_2W, only forty cases were included because, brain could not be accessed on the other cases. They all (100%) showed that mucus was hyperintense to brain (Appendix VI).

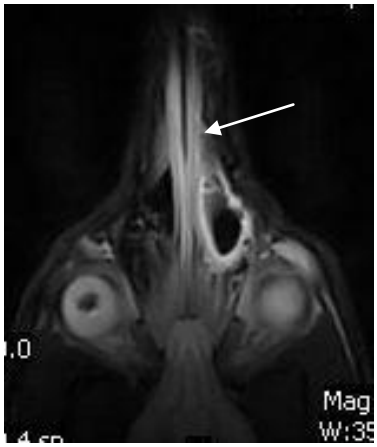
The following chart (Chart 7) shows all the results obtained for the T_2W sequence distributed according to the pathology present.

Chart 7 – Mucus intensity on T_2W



Some other variables were cross-tabulated with Mucus intensity on T_2W in comparison with fat because brain comparison only had mucus hyperintensity reported (Appendix VI). Those cross-tabulation, were shown to have associations not statistically significant: Inflammation final classification (p=0.55), Oedema (p=0.28), Epistaxis (p=1.0), Epithelial cell hyperplasia (p=0.98) and Nasal discharge (p=0.88).

Figure 22 – Mucus hyperintensity on T_2W when compared with fat and brain, on a dog with aspergillosis



4.4.2.3 Likely aspergillosis cases study

When cross-tabulated the MRI variables with the aspergillosis cases and the ones that were likely to be (Appendix VII), it was only found one association statistically significant (p=0.03). This statistical significant association was found between frontal sinus involvement and Aspergillosis (Table 14). The results showed that nine (56.3%) of the Aspergillosis cases had frontal sinus enhancement and none (0%) of the cases that were likely to have aspergillosis had it.

Table 14 – Classification * Frontal sinus involvement Cross-tabulation

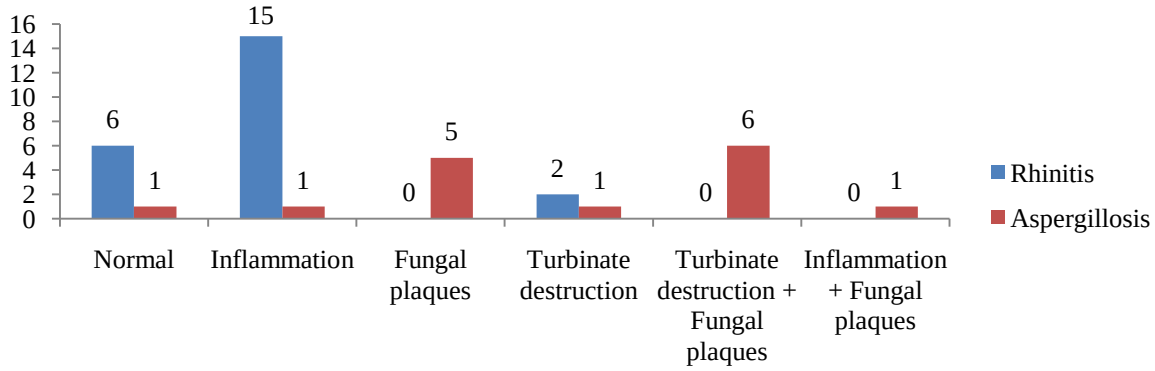
			Frontal Sinus Involvement		Total
			Present	Absent	
Classification	Aspergillosis	Count	9	7	16
		% within Classification	56.3%	43.8%	100%
	Likely aspergillosis	Count	0	5	5
		% within Classification	0%	100%	100%
	Total	Count	9	12	21
		% within Classification	42.9%	57.1%	100%

4.4.3 Rhinoscopy

Rhinoscopy was performed in forty three of the 46 dogs and revealed lesions in thirty five (81.4%) of the 43 dogs. Inflammation was detected in eighteen dogs (41.9%), although sixteen had only inflammation without any other abnormality found, the other two dogs had inflammation and fungal plaques seen. Turbinate destruction was detected in twelve dogs (28%), however only five had exclusively turbinate destruction seen, the other seven had turbinate destruction as well as fungal plaques seen. Fungal colonies were detected in fourteen (32.6%) of the 43 dogs, nevertheless only five dogs had exclusively plaques without any other abnormality detected.

The Aspergillosis and Rhinitis cases were distributed according to the Rhinoscopy results (Chart 8), and it was seen that fifteen (60%) cases of Rhinitis had only inflammation seen on rhinoscopy, six (24%) of them were considered to be normal and only two (8%) showed some turbinate destruction. Concerning the Aspergillosis cases, most of them (33.3%) showed turbinate destruction as well as fungal plaques but a considerable number (23.8 %) showed only plaques without any turbinate destruction seen (Appendix VIII).

Chart 8 – Rhinoscopy results distribution according to the Classification



The rhinoscopy results for statistical purpose were divided into two different groups, one with normal and inflammation only cases and another one with turbinate destruction and fungal plaques cases seen. All the five that were classified as Likely aspergillosis had turbinate destruction seen on rhinoscopy but they were excluded of the statistical analysis.

The rhinoscopy groups were cross-tabulated with Rhinitis and Aspergillosis (Table 15). The results were statistically significant ($p<0.001$), in fact most of the Rhinitis cases (91.3%) had a normal rhinoscopy result or presented only with inflammation, and most of the Aspergillosis cases (86.7%) were reported to have either turbinate destruction and/or fungal plaques seen during rhinoscopy.

Table 15 - Classification * Rhinoscopy Cross-tabulation

			Rhinoscopy		Total
			Normal /inflammation	Turbinate Destruction/ fungal plaques	
Classification	Rhinitis	Count	21	2	23
		% within Classification	91.3%	8.7%	100%
	Aspergillosis	Count	2	13	15
		% within Classification	13.3%	86.7%	100%
	Total	Count	23	15	38
		% within Classification	60.5%	39.5%	100%

The rhinoscopy results were also crossed with the MRI classification (Table 16). The results were statistically significant ($p=0.03$), and it was noticed that all the nineteen cases (100%) that showed turbinate destruction or fungal plaques on the rhinoscopy, had MRI scans classified as abnormal. Moreover, nineteen cases (79.2%) that were reported to be normal or with inflammation seen during rhinoscopy, had abnormal MRI scans and only five (20.8%) had normal MRI scans.

Table 16 - Rhinoscopy * MRI diagnosis Cross-tabulation

			MRI classification		Total
			Normal	Abnormal	
Rhinoscopy	Normal/inflammation	Count	5	19	24
		% within Rhinoscopy	20.8%	79.2%	100%
	Turbinate Destruction/ fungal plaques	Count	0	19	19
		% within Rhinoscopy	0%	100%	100%
	Total	Count	5	38	43
		% within Rhinoscopy	11.6%	88.4%	100%

4.4.4 Histopathology

Histopathology was performed on thirty eight (83.4%) patients out of the 46, including twenty one Rhinitis cases, fourteen Aspergillosis cases and three Likely aspergillosis cases. The histopathology study included for every single one of them, a classification according to the presence grade of lymphocytes, neutrophils, plasma cells and eosinophils, and also according to the final inflammation grade, epithelial cell hyperplasia, goblet cell hyperplasia, fungi hyphae and oedema presence. The cases that were Likely aspergillosis were excluded of the statistical association analysis.

4.4.4.1 Lymphocytes

Lymphocytes were found in all (100%) of the 38 cases from which histopathology was made (Appendix IX). Five (13.2%) cases were reported by the histopathologist to have a mild lymphocytic infiltration, twenty one (55.3%) had a moderate presence and twelve (31.5%) were classified as severe lymphocytic infiltration.

These results were table-crossed with Rhinitis and Aspergillosis cases (Appendix X) but this association had no statistical significance (p=0.30). It was assorted that the moderate presence of lymphocytes was the most common finding (66.7%) in the Rhinitis, while in Aspergillosis, moderate (38.5%) and severe (38.5%) infiltration were the most common findings. For both groups, mild infiltration was the least common feature with 9.5% of the Rhinitis cases and 23.1% of the Aspergillosis cases.

4.4.4.2 Neutrophils

Neutrophils were visualized in thirty two (84.2%) cases out of the 38 cases analyzed, with the rest of them classified as normal. Thirteen (34.2%) had a mild presence of neutrophils, twelve (31.6%) had a moderate presence, seven (18.4%) were classified as severe neutrophilic infiltration (Appendix IX).

Neutrophilic presence was table-crossed with the Rhinitis and Aspergillosis cases (Appendix X), but the association was shown not to be statistical significant (p=0.06). However, it was noticed that mild neutrophilic infiltration was the most common finding (47.6%) in Rhinitis and moderate infiltration was most commonly seen on Aspergillosis (46.2%). Normal cases had less expression in the Aspergillosis (7.7%) group than in Rhinitis (23.8%). Additionally, it was noticed that severe neutrophilic infiltration was most frequently seen on Aspergillosis (30.8%) than on Rhinitis (9.5%) cases.

4.4.4.3 Plasma cells

Plasma cells were found in thirty four patients (89.5%) out of the 38 reported cases, the other four cases did not show any signs of plasma cell infiltration (Appendix IX). The most common feature seen (47.4%) was the moderate infiltration of plasma cells in the analyzed tissues. Severe plasma cell infiltration was also reported in 28.9% of the cases and only 13.2% of the cases had mild infiltration. The cases that were Likely aspergillosis were excluded of the statistical analysis.

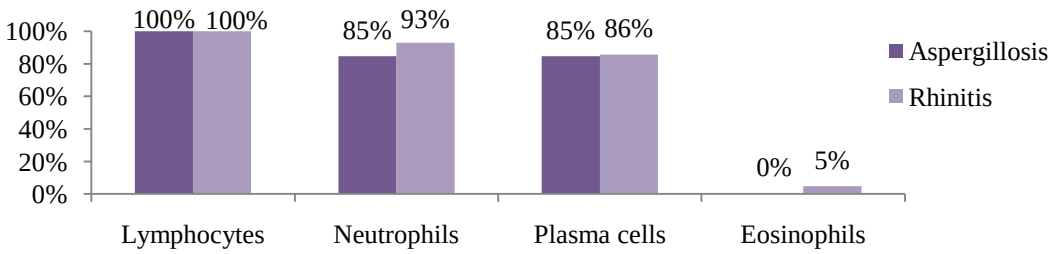
Rhinitis and Aspergillosis groups were cross-tabulated with the plasma cell results (Appendix X), this association was shown not to the statistically significant (p=0.23). It was seen that the most frequent features in Rhinitis were moderate (66.7%) and severe plasma cell infiltration (14.3%). Most of the Aspergillosis cases (38.5%) had severe infiltration of plasma cells, and only 15.4% were classified as normal.

4.4.4.4 Eosinophils

Eoshinophilic infiltration was found to be either normal or with moderate infiltration on the 38 cases. Thirty seven (97.4%) cases were reported to be normal and only one (2.6%) was classified as moderate eosinophilic infiltration (Appendix IX). The cases that were Likely aspergillosis were excluded of the statistical analysis. These results were cross-tabulated with Rhinitis and Aspergillosis, the association was shown not to be statistically significant (p=1.0) and the only case seen of eosinophilic infiltration belonged to the Rhinitis group (Appendix X).

The following chart (Chart 9) shows the frequency, in percentage, of the different cell types infiltration, described above. In the chart, we can see that all the cases of Aspergillosis and Rhinitis had lymphocytic infiltration and that only 4.8% of the Rhinitis and none of the Aspergillosis cases had eosinophilic infiltration. The frequencies of the other cell types infiltration was very similar for both pathologies.

Chart 9 – Cell type frequency distribution according to Rhinitis and Aspergillosis



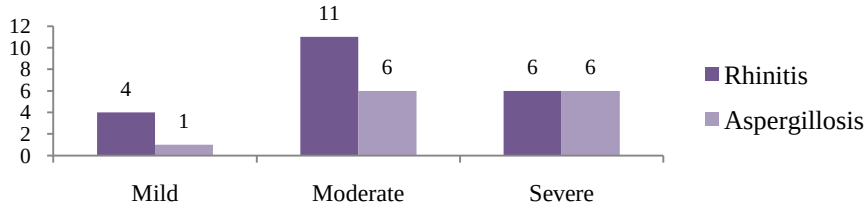
4.4.4.5 Final inflammation grade

The final cell infiltration classification was given by the most common cell type present. Twenty five (65.8%) out of the 38 samples were classified as lymphocytic rhinitis, eight (23%) as neutrophilic rhinitis and five (12.2%) as plasmacytic (Appendix IX).

A cross-tabulation between these results and Rhinitis and Aspergillosis was made (Appendix X). In both groups, the most common type cell found was the lymphocyte (sixteen [76.2%] Rhinitis and seven [53.8%] Aspergillosis cases), followed by the neutrophiles, which was found in three (14.3%) Rhinitis cases and in four (30.8%) Aspergillosis patients, and the least found type cell for both groups, was plasma cell type, which was more frequent in two (15.4%) cases of Aspergillosis and in other two (9.5%) of Rhinitis.

The inflammation final grade was given by the infiltration level of lymphocytes, neutrophils, plasma cells and eosinophils. It ranged from mild to severe, since no sample was found to be completely normal. In total, six (15.8%) out of the 38 cases were classified as mild inflammation, seventeen (44.7%) as moderate and fifteen (39.5%) as severe. Excluding the Likely aspergillosis group, the results were cross-tabulated with the Rhinitis and Aspergillosis cases (Chart 10) but the association was shown not to be statistically significant (p=0.46). It was seen that most (52.4%) of the Rhinitis cases had a moderate grade of inflammation and that Aspergillosis cases did not follow any specific distribution trend, although only one case (7.7%) had mild inflammation (Appendix X).

Chart 10 – Distribution of inflammation grade according to Rhinitis and Aspergillosis



This inflammation final grade was cross-tabled with Nasal discharge (present or absent), but it was shown not to be significant (p=0.05), but curiously thirteen (86.7%) out of the 15 samples classified as severe inflammation did not show any sign of nasal discharge (Appendix X).

4.4.4.6 Epithelial and goblet cell hyperplasia

Epithelial cell hyperplasia was analyzed on 38 samples. Epithelial cell hyperplasia was visualized in twenty seven (71%) cases, the other eleven (29%) cases were reported to be normal. Fifteen (39.5%) cases were classified as moderate epithelial cell hyperplasia, eight (21%) cases as mild and four (10.5%) as severe hyperplasia. The cases that were Likely aspergillosis were excluded of the statistical analysis (Appendix IX).

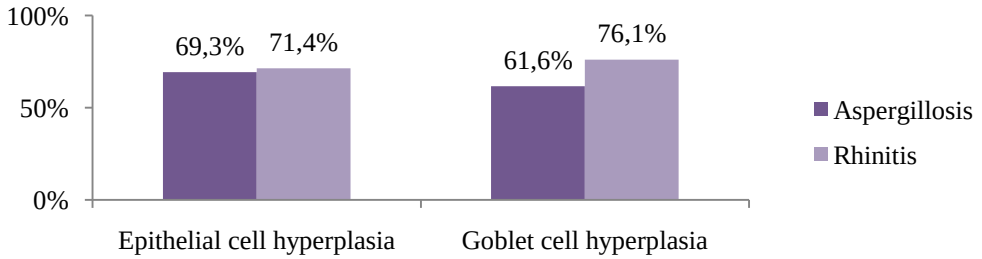
The cross-tabulation of epithelial cell hyperplasia with Rhinitis and Aspergillosis groups (Appendix X) was shown not to be statistically significant (p=1.00). Nevertheless, it was seen that the most part of the cases on both groups had moderate epithelial cell hyperplasia (38.1% of Rhinitis cases and 38.5% of Aspergillosis cases), severe hyperplasia was the less frequent finding with only two cases (9.5%) of Rhinitis and one case (7.7%) of Aspergillosis.

Goblet cell hyperplasia was found in twenty seven (71%) cases out of the 38 analyzed, the other eleven cases (29%) were classified as normal. Twenty (52.6%) cases had mild hyperplasia and the other seven (18.4%) had a moderate hyperplasia, no severe goblet cell hyperplasia was found (Appendix IX). The cases that were Likely aspergillosis were excluded of the statistical analysis**.**

Rhinitis and Aspergillosis cases were cross-tabulated with goblet cell hyperplasia (Appendix X) and the association was shown not to be statistically significant (p=0.58). It was seen that twelve (57.1%) Rhinitis cases and five (38.5%) Aspergillosis cases had mild goblet cell hyperplasia, being this grade the most frequent one in both cases.

The following chart (Chart 11) shows, in percentage, the epithelial and goblet cell hyperplasia distribution according to the Aspergillosis and Rhinitis cases. In this case, we can see that 69.3% and 71.4% of the Aspergillosis and Rhinitis cases, respectively had epithelial cell hyperplasia. While goblet cell hyperplasia was present in 76.1% of the Rhinitis cases and only in 61.6% of the Aspergillosis cases.

Chart 11 – Distribution of the different type cell hyperplasia according to Rhinitis and Aspergillosis



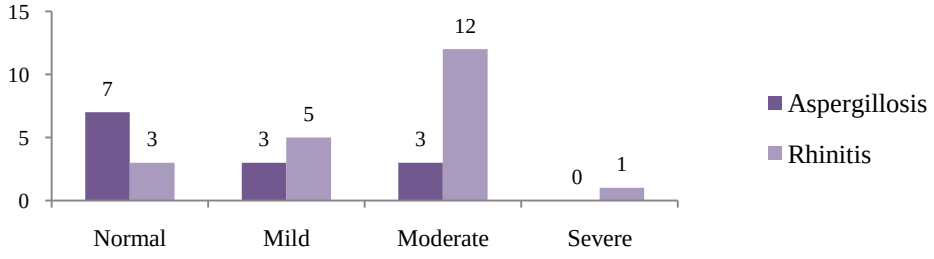
The epithelial and goblet cell hyperplasia was included in the final histopathology classification (Appendix XI), appearing first the name of the cell most frequently found. According to this, twelve (57.1%) Rhinitis cases were classified as epithelial and goblet cell hyperplasia and three (14.3%) as goblet and epithelial cell hyperplasia. In addition to this seven (50%) Aspergillosis cases were classified as epithelial and goblet cell hyperplasia and one (7.1%) as goblet and epithelial cell hyperplasia.

4.4.4.7 Oedema and Fungi

Oedema in the samples was also another variable considered and its presence was identified in twenty four (63.1%) cases out of 38. The presence ranged from mild to severe, eight (21%) cases were classified as mild, fifteen (39.5%) as moderate and only one (2.6%) as severe (Appendix IX). The cases that were likely to be aspergillosis were excluded of the statistical analysis.

These results were cross-tabulated with Rhinitis and Aspergillosis (Appendix X) but no statistical association was found (p=0.06). The results of this distribution (Chart 12) showed that twelve cases (57.1%) of Rhinitis had a moderate presence of oedema, five (23.8%) had a mild presence, three (14.3%) were normal and only one (4.8%) was described as a severe oedema presence. On seven (53.8%) Aspergillosis cases oedema was not visualized, nevertheless three cases (23.1%) were reported to have mild presence of oedema and no cases were reported to have severe oedema presence.

Chart 12 – Distribution of oedema according to Rhinitis and Aspergillosis



The presence of fungi was assessed on 38 samples, and it was seen that only four (10.5%) cases were reported to have fungi present. One was classified as mild fungal presence and the other three as severe presence (Appendix IX). The cases that were Likely aspergillosis were excluded of the statistical analysis.

This variable was cross-tabulated with Rhinitis and Aspergillosis (Table 17), and the association was shown to be statistically significant (p=0.04). It was seen that most of the Rhinitis cases (95.2%) did not show any sign of fungus, however one (4.8%) had a mild presence. The majority of the samples (76.9%) of the Aspergillosis group were also considered normal, nevertheless three cases (23.1%) were classified as severe fungal presence.

Table 17 - Classification * Fungi Cross-tabulation

			Fungi			Total
			Normal	Mild	Severe	
Classification	Rhinitis	Count	20	1	0	21
		% within Classification	95.2%	4.8%	0%	100%
	Aspergillosis	Count	10	0	3	13
		% within Classification	76.9%	0%	23.1%	100%
	Total	Count	30	1	3	34
		% within Classification	88.2%	2.9%	8.8%	100%

4.4.4.8 Other histopathology findings

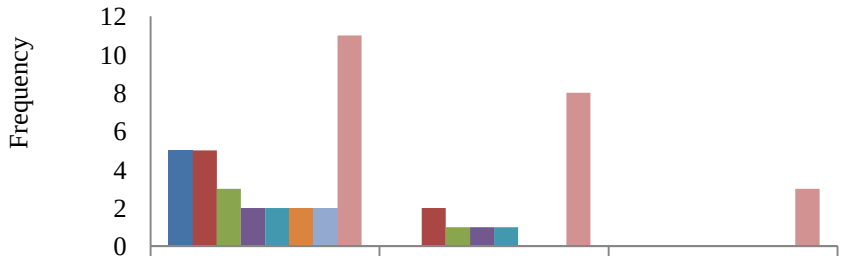
Whether the inflammation was subacute, acute or chronic was also studied, the most frequent one was subacute inflammation which was present in thirty one (81.6%) samples out of the 38 analyzed, followed by chronic inflammation that was seen in five (13.2%) cases and acute inflammation with only two (5.2%) cases (Appendix IX). These results were distributed according to the Classification (Appendix X) and it was seen that the two (15.4%) acute inflammation cases belonged to the Aspergillosis group, in this group nine (69.2%) cases were subacute and two (15.4%) were classified as chronic inflammation. Nineteen (90.5%) rhinitis cases were reported to have subacute inflammation and two (9.5%) to have chronic inflammation, in this group no sample was assessed to have acute inflammation present. The five cases that were Likely aspergillosis were excluded of this analysis.

After studying the samples, it was noticed that all the 38 samples (100%) presented diffused inflammation, so no sample had a localized inflammation present.

4.4.5 Bacteriology

Culture was performed in thirty six of the 46 dogs initially included in this study. The culture results (Chart 13) were negative for bacteria in twenty two dogs (61.1%) of the 36 dogs. Bacteria positive culture results were seen on fourteen dogs (38.9%), nine of them had been classified as Rhinitis (seven of which had more than one specie of bacteria present) and the other five cases as Aspergillosis (three of this five patients had bacterial and fungal positive culture at the same time). The bacteria species most commonly found were and *Pasteurella multocida* with seven positive cultures and coagulase negative *Staphylococcus spp.* with five positive cultures.

Chart 13 - Bacteriology results



	Rhinitis	Aspergillosis	Likely aspergillosis
Coagulase negative Staphylococcus sp.	5	0	0
Pasteurella sp.	5	2	0
Coagulase positive Staphylococcus aureus	3	1	0
Streptococcus sp.	2	1	0
Pseudomonas aeruginosa	2	1	0
Enterobacteriaceae	2	0	0
Staphylococcus intermedius	2	0	0
Negative	11	8	3

4.4.6 Mycology

Fungal culture of nasal specimens was negative, for *Aspergillus sp.*, in most of the cases (66.7%). However, it was seen that all the twelve positive results (33.3%), belonged to the Aspergillosis group (Appendix XI).

Serology testing for fungal antibodies was performed in thirty four (73.9 %) of the 46 dogs. Fifteen dogs, out of the 34, were seropositive (44.1%), the other nineteen (55.9%) were negative for *Aspergillus fumigatus* (Appendix XI). Nevertheless, one of the negative cases was positive for *Aspergillus niger*, which is not detected by normal serologic tests.

The serology results were crossed with the Rhinitis and Aspergillosis group (Table 18), the association was statistically significant (p=0.001), since 81.3% of the Rhinitis cases that did

serologic tests were negative and 78.6% of the Aspergillosis cases were positive. The five cases that were Likely aspergillosis were excluded of the statistical analysis.

Table 18 - Classification * Serology Cross-tabulation

			Serology		Total
			Negative	Positive	
Classification	Rhinitis	Count	13	3	16
		% within Classification	81.3%	18.8%	100%
	Aspergillosis	Count	3	11	14
		% within Classification	21.4%	78.6%	100%
	Total	Count	16	14	30
		% within Classification	53.3%	46.7%	100%

4.5 Statistic summary

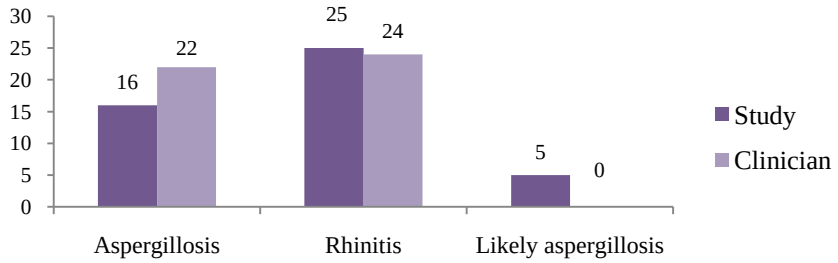
Cross-tabulation	N	Statistic	*p* value
Classification * Nasal Discharge	41	Fisher's Exact Test	0.084
Classification * Epistaxis	41	Pearson chi Square	0.002
Classification * Sneezing	41	Pearson chi Square	0.717
Classification * Nasal Hyperkeratosis	41	Fisher's Exact Test	0.390
Classification * Nasal Depigmentation	41	Fisher's Exact Test	< 0.001
Classification * Responsiveness to Antibiotics	15	Fisher's Exact Test	0.200
Classification * Leukogram	41	Monte Carlo Method	0.358
Classification * X-ray	20	Fisher's Exact Test	0.001
Classification * MRI	41	Fisher's Exact Test	0.137
MRI * X-ray	24	Fisher's Exact Test	0.091
Classification * Affected side	30	Fisher's Exact Test	0.642
Affected side (sign) * Affected side (MRI)	27	Fisher's Exact Test	0.280
Classification * Nasal septum/vomer destruction	41	Fisher's Exact Test	0.067
Classification * Cribriform plate destruction	41	Fisher's Exact Test	0.146
Classification * Frontal sinus enhancement	41	Pearson chi Square	0.070
Frontal sinus enhancement * Turbinate destruction	46	Pearson chi Square	0.208
Classification * Turbinate destruction	41	Monte Carlo Method	0.005
Turbinate destruction * Final Grade	38	Monte Carlo Method	0.01
X-ray * Turbinate destruction	24	Fisher's Exact Test	0.578
Serology * Turbinate destruction	34	Pearson chi Square	0.47
Rhinoscopy * Turbinate destruction (2)	43	Monte Carlo Method	0.002
Turbinate destruction (2) * Final Grade	38	Monte Carlo Method	0.016
Classification * Turbinate intensity T1W	41	Pearson chi Square	0.007
Epistaxis * Turbinate Intensity T1W	46	Monte Carlo Method	0.027
Inflammation final grade * Turbinate Intensity T1W	38	Monte Carlo Method	0.384
Classification * Turbinate intensity T2W (fat)	40	Monte Carlo Method	0.127
Classification * Turbinate intensity T2W (brain)	37	Monte Carlo Method	0.755
Classification * Mucus intensity T1W	40	Pearson chi Square	0.308

Classification * Mucus intensity T2W (fat)	38	Monte Carlo Method	0.161
Classification (2) * Frontal sinus enhancement	21	Fisher's Exact Test	0.027
Classification * Rhinoscopy	38	Pearson chi Square	<0.001
Rhinoscopy * MRI classification	43	Pearson chi Square	0.034
Classification * Lymphocytes	34	Monte Carlo Method	0.299
Classification * Neutrophils	34	Monte Carlo Method	0.059
Classification * Plasma cells	34	Monte Carlo Method	0.234
Classification * Eosinophils	34	Fisher's Exact Test	1.000
Classification * Final Grade	34	Monte Carlo Method	0.456
Final Grade * Nasal Discharge	38	Monte Carlo Method	0.046
Classification * Epithelial cell hyperplasia	34	Monte Carlo Method	1.000
Classification * Goblet cell hyperplasia	34	Monte Carlos Method	0.583
Classification * Oedema	34	Monte Carlo Method	0.060
Classification * Fungi	34	Monte Carlo Method	0.043
Classification * Serology	30	Pearson chi Square	0.001

4.6 Final diagnosis

All of the 46 dogs included in this study, were diagnosed as allergic rhinitis, non-specific chronic rhinitis and aspergillosis. Twelve (26.1%) of them were diagnose as allergic rhinitis, another twelve (26.1) as non-specific chronic rhinitis and twenty two (47.8%) as aspergillosis. Despite this number of aspergillosis cases, only sixteen (72.7%) of them were classified by the author as such, five (22.7%) were classified as Likely aspergillosis and one (4.5%) case as Rhinitis (Appendix XII).

Chart 14 – Comparison between study results and the clinician diagnosis



4.7 Treatment

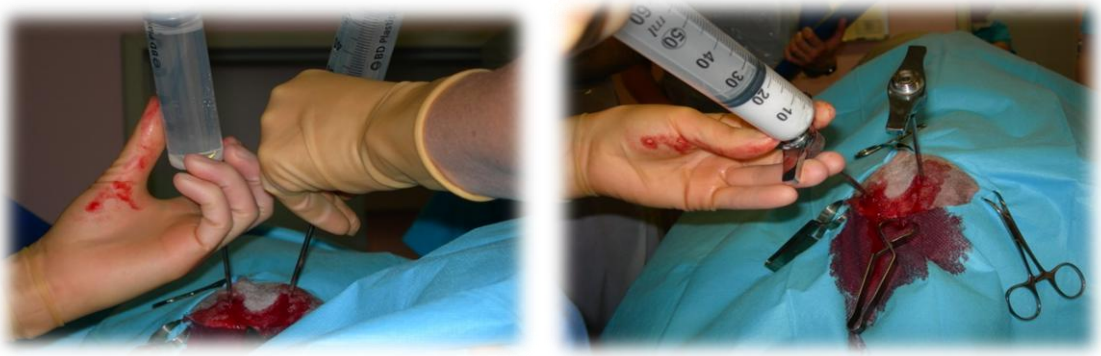
Treatment was performed on 44 of the 46 dogs included in this study. Twenty three (92%) of the 25 Rhinitis cases did a treatment; fourteen (60.8%) of the 23 cases were treated with antibiotics: four with amoxicillin and clavulanate acid, three with fluorquinolones (one with enrofloxacin and two with marbofloxacin), two had a tetracycline treatment with doxycycline, two cases with sulphonamides, another two cases were treated with a first generation cephalosporin (cefalexin) and one case with clindamycin. Two patients had a combination of two antibiotics at the same time, one with enrofloxacin and cefalexin and another with

marbofloxacin and clindamycin. Prednisolone was chosen in seven Rhinitis cases (30.4%), however three were combined with antibiotics (trimetropim sulfametoxazol, milbemycin or amoxicillin and clavulanate acid).

Other treatments less frequently prescribed for Rhinitis were: three cases (13%) of trephination with clotrimazole infusion and cream deposition, two cases (8.6%) received NSAID's and one case (4.3%) of drainage of frontal sinus and lavage with sterile saline with fluid draining.

All of the sixteen Aspergillosis cases received an antifungal treatment with clotrimazole, which consisted of a frontal sinus trephination and a 1% clotrimazole infusion followed by clotrimazole cream deposition (Figure 23). Like the Aspergillosis cases, all the five cases classified as Likely aspergillosis were treated with a frontal sinus trephination and a clotrimazole infusion followed by cream deposition.

Figure 23 – Aspergillosis treatment with clotrimazole infusion (left) followed by clotrimazole cream deposition (right).



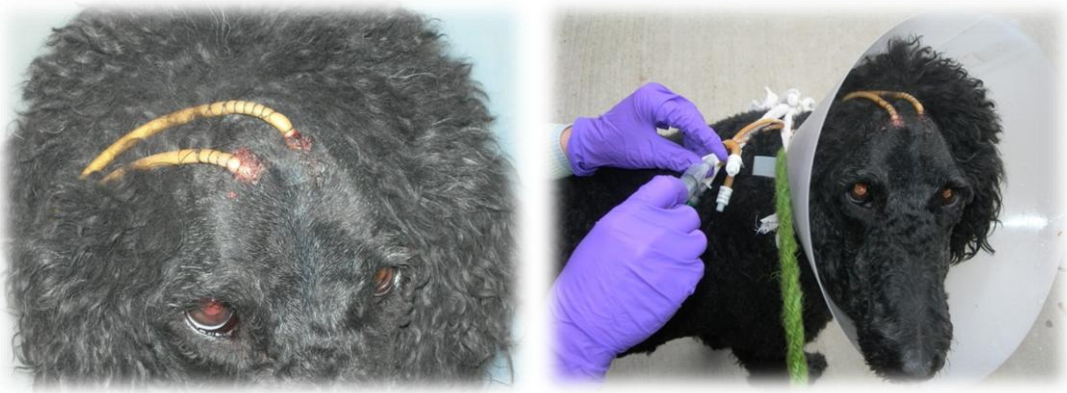
4.8 Follow-up

Twelve (48%) of the 25 Rhinitis cases did not have any information about the follow-up. The other thirteen cases were either resolved, recurrent or died. It was seen that eight (32%) cases resolved with only one treatment, four (16%) had a recurrence of the signs after one treatment and one (4%) patient died after the treatment due to aspiration pneumonia after surgery.

Three cases (18.75%) of the 16 Aspergillosis cases did not have any data about the follow-up. Two cases (12.5%) were resolved with only with one treatment with clotrimazole infusion, eleven (68.75%) had a recurrence after the first treatment with clotrimazole, from those, three of them had a second treatment with itraconazol capsules *per os* and other three with enilconazole through frontal sinus tubes (Figure 24), the remaining five cases were treated with clotrimazole again.

The cases that were classified as Likely aspergillosis, only one (20%) was resolved with a single treatment, one (20%) had a second trephination with a clotrimazole infusion and cream deposition and the other three (60%) did not have any information concerning new treatments or recurrences

Figure 24 –Aspergillosis treatment with enilconazol flush through frontal sinus tubes



5 Discussion

This study found a high frequency of inflammatory nasal disease in large (43.5%) and purebred (89.2%) dogs, which is in agreement with a previous study (Windsor, et al., 2004). Border Collies (10.9%) and Jack Russell Terriers (10.9%) were over-represented when comparing with other breeds. Other studies (Kuehn, 2009) reported German Shepherds and Rottweiler breeds to be predisposed to aspergillosis, while Dachshunds were predisposed to LPR. In the present study, different results may have occur because German Shepherds and Rottweiller might not be so frequently seen in Cambridge (England), which may influence the fact that no predisposition was found with such breeds.

Brachycephalic breeds represented only 13% of nasal inflammatory disease cases, while mesocephalic were over-represented with 63% of the population. This is in agreement with the literature, where brachycephalic breeds are reported to be uncommonly affected by nasal diseases, compared to dolicocephalic and mesocephalic breeds (Knotek, et al., 2001; Mathews & Sharp, 2006; J. Saunders, et al., 2004; Sullivan, 1987).

The dog's age ranged from 18 months to 14 years (mean 6.7 years), being this a large spectrum of years, it indicates that not only young dogs are affected by nasal inflammatory diseases but also older dogs.

Male dogs were over-represented (63%) comparing with female dogs (37%), but no sex predispositions cross-tabulations were made. Sex predisposition, in nasal aspergillosis, was shown in a study (J. Saunders, et al., 2004), in which male dogs appeared to have a higher incidence than female dogs. Most of the male dogs (18 dogs, 62%) were castrated, as well as most of the female dogs (12 dogs, 70.6%) were spayed. These results reflect the increase number of castrated male dogs and spayed female dogs and not necessarily a predisposition of castrated male dogs and spayed female dogs to inflammatory nasal diseases.

Body weight characteristics were also studied, and it was seen that it ranged from 2.8 kg. to 46 kg., with the mean 23.4 kg. Being large breeds the most represent on this study, it was with no surprise that heavier dogs were over-represented.

Most of the cases (28.3%), in which the duration of clinical signs was reported, had signs for 1 to 3 months prior to the first appointment at the QVSH. Nevertheless, a large percentage (37%) did not have any information about this feature so no conclusions could be made based on these results.

Nasal discharge was the most common clinical complaint (70%), which was in agreement with previous studies (Ford, 2005; Tasker, et al., 1999). Since it was common in aspergillosis (87.5%) and in rhinitis (60%), it was expected that there would be no statistical association (p=0.08) between these two diseases and nasal discharge.

Mucopurulent was the most common type of discharge (34.5%). This is in agreement with other studies that showed this type of discharge as the most common in rhinitis (Windsor, et al., 2004) and aspergillosis (Haagen & Herrtage, 2010). This finding is compatible with inflammation (Hawkins, 2009) or/and secondary bacterial invasion (Kuehn, 2009).

Nasal discharge was unilateral in 62.5% of the cases and bilateral in 35.5% of the cases. This feature was not cross-tabled with the pathologies. However, the literature (Sullivan, 1987; Tasker, et al., 1999) says that aspergillosis is usually unilateral and LPR is usually reported to be associated with bilateral nasal discharge but not invariably. It must be taken in consideration, that in some aspergillosis cases, it can become bilateral, indicating that the continuity of the nasal septum has been lost (Sullivan, 1987). Other studies (Tasker, et al., 1999; Windsor, et al., 2004), contradict these findings saying that there is not a big discrepancy between the incidence of bilateral and unilateral nasal discharge in dogs with rhinitis and aspergillosis. More recent studies (Miles, et al., 2008; Petite & Dennis, 2006; J. Saunders, et al., 2004; Windsor, et al., 2004) showed that lesion extension (uni- or bilateral) was shown not to be associated with diagnosis of rhinitis or aspergillosis.

The second most common complaint was epistaxis (37%), this sign was shown to be statistically associated (p=0.002) with Aspergillosis. References to this association were not found in the literature, by the author, however it is known to be a common sign in dogs with aspergillosis due to the destruction of the turbinates (Bissett, et al., 2007; Haagen & Herrtage, 2010; Mathews, et al., 1998; Strasser & Hawkins, 2005). Since this is not a common complaint in dogs with rhinitis, when it is found, it may be due to paroxysmal sneezing or to the fact that some cases may have had undiagnosed aspergillosis.

Sneezing was another common sign (34.8%) reported in this study. It may be related to mucosal irritation or inflammation (Tilley & Smith, 2007), so it was not surprisingly that no statistical association (p=0.72) was found between this sign and Aspergillosis or Rhinitis, since in both nasal irritation and inflammation are present.

Nasal depigmentation was found in nine dogs (19.6%) all of them classified as Aspergillosis cases. This sign was shown to be statistical associated (p<0.001) with aspergillosis. This finding is in agreement with the bibliography published (Haagen & Herrtage, 2010; Mathews & Sharp, 2006; Tilley & Smith, 2007), in which a high incidence of nasal depigmentation was found in dogs with nasal aspergillosis.

In the present study, only three dogs, all of them with aspergillosis, were reported to have nasal pain. This is agreement with other studies (Haagen & Herrtage, 2010; Tasker, et al., 1999) that referred to nasal pain as being frequent in aspergillosis cases. Whether this is

statistically associated with aspergillosis was not evaluated because more cases would have been needed to establish an accurate result.

Nasal hyperkeratosis was only present in one dog (2.2%) with aspergillosis, but it was found not to be statistically associated (p=0.39) with this disease. Reverse-sneezing was seen in five cases, all of them classified as Rhinitis, however no statistical association was made. Further studies are necessary in order to see if this sign is associated or not to rhinitis.

Cough, lymphoadenopathy and exophthalmus were not associated to rhinitis and aspergillosis cases because they had a uniform distribution between the two diseases and the number of dogs with these signs was not high enough to give significant results.

In the present study, no statistical association (p=0.20) was found between antibiotic response and rhinitis and aspergillosis, however all the aspergillosis cases included showed no response and more than half of the rhinitis cases (58.3%) showed some improvement after antibiotic therapy. This improvement may be due to the fact that secondary bacterial infection is common in rhinitis patients, so clinical signs often partially or completely resolve with a complete antibiotic therapy. In order, to find a statistical association in future studies, more cases should be included to provide a more reliable result.

In the present study, most of the cases (54.1%) had an abnormal leukogram, with neutrophilia (70%) and eosinophilia (35%) being the most common abnormalities found. This is in agreement with other published studies (Mathews & Sharp, 2006; Tilley & Smith, 2007) in which neutrophilia was the most common finding, but contrary in our study, leucocytosis (20%) and/or monocytosis (0%) were not found to be very frequent. Lymphopenia was found in two aspergillosis cases, and although not common, it has been associated with systemic aspergillosis in dogs (Schultz, et al., 2008). Whether or not this was true in the present study, it was not studied, because the population of aspergillosis cases with lymphopenia was too low to give significant conclusions. No statistical association (p=0.36) was found between leukogram results and aspergillosis and rhinitis. So like another study published (Sullivan, 1987) this was shown to have no diagnostic value, reflecting only chronic inflammation.

Radiographic evaluation was also studied and lesions were found in most of the cases (62.5%), however 37.5% were considered to be normal. This is not in agreement with the literature (Russo, et al., 2000), in which it is reported a prevalence of 100% of abnormal radiographs in dogs with nasal disease. The possible explanations for the lower prevalence observed in the present study, are that the frequency of mild changes was higher than in the cited study and/or since x-ray does not localize early nasal disease (Haagen & Herrtage, 2010), those were classified as normal.

All the Aspergillosis cases had abnormalities seen on x-ray, with turbinate destruction the most common one (88.9%). Most of the Rhinitis cases (72.3%) had normal x-rays, with soft tissue opacities the most common abnormality found. On the Likely aspergillosis group, turbinate destruction was the most common finding (75%). This indicates that turbinate destruction is more frequently seen on x-rays in dogs with aspergillosis, when compared to a dog with rhinitis. For statistical proposes only two groups were made: abnormal and normal and the Likely aspergillosis group was excluded. The x-ray groups were table-crossed with the Rhinitis and Aspergillosis, and the association was shown to be statistically significant (p=0.001). Abnormal x-rays were statistically associated with Aspergillosis while normal x-rays were to Rhinitis.

Even though only soft tissue/fluid density and loss of turbinates were studied and lysis of facial bones and radiodense/radiolucency of the structures were not evaluated, it was noticed that all (100%) the aspergillosis cases had some kind of abnormality present. These findings are in agreement with other studies (Saunders & Bree, 2003; Saunders, et al.,2002), which obtained a higher sensitivity in dogs with generalized lesions. On the other hand, 72.7% of the rhinitis cases did not show any abnormality on x-ray, which is in agreement with the literature (Mackin, 2004) where most of the rhinitis cases were reported to have normal nasal radiographs or mild to moderate increase in fluid/tissue radiopacity associated with excessive intraluminal nasal discharge.

The cases studied were submitted to an MRI scan, which showed that only 13% had normal nasal passages and 87% were classified as abnormal. This MRI classification was cross-tabulated with the x-ray classification (normal/abnormal), but it was shown not to be statistically significant (p=0.09). It was seen that an abnormal MRI scan does not necessarily corresponds to an abnormal x-ray, in fact 25% of the cases, which had an abnormal MRI were classified as normal on x-rays. Like other studies (Haagen & Herrtage, 2010; Petite & Dennis, 2006; Rycke, et al., 2003), this one showed that the sensitivity of MRI is higher than the x-ray and that subtle changes may be easier spotted on MRI than on x-ray. Probably, this happens because of superimposition of bone structures and the fact that some nasal structures like the nasal septum are not visible on x-ray.

The MRI classification (normal/abnormal) was cross-tabulated with Rhinitis and Aspergillosis, but the association was shown not to be statistically significant (p=0.14). Nevertheless, all the Aspergillosis cases and 80% of Rhinitis cases were found to have abnormal nasal MRI scans. This shows that an abnormal MRI does not necessarily relate to a specific inflammatory nasal disease.

MRI evaluation showed that concerning the affected side, 65.2% of the cases had bilateral changes and only 10.8% had unilateral changes. The cross-tabulation of this feature with the Classification, was shown not to be statistically significant (p=0.64). These results are not in agreement with a study (J. Saunders & Bree, 2003), which reported that in the presence of localized turbinate destruction aspergillosis should be the first diagnosis. According to these study findings, when in presence of unilateral/localized turbinate destruction no pathology should be thought as the most probable one.

The affected side (nasal discharge) was cross-tabulated with the affected side changes seen on MRI. The association was not statistically significant (p=0.28), but all the cases with bilateral nasal discharge had bilateral changes on MRI, and 73.7% of the cases with unilateral nasal discharge had bilateral changes seen on MRI scans. This shows that nasal discharge is more frequently seen when bilateral changes are seen on MRI, however, unilateral discharge can also be seen with only unilateral nasal changes on MRI scans.

Nasal septum/vomer destruction was seen on 10.9% of the cases, but surprisingly, it was not statistically associated with aspergillosis (p=0.07). Nevertheless, 80% of the cases were classified as aspergillosis, which indicates a higher frequency of this abnormality in dogs with aspergillosis. Destruction of the cribriform plate was only seen in two dogs (4.3%), both of them had aspergillosis. Although not statistically associated (p=0.15) with any specific pathology, cribriform plate destruction is more frequently seen in aspergillosis than in rhinitis. Previous studies (Miles, et al., 2008) showed a significant association between cribriform plate destruction and vomer bone lysis and neoplasia, however, those abnormalities on MRI were also seen in non-neoplastic cases, which included aspergillosis. This is in agreement with the present study, because no statistical association was found between cribriform plate destruction or vomer bone lysis and rhinitis and aspergillosis, meaning that this type of destruction is not associated with inflammatory nasal diseases.

Frontal sinus involvement was seen in 34.8% of the cases, but its association with a specific disease was shown not to be statistically significant (p=0.07). Nevertheless, this abnormality was more frequently seen in Aspergillosis cases (56.3%). Frontal sinus involvement was, also, shown not to be associated with turbinate destruction (p=0.21). This result was expected since it was noted before, in this study, that the frontal sinus involvement was not associated with Aspergillosis. These results show that turbinate destruction does not increase the risk of frontal sinus involvement, and that cases with no destruction may have frontal sinus involvement present.

Turbinate destruction was seen in 69.6% of the cases, and when cross-tabulated with Rhinitis and Aspergillosis cases, it was shown that turbinate destruction was associated with

Aspergillosis (p=0.005). In fact, 87.5% of the aspergillosis cases exhibited turbinate destruction that ranged from mild to severe. The other 12.5% that did not show any turbinate destruction, could be cases of recent aspergillosis that had not yet developed turbinate destruction or maybe the destruction was not severe enough to be visible on MRI. The percentage of Rhinitis cases that had turbinate destruction was also relatively high (52%), but the destruction seen in such cases was majoritarily mild (40%). This may indicate that even though turbinate destruction is not associated with rhinitis, mild destruction can be present. So, turbinate destruction should not be used as a single diagnostic feature on MRI, it should be used instead as indicative of aspergillosis. The results are in agreement with other studies published (Haagen & Herrtage, 2010; Miles, et al., 2008; J. Saunders, et al., 2004) that describe moderate to severe turbinate destruction characteristic of aspergillosis, while mild to moderate is seen in severe LPR cases, however no statistical significance was ever proven.

Turbinate destruction was not statistically associated with x-ray results (p=0.58), proving that turbinate destruction seen on MRI does not necessarily appear abnormal on x-ray. However, it should not be forgotten that x-rays were only classified as normal and abnormal, and specific changes were not taken in consideration. More studies are needed to prove if the changes seen in the turbinate area, on x-rays, are or are not associated with the turbinate destruction seen on MRI.

Since the turbinate destruction was associated with Aspergillosis, it seemed logical to associate this feature with serology. However no statistical association was found (p=0.45), 68.4% of the cases with negative serology had turbinate destruction, and 80% of the cases with positive serology had turbinate destruction. The statistical results may be due to the fact that rhinitis or non-seroconverted aspergillosis cases which were negative on serology, showed turbinate destruction on MRI. No literature was found concerning which appears first out of turbinate destruction or seroconversion, so no real causes for these results were found.

Whether turbinate destruction was or was not associated with the final inflammation classification was seen by two different perspectives, which were both shown to be statistically significant. On the first one, turbinate destruction (present or absent) was table-crossed with the different grades of inflammation (p=0.01), while in the second perspective different grades of turbinate destruction (no destruction, mild, moderate and severe destruction) were table-crossed with the same grades of inflammation (p= 0.02).

The first cross-tabulation showed that most of the cases (80%) without turbinate destruction had moderate inflammation grade, while most of the cases (53.6%) with turbinate destruction seen on MRI had severe mucosal inflammation seen. The second association showed that most of the cases (57.9%) with severe inflammatory infiltration of the mucosa were seen on

MRI as mild turbinate destruction, while moderate inflammatory infiltration was more commonly seen (57.1%) on cases that on MRI did not show any turbinate destruction. When turbinate destruction was not present the grade of inflammation was classified as mild (20%) or moderate (80%) but never severe. All these results may indicate that when destruction is seen the possibility of having severe inflammation on the samples is low. Mild destruction does not indicate a mild inflammation degree and severe destruction can be seen with several grades of inflammation.

Turbinate destruction seen on MRI was also cross-tabulated with the rhinoscopy results, and the results were shown to be statistically associated (p=0.002). Dogs with no destruction or mild destruction seen on MRI were classified as normal/inflammation on rhinoscopy, showing that when no or mild destruction was seen on MRI prior to rhinoscopy, there was a high possibility that no changes would be seen on rhinoscopy. On the contrary, when moderate to severe destruction was seen on MRI, the possibility of seeing turbinate destruction and/or fungal plaques was higher.

Turbinate intensity was evaluated on T_1W and T_2W. When compared with muscle on T_1W, the turbinate hypo- or isointensity was shown to be associated with rhinitis and hyperintensity with aspergillosis (p=0.007). To the author's knowledge no study has published results concerning turbinate intensity, only mucus intensity, so further studies should be done in order to confirm this association.

The association between epistaxis and turbinate intensity on T_1W was shown to be statistically significant (p=0.03). These results reinforce the fact that turbinate hyperintensity is associated with aspergillosis; since it was shown previously that Aspergillosis is associated with epistaxis. On T_2W, epistaxis was not statistically associated with any specific turbinate intensity (p=1.00 [fat], p=0.58 [brain]).

On T_2W, turbinate intensity was compared with fat and brain intensity. Both were cross-tabulated with Aspergillosis and Rhinitis, and were shown not to be statistically associated (p=0.13 [fat]; p=0.76 [brain]). Nevertheless, the majority of Rhinitis cases were hypointense (62.5%) to the fat and iso-intense (56.5%) to the brain, while 56.3% of Aspergillosis cases were iso-intense to the fat and 50% were hyperintense to the brain. These results may suggest that T_2W is not the best sequence to evaluate turbinate intensity, since it seems not to be associated with either pathology. Although T_2W should always be evaluated, conclusions about the turbinate intensity should not be made based only on this sequence.

It was also presumed that the turbinate intensity, on T_1W and T_2W, could be due to the inflammation grade seen on histopathology, however, no statistical association was found using either sequence (p=0.38 [T_1W]; p=0.52 [T_2W, fat]; p=0.59 [T_2W, brain]). Nevertheless,

on T_1W most of the cases (53.3%) with severe inflammation had hypointense turbinates, and 47.1% of the cases with moderate inflammation had hyperintense turbinates.

Mucosal oedema was shown not to be statistically associated with the turbinate intensity on either sequences (p=0.80 [T_1W]; p=0.38 [T_2W, fat]; p=0.15 [T_2W, brain]), which means that the presence of oedema does not influence the intensity seen on MRI. Epithelial cell hyperplasia was not associated with turbinate intensity (p=0.58 [T_1W]; p=0.97 [T_2W, fat]; p=0.71 [T_2W, brain]). Since all the histopathology results, were not associated with the turbinate intensity, we can conclude that in reality there is no statistical association between the two features, probably because rhinoscopy was not done immediately after the scan evaluated, or histopathology samples were not taken from the brightest area on MRI, which was the chosen to be evaluated.

Nasal discharge was associated with the turbinate intensity on both sequences, but the associations were not statistically significant (p=0.16 [T_1W]; p=0.51 [T_2W, fat]; p=0.78 [T_2W, brain]). This suggests that hyper- or hypointensity are not related with the presence or absence of nasal discharge.

Mucus intensity was also evaluated on T_1W and T_2W. In this case, all the cross-tabulations between this finding and Rhinitis and Aspergillosis, were not to statistically significant (p=0.31 [T_1W]; p=0.16 [T_2W, fat]). Since all the cases were hyperintense on T_2W when compared with the brain, no statistical association was made. Nevertheless, on T_1W and T_2W mucus appeared hyperintense in most of the cases of rhinitis (87.5% [T_1W]; 69.6% [T_2W]) and aspergillosis (75% [T_1W]; 46.7% [T_2W]). These results showed that independently of the sequence and of the pathology present, mucus, when present, is hyperintense in most of the times. These results are not in agreement, with other studies (Miles, et al., 2008; J. Saunders, et al., 2004) that postulated that the protein content of secretions can significantly affect the signal intensity. Mucus intensity can be misleading because the signal can overlap between secretions and fungal colonies, and differentiating both can sometimes be impossible. However, in the present study, the results showed were not similar to others (Miles, et al., 2008; J. Saunders, et al., 2004) where thick mucus had low T_1W signal and low T_2W signal.

A possible explanation for the results seen, is that the mucus examined in the present work was, in most of the cases, desiccated due to chronic nasal disease, which is known to have an hyperintense appearance when compared with other types of mucus (Kuehn, 2009). Another aspect that should be taken into consideration is that fungal plaques can also produce signal voids on both sequences (J. Saunders, et al., 2004), and this may explain why mucus appeared hyperintense in aspergillosis cases.

A group of cases (n=5) which was excluded from most part of the statistical analysis, called Likely aspergillosis, included dogs where aspergillosis was the most likely pathology present, but did not have 3 positive ancillary diagnostic tests to prove it. All the cross-tabulations, but one, made between this group and the Aspergillosis cases were shown not to be statistically significant, which means that no differences exist between these groups. Whether these cases could be included in the Aspergillosis cases, was also debated during the research time, however it was considered to be appropriate to see if differences existed between both groups. It seems that considering MRI features, Likely aspergillosis cases could not be differentiated from those with aspergillosis. Curiously, the only feature in which a statistical association was found (p=0.03), was the frontal sinus involvement, which was not present in all the Likely aspergillosis cases. This seems to be a coincidence, since the same feature was shown not to be associated with Rhinitis or Aspergillosis. A possible reason for this, results on the fact that because the frontal sinus was not involved in these cases, maybe the samples that were taken for other diagnostic tests were not representative. These cases could have had aspergillosis but because the focus of the disease was not in the frontal sinus, the pathology was not confirmed by three diagnostic tests.

Since the Likely aspergillosis group identified for this study was considered by the clinicians to be an aspergillosis case, another comparison was made to see whether the final diagnosis matched the groups made. The results showed that, as expected, all the Likely aspergillosis cases were diagnosed by the clinicians as aspergillosis, and curiously, one case that was classified as Rhinitis was diagnosed and treated as aspergillosis.

Rhinoscopy revealed lesions in 81.4% of cases, with inflammation the most common abnormality found (41.9%). Turbinate destruction was seen in 28% of the cases and fungal colonies were seen in 32.6% of the cases. Compared with a previous study (Johnson, et al., 2006) where 83% of the cases had lesions, fungal plaques were seen less frequently in this study. This may be due to the fact that in the rhinoscopy report it was not mention that plaques were seen or because the areas where the plaques were, were not visualized. Turbinate destruction was also found in fewer cases than the referred 74% in a previous study (Johnson, et al., 2006). This may be due to technique problems or insufficient data reported. Nevertheless, previous studies only included aspergillosis cases data, since the present study includes rhinitis and aspergillosis cases, the turbinate destruction frequency was expected to be lower than in the referred study.

The rhinoscopy results were cross-tabulated with Rhinitis and Aspergillosis, and the association was shown to be statistically significant (p<0.001), which means that normal or inflammation results were associated with Rhinitis, while turbinate destruction and/or fungal

plaques seen were associated with Aspergillosis. However, in previous studies (Harcourt-Brown, 2006b) the endoscopic appearance on its own was not usually diagnostic of a particular condition, and clinical examination plus biopsy for cytology and histology were also vital to achieve the final diagnosis. In the present study, two cases of Rhinitis showed turbinate destruction typical of aspergillosis, but did not show any other positive examination to *Aspergillus sp.*, so they were considered to be cases of severe destructive rhinitis.

Rhinoscopy findings were also table-crossed with MRI classification (normal or abnormal), and the association was shown to be statistical significant (p=0.03). These results showed that an abnormal MRI is associated with turbinate destruction or fungal plaques seen on rhinoscopy, so when in presence of an abnormal MRI nose scan the clinician should expect to see visible nose changes during rhinoscopy.

Histopathology revealed that all the cases (100%) had lymphocytic infiltration in the mucosa, from which most of them (55.3%) had moderate infiltration. The literature (Lorenzi, et al., 2006) points out neutrophilic infiltration as the primarily inflammatory cellular response in aspergillosis cases. However in this study neutrophilic infiltration, although very common (84.2%), was the second most common finding in aspergillosis cases.

When distributed through the Rhinitis and Aspergillosis cases, it was noted that in most Rhinitis cases, a mild neutrophilic infiltration was the most common finding (47.6%), while in Aspergillosis a moderate infiltration was the predominant one (46.2%). Neutrophilic inflammation is considered to be characteristic of a catarrhal and purulent rhinitis (Mathews, 2004), when inflammation is more severe, which may indicate that rhinitis cases are more frequently associated with advanced states of inflammation.

Plasma cell infiltration was found in 89.5% of the cases. Moderate plasma cell infiltration (66.7%) was most common in rhinitis cases, while in aspergillosis severe infiltration was the predominant (38.5%) finding. Eosinophilic infiltration was the least common finding (2.6%), with only one case of rhinitis with moderate eosinophilic infiltration. Eosinophilic infiltration of the nasal mucosa has been reported in cases of allergic rhinitis (Terada, et al., 2001) in humans. In the present study, a hypothesis that should be taken in consideration is that the rhinitis case with eosinophilic infiltration may have been a case of allergic rhinitis.

The final inflammation grade was given by the degree of infiltration with lymphocytes, neutrophils, plasma cells and eosinophils, and was cross-tabled with rhinitis and aspergillosis. The association was not statistically significant (p=0.46), but showed that both pathologies have similar distributions according to the degree of inflammation, with moderate inflammation the most common finding (44.7%), followed by severe inflammation degree (39.5%). These results were expected since both pathologies are inflammatory nasal diseases.

The Final inflammation grade was also cross-tabulated with Nasal discharge, but the association was shown not to be statistically significant (p=0.05). Curiously, 86.7% of the cases with inflammation did not show any sign of nasal discharge, which means that the presence of nasal discharge does not necessarily mean that nasal inflammation is present.

Epithelial cell hyperplasia was visualized in most of the cases (71%) in which the majority (39.5%) had moderate hyperplasia, independently of being classified as Rhinitis or Aspergillosis. Goblet cell hyperplasia was found in 71% of the cases analyzed, with mild hyperplasia the most frequent grade in both Rhinitis (57.1%) and Aspergillosis (38.6%) groups. Epithelial cell and goblet cell hyperplasia were more frequently seen in Rhinitis, however no statistical association was found between these variables, so further studies are needed to show if these characteristics are associated or not with this pathology. Epithelial cells are considered to be more resistant to all forms of injury than goblet cells (López, 2007). The possible explanation for the epithelial cell hyperplasia be more frequent in rhinitis, is related to the fact that in rhinitis cases the inflammation is more severe so epithelial cells are damaged more frequently. Goblet cell hyperplasia is known to result from mucociliary damage, which leads to excessive mucus production (López, 2007). In this study, it can be assumed that rhinitis has more frequently mucociliary damage than aspergillosis, but possible causes are not reported on the literature, so further studies should be made in order to obtain more conclusive data.

Oedema was found in 63.1% of the cases. This variable was cross-tabulated with Rhinitis and Aspergillosis but the association was proven not to be statistically significant (p=0.06). Most of the Aspergillosis (53.8%) cases did not show any sign of oedema in the samples, however most of the Rhinitis (57.1%) cases show a moderate presence of oedema, with the only case of severe oedema was seen in a case of Rhinitis. Although not diagnostic, these results may tell us that samples without oedema are more frequently seen in aspergillosis cases while the presence of oedema is more frequently seen in rhinitis cases.

Fungal presence on histopathology was also assessed, only four cases (10.5%) were shown to have fungal presence on the samples, one was classified as Rhinitis and the other three (severe infiltration) as Aspergillosis. Aspergillosis cases, where no *Aspergillus sp.* were found, may result from the fact that the samples were not representative of the lesions found on rhinoscopy. The explanation for the presence of fungus in Rhinitis cases, is that most probably this case did not have three positive results for aspergillosis and were not classified as such; or being *Aspergillus sp*. part of the normal flora of the nose it is accepted that it can be seen on histopathologic examination of dogs without fungal disease present.

The association between fungal presence and Aspergillosis and Rhinitis, was shown to be statistically significant (p=0.04), with *Aspergillus sp.* presence associated with Aspergillosis cases. Although histologic evidence of aspergillosis is often used as a diagnostic criteria (Mathews, et al., 1998), a study (Peeters, et al., 2005) showed that given the superficial nature (no mucosal invasion) of fungal hyphae in dogs with nasal aspergillosis, detection of fungal hyphae in the nasal cavity would not always be expected as observed in the present study. Another aspect that was taken into consideration was whether the inflammation was subacute, acute or chronic. The results were table-crossed with Rhinitis and Aspergillosis cases, and it was showed that the majority of both groups were classified as subacute (81.6%), with acute inflammation the least frequent type (5.2%). All the cases were found to have diffuse inflammation present in the samples submitted. This result is in agreement with the literature (Mackin, 2004) where it is reported that the histopathologic changes in LPR are typically diffuse and distributed throughout the nasal cavity. Although in aspergillosis the fungal plaques are localized, in this study it was shown that the inflammation is, on the contrary, more frequently generalized.

To the author's knowledge, this type of detailed histopathology classification has not been yet published in any other study. Classification of chronic nasal inflammatory diseases into distinct categories based on the predominant inflammatory cell and cell hyperplasia has been somehow arbitrary (Mackin, 2004). So in this study, a more objective classification was proposed because it gave more information than the simple designation of chronic rhinitis or LPR, and aspergillosis.

It is suggested that the classification of rhinitis should include first the most abundant cell type present, for instance neutroplasmacytic rhinitis. Nevertheless, most of the cases (65.8%), in the present study were predominantly a lymphoplasmacytic inflammatory process. After the name of the two most abundant cell types present in the sample, the classification should include if the inflammation was subacute, acute or chronic, whether it was diffuse or localized, the level of epithelial and goblet cell hyperplasia present and finally the presence of oedema and fungus.

In fact, cases of LPR may not have lymphocytes or plasma cells as the most prevalent type of cell, so the classification suggested will give more information about the type of inflammation present. An example of such a classification is: plasmalymphocytic, subacute and diffuse rhinitis with moderate hyperplasia of epithelial and goblet cells and fungus present. All the cases, in this study, were described according to this classification and the results are shown in attachment (Attachment I).

Bacterial culture was positive in only 38.9% of the cases that underwent a culture test. In the cases with rhinitis the most common bacteria species found were coagulase negative *Staphyloccoccus sp.* (15.6%) and *Pasteurella sp.* (15.6%). In Aspergillosis cases *Pasteurella sp.* was the most common bacteria cultured (15.3%); however most of the cases (84.6%) were negative. The species of bacteria isolated were in agreement with previous studies (Harvey, 1984; Padrid, 2007) that showed *Staphyloccocus sp.* and *Pasteurella sp.* were two of the normal intranasal commensal bacterial flora, and so they were expected to be frequently found in culture results. Perhaps in the present study, the results were due to sample collection problems or in the case of aspergillosis, the samples were collected from an unaffected region of the nasal cavity.

Fungal culture was negative in most of the cases (66.7%), nevertheless is worth referring that all the positive results were classified as Aspergillosis. These results are in agreement with other published (Mathews, et al., 1998; Pomrantz, et al., 2007; J. Saunders & Bree, 2003) that showed a high sensitivity for culture in the diagnosis of aspergillosis.

Serologic positive results for aspergillosis were more frequent (78.6%) in dogs that actually had the disease, and on the other hand most of the rhinitis cases (81.3%) had a negative result on *Aspergillus* serologic test. Serology positive results were shown to be statistically associated (p=0.001) with Aspergillosis. According to several studies (Billen, Peeters, et al., 2009; Pomrantz, et al., 2007), that showed that the sensitivity of the serologic test for detection of *Aspergillus* infection was around 67%, it may be concluded that although most of positive results are actually aspergillosis cases, the diagnosis cannot be ruled out on the basis of a negative serologic result.

In the same way, studies reported a specificity of 100% (Billen, Peeters , et al., 2009) and 98% (Pomrantz, et al., 2007). In this study, some Rhinitis cases had a positive serologic test, which may be due to the lack of data, small case number included in this study and because some of the dogs classified as Rhinitis could have had aspergillosis not yet diagnosed by other ancillary diagnostic methods or also because dogs diagnosed now with rhinitis could have had, previously, aspergillosis.

The treatments prescribed were in agreement with the literature. Most of the Rhinitis cases received a treatment, being antibiotic therapy the most frequent treatment prescribed, followed by prednisolone. All the Aspergillosis cases received the most effective antifungal treatment currently known, which consists of frontal sinus trephination and a clotrimazole infusion followed by clotrimazole cream deposition (Bray, et al., 1998; Friend, et al., 2002; Haagen & Herrtage, 2010; Mathews, et al., 1998; Mathews, et al., 2009). All the likely

aspergillosis, also received the same antifungal treatment because most of them were diagnosed as aspergillosis.

When available the follow-up shown that most of the rhinitis cases resolved with only one treatment with antibiotics, which suggests that the diagnosis was correct and sensitivity tests were done in most of the cases before treatment was prescribed. The cases that were not resolved may be due to the fact that empirical antimicrobial treatment was prescribed (Haagen & Herrtage, 2010), persistent nasal discharge and inadequate drainage contributed to the recurrence of the disease (Chandler, et al., 1991), the antibiotic treatment was discontinued or tapered (Mackin, 2004), or due to the fact that no oral glucocorticoids were added to the treatment.

The Aspergillosis scenario was quite different, since most of them (68.75%) had a recurrence after the first treatment with clotrimazole. Recurrence was also seen in the Likely aspergillosis cases; however more data was needed to draw conclusions about this group recurrence when compared with the aspergillosis cases.

6 Conclusions

To determine the underlying cause in dogs with inflammatory nasal disease can be a challenging and frustrating task, which often necessitates multiple diagnostic procedures and substantial cost to the client. With the increased availability of MRI, this technique has been used increasingly often to evaluate dogs with nasal disease, but even though the MRI findings were published in other studies, no statistical study association of two different inflammatory nasal diseases has ever been made.

In the present study, brachycephalic breeds were found to be uncommonly affected by nasal diseases when compared to dolicocephalic and mesocephalic breeds and male dogs appeared to have a higher frequency of nasal diseases than female dogs. The most common sign reported was nasal discharge, which was in most of the cases unilateral and mucopurulent. The second most common sign was epistaxis, which was shown to be statistically associated with Aspergillosis. Other signs reported included sneezing, nasal depigmentation (associated with aspergillosis), reverse-sneezing, coughing, nasal pain, lymphoadenopathy, exophthalmus and nasal hyperkeratosis.

The response to antibiotics was also described, but no statistical association was found between the antibiotic response and Rhinitis and Aspergillosis, nevertheless all the Aspergillosis cases did not have any response reported comparing with more than half of the Rhinitis cases which showed some improvement after antibiotic therapy.

The leukogram was cross-tabulated with Rhinitis and Aspergillosis but no statistical significance was found. However, most of the cases had an abnormal leukogram, with neutrophilia and eosinophilia the most common abnormalities found. Leukogram was found not to be reliable diagnostic test, unless systemic aspergillosis is considered likely.

At the start of this study, six aims were set in order to prove that MRI was a good and reliable imaging study for the diagnosis of inflammatory nasal diseases.

The first aim was to determine whether a statistical association existed between MRI abnormalities seen and Aspergillosis and Rhinitis. It was shown that the affected side seen on MRI was not necessarily associated with any specific inflammatory nasal disease; nasal septum and cribriform plate destruction were not statistically associated with Aspergillosis although most of the cases belonged to this group, and frontal sinus involvement was not seen frequently and was not associated with any particular disease.

Turbinate destruction ranged from mild to severe and was shown to be associated with Aspergillosis, however since Rhinitis cases also had turbinate destruction seen on MRI, it should not be used as a single diagnostic MRI feature. Turbinate destruction was statistically associated with rhinoscopy results, proving that when turbinate destruction is seen, the

clinician should expect to see turbinate destruction or fungal plaques on rhinoscopy, rather than only mucosal inflammation. The histopathology of the biopsy samples taken during rhinoscopy, was also associated with the turbinate destruction seen on MRI scan, this finding was statistically significant, indicating that turbinate destruction was present in the cases of severe inflammatory infiltration, while no turbinate destruction tend to show mild to moderate signs of inflammatory response.

Turbinate intensity was evaluated on T_1W and T_2W sequences, but only on T_1W images were statistically relevant associations found with Rhinitis or Aspergillosis. On T_1W, the turbinates when hypo- or iso-intense were associated with Rhinitis while hyperintense were associated with Aspergillosis. These findings may help the clinician deciding, when looking at an MRI scan, what nasal pathology is likely to be present. Nevertheless, this feature should always be evaluated as part of a diagnostic plan and not as a single diagnostic feature. Since epistaxis is statistically associated with Aspergillosis, it was also found that there was an association between epistaxis and turbinate intensity, which showed that dogs with epistaxis have a hyperintense turbinates.

Mucus intensity was also evaluated on T_1W and T_2W, but all the cross-tabulations were shown not to be statistically significant when associated with Rhinitis and Aspergillosis. Mucus was hyperintense in most of the cases on both sequences, so independent of the sequence used and of the pathology present, mucus appeared mostly hyperintense.

Besides the Rhinitis and Aspergillosis groups, another group was made with cases that were likely to have aspergillosis, but did not have 3 ancillary positive tests to prove it. The findings of this group were compared with the Aspergillosis group findings in order to see if any difference was apparent. The results showed that there were no statistical significant differences between the two groups in all the features except in the frontal sinus involvement, where Aspergillosis dogs were shown to be statistically associated with the involvement of the frontal sinus. These results showed that Likely aspergillosis cases should be considered as aspergillosis from an imaging point of view and that maybe the reason why other tests were not positive, was due to the fact that the focus of the disease was not primarily on the frontal sinus, so no representative samples could be taken.

The second aim of this study was to compare the MRI findings with the rhinoscopy findings. The only feature analysed was the turbinate destruction seen on MRI, which was compared with the rhinoscopy findings. This association was statistically significant, the result showed that dogs with no turbinate destruction or mild destruction seen on MRI were associated to normal or mucosal inflammation seen on rhinoscopy, and on the contrary, that dogs with moderate to severe destruction seen on MRI were associated to turbinate destruction and/or

fungal plaques seen during rhinoscopy. Whether the rhinoscopy results were associated with other MRI features was not assessed because case numbers were not sufficient to give significant results.

The third aim of this study was to determine whether MRI features were associated with the histopathology findings. The most relevant histopathologic findings were cross-tabulated with turbinate intensity on T_1W and T_2W. Mucus intensity on T_1W and on T_2W was not cross-tabled with the histopathology findings because mucus was hyperintense in all the cases. Turbinate intensity, on both sequences, was shown not to be statistically associated with mucosal inflammation grade, mucosa oedema or epithelial cell hyperplasia. This means that turbinate intensity is not influenced by these histopathologic findings, or maybe because histopathology samples were not taken from the brightest area seen on MRI, which was the chosen area to be evaluated. Further studies should be done, concerning this association, with bigger populations, in order to confirm these findings

The final inflammation grade was also cross-tabulated with the turbinate destruction seen on MRI in two different ways; both of them were shown to be statistically significant. It was noted that most of the cases with severe inflammatory mucosal infiltration were seen on MRI as mild turbinate destruction, while moderate inflammatory infiltration was more commonly seen in cases where no turbinate destruction was seen. This proved that even though turbinate destruction was statistically associated, severe turbinate destruction seen on MRI does not necessarily indicate severe inflammation degree on the biopsies taken during rhinoscopy.

The forth aim of this study was to purpose a new histologic inflammation score in dogs with inflammatory nasal disease and determine the statistical association between this score and the MRI findings. This new histologic inflammation score included the name of the two most common cell type found, acuteness of the disease (subacute, acute or chronic), diffusion (diffuse or localized), the level of epithelial and goblet cell hyperplasia and the presence of oedema and fungus. This new classification was done for most of the cases and has proved to be descriptive, giving more information to the clinician than other classifications. The association, between this classification and MRI findings, was not done, because the cases, in this study, were too few to draw conclusive results. Further studies, with more cases, should be done in order to determine the statistical association between this histologic inflammation score and MRI findings.

Since the association with MRI findings was not done, a new association was made in order to see the association between each histological finding and Rhinitis and Aspergillosis. All the cases had plasma cell infiltration, but moderate infiltration was more commonly seen in Rhinitis cases, while in Aspergillosis severe infiltration was the predominant one. Epithelial

cell and goblet hyperplasia were more frequently seen in Rhinitis cases, however no statistical association was found. Most of the cases in both pathologies were classified as subacute, with chronic inflammation the least frequent type. All the cases had diffuse inflammation present throughout the samples. Oedema presence was more commonly seen in Rhinitis cases, however no statistical association was seen. Fungal presence results were statistically associated with Aspergillosis, however not all the Aspergillosis cases had fungus present throughout the samples. Possible explanations for this are the superficial nature (no mucosal invasion) of fungal hyphae or the samples taken during rhinoscopy were not representative of the lesions in the nasal cavity.

The fifth and last aim set in the beginning of this study was to assess the statistical association between serology and culture, and MRI findings and Rhinitis and Aspergillosis. Serology positive results were statistically associated with Aspergillosis, showing that positive results were more frequently seen in dogs with aspergillosis. Nevertheless, Rhinitis cases also had positive results, so the final diagnosis should not only be made based on this serology results. Fungal culture results were negative in most of the cases, the positive results were only found in cases of Aspergillosis. Bacterial culture was negative in most of the cases, the most common bacteria species isolated were coagulase negative *Staphyloccoccus sp.* and *Pasteurella sp*.. In the present study, the negative results may be due to sample collection problems in the case of fungal culture maybe the samples were collected from an unaffected region of the nasal cavity. The cross-tabulation between culture, serology and the MRI findings was not done, because the number of cases was not sufficient to produce significant results.

Most of the aims in this study were achieved, confirming that MRI findings are important in the diagnosis of the different inflammatory nasal diseases such as LPR and aspergillosis. Clinicians should consider an MRI scan for all dogs with inflammatory nasal disease, but its final interpretation should always consider other ancillary diagnostic tests like rhinoscopy, histopathology, serology and culture. Since nowadays, more clinicians are showing an interest in this particular field; it is advised that further studies are done in order to evaluate more MRI findings in order to permit a more accurate diagnosis.

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