MYCOTIC RHINOSINUSITIS IN DOGS

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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1. CHAPTER ONE: BACKGROUND AND REVIEW OF THE LITERATURE

1.1. Pathogenesis

1.1.1. Environmental contamination and distribution

Fungal infections of the nasal cavity are relatively uncommon in dogs and neoplasia is considered a much more frequent cause of nasal disease.\textsuperscript{1-4} A variety of infections have been reported in the veterinary literature with \textit{Aspergillus spp.}, particularly \textit{Aspergillus fumigatus}, the most commonly identified.\textsuperscript{3} These ubiquitous soil saprophytes play an important role in recycling environmental debris and sporulate abundantly producing vast quantities of conidia.\textsuperscript{5} Elaborate mechanisms to release conidia are absent and dissemination is reliant upon environmental disruption and air currents.\textsuperscript{5} The conidia released are small enough (2-3 μm) to reach pulmonary alveoli and exposure of humans and animals occurs with subsequent inhalation, but disease does not develop in every individual.\textsuperscript{5}

1.1.2. Innate immunity

The capacity of fungal elements to result in infection depends upon both host immunocompetency and specific virulence factors associated with the fungal organism. Host defence against fungal infection is initially via the innate immune system, initially via physical mucosal barriers.\textsuperscript{6, 7} Inhaled fungal elements are trapped and repelled by the respiratory tract mucociliary defenses thus preventing further access to the host. To stop those that are not repelled from gaining further access, additional innate immune system mechanisms are employed. These comprise of the alternative complement system, key professional phagocytic cells (neutrophils, macrophages and dendritic cells), natural killer and γδ T-cells which work to destroy pathogens either intracellularly or by secretion of compounds extracellularly.\textsuperscript{6, 7} Reduced mucociliary clearance (as occurs with cystic fibrosis), decreased numbers of phagocytic cells (ie granulocytopenia) or a reduction in the
capacity of these cells to destroy organisms all may result in infection. Long term
administration of corticosteroids causes decreased production of reactive oxidant
intermediates important for phagocytic killing, also increasing the risk of infection.
Phagocytic cells also possess surface receptors, including toll-like receptors (TLRs), which
are designed to recognise conserved molecular patterns on microbial and fungal products.  
Not only is the fungal organism that triggers these receptors important, but also the
particular form of that organism, as the resultant signalling pathways and subsequent
outcomes differ significantly. For example stimulation of TLR-4 by Aspergillus spp. conidia
results in macrophage production of pro-inflammatory cytokines such as TNF-α, IL-1α and
IL-1β, whilst hyphae result in IL-10 production via TLR-2 mediated pathways. These factors
are important as the particular pathway initiated may result in counteraction of host
protective mechanisms. Most importantly, by processing and presenting antigen and aiding
production of inflammatory mediators, the innate immune system has a means by which to
instruct the adaptive immune system.

1.1.3. Adaptive Immunity

The aim of the adaptive immune system is to eradicate infection beyond the capabilities of
the innate system and provide long term protection. Phagocytic cells of the innate
immune system, particularly dendritic cells, play an important linking role between the
innate and adaptive immune systems. Not only do these cells capture and process fungal
antigen, but they also express lymphocyte co-stimulatory molecules and migrate to
lymphoid organs where they secrete a variety of cytokines and initiate a variety of other
adaptive immune responses.
The array of cytokines released determines protection or susceptibility to infection by
resulting in differentiation of CD4+ T-lymphocytes to either a T-helper (Th)-1 or Th-2 cell
response. Interferon (IFN)-γ, interleukin (IL)-6, tumour necrosis factor (TNF)-α and IL-12
result in a Th-1 response while a Th-2 response is seen when IL-4 and IL-10 are the
predominant cytokines. In normal circumstances these responses are balanced which is important not only for the elimination of infectious agents, but also to limit autoimmune injury. Immunological studies demonstrate that a Th-1 biased response conveys protection from fungal infection with mild or asymptomatic infection occurring, whilst a Th-2 biased response results in severe or allergic disease. In people, defects within this system are recognised as contributing to susceptibility to invasive fungal infection, however the pathogenesis of less invasive disease is much more poorly understood.

1.1.4. Virulence factors

Despite frequent exposure and similar biological characteristics between different fungal species, fungal infection is infrequent, which likely reflects variability in pathogenicity. The ability of an individual species to cause disease must, in addition to the immune status of the host, depend upon the particular array of virulence factors it possesses. Fungal metabolites and secretory products may convey a survival advantage for the fungal organism by suppressing localised host immune factors or allowing evasion of the immune system. This protection from immune defences may result in a range of disease including localised or disseminated infection as well as allergies. *Aspergillus fumigatus* specifically has been demonstrated to possess a number of virulence factors. Slowed ciliary beat frequency and epithelial damage are seen in human respiratory epithelium during *in vitro* assessment using culture filtrates obtained from clinical isolates of *Aspergillus* spp. A variety of fungal toxins and metabolites have been implicated with gliotoxin in particular recognised as a cause of reduced mucociliary function, although fumagillin and helvolic acid, amongst others, appear to have similar effects. Reduced removal of inhaled particles via mucociliary clearance provides an opportunity for fungal elements to reach epithelial surfaces, resulting in further damage and potentially invasion. This invasion of host tissues may also be enhanced by improved adherence of fungal
elements to host tissues via extracellular matrix and serum proteins including laminin, fibronectin, collagen, fibrinogen and complement component C3.\textsuperscript{9} As well as these factors, fungal metabolites have been shown to interfere with alveolar macrophage and polymorphonuclear cells by impairing phagocytic functions that normally act together to destroy both conidial and hyphal forms.\textsuperscript{9} Gliotoxin reduces adherence and phagocytosis of fungal elements, while alfatoxins not only affect phagocytosis but also intracellular killing and spontaneous superoxide production by macrophages. Complement binding and activation of bound opsonins, processes which normally enhance phagocytosis, are affected too making fungal elements less susceptible.\textsuperscript{9} Localised inhibition of host cell growth and subsequently enhanced fungal growth may also be facilitated by toxins and again gliotoxin is largely implicated.\textsuperscript{9} In addition gliotoxin reduces T-cell proliferation and activation of cytolytic T-lymphocytes, blood monocytes, fibroblasts and other cells. Other toxins such as ribotoxins inhibit protein synthesis and as such are also highly toxic to eukaryotic cell lines.\textsuperscript{9}

1.2. Mycotic rhinosinusitis in people – the parallels

In people, a number of upper and lower respiratory tract conditions resulting from fungal infection are reported and are grouped into those affecting immunocompromised or immunocompetent patients.\textsuperscript{10} Immunocompetent people are typically diagnosed with non-invasive forms such as allergic rhinosinusitis / bronchopulmonary disease or chronic erosive rhinosinusitis.\textsuperscript{11, 12} Granuloma formation, particularly aspergillomas, has also been reported in chronically obstructed paranasal sinuses and in patients with pre-existing pulmonary bullae due to tuberculosis and sarcoidosis.\textsuperscript{5, 11, 13} On the other hand, invasive fungal sinusitis, pulmonary infection and disseminated systemic fungal disease are mainly identified in immunocompromised patients particularly subsequent to organ transplant, due to haematological malignancies or in those with concurrent disease such as diabetes mellitus.\textsuperscript{5, 11, 13}
1.3. Canine mycotic rhinosinusitis

In previous studies of dogs with chronic nasal disease, mycotic rhinosinusitis occurs with a frequency of 7-34%. Aspergillus fumigatus is most frequently isolated, although a variety of other species including A. niger A. nidulans and A. flavus have also been reported. Penicillium spp. and other fungal species are rare causes.

Despite the lack of invasion observed in the dog, marked destruction of the nasal turbinates is almost always identified in dogs with extension of disease into periorbital soft tissue and destruction of the cribriform plate seen in severe cases. Because invasive disease is not seen, destruction is attributed to the inflammatory response of the host as well as dermonecrotic fungal toxins, rather than direct action of the fungal species involved.

Why only a small number of dogs develop mycotic rhinosinusitis is unclear and as is the case in people the pathogenesis of such non-invasive fungal infections is poorly understood. Although susceptibility to invasive fungal infection is often associated with particular defects of innate and adaptive immune mechanisms, these same defects are not usually noted in dogs with disease limited to the nasal cavity. Dogs with sinonasal aspergillosis (SNA) do not appear to have concurrent disease or systemic immunodeficiency, however facial trauma, nasal foreign bodies and dental disease may be contributing factors. Localised immune dysfunction and fungal virulence factors are very likely to contribute to disease, but other factors may also be important.

Development of particular adaptive immune responses has been demonstrated to be important for protection against disease in humans. A dominant Th-1 response appears to convey protection against fungal infection, whilst development of a Th-2 response may be an important reason for progression to more invasive and severe disease. Limited evaluation of adaptive immunity has occurred in the dog. Cytokine profiling in dogs with SNA identifies significant upregulation of mRNA for IL-6, IL-8, IL-10, IL-12, IL-18, IFN-γ, TNF-α
and monocyte chemoattractant proteins (MCP)-1, -2, -3 and -4 compared with normal control dogs. This was considered compatible with a Th-1 cell response.\(^8\),\(^14\) Upregulation of mRNA encoding cytokines and chemokines including IL-8, IL-18 and TNF-\(\alpha\) in dogs with SNA may promote phagocytic killing and recruitment of a variety of immune cells.\(^8\) The local immune response seen in dogs with SNA may therefore be adequate enough to prevent invasive infection and dissemination, but not sufficient enough to prevent inflammation. Up-regulation of mRNA encoding IL-10 and TGF-\(\beta\) is proposed to relate to failure to clear infection, despite significant inflammation.\(^14\) However increased IL-10 may also limit excessive inflammation and thus be beneficial.\(^8\) Why the various monocyte chemoattractant proteins are upregulated is unclear. Additional evaluation of cytokines and chemokines in dogs with SNA is required, particularly before adjunctive immunotherapeutics are developed.

### 1.3.1. Signalment and Clinical Course

Mesocephalic and dolicocephalic dogs are most commonly diagnosed with sinonasal aspergillosis. In contrast to canine systemic aspergillosis where German Shepherd dogs are most commonly diagnosed, no specific breed predisposition is observed and brachycephalic breeds may also be diagnosed with mycotic rhinosinusitis.\(^1\),\(^15\) Affected dogs are generally young to middle-aged with very young or very old dogs occasionally reported and some studies report that male dogs are more frequently affected, however this is not consistently supported.\(^1\),\(^15\),\(^16\)

The clinical course of disease is frequent prolonged and dogs may be presented with nasal signs that have been present for weeks to months or even years. Chronic mucopurulent to purulent nasal discharge, nasal pain and nasal planum ulceration/depigmentation are most commonly reported (Figures 1 & 2). Sneezing, epistaxis, decreased appetite and signs of depression may also be observed, while in severe disease facial deformity, epiphora and seizures may even be identified.
1.3.2. **Diagnostics**

Although the clinical findings and course of disease may increase the suspicion of mycotic rhinosinusitis a combination of diagnostics encompassing diagnostic imaging (computed tomography (CT) or radiography), rhinoscopy / sinuscopy, histopathology, cytology, fungal culture and serology are often required for definitive diagnosis as discussed below.

1.3.2.1. **Diagnostic imaging**

Imaging studies are an important diagnostic tool in investigating chronic nasal disease of any aetiology. The radiographic features of mycotic rhinosinusitis are well described and are detectable in the majority of cases where this method is utilized.\(^1\) Accurate positioning is vital for diagnosis and a variety of radiographic views should be obtained for evaluation including dorsoventral and lateral views of the skull, intraoral radiographic views of the nasal cavities and maxilla as well as a rostro-caudal view of the frontal sinuses. Intra-oral, dorsoventral and rostrocaudal views of the frontal sinuses are considered the most important as these allow the best evaluation of nasal cavity symmetry and frontal sinus involvement.\(^1\)

Turbinate destruction within the nasal cavity is evident as either wide-spread punctuate lucencies or a general increase in radiolucency. Mixed density patterns or an overall increase in opacity may be seen with accumulation of fungal plaques, debris or discharge. Inclusion of rostrocaudal views of the frontal sinuses is particularly important as disease within this area may be otherwise easily overlooked resulting in misdiagnosis or implementation of an inappropriate choice of anti-fungal treatment. Soft tissue density, hyperostosis and punctuate lucencies may also be seen within the frontal bones on these views.\(^1\)
Figure 1 Mucopurulent Nasal Discharge in a dog affected by sinonasal aspergillosis. Image courtesy of Dr Bruce MacKay, Veterinary Specialist Services, Queensland, Australia.

Figure 2 Nasal planum ulceration and depigmentation in a dog affected by sinonasal aspergillosis. Image courtesy of Dr Bruce MacKay, Veterinary Specialist Services, Queensland, Australia.
Improved diagnostic accuracy can be gained by use of computed tomography and magnetic resonance imaging (MRI) compared with radiographs. These techniques allow more extensive evaluation by eliminating the typical superimposition of normal nasal structures that occurs and is problematic in radiographic examination. When computed tomography (CT) is used to detect mycotic rhinosinusitis, improved sensitivity is seen with CT (88-92%) compared with radiographs (72-84%). The typical findings of cavitary destruction of the turbinates (Figure 3), mucosal thickening and thickened and reactive maxillary, vomer and frontal bones are much more evident using CT, particularly when subtle or unilateral changes are present (Figure 4). CT is also more sensitive at detecting cribiform plate changes.

Magnetic resonance imaging (MRI) has been evaluated for the diagnosis of mycotic rhinosinusitis in dogs and is considered more sensitive than CT for soft tissue change, whilst the opposite is true when evaluation of hyperostosis and lysis within the bones surrounding the nasal cavity is required. Despite these differences neither CT or MRI has been shown to be particularly superior to the other for the purpose of diagnosing nasal cavity mycoses.

1.3.2.2. Rhinoscopy and Sinuscopy

Direct visualisation of the nasal cavity is achievable with rhinoscopy and typical abnormalities include turbinate destruction, mucoid nasal discharge, irritation to the mucosal surface and the presence of fungal plaques (Figures 5 & 6). In some cases where significant destruction of the nasal turbinates is present, direct access to the sinuses can be obtained by use of rigid rhinoscopy via the nasal cavity or with flexible endoscopy. In a recent study 8/46 dogs (17%) had disease confined to the frontal sinuses that was only identified with the benefit of sinuscopy. Trephination of the frontal sinuses may therefore be advantageous where nasal cavity disease is minimal and allow confirmation of fungal disease. It also may provide access so that debridement of fungal plaques can be
1.3.2.3. Cytology and Histopathology of nasal specimens

Cytology is generally considered to have poor diagnostic accuracy in nasal disease and as a result is a poorly utilized diagnostic technique. Fungal organisms found on nasal cytology may reflect normal colonization of the nasal cavity or be present secondary to other causes of chronic nasal cavity disease which reduce mucociliary clearance, rather than represent infection. Positive cytological examination results may therefore not, on their own be considered diagnostic for nasal aspergillosis. Comparison of four collection techniques including direct smears of nasal discharge, blind endonasal swabs, mucosal brushings of suspected lesions under endoscopic guidance and squash preparations from nasal biopsies collected under endoscopic guidance showed the greatest accuracy when samples were collected under direct visual guidance. Fungal elements were detected in 14/15 (93.3) of cases using mucosal brushings and in 15/15 (100%) using squash preparations of nasal biopsies. Blind endonasal swabs identified fungal elements in only 3/15 (20%) cases while the poorest detection rates were seen with samples prepared directly from nasal discharge which identified fungal elements in two cases only (13.3%).

Histopathological examination of nasal biopsies is considered a much more accurate method of confirming mycotic rhinosinusitis. In one previous histological study of 15 dogs with SNA, fungal elements were demonstrated in only six cases (40%), although in none of these were hyphae identified within or below the mucosal surface leading to the reasoning that infection is non-invasive in SNA (Figure 7). A more recent study detected fungal hyphae in 8/22 (82%) nasal biopsy samples collected under direct endoscopic guidance.

more easily performed prior to treatment which is likely important for treatment outcome. In addition direct visualisation of the nasal cavity and sinuses via rhinoscopy allows guided sample collection for cytology, histopathology and fungal culture and as such it is regarded as a vital diagnostic tool.
Figure 3 Destruction of the nasal turbinates identified on CT of the nasal cavity. Image courtesy of Murdoch University Veterinary Hospital, Western Australia.

Figure 4 Marked hyperostosis of the frontal bone associated with severe involvement of the right frontal sinus, as identified using CT. Image courtesy Murdoch University Veterinary Hospital, Western Australia.
Figure 5 Severe nasal turbinate destruction and fungal plaques within the nasal cavity of a dog affected by sinonasal aspergillosis. Image courtesy of Dr Aitor Arteaga, University of Sydney Veterinary Teaching Hospital, New South Wales, Australia.

Figure 6 Fungal plaques within the nasal cavity of a dog with sinonasal aspergillosis. Image courtesy of Dr Aitor Arteaga, University of Sydney Veterinary Teaching Hospital, New South Wales, Australia.
Although both of these studies utilized perendoscopic biopsy techniques the variation in detection of fungal elements is interesting. Most likely lower detection results from sampling of areas adjacent to plaques rather than the plaques themselves, but erroneous identification of mucus or secondary mucosal irregularities could also contribute to this lower than expected rate of detection.

Other common histopathological findings include ulceration and inflammation of the mucosal surface with the inflammatory infiltrate identified usually comprising of a mix of neutrophils and mononuclear cells although predominantly lymphoplasmacytic infiltrates may be seen. These histopathological findings described in canine SNA are consistent with those seen in human chronic, erosive, non-invasive mycotic rhinosinusitis.

1.3.2.4. Fungal culture and DNA quantification

Definitive diagnosis of sinonasal aspergillosis by fungal culture of nasal biopsy samples is also considered to lack sensitivity and false positive results have also been recorded in dogs with other primary nasal cavity disease including neoplasia. Failure to record growth is attributed to a failure to collect appropriate samples (blind endonasal swabs, culture of direct smears of nasal discharge) or failure to collect samples directly from fungal plaques. The use of endoscopic guidance for the collection of samples appears to improve sensitivity (75 – 96%) in more recent studies.

Laboratory methodology is also likely to contribute to the disparate and often poor yields reported. Different culture mediums and incubation temperatures influence fungal growth and as these differ between studies directly comparison is often difficult. In a recent study, yield of fungal cultures from dogs with mycotic rhinosinusitis was not only influenced by the type of sample submitted (blind endonasal swabs, blind mucosal biopsies, perendoscopic mucosal biopsies), but was also greatly enhanced at 37°C compared with samples incubated at room temperature. In addition to an enhanced yield the time till positive culture results were obtained was reduced at 37°C. Inability of the fungal elements to
adapt from the host environment has been demonstrated in samples from humans with invasive bronchopulmonary aspergillosis and fungal hyphae recovered from infected tissue appear energy starved and unable to rapidly adapt to laboratory conditions. Providing appropriate conditions, as similar to those in the host from which samples are recovered as possible, is therefore likely to greatly enhance the likelihood of obtaining a positive result where infection is truly present.

Blind endonasal swabs lacked sensitivity regardless of incubation temperature with only 1/16 (6.25%) and 3/16 (18.75%) positive cultures obtained at room temperature and 37°C respectively. None of the samples from normal dogs or those with non-fungal nasal disease produced fungal growth in two recent studies.26, 27 This suggests that the positive predictive value is high and likely to be more specific to nasal infection than previously thought. Therefore it appears important not only to obtain appropriate samples, preferably utilizing endoscopic visualisation of fungal plaques, but also to provide an adequate environment similar to that in the host, such that optimal growth can occur. Where these factors are optimized fungal culture can be considered highly sensitive and specific for the detection of fungal disease.

In humans quantification of fungal DNA by real time Polymerase Chain Reaction (rtPCR) in whole blood or serum has been demonstrated to be useful for diagnosis and monitoring of invasive fungal disease. Recent studies in dogs with sinonasal aspergillosis have found limited benefit in quantifying DNA in tissue samples using an A. fumigatus specific assay due to the degree of overlap between affected dogs, controls and those with lymphocytic plasmacytic rhinitis (LPR) or nasal neoplasia.24 This detection of fungal DNA in the control groups likely represent filtration of airborne fungal organisms or colonisation of the nasal cavity.
Figure 7 Histologic section of a nasal cavity biopsy showing an *Aspergillus spp.* conidial head and many small conidia. Many septate hyphae are also seen (arrow). H&E, scale bar = 10.08 μm. Image courtesy of Murdoch University Veterinary Hospital, Western Australia.
**Figure 8** Indwelling catheters in the frontal sinuses and nasal cavity of a dog receiving topical treatment with enilconazole. Image courtesy of Associate Professor Peter Irwin, Murdoch University, Western Australia.

**Figure 9** Temporary trephination of the frontal sinuses for treatment with either clotrimazole or enilconazole as used in the study included.
Detection of fungal DNA in whole blood was also of little benefit. Rather than this being due solely to poor sensitivity (21%) alone because of low detectable levels however, as expected with non-invasive disease, it was instead also due to poor specificity (45%) secondary to a high frequency of positive results obtained from blood samples in the control groups. Positive predictive value (15%) is poor, but negative predictive values (82%) also indicate that a negative result cannot truly be relied upon to rule out disease.

Extraction of DNA from fungal organisms is difficult and expensive because of the complex and sturdy nature of their cellular walls which further complicates the development of a reliable and commercially viable rtPCR assay for routine use in the dog.

1.3.2.5. Serology

A number of different serological tests have been used to evaluate aspergillosis in dogs including agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA) and counter immunoelectrophoresis, although the latter is not widely available. Serology is widely regarded as a less reliable diagnostic tool however due to variability in sensitivity and specificity of the available tests in previous reports. Two studies of the more commonly utilized AGID test showed it to be a useful diagnostic tool with a sensitivity of 67-76.5% and specificity of 98-100%, although sinonasal aspergillosis could not be ruled out on the basis of a negative test results. Serial monitoring throughout therapy using AGID also has not proved to be a useful indicator of disease status as results of serological testing appear unpredictable. No significant difference has been found when AGID is compared to ELISA for sensitivity (88.2%), specificity (96.8%), PPV (88.2%) or NPV (96.8%). ELISA is considered advantageous however as it offers the advantage of quantification of the immune response, although the benefit of this has not been established in canine disease.

In people with invasive aspergillosis antibody detection is not reliable for diagnosis of disease. Most patients fail to mount an appropriate immune response and therefore have
low or undetectable antibody concentrations, similar to that reported in dogs with invasive aspergillosis.\textsuperscript{29, 31} Diagnosis therefore relies upon detection of fungal antigens and measurement of galactomannan (GM), an \textit{Aspergillus} spp. cell wall component, is much more widely utilized for diagnostic and therapeutic monitoring purposes.\textsuperscript{32, 33} Limited evaluation of GM in dogs with SNA fails to consistently detect elevated levels and this most likely reflects the non-invasive nature of the disease making it a less useful test for this particular presentation.\textsuperscript{28, 29}

1.3.3. Treatment

Despite the similarities seen between human chronic, erosive rhinosinusitis and the disease described in dogs, treatment is significantly different. In people treatment with endoscopic surgery to removal fungal balls and plaques is curative without the need for topical therapy or ongoing medical management with antifungal agents.\textsuperscript{12} In contrast treatment of dogs remains challenging despite a range of available therapeutics. The reason for this is unclear and may be the result of differences in the pathogenesis of disease or reflect the more complex anatomy of the canine nasal cavity which makes endoscopic removal more challenging.

The most widely used antifungal agents in the treatment of canine sino-nasal aspergillosis are the azole group which comprise of the benzimidazoles (thiabendazole), imidazoles (ketoconazole, clotrimazole, enilconazole, miconazole) and the triazoles (fluconazole, itraconazole, posaconazole, voriconazole). These impede ergosterol biosynthesis, an integral component of the fungal membrane and this occurs via the p450 enzyme system by blocking 14α-sterol demethylase resulting in accumulation of lanosterol within fungal membranes.\textsuperscript{34} Topical azoles such as clotrimazole and miconazole also have a direct lytic effect on fungal membranes.\textsuperscript{34} Although selective for fungi, individual azoles vary in their interaction with mammalian cytochrome p450 which can result in hepatotoxicity and cause
a wide-range of drug interactions of varying importance. Degree of protein binding, oral bioavailability and solubility also vary between azoles and these factors frequently dictate the way they are used. For example itraconazole, which is highly protein bound, is less useful for CNS mycoses than fluconazole which is able to cross the blood-brain-barrier due to high water solubility and low protein binding. Oral bioavailability may also influence route of application and azoles such as clotrimazole and enilconazole which have poor oral bioavailability are instead administered topically.

Clotrimazole has been associated with severe, life-threatening side-effects in dogs including oral ulceration and nasopharyngeal swelling severe enough to necessitate temporary tracheostomy placement. This appears to be related to the propylene-glycol base used rather than the clotrimazole itself and when a base of polyethylene glycol is used, these severe effects are not observed.

Other classes of antifungal agents include the allylamines, which inhibit ergosterol synthesis at an earlier step in the pathway than azoles; and the echinocandins, which interfere with β-1,3-glucan synthesis, another important component of fungal cell walls in some species. These groups of antifungals have not been extensively evaluated in the treatment of canines, but may in the future prove useful adjunctive therapies by offering additional mechanisms by which to attack fungal infections.

1.3.3.1. Oral therapy

Generally a poor clinical response is seen when oral azole antifungal agents alone are prescribed. Benzimidazole and imidazole compounds such as thiabendazole and ketoconazole were initially investigated in dogs in the 1980s and although resulted in significant improvement without the need for surgical resection, cure rates of only approximately 50% were achieved. Newer triazole compounds such as fluconazole and itraconazole have also been investigated and appear to give improved success (70%).
The positive clinical response with fluconazole is interesting as its efficacy against *Aspergillus spp.* and other filamentous fungi has been questioned and it is generally regarded as ineffective based upon *in vitro* data. Improved effect is seen *in vitro* against *Aspergillus spp.* when fluconazole is used in combination with the allylamine terbinafine, however this has not been widely investigated and is not a combination commonly prescribed to dogs with mycotic rhinosinusitis. The benefit of agents such as voriconazole or posaconazole over the more commonly used azoles is unknown. Very little, if any, investigation has been performed to evaluate their efficacy in canine disease and these particular agents are likely to be prohibitively expensive.

The limited response seen with oral antifungal agents in dogs is not surprising given that histopathological studies have identified no evidence of fungal organisms invading the mucosa and systemic distribution will be confined to the mucosa itself. Regardless of the particular oral agent prescribed the duration of therapy required to obtain a cure is often markedly prolonged, increasing costs.

### 1.3.3.2. Topical Therapy

Topical therapeutic techniques, which allow direct penetration and therefore direct action on fungal plaques have been developed, however frequently even with these methods multiple treatments are required for a cure. Topical administration of anti-fungal agents is currently the most widely used method of treatment in dogs and a variety of different techniques have been assessed including surgically implanted administration catheters in the frontal sinuses, endoscopic placement of temporary frontal sinus catheters, a non-invasive nasal technique, and trephination of the frontal sinuses with instillation of depot therapy.
1.3.3.2.1. **Indwelling catheters and enilconazole**

The original topical therapeutic technique was described in dogs in 1986 and involved instillation of enilconazole (10mg/kg) twice daily for 7-14 days, via catheters surgically implanted into the nasal cavity (Figure 8). In the initial study five dogs were treated and this therapy was 100% successful with resolution of nasal signs in all cases.\(^\text{39}\) In a subsequent study of 24 dogs, cures were reported in 83% of cases, 50% based on reassessment with radiography, rhinoscopy and mycological examination and 33% based upon clinical scoring alone.\(^\text{42}\)

Although this treatment protocol was considered successful, the prolonged duration of hospitalisation and degree of morbidity led to its declining popularity. Complications such as catheter dislodgement, inappetance, profuse pytalism and aspiration pneumonia have been reported and dogs also become intolerant of the daily administrations requiring sedation for twice daily treatments. In addition, dogs with disease extension into the periorbital regions have been reported as refractory to treatment presumably due to inadequate penetration of antifungal agents to this region.\(^\text{42}\)

1.3.3.2.2. **Temporary trephination and non-invasive Infusion**

Alternative, less invasive techniques were developed to avoid the prolonged period of hospitalisation and potential complications associated with indwelling catheters. The first of these involved administration of clotrimazole by trephination of the frontal sinuses for placement of temporary catheters (Figure 9).\(^\text{43}\) This was well tolerated with only mild side effects and an excellent outcome observed in all dogs\(^\text{43}\) and further evaluation of this technique gave a first and overall treatment success of 62% and 83.7% respectively.\(^\text{44}\)

Friend et al. (2002) also investigated whether a routine second treatment greatly enhanced overall treatment outcome and found no significant difference compared with dogs that received only one.\(^\text{45}\)
In an experimental study of normal dogs, a non-invasive technique was found to have superior distribution of coloured dyes to the frontal sinuses and it was concluded that this technique would likely result in improved clinical outcomes. Development of non-invasive protocols eliminated the need for surgical trephination of the sinuses and the simplest technique involved blind placement of catheters into the nasal cavity. As such minor side-effects seen with the trephination technique including emphysema and the potential for incision site infection were avoided. Both enilconazole and clotrimazole have been evaluated and at varying concentrations with outcomes varying from 46.6 – 69.5% for first treatment outcome and 89.5 – 94.4% for overall treatment success, albeit in small numbers of dogs in some of these studies.

Although experimental models demonstrate improved distribution to the frontal sinuses using a non-invasive technique, only one previous study has evaluated distribution in clinical disease. In this study six dogs treated with 1% clotrimazole (mixed with ioxaglate to a 20% solution) using a non-invasive technique were assessed with CT at completion of a 1 hour infusion. Evaluation of post-treatment CT images established that good distribution throughout the nasal cavity and frontal sinuses was achievable, although the volume of antifungal agent within the sinuses was variable. Frontal sinus disease was only identified in a small number of cases included in this study, however in one case was felt to diminish the volume of infusate that was able to enter this region. In other cases caudal nasal cavity disease or frontal sinus involvement did not appear to impede distribution. Although this study demonstrated distribution to the frontal sinuses subsequent studies identified high first treatment failures in larger numbers of dogs. This high requirement for further therapy was theorised to be the result of inadequate distribution to the frontal sinuses in dogs with significant disease in this location and led to modification of the technique. Modification primarily involved the use of flexible endoscopy to place additional catheters within the frontal sinuses in order to improve
distribution. Although improved distribution has not been confirmed with imaging studies, evaluation of this modified technique appears to improve first treatment outcome, albeit in small numbers of dogs. ¹⁵,⁴⁸

Despite increased invasiveness techniques utilizing a trephination approach do have the additional advantage of offering an opportunity to significantly debride disease within the frontal sinuses and ensure patency of the nasal ostium which are likely key factors that improve distribution and therefore influence outcome. No studies have been performed to evaluate distribution when a trephination technique is used compared to that seen with a non-invasive soak in clinical cases.

1.3.3.2.3. Short infusion and depot therapy

Although reasonable success is achieved with these techniques, both the non-invasive and temporary trephination techniques require a long duration of anaesthesia. This long duration of infusion is felt to be necessary in order to achieve adequate distribution of antifungal agent throughout the nasal cavity and sufficient contact time.

In order to improve retention and contact time Sisener et al. (2006) described a technique which encompassed trephination of the frontal sinuses, a short clotrimazole (1%) soak of the sinuses and nasal cavity, followed by application of clotrimazole (1%) cream to the frontal sinuses (10-20g per sinus).⁵⁰ The application of this more viscous cream within the frontal sinuses was postulated to improve retention of antifungal agents in this region thus improving contact time and success of treatment. In addition clotrimazole cream slowly moved from the sinuses into the nasal cavity theoretically allowing improved contact in this region also. This protocol greatly reduced treatment duration and hospitalisation and was also associated with a good success rate with 12/14 dogs (86%) achieving clinical cures.⁵⁰

In an experimental study, retention of commercially available cream in vitro using glass funnels was poor compared to compounded 1% clotrimazole gels made from
hydroxypropyl cellulose, poloxamer and carboxymethylcellulose in various concentrations. Only the compounded gels were evaluated in vivo.\textsuperscript{51} Although it is logical that improved retention and therefore contact may improve outcomes, ideal timeframes have not been established and the degree to which commonly utilized antifungal agents are distributed and retained has also not been extensively evaluated.

\textbf{1.3.3.2.4. Other topical therapies}

A number of other topical protocols have also been trialled in small numbers of dogs. Povidone-iodine (10\%) was painted onto turbinates in five cases following excision of diseased tissue, three times daily for 6-8 weeks and was successful in all cases.\textsuperscript{52} Application of povidone-iodine impregnated cadexomer dressings (Iodoflex; Smith and Nephew), following rhinotomy and debridement, was successful in three other reported cases.\textsuperscript{52, 53} Dressings were changed approximately every three days over a 15-21 day period until all bone surfaces were covered with healing granulation tissue. Combination treatment with surgical rhinotomy, followed by clotrimazole soak and application of clotrimazole cream has also been evaluated.\textsuperscript{54} Three dogs initially treated with this protocol all had recurrence of disease identified although this was 15 months after therapy in two cases.\textsuperscript{54} Recurrence in these dogs was associated with the bone flap and associated cerclage wires in two of these. Four further cases were treated and the bone flap discarded and three of these were successful without the need for further therapy.\textsuperscript{54} While these protocols resulted in good success in a small number of dogs it is more likely that the ability to more easily and extensively debride disease improved success rates, rather than the type or way in which the topical agent was applied. These methods are also markedly invasive and therefore unlikely to be useful as routine therapies.
1.3.3.3. Predicting treatment outcome.

Treatment failure is often attributed to the severity of disease and development of specific criteria designed to assess severity including clinical, rhinoscopic and radiographic scoring systems have been established in order to evaluate their influence on outcome. Initial evaluation of a CT scoring system to predict first treatment outcome, using a 1 hour clotrimazole (1%) infusion, found reasonable sensitivity (71-78%) and specificity (79-93%). These results were contradicted in a subsequent study that used the same scoring system. This second study, using a 1 hour enilconazole (1%) infusion, found that although a high sensitivity was achievable (100%), the specificity was low (30%). CT was therefore concluded not to be useful to predict therapeutic success although it is possible that variation in the application of the scoring system between different investigators, or the use of a different antifungal agent may have contributed. Given that overall treatment outcomes were similar between the studies the latter is less likely. Inadequate distribution and retention are also proposed reasons for failure. Experimental models show improved distribution in clinically normal dogs using a non-invasive technique. One study has evaluated distribution in clinical disease using a non-invasive treatment technique and no comparisons with other techniques were made. Theoretically, improved retention of antifungal agents should improve treatment outcome by increasing contact time and penetration of fungal plaques. However ideal retention times have not been developed and whether retention of agents within the frontal sinus and nasal cavity of days or weeks are better than hours is unknown.

The inability to predict first treatment success rate despite comparable treatment protocols is frustrating, but is likely to be multifactorial in origin. As well as severity of disease, minor modification of technique, the particular antifungal agent used, the experience of the treating clinician and the ability and extent to which debridement is performed are potential factors.

2.1. Introduction.

Mycotic rhinosinusitis is an uncommon cause of chronic nasal disease in dogs with a frequency of 7-34% reported.\textsuperscript{1-5} Environmental dissemination of ubiquitous soil saprophytes such as \textit{Aspergillus} spp. via air currents allows exposure of humans and animals through inhalation. These fungal elements are usually eliminated by a range of host defence mechanisms and disease does not occur.\textsuperscript{5} It is unclear why only small numbers of dogs develop mycotic rhinosinusitis. Facial trauma, nasal foreign bodies and dental disease have been implicated in some cases\textsuperscript{1,3} and localised immunodeficiency has also been proposed to be a predisposing factor, with no evidence of systemic immunodeficiency existing.\textsuperscript{3,13}

Marked destruction of the nasal turbinates occurs and disease may extend into the periorbital structures and cranial vault causing structural deformity and neurological signs. Turbinate destruction occurs in the absence of tissue invasion as a result of host inflammatory response in conjunction with dermonecrolytic fungal toxins.\textsuperscript{3,13,22} \textit{Aspergillus fumigatus} is the most frequently isolated organism, although a variety of species including \textit{A. niger}, \textit{A. nidulans} and \textit{A. flavus} have also been implicated.\textsuperscript{3} \textit{Penicillium} spp. and other fungal organisms such as \textit{Scedosporium} are rare causes.\textsuperscript{37,55-58}

Treatment of canine mycotic rhinosinusitis remains challenging. Oral administration of antifungal agents such as thiabendazole and ketoconazole result in cure rates of approximately 50%,\textsuperscript{37,38,59} while sole treatment with itraconazole and fluconazole have reported cure rates of 70%.\textsuperscript{3,40} Topical therapy is considered to provide better clinical outcome via direct effects on fungal plaques.\textsuperscript{22} A number of topical treatment techniques have been described and are reported to be associated with greater success rates than oral
treatment, but multiple treatments are often required to achieve cures with these methodologies (Table 1). 15, 39, 42, 44, 45, 47, 48, 50

This study retrospectively reviewed the treatment response of mycotic rhinosinusitis to commonly utilized techniques in multiple veterinary referral centres.

2.2. Materials and Methods

Medical records of dogs treated for mycotic rhinosinusitis were obtained via a manual search of clinical databases from six veterinary referral centres [Murdoch University Veterinary Hospital (MUVH), Veterinary Specialist Services (VSS), Veterinary Specialist Centre (VSC), Queensland Veterinary Specialists (QVS), University of Sydney Veterinary Centre (UVSC) and Adelaide Veterinary Specialist and Referral Centre (AVSRC)] for the time period January 1998 to June 2008.

Dogs with complete medical records and a confirmed diagnosis of mycotic rhinosinusitis that was treated with topical or systemic antifungal therapy were included. Mycotic rhinosinusitis was considered confirmed when more than two ancillary diagnostic tests were positive (diagnostic imaging, rhinoscopy, mycology, serology, cytology or histopathology).

The following information was recorded for each case: age; gender; breed; body weight; clinical signs; duration of clinical signs; whether unilateral or bilateral nasal disease was present; diagnostic imaging findings; rhinoscopy findings; histopathological findings; fungal culture results; serology results; presence of a nasal foreign body; frontal sinus involvement; type of treatment administered, duration of therapy and treatment outcome. Treatment success was defined as complete resolution of nasal disease as determined by physical examination and clinical history for at least a 6-month period following treatment. Any dog that had recurrent signs of nasal disease in which fungal organisms were not identified and therapy for lymphoplasmacytic rhinitis or secondary bacterial rhinitis was
successful were classified as treatment successes. Treatment failure was defined as any
dog with continued signs of nasal disease where mycotic rhinosinusitis was confirmed or
could not be ruled out.

Data was analysed using the Statistical Package for Social Scientists (SPSS Version 16.0, Inc.
Chicago, Illinois). Chi square tests for independence or Fisher’s exact tests were used to
determine the significance of first treatment success and categorical variables. Odds ratios
and their 95% confidence intervals were also calculated. For continuous variables the
homogeneity of variances was initially assessed. For those with significantly different
variances a Mann-Whitney test was conducted and where variances were similar an ANOVA
was calculated and means compared. Significance was defined as $p < 0.05$.

2.3. Results

116 dogs were initially identified in which a diagnosis of mycotic rhinosinusitis and/or
treatment with anti-fungal agents had been administered and these were reviewed. Thirty-
one dogs were excluded from analysis as they did not match the inclusion criteria. The
remaining 85 cases were included in the assessment of treatment outcome. The most
common breeds were Rottweilers (n=10), Golden Retrievers (n=7), Australian Cattle Dogs
(n=6), Staffordshire Bull Terriers (n=6), Miniature Schnauzers (n=5), Bull Terriers (n=4),
German Shepherds (n=4), Malamutes (n=4), Labradors (n=3) and Australian Kelpies (n=2).
Twenty-two were crossbred dogs and 12 other breeds were represented all of which were
mesocephalic or dolicocephalic breeds. Twenty-six (30.6%) dogs were neutered females
and 59 (69.4%) were male (13 entire). The mean age at presentation was 5.57 years
(median 4.5 years; Range 1 to 14 years). Mean bodyweight was 29.2kg (range 6.6 to
70.4kg). Twenty-one cases were included from VSS, 23 from VSC, 16 from MUVH, 10 from
UVSC, 8 from QVS and 7 from AVSRC.
2.3.1. Treatment

Ten dogs were treated with a short five minute clotrimazole soak (1% Clotrimazole in polyethylene glycol) followed by administration of clotrimazole cream (Clotrimazole 1% cream; Canesten®, Bayer) as a depot therapy into the trephined frontal sinuses. Seven (70%) of these dogs were considered cured after first treatment.

Forty-five dogs were treated with a 1-hour non-invasive soak, via tubes inserted into the external nares, with clotrimazole (Clotrimazole 1% solution as Canesten®, Bayer; Clotrimazole 1% solution compounded in polyethylene glycol), as described by Matthews. Eighteen (40.0%) of these dogs were considered cured after their first treatment.

Frontal sinus trephination for temporary catheter placement and a 1-hour soak was performed in 24 dogs given clotrimazole (Clotrimazole 1% solution as Canesten®, Bayer; Clotrimazole 1% solution compounded in polyethylene glycol) and in two dogs given enilconazole (Imaverol®; Austrichter). Thirteen (50%) of these dogs were considered treatment successes after the first treatment.

There was no statistical difference in first treatment outcome between these treatment groups (p=0.21) (Table 2). Four dogs received modified treatments protocols or other topical treatments and outcome was not compared due to low numbers. Dogs requiring multiple treatments to obtain a cure received a range of topical treatments often different from the initial protocol.

When all topical treatment cases were considered together (n=85) 39 (45.8%) were successful after the first treatment and 59 (69.4%) were successful following multiple treatments (Table 2). The number of topical treatments given ranged from 1 to 6 (mean 1.5; median 1). Eleven (12.9%) dogs were euthanased or died due to failure of treatment or due to progressive neurological signs. Fifteen (17.6%) dogs that failed first treatment were lost to follow-up following multiple treatments or based upon their available follow-up were categorised as treatment failures.
Analysis of age, sex and body weight data indicated that treatment success was associated with a younger age (56.3 months vs. 75.8 months; p = 0.02), with no difference in the distribution of sex (Females = 28.2% failure group (13/46), vs. 33.3% success group (13/38); p = 0.352) or body weight (28.9kg failures vs. 29kg successes; p = 0.96) of dogs between treatment outcome groups.

Successful first treatment was 2.7 times more likely in dogs with unilateral nasal discharge compared to those with bilateral discharge but this was not significant (p=0.07). Although prior duration of clinical signs was longer in the failure group, this also was not significant (Failures - median = 15.3 weeks, mean = 8 weeks; Successes - median = 8.85 weeks, mean = 6 weeks; p = 0.11).

Adjunctive treatment with oral systemic antifungal agents was associated with treatment failure (41.3% (19/46) of treatment failures received systemic antifungals vs. 10.2% (4/39) successes; p = < 0.01) and was implemented at the time of diagnosis in 18/23 cases. Dogs in the treatment failure group received a longer course of adjunctive systemic antifungal therapy than those in the treatment success group (median = 19.5 weeks (mean = 8.5) failures vs. Median = 4.87 weeks (mean = 4) successes). One dog in the treatment failure group was still receiving fluconazole for *Curvularia* spp. infection at the time of data collection, thirteen months after diagnosis.

Treatment outcome was not associated with frontal sinus involvement (p = 0.76), the presence of a nasal foreign body (p = 0.37), results of serological testing (p = 0.59), fungal culture (p = 0.62) or histopathological demonstration of fungal elements (p = 0.92). Although considered important for treatment outcome, the extent of debridement of fungal disease in each individual case was not able to be assessed retrospectively.
2.4. Discussion

Young to middle-aged, mesocephalic and dolicocephalic dogs were diagnosed with mycotic rhinosinusitis in this study, similar to that which has been reported previously in other studies. A distinct male predisposition has also been reported, and this was observed in the overall population of dogs diagnosed in this study (69.6%) but cannot be conclusively proven due to the different hospital populations studied.

Although previous studies have reported encouraging first treatment success in 69-86% of dogs, the reports often only include small case numbers. No significant difference in first treatment outcome between treatment methodology was observed in the current study. Two or more treatments were required in 54.2% of cases, and two dogs required six treatments before a cure was obtained. Treatment success following multiple treatments in the current study was 69.4%, less than previously reported. Given the multi-institutional design of the current report the authors feel this is likely a more realistic indication of clinical outcome. This may be an overestimation of treatment success given that recurrence beyond six months has been recorded. Conversely treatment success could also be underestimated in this study as some dogs with persistent nasal signs may have actually been free of fungal infection. The authors would suggest however that the severity and persistence of the signs reported in these cases would constitute treatment failure in the opinion of the owner regardless of the presence or absence of fungal infection was confirmed.

The lack of treatment response at first attempt is likely to be multifactorial in origin, making it difficult to predict which dogs are likely to have a superior prognosis regardless of the treatment type chosen. Despite comparable treatment protocols the rate of first treatment success varies in previous reports (Table 1). Minor modification of technique alone between these studies is less likely to contribute significantly, than the experience of the treating clinician and the degree to which fungal plaques are debrided may.
In the current study failure was significantly associated with age, but was not associated with a longer duration of clinical signs. Intuitively it would be reasonable to assume dogs failing treatment had more extensive disease. Although the retrospective nature limits conclusive assessment of duration of disease, the presence of large amounts of fungal plaques and frontal sinus involvement failed to have a statistical impact on treatment success or failure. However, bilateral changes identified on rhinoscopy and prescription of adjunctive systemic antifungal therapy, both of which might suggest clinically worse disease, were more frequently recorded in the failure group. Objective stratification of disease severity was not possible as not all dogs had rhinoscopic or CT images available for review. CT severity scoring may not have been helpful in assessing response, as studies assessing the value of CT in predicting outcome have produced conflicting results.\textsuperscript{44, 47}

Outcome may also be influenced by the distribution and retention of antifungal agents used. It has been suggested that more effective penetration of affected areas may be achieved by physical debridement of fungal plaques to reduce extent of disease.\textsuperscript{36} Variation in ability to perform this rhinoscopically and the degree to which debridement is performed is a very likely explanation for the differences observed. The retrospective nature of the current report makes it difficult to assess the degree to which debridement of fungal plaques occurred in each of the current cases, but this remains a potential factor. There was no difference in outcome between institutions, making specific techniques less likely contributors to overall success rates. In one study however meticulous removal of fungal plaques did not significantly improve outcome, regardless of the treatment type utilized.\textsuperscript{48} Distribution and retention of antifungal agents may also be influenced by the viscosity of the drug and this has previously been demonstrated in experimental models.\textsuperscript{46, 51}

Whether longer retention times are required for improved efficacy is not clear although reasonable distribution of agents is likely to be imperative for successful outcome. Two described techniques of deposition of clotrimazole cream and administration of 2%
enilconazole via endoscopically inserted catheters originally have reported high first treatment success rates, albeit in small numbers of dogs, compared to less viscous agents, but this rate of success was not perpetuated in this study.\textsuperscript{15, 50}

Where local penetration of fungal plaques is adequate, antifungal drug resistance could also contribute to treatment failure, but this has not been documented apart from the poor activity of fluconazole against \textit{Aspergillus} spp.\textsuperscript{34} While \textit{in vitro} susceptibility testing can easily be performed for fungal isolates, controversy remains as to what this means \textit{in vivo}.\textsuperscript{34, 60} Pharmacokinetic studies of systemically administered antifungal agents are lacking in domestic animals, however given that mycotic rhinosinusitis is considered a non-invasive disease for which topical drug administration is preferred, this is likely to be irrelevant.\textsuperscript{22} Newer antifungal agents such as posaconazole or voriconazole may prove to be more efficacious however both are administered orally and have not been evaluated in this disease to date.

While every effort was made to include only dogs that satisfied sufficient criteria for a diagnosis of mycotic rhinosinusitis, it is not possible to completely rule out the probability that some of the dogs for which there was treatment failure were mis-diagnosed or had underlying or pre-existing disease.

In conclusion mycotic rhinosinusitis is an uncommon cause of chronic nasal disease that is usually the result of infection with \textit{Aspergillus} spp. Although previous studies may report promising first treatment results the inclusion of low case numbers is likely to be a confounding factor. First and overall treatment successes in this study were less than previously reported, particularly when compared to controlled prospective studies and over half of the dogs in this study required more than one treatment in order to achieve a cure. Reasons for treatment failure are likely numerous and multifactorial. Although difficult to prove definitively, duration and severity of disease, inadequate debridement of fungal plaques and poor distribution of anti-fungal agents are likely to be major factors.
Table 1. First and overall treatment success rates in previously published studies of mycotic rhinosinusitis.

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Number of Dogs</th>
<th>First Treatment Success (%)</th>
<th>Overall Treatment Success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indwelling Catheters - Enilconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Sullivan &amp; Sharp (1986)</td>
<td>5</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>○ Enilconazole (10mg/kg) + Ketoconazole (10mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Sharp (1993)</td>
<td>7 *</td>
<td>85.7</td>
<td>100</td>
</tr>
<tr>
<td>○ Enilconazole (10mg/kg) + Ketoconazole (10mg/kg)</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>○ Enilconazole (10mg/kg) only</td>
<td>24</td>
<td>50 (83) **</td>
<td></td>
</tr>
<tr>
<td>Temporary Trephination –1% Clotrimazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Matthews and others (1998)</td>
<td>37</td>
<td>62</td>
<td>83.7</td>
</tr>
<tr>
<td>• Friend and others (2002)</td>
<td>23</td>
<td>76.9 ***</td>
<td>86.9</td>
</tr>
<tr>
<td>Non-invasive catheter soaks – 1% Clotrimazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Matthews and others (1998)</td>
<td>23</td>
<td>69.5</td>
<td>91.3</td>
</tr>
<tr>
<td>Non-invasive catheter soaks – 1% Enilconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Zonderland and others (2002)</td>
<td>19</td>
<td>47.3</td>
<td>89.5</td>
</tr>
<tr>
<td>• Saunders and others (2003)</td>
<td>36</td>
<td>55.5</td>
<td>94.4</td>
</tr>
<tr>
<td>• Schuller &amp; Clercx (2007)</td>
<td>15</td>
<td>46.6</td>
<td>93.3</td>
</tr>
<tr>
<td>Endoscopically inserted Catheters 2% Enilconazole</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>• Zonderland and others (2002)</td>
<td>7</td>
<td>85.7</td>
<td>100</td>
</tr>
<tr>
<td>• Schuller &amp; Clercx (2007)</td>
<td>12</td>
<td>58.3</td>
<td>83.3</td>
</tr>
<tr>
<td>Depot Clotrimazole Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Sissener and others (2006)</td>
<td>14</td>
<td>86</td>
<td>86</td>
</tr>
</tbody>
</table>
Includes five dogs from Sullivan & Sharp (1986)

**12 dogs (50%) were considered cured based upon re-examination which included radiologic examination and rhinoscopy. A further eight dogs (33%) were possibly cured based upon the absence of clinical signs (n=7) or the absence of disease at post-mortem following death from unrelated disease (n=1). Two dogs failed treatment and did not receive further therapy.**

***A total of 23 dogs were included in the study. For Group A dogs (n=13) 10 had a successful 1st treatment (76.9%) and 12 dogs were successful following multiple treatments. As Group B dogs routinely received a 2nd treatment regardless of whether disease was determined to be present upon reassessment, first treatment outcome in this group could not be assessed. Twenty dogs (86.9%) were reported as having successful outcomes when all treatments were considered.***
Table 2. First treatment response of mycotic rhinosinusitis to commonly utilized techniques in multiple veterinary referral centres (1998 – 2008)

<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Number of dogs</th>
<th>Referral Centre Case Breakdown</th>
<th>First Treatment Success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depot Clotrimazole Therapy</td>
<td>10</td>
<td>AVSARC = 4 MUVH = 1 UVSC = 5</td>
<td>70%</td>
</tr>
<tr>
<td>Non-invasive catheter soaks – 1% Clotrimazole</td>
<td>45</td>
<td>AVSARC = 3 MUVH = 7 QVS = 6 UVSC = 2 VSC = 18 VSS = 9</td>
<td>40%</td>
</tr>
<tr>
<td>Temporary Trephination –1%</td>
<td>26</td>
<td>MUVH = 8 QVS = 1 VSC = 5 VSS = 12</td>
<td>50%</td>
</tr>
<tr>
<td>Clotrimazole (n=24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enilconzole (n=2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All topical treatments combined</td>
<td>85</td>
<td></td>
<td>43.5%</td>
</tr>
<tr>
<td>Depot Therapy (10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-invasive Soak (45)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Temporary Trephination (26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indwelling Catheters (2)</td>
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<td>Rhinotomy and Soak (1)</td>
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<td>Topical nasal cream (1)</td>
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</table>
3. CHAPTER THREE: DISTRIBUTION OF CLOTRIMAZOLE AND ENILCONAZOLE IN THE FRONTAL SINUSES AND NASAL CAVITY OF DOGS WITH SINONASAL ASPERGILLOSIS AND ASSOCIATED OUTCOME

3.1. Introduction

Treatment of mycotic rhinosinusitis in dogs remains challenging despite a number of existing therapeutic options. Oral administration of antifungal agents is considered suboptimal due to the requirement for prolonged treatment, high cost and poor clinical response.\textsuperscript{38-40} Failure of oral systemic therapy is likely explained by previous histopathologic evaluation which indicates that disease in dogs is non-invasive.\textsuperscript{22} Penetration of fungal plaques is therefore limited. The ability of topical therapy to directly penetrate and act on fungal plaques is presumably the reason for improved outcomes with this modality. A number of different topical treatment protocols have been described; however multiple treatments are frequently required to attain success.\textsuperscript{15, 39, 42-45, 47, 48, 50, 54} Predicting which dogs are likely to have good outcomes with topical treatment is challenging, regardless of the treatment protocol selected.

Intuitively it is reasonable to assume that failure of initial treatment is because of clinically more severe disease, however previous studies have failed to associate treatment failure with disease severity as assessed by clinical, rhinoscopic and CT scoring.\textsuperscript{44, 47, 61} In reality treatment failure is likely multifactorial and influenced by factors other than severity of disease.\textsuperscript{61} Ability to adequately debride plaques, the type and viscosity of antifungal agent chosen and the experience of the treating veterinary surgeon are also likely to influence treatment outcome due to their effects on distribution, retention and penetration of the antifungal agent within affected areas.\textsuperscript{61} An experimental study showed distribution of colored dyes to the frontal sinuses in normal cadavers was greatest with a non-invasive infusion protocol compared with other described techniques.\textsuperscript{46} Computed tomographic evaluation of the distribution of
antifungal agents using non-invasive, intranasal technique in a small number of dogs with
disease has also been described.\textsuperscript{49} Infusate was distributed throughout all areas of the nasal
cavity and sinuses in this study and as such non-invasive treatment was considered a viable
treatment option.\textsuperscript{49} Evaluation of distribution in clinical disease using other described
techniques has not been performed.

A more recent study using normal dogs evaluated retention of, and inflammatory response to,
a variety of compounded gel formulations and commercially available creams.\textsuperscript{51} This study
concluded that compounded poloxamer gels held the most promise for treatment due to their
unique handling characteristics (reverse gelation) and because they induced less localized
inflammatory change. Poloxamer gels were rapidly cleared from the frontal sinuses, however
the impact of this is unknown as ideal retention times for successful treatment of mycotic
rhinosinusitis have not been determined.\textsuperscript{51}

Studies in normal dogs fail to assess the influence that turbinate destruction and nasal ostium
patency have on distribution and retention of antifungal agents. Although distribution
throughout the nasal cavity and frontal sinuses can be achieved with a non-invasive, intranasal
technique, debridement of significant disease within the sinuses cannot be accomplished and
this may impede distribution of the agent and subsequently affect outcome. The purpose of
this prospective study was to describe the distribution and retention of two antifungal agents
using a temporary trephination protocol and to determine whether this was associated with
initial treatment outcome.
3.2. Materials and Methods

3.2.1. Preliminary Study

A preliminary study was performed to optimise the CT protocol used to assess distribution of antifungal agents. The frontal sinuses of two normal dolichocephalic cadavers were trephined and the nasopharynx and nares occluded using foley catheters. Clotrimazole (1% compounded in polyethylene glycol) and enilconazole (100mg/ml as Imaverol®; Austrichter), mixed for five minutes using a laboratory mixer with iohexol to a 2% solution, were administered via catheters in the frontal sinuses to the left and right sides respectively of dog number one. Computed tomography was then performed to assess whether the contrast enhancement of each antifungal agent was sufficient to allow easy differentiation from other nasal structures. The process was repeated on the second cadaver using a 5% iohexol solution which enhanced visualisation of the antifungal agent.

3.2.2. Animals

Between March 2008 and December 2009 nine dogs were diagnosed with mycotic rhinosinusitis and were recruited for this study. The ninth dog was recruited to replace case eight which was euthanased due to neurological and respiratory complications that developed immediately post-treatment. Mycotic rhinosinusitis was confirmed in all cases based upon clinical history, physical examination and compatible computed tomography, rhinoscopy, mycology, cytology and histopathology results. Serology was also performed in a small number of cases. Dogs were excluded if there was insufficient positive diagnostic criteria to confirm disease, obvious extension of disease into the intracranial vault or if systemic antifungal therapy had been administered prior to inclusion. The Murdoch University Animal Ethics Committee approved all animal use according to National Health and Medical Research Council guidelines and signed, informed owner consent was obtained at the time of recruitment.
3.2.3. Rhinoscopic Scoring
Rhinoscopy was performed using a rigid rhinoscope (Karl Storz). Sinuscopy was performed when possible at the time of treatment to assess the degree of fungal infection in dogs with definitive identification or suspicion of disease within the sinuses based upon computed tomographic assessment. At the conclusion of the study rhinoscopy images were reviewed by one investigator (MJS) and a score calculated based upon that previously described (Appendix 1). As sinuscopy was inconsistently performed, scoring of the frontal sinuses was not included.

3.2.4. Computed Tomographic Scoring
Diagnostic CT images were acquired using a dual-slice helical scanner (Siemens Emotion Duo) in 8 dogs and a 6-slice helical scanner (Philips Brilliance 6 Dunlee) in one dog. The acquisition parameters were 3mm overlapping slices (100 kVp and 209 mA) for the Siemens scanner and 2mm overlayer slices (120 kVp 160 mA) for the Philips scanner with the latter reconstructed into 1mm contiguous slices. Both bone (W2000, L 400) and soft tissue (W 350, L 50) reconstructions were performed.
Computed tomographic images for diagnosis and reassessment were assessed retrospectively at the conclusion of the study by a board-equivalent radiologist (ZL). Bone windows were used to assign scores according to the scheme described previously (Appendix 1).

3.2.5. Treatment
Dogs were sequentially allocated to receive either clotrimazole (Clotrimazole 1% solution compounded in polyethylene glycol) or enilconazole (100mg/ml as Imaverol®; Austrichter). Both groups were administered the allocated antifungal agent via trephination of the frontal sinuses similar to that described previously. Dogs were anaesthetised and positioned in sternal recumbency. Suture material (0-PDS) was inserted into the ventral meatus of the least
or unaffected nasal cavity, as assessed by CT, until it was observed to pass into the pharynx. The end of the suture material was retrieved using forceps and secured to a 22-24-F Foley catheter by its distal fenestrations. By gentle traction of the suture material at the nares the catheter was positioned within the caudal nasopharynx. The foley balloon was then inflated for occlusion of the nasopharynx with sterile saline until it was palpable at the junction of the hard and soft palate and was firmly secured. Rolled cotton gauze was then packed around the catheter to prevent aspiration. Following aseptic preparation separate incisions were made over each frontal sinus followed by trephination and sinuscopy where indicated. Debridement of fungal disease was performed in all cases and was achieved via the nasal cavity and frontal sinuses where necessary with a combination of forceps, suction catheters and copious saline irrigation. Once visual debridement was completed, the nares were occluded using 8F Foley catheters and nelaton-Ryles catheters (14-F) were inserted into each frontal sinus. Needles (18g) were positioned adjacent to these catheters to prevent airlocks from limiting filling during infusion. Each antifungal agent (mixed with iohexol to a 5% solution) was administered via the frontal sinus catheters. Infusion was considered complete when agent was noted to leak from the nares as well as the needles inserted adjacent to the frontal sinus catheters. Computed tomography was performed five minutes later to allow assessment of distribution and retention of antifungal agent within the frontal sinus and nasal cavity. Following CT each dog received additional intermittent infusion of their allocated antifungal agent via the frontal sinus catheters every 5-10 minutes for a further hour.

3.2.6. Distribution Scoring

Distribution of antifungal agent within the nasal cavity and frontal sinuses was assessed retrospectively at conclusion of the study by one of the investigators (ZL), who was blinded to treatment group. All distribution CT scans were performed using a dual slice scanner (Siemens
Emotion Duo) using a similar protocol to that for diagnosis. Distribution and retention of contrast enhanced fluid was assessed using a soft-tissue window and scored from 1-4 (<\= 25% filled =1, 26–50% =2, 51–75% =3, 76–100% =4) at the canine tooth, premolar four, cribiform plate both on the left and the right as well as each of the frontal sinuses to a maximum score of 32.

3.2.7. Reassessment

Reassessment was performed at 3-4 weeks for each patient and comprised of clinical history, physical examination, computed tomography, rhinoscopy and where indicated biopsy. Treatment success was defined as complete resolution of nasal disease based on clinical history and physical examination in combination with the absence of identifiable disease on computed tomography and rhinoscopy. Treatment failure was defined as any dog with continued nasal signs and/or identifiable fungal disease on reassessment. Dogs that failed initial treatment received further therapy at the discretion of the treating veterinarian. All owners were contacted following conclusion of the study to obtain follow-up information.

3.2.8. Statistical Analysis

Association between duration of clinical signs, rhinoscopy score, CT score and treatment type with distribution was explored using logistic regression with backwards selection. Association between duration of clinical signs, rhinoscopy score, CT score, treatment type and distribution score with outcome (success/failure) was also explored. Variables were retained in the model if they were significant at p<0.05 based on assessment of the Wald statistic. The odds ratio and 95% confidence interval of the odds ratio were reported for significant variables. PROC LOGISTIC (SAS v 9.1, SAS Institute Cary, NC) was used for the analysis. Based on the outcome achieved with the treatments in this study, a power analysis was performed when there was no significant result. Sample size required to prove a significant
difference in outcome with treatment was estimated with 95% confidence at a power of 80%. Sample size estimation was also performed for the distribution scores in order to prove a difference between treatments with 95% confidence at 80% power. The current findings were used as estimates of the difference.

3.3. Results

3.3.1. Animals

Nine dogs were enrolled including two Rottweilers, two Bull Terriers and one each of the following breeds - Australian Cattle Dog, Poodle, Mastiff X, Kelpie and Labrador (Table 3). No dogs diagnosed with sino-nasal aspergillosis were excluded during the study period. Five dogs were male (3 MN, 3ME) and four dogs were neutered females. Mean age at presentation was 6.25 years (median 4 years; Range 2 to 14.5 years). Mean body weight was 27.8kg (range 7.9 – 40kg). Clinical signs of nasal disease had been present for a mean of 9.7 weeks (median 8; range 2 to 26 weeks). *Aspergillus fumigatus* was cultured from 8/9 dogs and the remaining dog was positive for fungal elements consistent with *Aspergillus spp* on histopathology. Four dogs received treatment with clotrimazole and five dogs received enilconazole.

3.3.2. Rhinoscopic and Computed Tomographic Scores

Rhinoscopic scores (mean 6.77, median 6; range 4-11) and pre-treatment CT scores (mean 9.55, median 11; range 2-15) varied between cases, but were similar overall between the treatment groups (Table 3).

3.3.3. Distribution and Retention

Both antifungal agents were distributed to all regions of the nasal cavity and frontal sinuses (Table 4). Review of the post-infusion CT images (Figure 10 and Table 5) showed excellent filling of the rostral nasal cavity at the level of the canine with fluid in the majority of cases,
although distribution of the contrast enhanced antifungal agent was poor and limited to <25% of the fluid filling in 12/18 regions and 26 – 50% in 3/18. One region scored 51-75% and only 2/18 rostral nasal cavity regions scored >76%. Mid nasal cavity distribution, scored at the level of premolar four, was variable, but consistently higher than the rostral cavity with 6/18 regions scoring <25% filling, 10/18 regions scoring 26-50% and only one region each scoring 51 – 75% and > 76%. Filling in the caudal nasal cavity at the level of the cribriform plate was also poor with 10/18 regions scoring <25% and 5/18 scoring 26 – 50%. One caudal nasal cavity region scored 51 – 75% and 2/18 regions score >76%. A greater proportion of the filling within the nasal cavity appeared to be with non-contrast enhanced fluid, presumably saline, from the lavage performed prior to antifungal infusion.

Retention of antifungal agent in the sinuses was poor and 10/18 sinuses were < 25% filled or empty five minutes after completion of infusion. Four sinuses scored 26 – 50%. Where frontal sinus filling was improved, frontal sinus disease impaired drainage in 2 sinuses that scored 51-75%. One dog with localized rostral right nasal cavity disease scored 51-75% and >76% retention for the right and left sinuses respectively. The total score for each individual dog indicated that overall distribution was generally poor (mean = 13.8, median = 13, range 9 – 21). Three cases had no evidence of leakage of agent into the pharyngeal packing region on CT evaluation, four cases had mild leakage, one case had a moderate leak and in one case severe leakage was observed.

3.3.4. Treatment Outcome

Three dogs were considered treatment successes two of which were treated with clotrimazole and one with enilconazole (Table 3). One dog (case 8) developed seizures and aspiration pneumonia in the immediate post-treatment period although no evidence of intracranial involvement was observed on either the pre-treatment or treatment CT. No gross evidence of
fungal invasion was identified on post-mortem and histopathologic sections identified mild to focally marked, subacute meningioencephalitis with scattered neuronal necrosis only. No fungal elements were identified.

Five dogs failed treatment and went on to receive further therapy. Four of these dogs (Cases 2, 3, 4 and 7) received a second treatment via temporary trephination of the frontal sinuses followed by a short clotrimazole infusion and instillation of clotrimazole cream into the sinuses50 and two (Cases 2 and 7) were considered cured with this treatment. Of the remaining three dogs, Case 3 received a further three treatments with depot therapy and was then treated with indwelling catheters and enilconazole which was complicated with aspiration pneumonia. The dog had a complete recovery and at the time of writing 18 months later remains disease free. Case 4 had a third treatment with depot therapy and subsequently underwent rhinotomy after which mild unilateral nasal signs continued, but repeat rhinoscopy was not permitted. Case 9 had no clinical signs of nasal cavity disease at initial follow up, but rhinoscopy identified fungal plaques in a localised area of the rostral nasal cavity as initial described. This case underwent a second treatment with a non-invasive nasal soak at the request of the owner and in addition clotrimazole cream was infused into the affected region.

There was no significant association between duration of clinical signs, rhinoscopy score, CT score or treatment type with distribution of antifungal agent. There was also no significant association between any of these factors and outcome. Based on the results of 50% success with clotrimazole and 25% success with enilconazole, the power of this comparison with a sample size of 8 is 6%. A sample size of 66 (33/treatment) would be required to prove this difference significant with 95% confidence at 80% power. Based on the distribution scores with an estimated difference in the means of 2.2 and a standard deviation of 3.98 between
treatments, a sample size of 52 (26/treatment) would be needed to prove this difference significant with 95% confidence.

3.3.5. Follow-up

Follow-up ranged from one and a half months to 23 months. One dog (Case 2) considered cured of mycotic infection was diagnosed with lymphoma nine months after the last rhinoscopy. No signs of nasal disease were identified during the follow up period and the dog was euthanased 2 months after the diagnosis of lymphoma. Another dog (Case 4) was diagnosed with cutaneous lupus erythematosus 18 months after the diagnosis of mycotic rhinosinusitis was made. Mild unilateral nasal signs had persisted since rhinotomy, but these signs improved with immunosuppressive therapy implemented for treatment of dermatologic disease. The last dog recruited into the study (Case 9) at the time of writing was free from nasal signs and repeat rhinoscopy was recommended, but not permitted. The dog was subsequently re-homed due to changes in family dynamics and lost to follow-up.

3.4. Discussion

In the current study distribution of both topical agents to all regions was identified using CT following treatment with a technique encompassing trephination of the sinuses. The degree of filling and retention of antifungal agent was variable between individual dogs. A single explanation for the variability in filling is not apparent, but the combination of degree of nasal turbinate destruction, patency of the nasal ostia, leakage into the nasopharynx, presence of obstructive fungal granulomas, viscosity of the antifungal agent and technical skill of the treating veterinary surgeon could potentially contribute.

Extensive nasal turbinate destruction for example may be expected to result in less restricted movement of antifungal agent from the sinuses and throughout the nasal cavity therefore
enhancing distribution, particularly where a trephination technique is used. This did not appear to be the case in this study and some dogs with very severe nasal turbinate destruction had poor filling. Frontal sinus filling at the time of CT was poor and the sinuses were <25% filled or were empty in the majority of dogs (10/18) and were 25-50% in a further 4/18. Overflow of antifungal agent via needles inserted adjacent to the frontal sinus filling catheters was observed in all cases in this study suggesting that complete filling of the sinuses was achieved. The lack of filling seen at CT five minutes after infusion is therefore more likely to represent a lack of retention rather than failure to appropriately fill the sinuses. Widened nasal ostium as a result of disease may contribute to poor sinus retention. Markedly widened nasal ostium, as identified on rhinoscopic examination, are expected to have contributed to very poor retention identified on CT in one dog (Case 2, Figure 2). Poor retention of antifungal agents in the frontal sinus may in turn be expected to improve caudal nasal cavity filling, but this was not seen. Leakage into the caudal nasopharynx may explain this, but this did not appear to affect distribution and the dog with severe leakage achieved the highest distribution scores in the mid and caudal nasal cavity (Case 5, Table 4).

Conversely, fungal granulomas may impair drainage as has been reported in a previous study of distribution in disease. Copious lavage via the frontal sinuses was performed in the current study following visual debridement to confirm patency of the nasal ostium in order to ensure antifungal agent would flow from the sinuses to the nasal cavity. In two cases (3 and 6) with severe unilateral frontal sinus involvement, dwell times within the affected sinuses did appear improved (compared to the contralateral side), despite confirmed patency of the nasal ostia (Figure 1 (d)). This most likely resulted from partial outflow obstruction and subsequent impaired drainage as has been reported previously. Except in these two cases, distribution
did not otherwise appear to correlate with severity of disease as determined by rhinoscopic or CT scores (Table 3).

Retention has additionally been shown to be affected by viscosity in experimental *in vitro* and *in vivo* studies using normal dogs.\textsuperscript{51} Prolonged retention of compounded gels has been identified in experimental studies using CT, with up to 100% of the agent retained at 28 days in some dogs.\textsuperscript{51} Prolonged retention of antifungal agents allows long-term contact with fungal elements. Whether or not this is beneficial in a clinical sense however is unknown and minimal required retention times for successful treatment have not yet been established. Prolonged retention may in fact be detrimental and compromise longterm outcome by trapping viable fungal elements beyond a time-point when the remaining material contains active antifungal agent. Retained agents may also cause marked inflammation and longterm impairment of nasal function resulting in residual rhinosinusitis and recurrent secondary bacterial infections.\textsuperscript{49} Where appropriate debridement of fungal disease is performed, prolonged retention is presumably less important.

Endoscopic debridement alone is often a successful treatment in people with chronic, erosive mycotic rhinosinusitis, a disease with reported similarities to canine sinonasal aspergillosis.\textsuperscript{12} This is also likely to be one of the most important aspects of therapy in dogs, but is technically difficult due to their more complex nasal cavity. Trephination of the frontal sinuses offers the advantage of allowing improved access to any fungal plaques in that region and is therefore warranted in cases where frontal sinuses disease has been identified or is suspected. Degree of debridement, as assessed visually, in this study was considered adequate on visual inspection but was less extensive by CT evaluation. This may have contributed to initial treatment failure and relying on endoscopic assessment of debridement in the dog may not be reliable.
In some regions of the nasal cavity it was interesting to note that fluid filling was 100%, however less than 25% of this was comprised of contrast enhanced filling agent. The non-enhanced fluid seen is presumed to be residual saline from the lavage performed during debridement. Failure to allow adequate drainage post lavage may have impeded filling with the antifungal agent in the current study.

High nasal cavity pressures (15mm H$_2$O) have also been reported to be important in order to obtain adequate distribution, particularly to the frontal sinuses, with a non-invasive technique. Whether similar filling pressures are required with a trephination protocol could not be determined in the current study. High filling pressures are likely to be less important for distribution using a trephination technique given that direct administration of agent to the sinus is accomplished. Retention within the sinuses may however be improved where higher filling pressures are obtained, but simply ensuring that ongoing loss from the nares or nasopharynx is minimised is likely to be sufficient.

Initial treatment failure was high in this study and only three cases (33.3%) were determined to be free of disease following the first treatment. Case numbers are too small to draw specific conclusions for this high rate of failure or statistically compare the two compounds. It is interesting to note however that despite the use of a much higher concentration of enilconazole (10%) than has previously been reported, only one case treated with this antifungal agent had initial treatment success.

None of the scoring systems used in the current study were able to predict which cases were likely to achieve a successful first treatment. Some dogs with moderately elevated CT scores, indicative of worse disease, had successful first treatment outcomes, whilst others with very low scores, due to localised disease, failed. Seven dogs (77.7%) were determined to be free of disease following multiple treatments and one dog (Case 9) had insufficient follow up, after a
second treatment, to determine whether a successful outcome was achieved. Clinical signs alone were not reliable to decide whether or not disease was present. One dog with localised disease confirmed at recheck had no clinical signs, whilst another dog had persistent unilateral nasal signs for 18 months after last treatment, despite being free from fungal infection. Three other dogs had intermittent nasal signs during the follow up period which resolved without further treatment.

Complications in the peri-treatment period were identified in one dog and included generalised ataxia as well as generalised and focal facial seizures. These were deemed severe enough by the owners to warrant euthanasia despite improvement being observed over subsequent days. A definitive cause of the neurological signs was not identified on post-mortem in this case, but may be the result of direct irritation by the antifungal agent used. Enilconazole is generally considered less irritant than clotrimazole however it was administered at a much higher concentration here than has previously been reported. Fungal meningioencephalitis would seem less likely given the non-invasive nature of disease, but cannot be entirely ruled out.

In conclusion, distribution of clotrimazole and enilconazole throughout the nasal cavity was achievable, but variable in dogs with sinonasal aspergillosis treated via temporary trephination. Retention within the frontal sinuses tended to be poor except where significant frontal sinus involvement was present which improved dwell times. Otherwise there was no association between duration of clinical signs, disease severity as determined by rhinoscopy and CT scoring, or treatment type with distribution. Equally there was no association between any of these factors including distribution with treatment outcome, however inclusion of a larger number of dogs would be required for more comprehensive evaluation. Poor retention presumably related to ongoing loss of agent via widened nasal ostia and the nares. Inadequate distribution and retention of antifungal agents is likely to affect treatment outcome. Where
significant frontal sinus involvement is present techniques which allow direct access to this location should be considered preferential to non-invasive protocols because adequate debridement and application of antifungal agents can occur more easily.
Table 3. Diagnostic data, distribution and outcome for each patient.

<table>
<thead>
<tr>
<th>Case</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (months)</th>
<th>Body Weight (kg)</th>
<th>Duration of Clinical Signs Prior to Treatment (weeks)</th>
<th>Mycological isolate</th>
<th>Rhinoscopy Score Pre-Tx (max = 14)</th>
<th>CT Score (max = 24)</th>
<th>Type of Treatment</th>
<th>Distribution Score (max = 32)</th>
<th>First Treatment Outcome</th>
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<td>Success</td>
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<td>7</td>
<td>Enilconazole</td>
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<td>Failure</td>
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<td>38.3</td>
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</tr>
<tr>
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<td>Red Heeler</td>
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<td>26</td>
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<td>15</td>
<td>Enilconazole</td>
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<td>Failure</td>
</tr>
<tr>
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<td>144</td>
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<td>8</td>
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<td>Success</td>
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</tr>
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Table 4. Breakdown of distribution scores and degree of pharyngeal leakage for each patient.

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<th>Canine (right)</th>
<th>Canine (left)</th>
<th>Premolar Four (right)</th>
<th>Premolar Four (left)</th>
<th>Cribriform plate (right)</th>
<th>Cribriform plate (left)</th>
<th>Frontal sinus (right)</th>
<th>Frontal Sinus (left)</th>
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<td>2</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td>2</td>
<td>Enilconazole</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Moderate</td>
</tr>
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<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
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<td>Mild</td>
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<td>Enilconazole</td>
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<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Mild</td>
</tr>
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(a) Canines

(b) Pre-molar Four
Figure 10. Distribution of contrast enhanced clotrimazole in case 3, at the level of the canine (a), pre-molar four (b), cribriform plate (c) and within the frontal sinuses (d). A large amount of non-contrast enhancing material is seen within the left frontal sinus, presumably fungal granulomas, despite visual debridement being considered complete.
Table 5: Individual Case Scores and Images

<table>
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<th>Case</th>
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<th>Age (months)</th>
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a. Level of the Canines

b. Level of Premolar Four
### Case Treatment

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<tr>
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**Images:**

- **a.** Level of the Canines
- **b.** Level of Premolar Four
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a. Level of the Canines

b. Level of Premolar Four
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1. **Level of the Canines**

2. **Level of Premolar Four**

![CT Scan Image 1](#)

![CT Scan Image 2](#)
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**Images:***

- **a. Level of the Canines**
- **b. Level of Premolar Four**
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**Images:**

- ![Image 1](image1.png) - Level of the Canines
- ![Image 2](image2.png) - Level of Premolar Four
<table>
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c. Level of the Cribriform Plate
d. Level of the Frontal Sinuses
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![CT Scan Images](image1.png)

- a. Level of the Canines

![CT Scan Images](image2.png)

- b. Level of Premolar Four
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c. Level of the Cribriform Plate
d. Level of the Frontal Sinuses
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![CT Scan Images](image1.png)

- **a.** Level of the Canines
- **b.** Level of Premolar Four
Case | Treatment Type | Canine (right) | Canine (left) | Premolar Four (right) | Premolar Four (left) | Cribriform plate (right) | Cribriform plate (left) | Frontal sinus (right) | Frontal sinus (left) | Pharyngeal Leakage
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
8 | Enilconazole | 3 | 1 | 2 | 2 | 3 | 2 | 1 | 1 | None

c. Level of the Cribriform Plate

d. Level of the Frontal Sinuses
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**Images**

- **a. Level of the Canines**
- **b. Level of Premolar Four**
<table>
<thead>
<tr>
<th>Case</th>
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<th>Canine (right)</th>
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4. SUMMARY

Mycotic rhinosinusitis is an uncommon, yet debilitating condition in dogs that most resembles non-invasive, chronic rhinosinusitis in humans. Whilst various ubiquitous soil saphrophytes have been reported causes of infection *Aspergillus spp* organisms are most commonly isolated leading to the term sinonasal aspergillosis (SNA). This finding was reflected in the retrospective population of dogs with mycotic rhinosinusitis included here where only a small number of dogs had infection other than *Aspergillus spp.* detected.

Why only a small number of dogs exposed to inhaled fungal elements develop SNA is expected largely to be due to the limitations innate and adaptive immune mechanisms impose upon the organisms ability to invade and induce infection. Mechanisms for failure of these responses have been proposed, however no particular deficits have been detected in dogs with SNA and those affected appear immunocompetant. Limited evaluation of cytokine and chemokine expression has been performed in dogs with SNA, but where this has been evaluated the local immune response appears sufficient to limit infection to the nasal cavity, but inadequate to prevent inflammation or completely eliminate infectious fungal organisms.\(^8\) This may indicate that virulence factors and toxins produced by the infecting fungal organism reduced the ability of the host to mount effective immune responses, but further evaluation is required before robust conclusions can be made. In addition facial trauma, nasal foreign bodies and dental disease, may contribute but are not always present and were rarely identified in either study reported here.\(^1,3,13\)

Of the numerous treatment options described, topical antifungal administration is generally preferred and considered most successful. Previous studies have reported excellent first and overall outcomes for a variety of topical protocols, but multiple treatments are frequently required.\(^15,39,42,44,45,47,48,50\) This study compared first treatment outcome between three commonly used topical treatment protocols in a multi-centre retrospective study and found no significant difference.
First and overall treatment outcome in both of the studies included were also reduced compared with the previous studies. Two or more treatments were required in 54.2% of dogs included in the retrospective analysis whereas previous studies report first treatment success of up to 86%. In addition only 69.4% achieved overall treatment success, also lower than that reported previously. This lower rate of success in a multi-centre setting may reflect a more realistic expectation of outcome particularly in comparison to studies where treatment and re-evaluation were more controlled. In the latter prospective study only 33% of dogs achieved first treatment success. Success following multiple treatments was higher at 77%, with one dog having insufficient follow-up after a second treatment to confirm outcome. As this latter study included only a small number of patients in each treatment group it is difficult for strong conclusions to be made. Attempts have been made to evaluate factors that may help predict first treatment. Most of these utilize subjective scoring systems based upon severity assessed via advanced imaging (CR and MRI) or rhinoscopy. While these systems are useful guides they assume severity alone is most important for outcome. Failure of severity scores to predict outcome absolutely reflects either an inability to assess subtle change that may have a significant impact or that rather more likely that additional factors are also important for outcome.

Factors with the potential to influence outcome were assessed in the retrospective study and only age was significantly associated with success. Factors considered to reflect severity were not associated with outcome although dogs with unilateral disease were 2.7 times more likely to have a successful treatment outcome. Previously described severity scoring systems were unable to be applied to all dogs in this study as not all cases underwent the required procedures or had sufficient detail recorded to allow their application. Interestingly, oral antifungal agent administration was significantly associated with treatment failure and in the majority of cases was prescribed at diagnosis or initial
treatment. These dogs were not able to be definitively established as having worse disease, however prescription of oral antifungal agents may have acted as a surrogate marker for severity based on subjective assessment at the time of diagnosis in these cases. In the prospective study severity as assessed by rhinoscopic or CT scoring systems was not associated with outcome. Focal disease on occasion had a much poorer outcome compared with dogs whose disease resulted in severe turbinate destruction, despite the latter having a higher severity score with currently reported schemes. This may result from more difficult access with the former and in therefore insufficient debridement of disease as adequate reduction in the amount of disease does appear to be a key factor for outcome in humans with chronic erosive rhinosinusitis and may also be important for outcome in dogs. Improved outcome is reported in dogs with some protocols that improve exposure to the nasal cavity (Table 1), which may primarily reflect improved access and therefore debridement. Severe turbinate destruction also likely improves ability to adequately debride disease and in turn adequate debridement limits potential obstruction and therefore improves distribution to key regions of the nasal cavity and/or frontal sinuses. In addition severe turbinate destruction itself may enhance distribution by reducing potential obstruction.

Adequate distribution of appropriate antifungal agents to all affected regions of the nasal cavity and frontal sinuses is important. Aside from regional obstruction secondary to insufficient debridement, and degree of turbinate destruction, distribution and retention of antifungal agents is also probably affected by the particular treatment protocol used, the skill of the treating veterinary surgeon and the viscosity of the agent chosen. Limited evaluation of distribution has been performed in dogs, particularly in those with disease, although experimentally a non-invasive technique appears to provide better distribution to the frontal sinuses of normal dogs.46
We evaluated distribution and retention of two antifungal agents using a trephination technique. Distribution of contrast enhanced antifungal agent to all regions of the nasal cavity and frontal sinuses was achievable using this technique, but filling and retention was quite variable and generally poor. This appeared independent of severity assessed by rhinoscopy and computed tomography. Although only a small number of dogs were included in this study there appeared no association between distribution and the type of antifungal used, despite the difference in viscosity.

It was interesting to note in the prospective study that the nasal cavity of many of the dogs appeared 100% filled with fluid, with a much lower percentage of this filling due to contrast enhanced antifungal agent. Presumably this non-contrast enhancing fluid was saline from the pre-treatment flush. This may have impaired subsequent filling with antifungal agent, reducing distribution and given this finding it could be theorized that flushing is detrimental with the potential to impair outcome and therefore should not be performed. The benefit of ensuring patency of the frontonasal ostia and the assistance that flushing provides to debriding the nasal cavity likely outweigh this. We would therefore suggest on the basis of our findings that providing sufficient time for drainage of the saline should be provided prior to infusion.

We were also able to demonstrate that retention of antifungal agent in the frontal sinuses was poor. Five minutes after initial infusion was completed filling of the sinuses was limited in the majority of cases. We propose that this is due to ongoing losses to the nasal cavity rather than failure to provide adequate filling as overflow from the needles adjacent to the filling catheters was noted in all cases suggesting adequate filling.

In a small number of sinuses, retention was enhanced by residual fungal disease. This was despite debridement being considered complete based on visual assessment. We would therefore propose that reliance on endoscopic visual assessment of debridement in the dog is insufficient.
Outcome of first treatment was poor in the second study, but did not appear to be associated with signalment, severity assessed by rhinoscopy and CT scores, treatment type ( clotrimazole vs. enilconazole) or distribution. Aside from these factors it is likely that other less easily measurable factors are important. For example virulence factors of and toxins produced by small numbers of remaining fungal elements for the infectious organism involved may be sufficient to overcome immune responses, despite seemingly adequate debridement, appropriate treatment and good distribution.

Antifungal resistance may be important. Susceptibility testing to available antifungal agents is not routinely performed in veterinary medicine, but this is less likely to be important in canine SNA as high concentrations of antifungal agents are achieved locally.

In conclusion the studies included here demonstrate the difficulty in predicting initial treatment outcome regardless of subjectively assessable criteria, rhinoscopy or CT severity score, treatment protocol or antifungal agent used. A variety of factors are likely to be important for outcome, some of which aren’t likely to be easily assessed. Although distribution was not associated with outcome in the second study, this included only a small number of cases. Achieving adequate distribution is still likely to be important and may prove significant in larger studies, but itself is likely to be affected by a variety of factors. Flushing of the frontal sinuses and nasal cavity to ensure patency of the frontonasal ostia and provide debridement is important, but adequate drainage should follow this process so that saline retention is reduced and impairment of filling with antifungal agent is limited. Enhanced retention in the frontal sinuses due to remaining disease has equal consequences for reducing distribution regardless of whether a non-invasive nasal technique is used. Therefore when disease is observed or suspected in this location use of a protocol which directly access sinuses is likely to be preferable.
REFERENCES


APPENDIX ONE: Scoring Systems

CT Scoring System

The nasal cavity was classified into 6 anatomic regions:
- Right and left nasal turbinates rostral to the maxillary recess
- Maxillary turbinates at the level of the maxillary recess
- Ethmoid turbinates caudal to the maxillary recess

A score of 0-3 was assigned to each region for each dog depending upon severity of disease:
- 0 = no detectable abnormalities
- 1 = mild turbinate atrophy or fluid accumulation
- 2 = moderate disease
- 3 = severe disease

A total score was then calculated for each dog by summing the scores of the 8 regions to a maximum score of 24.

Rhinoscopic Scoring System

Turbinate destruction was scored for both the left and right nasal cavity
- 0 = absent
- 1 = moderate
- 2 = severe

Destruction of the nasal septum – 0 OR 2

Severity of intranasal or sinusal fungal plaques –
- 0 = absent
- 1 = mild
- 2 = moderate
- 3 = severe

Unilateral or bilateral presence of mucopurulent material
- 0 = absent
- 1 = unilateral
- 2 = bilateral

Damage to the nasal mucosa –
- 0 = absent
- 1 = moderate
- 2 = severe

A total score was then calculated for each dog by summing the scores for each of these factors to a maximum score of 14.
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