DIAGNOSIS OF NASAL ASPERGILLOSIS IN THE DOG

De diagnose van nasale aspergillose bij de hond

J.H. Saunders, H. van Bree

Department of Medical Imaging, Faculty of Veterinary Medicine,
Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ABSTRACT

Canine nasal aspergillosis is a common disease that continues to present a diagnostic and therapeutic challenge. On physical examination, a profuse mucopurulent/hemorrhagic discharge, ulceration of the external nostrils, and facial pain or discomfort are the three most commonly encountered features. Hematology/chemistry is unrewarding. Serology, most commonly an agar gel double diffusion test, is easily performed and it has a very low rate of false positives (0-6%), but it may be falsely negative in the early stage of the disease. Imaging diagnosis of nasal aspergillosis is based on turbinate destruction, mucosal thickening and hyperostosis. Radiography is currently the most commonly used imaging technique, though its diagnostic value and reliability are still controversial. Computed tomography and magnetic resonance are promising emerging techniques. Rhinoscopy permits direct visualization of fungal colonies in 80 to 100% of the dogs and also has a therapeutic role. Culture is very hard to interpret, as 40% of nasal swabs of normal dogs and those with nasal neoplasia will yield *Aspergillus*. Cytology and histology show aspecific features in the absence of visualization of fungal hyphae, which occur in approximately 50% of the dogs. A definite diagnosis of nasal aspergillosis should reasonably be based on at least three positive diagnostic tests, including direct visualization of fungal colonies with rhinoscopy. Differentiation from other causes of chronic nasal disease is mainly based on the imaging findings, rhinoscopy and histology.

SAMENVATTING

Nasale aspergillose is een vaak voorkomende aandoening bij de hond. De diagnose en behandeling ervan blijven echter een uitdaging. Bij klinisch onderzoek zijn een uitgesproken mucopurulente/hemorragische neusvloei, ulceratie van de neusspiegel en fasciale pijn of ongemak de drie meest voorkomende klachten. Hematologie en biochemie zijn niet erg lonend. Serologie, meestal agar gel dubbele diffusietest, is daarentegen gemakkelijk uit te voeren, heeft een zeer lage kans op een vals positief resultaat (0-6%), maar kan een vals negatieve uitslag geven in een vroeg stadium van de aandoening. De beeldvormende diagnose van nasale aspergillose is gebaseerd op de destructie van de turbinalia, een verdikking van de mucosa en op hyperostose. Radiografie is steeds de meest gebruikte, beeldvormende techniek maar de diagnostische waarde en de betrouwbaarheid van radiografie zijn steeds controversieel. Computer tomografie and magnetische resonantie zijn veelbelovende technieken. Rinoscopie heeft, naast een rechtstreekse visualisatie van schimmelkolonies bij 80 tot 100% van de honden, ook nog een therapeutische rol. Culturen van de schimmel zijn erg moeilijk te interpreteren gezien 40% van de neusstalen van normale honden en eenzelfde percentage honden met een nasale tumor positief zijn voor *Aspergillus*. Cytologie en histologie tonen enkel aspecifieke kenmerken bij afwezigheid van zichtbare hypha, en dit bij ongeveer 50% van de honden. Een definitieve diagnose van nasale aspergillose moet gebaseerd zijn op ten minste drie positieve diagnostische testen, waaronder directe visualisatie van de schimmel kolonies met rinoscopie. Een differentiatie met andere oorzaken van chronische neusaandoeningen berust voornamelijk op de bevindingen met beeldvorming, rinoscopie en histologie.

INTRODUCTION

The most common causes of chronic nasal disease (CND) in the dog are nasal neoplasia and nasal aspergillosis (Harvey *et al.*, 1979). Foreign body (FB) rhinitis and non-specific rhinitis (including lymphoplasmocytic, allergic and hyperplastic rhinitis) occur occasionally, whereas nasal polyps, rhinitis secondary to dental disease, traumatic rhinitis, parasitic rhinitis, congenital abnormalities and idiopathic necrosis of the conchae are rarely encountered (Harvey *et al.*,

1979; Gartrell *et al.*, 1995; Schwarz, 1997; Davidson *et al.*, 2000).

Nasal aspergillosis accounts for 12-34% of all nasal disorders in the dog (Lane and Warnock, 1977; Harvey and O'Brien, 1983). The disease is most commonly caused by the fungus *Aspergillus fumigatus,* although *A. niger, A. flavus, A. nigrans and Penicillium sp.* have also been reported (Lane and Warnock, 1977; Gibbs *et al.*, 1979; Harvey *et al.*, 1981; Harvey and O'Brien, 1983; Harvey, 1984a; Sharp *et al.*, 1984; Sharp *et al.*, 1991). All these organisms are ubiquitous saprophytes and are considered opportunistic pathogens (Sharp *et al.*, 1991). In this paper, the term "nasal aspergillosis" was preferred to "fungal rhinitis" in view of the low prevalence of fungal rhinitis due to *Penicillium* species.

The pathogenesis of nasal aspergillosis is still not fully elucidated. Fungal colonization of the nasal cavities occurs following inhalation of spores from the environment (Wolf, 1992). The disease tends to be invasive, causing erosion of the external nares, roughening of the mucosa and marked destruction of the turbinate bones by production of hemolytic and dermonecrotic toxins (Sharp *et al.*, 1991). It has been postulated that nasal aspergillosis may result from continuous foreign body irritation (Suter, 1985; De Foer *et al.*, 1990; Saunders *et al.*, 2000). Foreign bodies are considered to provide a hiding place for the ubiquitous *Aspergillus* spores and to alter the respiratory epithelium by cilia paralysis so that the fungi cannot be removed (Lenglinger *et al.*, 1986; De Foer *et al.*, 1990). Moreover, certain substances such as heavy metals can also be used by *Aspergillus* species in their metabolism (Lenglinger *et al.*, 1986).

Nasal aspergillosis has occasionally been reported in dogs with debilitating conditions such as chronic metabolic diseases, or in those receiving immunosuppressive drugs, but most of the dogs with nasal aspergillosis are otherwise clinically healthy (Soltys and Summer-Smith, 1971; Dawson *et al.*, 1973; Sharp *et al.*, 1984; Sharp *et al.*, 1991; Sharp *et al.*, 1993). Evidence of immunosuppression has been reported, but it seems to be caused by the disease and not by a preexisting abnormality. Indeed, products of *Aspergillus fumigatus* have been shown to inhibit lymphocyte transformation of both B- and T-cells in vitro (Barrett *et al.*, 1977; Harvey and O'Brien, 1983; Chaparas *et al.*, 1986; Sharp, 1990).

The aim of this paper is to review the diagnostic modalities of nasal aspergillosis in the dog. For each modality, the procedure, findings in dogs with aspergillosis and criteria for differentiation from other chronic nasal diseases, particularly nasal neoplasia and non-specific rhinitis, are described.

OCCURRENCE

Nasal aspergillosis affects predominantly mesaticephalic and dolichocephalic breeds (Sharp *et al.*, 1992). A breed predisposition has not been clearly established (Sharp *et al.*, 1987). Male dogs appear to be at greater risk than females (Wolf, 1992).

The age range of dogs affected with nasal aspergillosis is 6 months to 15 years. However, 80% of the infections occur in young to middle-aged dogs (1-7 years), and the mean age is between 4 and 5 years (Norris and Laing, 1985; Sharp *et al.*, 1991; Tasker *et al.*, 1999). This differs from nasal neoplasia, which most often occurs in dogs of more than 8 years of age (Confer and DePaoli, 1978; Norris, 1979; Legendre *et al.*, 1983).

PHYSICAL EXAMINATION

After a general physical examination, the nasal cavities, frontal sinuses and surrounding areas should be carefully inspected. Abnormalities are usually confined to the nasal cavities and sinuses, thus reflecting the localized nature of the upper airway disease. The lateral movement of the nasal alae during inspiration, patency of the nasal cavities (by use of a clean glass, a thin piece of cotton or a thread placed at a nostril opening) and pigmentation of the nares should be evaluated (Norris and Laing, 1985). The type of nasal discharge should be determined (serous, mucoid, mucopurulent, hemorrhagic), as well as its duration, volume, color, odor, and uni- or bilaterality (Gartrell *et al.*, 1995). The eyes should be inspected for epiphora and exophtalmos, and the surrounding soft tissue and osseous shell for areas of pain, deformity, swelling, masses, bony defects and symmetry (Norris and Laing, 1985; Gartrell *et al.*, 1995). Finally, the hard and soft palate, tonsils and dental arcade should also be evaluated (Norris and Laing, 1985).

The main clinical features of nasal aspergillosis recorded in a series of 35 dogs were a profuse nasal discharge (91%), nasal discomfort (85%), ulceration of the external nares (77%), sanguinopurulent discharge (76%), frontal sinus osteomyelitis (65%), epistaxis (63%), bilateral (52%) / unilateral (48%) nasal discharge, mucopurulent discharge (24%), sparse nasal discharge (9%) and chemosis (7%) (Sharp *et al.*, 1991). On the basis of this study, three hallmarks of

nasal aspergillosis were defined: a profuse sanguino-purulent discharge, ulceration of the external nares, and pain or discomfort in the facial region (Bright, 1979; Sharp *et al.*, 1998). In an advanced stage, the general condition of the dog may be altered (Sharp *et al.*, 1984). Extension of the pathological process into the cranial vault and orbit, secondary focal meningo-encephalitis and systemic dissemination are rarely reported complications (Cadwallader *et al.*, 1973; Bright, 1979; Hotston Moore and Hanna, 1995).

The profuse, purulent nature of the discharge in aspergillosis may help to differentiate this condition from nasal neoplasia, in which the discharge tends to be more serous and intermittent (Sharp *et al.*, 1992). Ulceration of the external nares, probably caused by fungal toxins in the nasal discharge, and a debilitated status are also rarely observed in dogs with nasal neoplasia (Sharp *et al.*, 1992). Conversely, soft tissue masses caused by extension of the disease outside the nasal chambers as well as facial deformity or severe exophtalmos are usually restricted to nasal neoplasia (Harvey, 1984b). Aspergillosis may be differentiated from other non-neoplastic conditions of the nasal cavities by the occurrence of blood in the discharge, nasal pain and ulceration of the external nares (Sharp *et al.*, 1992).

HEMATOLOGY AND BIOCHEMISTRY

Routine hematology and serum biochemistry profiles are usually normal in dogs with nasal aspergillosis. Peripheral eosinophilia and lymphopenia have been observed in a few patients (Goring *et al.*, 1983).

SEROLOGY

Agar gel double diffusion (AGDD), counterimmuno-electrophoresis (CIE) and enzyme-linked immunosorbent assay (ELISA) techniques to detect serum antibodies against *Aspergillus* have been used for diagnosis (Lane *et al.*, 1974; Chandler, 1975; Thoday, 1975; Lane and Warnock, 1977; Poli *et al.*, 1981; Harvey and O'Brien, 1983; Goodall *et al.*, 1984; Kahn *et al.*, 1984; Norris and Laing, 1985; Sharp *et al.*, 1984; Sharp *et al.*, 1991; Sharp *et al.*, 1993). AGDD is still the most widely used technique for the serodiagnosis of aspergillosis due to its simplicity and ease of performance (Kurup and Kumar, 1991). It is available in many veterinary teaching hospitals and public health laboratories. False positive rates range from 0% to 6% for AGDD (Lane and Warnock, 1977; Poli *et al.*,

1981; Cervantes-Olivaries, 1983). False negatives are rare except in the early course of the disease, and these will usually seroconvert later (Norris and Laing, 1985). Animals with other nasal diseases, such as neoplasms, may be serologically positive owing to a concurrent fungal infection in 15% of the cases (Lane and Warnock, 1977). Therefore, the results of serology for *Aspergillus* should always be correlated with the clinical findings and the results of other diagnostic procedures. The number of studies about the use of the CIE and ELISA techniques for diagnosis of nasal aspergillosis in dogs is limited and these techniques are not routinely used (Richardson *et al.*, 1982; Cervantes-Olivaries, 1983; Sharp *et al.*, 1991).

DIAGNOSTIC IMAGING

Imaging studies should precede rhinoscopy and biopsy procedures to avoid the resultant hemorrhage that may obscure subtle lesions and result in focal areas of increased opacity (Norris and Laing, 1985).
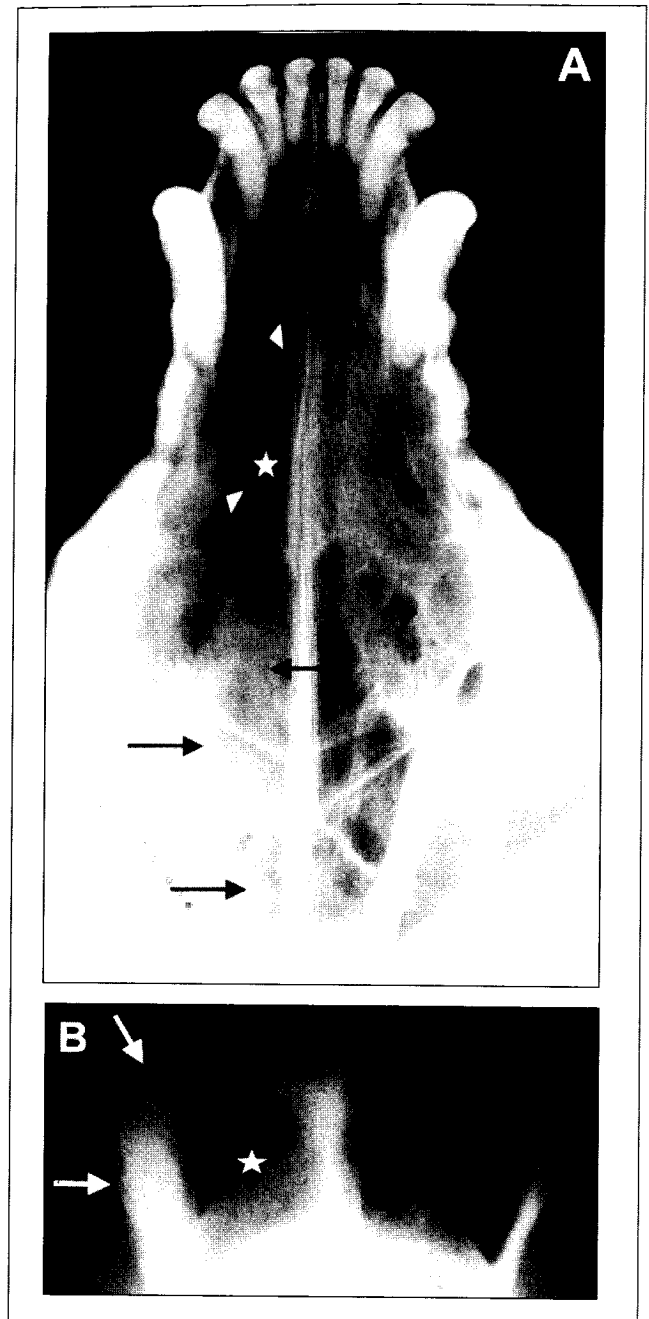
Radiography

The radiographic anatomy of the nasal cavities and frontal sinuses has been described in detail (Harvey, 1979; Schmidt and Voorhout, 1992; Schwarz *et al.*, 2000a; Schwarz *et al.*, 2000b). All radiographic examinations should be performed under general anesthesia to obtain adequate positioning. A complete radiographic study of the nasal cavities and frontal sinuses includes at least a dorsoventral (DV) and a lateral projection of the entire skull, a DV (intra-oral) projection of the nasal cavities and maxilla, and a rostrocaudal (RCd) or RCd (horizontal beam) projection of the frontal sinuses. Alternatively to the DV (intraoral) projection, a ventro-70°-rostro-dorsocaudal oblique (V70R-DCdO) projection may be obtained (Douglas and Williamson, 1972; Norris and Laing, 1985; Sullivan *et al.*, 1986.). The use of nonscreen films for the DV (intra-oral) projection or detail (fine) rare-earth screen-film combinations for the V70R-DCdO projection is mandatory as fast screen-film systems have been demonstrated to result in misinterpretation of nasal turbinate integrity and of abnormal soft tissue opacities (Miyabayashi *et al.*, 1994). Additional oblique projections may be used for evaluation of the teeth or to show selected areas (Gibbs *et al.*, 1979). The DV (intra-oral) and the RCd projections are the most informative for diagnosis of nasal aspergillosis (Sullivan *et al.*, 1986).

The radiological features of nasal aspergillosis were described in the 1970s on a small number of dogs; this description showed a characteristic loss of radiopacity within the affected nasal cavities (Otto, 1970; Soltys and Summer-Smith, 1971; Dawson *et al.*, 1973; Lane *et al.*, 1974; Barrett *et al.*, 1977). Gibbs *et al.* (1979) (on 19 dogs) and Harvey *et al.* (1979) (on 15 dogs) reported an increased lucency of the affected nasal cavity(ies) rostrally and ill-defined areas of opacity caudally. Sullivan *et al.* (1986) described the main radiological features of nasal aspergillosis in more detail on the basis of a series of 45 dogs. Turbinate loss with punctate lucencies of the supporting bones, increased lucency rostrally, increased opacity caudally in the nasal cavity and increased opacity in the frontal sinuses with thickened, mottled frontal bone were the main features (Sullivan *et al.*, 1986) (Figure 1A,1B).

The technique of positive-contrast rhinography has been described using unilateral intranasal administration of a 30% aqueous barium sulphate suspension (Goring *et al.*, 1984a). When used in dogs affected with nasal aspergillosis, it proved to be useful for the evaluation of the extension of the disease process and diagnosis, yielding information not available by survey radiographs (Goring *et al.*, 1983; Goring *et al.*, 1984b).

Nasal neoplasia shows a combination of an increased opacity with turbinate destruction (mass-like process) instead of an increased lucency with turbinate destruction (cavitated-like process) for nasal aspergillosis (Gibbs *et al.*, 1979; Sullivan *et al.*, 1987). Nasal neoplasia is also frequently associated with bone destruction, while nasal aspergillosis most commonly shows bone hyperostosis (Gibbs *et al.*, 1979; Sullivan *et al.*, 1987). The main criterion for differentiating a non-specific rhinitis from nasal aspergillosis and nasal neoplasia is the absence of turbinate destruction (Gibbs *et al.*, 1979) (Figure 2A-D).

However, even with an accurate description of the radiological signs of these three main causes of chronic nasal disease, it is generally acknowledged that the differential diagnosis of chronic nasal disease is difficult and unreliable, and the agreement between observers in terms of their evaluation of the radiographic signs and their final diagnosis is only moderate (Morgan *et al.*, 1972; Delmage, 1973; Harvey *et al.*, 1979; Russo *et al.*, 2000). In one study involving 40 dogs with CND, nasal neoplasia was correctly diagnosed in 83% of the dogs, nasal aspergillosis in 60% and non-specific rhinitis in 52% (Harvey *et al.*, 1979).

Figure 1. Radiographic images of nasal aspergillosis. (A) DV (intra-oral) projection showing severe turbinate destruction, which left an 'empty' nasal cavity (asterisk), a rim of soft tissue along the nasal bones (white arrows), opacification of the caudal nasal cavity (black arrows), and the absence of the left orbital lamina of the ethmoid bone (black arrowheads) in the right nasal cavity. (B) RCd projection of the frontal sinuses (asterisk) in the same dog. There is an increased opacity of the right frontal sinuses and thickening of the right frontal bone (arrows).

Computed tomography

Computed tomography (CT) is becoming more available to veterinarians. CT makes it possible to avoid superimposition and obtain reconstructions in different anatomic planes, thus reducing the com-
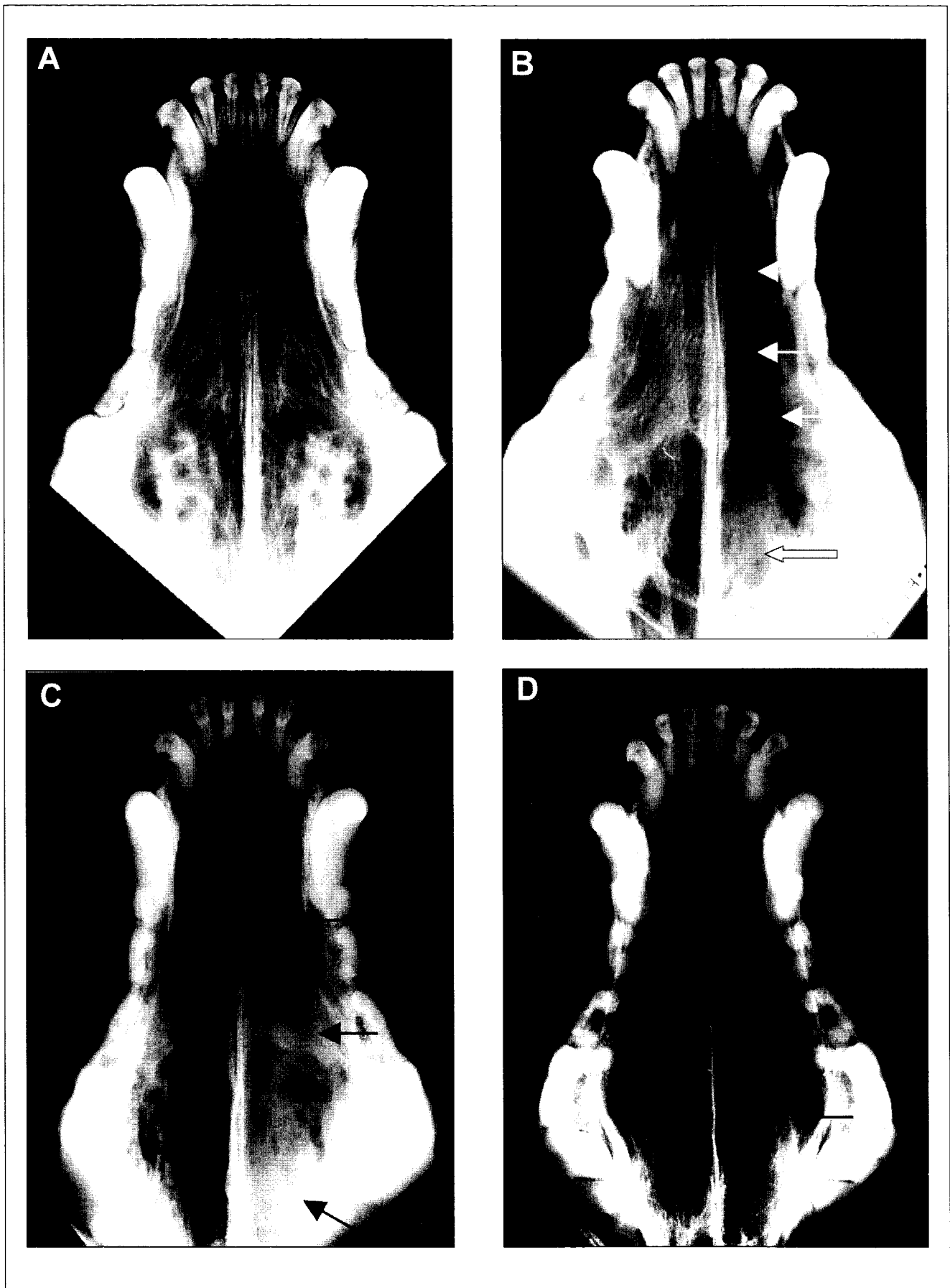
Figure 2. DV (intra-oral) projections of the nasal cavities and maxilla. (A) Normal nasal cavities. (B) Nasal aspergillosis showing an increased lucency (arrows) of the left nasal cavity with a rim of soft tissue along the facial bones and increased opacity caudally (large arrow). (C)Nasal neoplasia showing increased opacity and turbinate destruction of the right nasal cavity (arrows). (D) Non-specific rhinitis showing multiple areas of increased opacity (arrows). The turbinates are intact.

plexity of nasal cavities, which comprise many structures of different densities. Recent developments in the technique of CT are related to the reduction of the acquisition time and post-processing capabilities. Helical CT is a new modality that significantly reduces the examination time compared with axial CT.

The CT anatomy of the nasal cavities and frontal sinuses has been described, but no definite scanning technique has been established for CT examination of the nasal cavities and frontal sinuses in the dog (Burk, 1992a; Assheuer and Sager, 1997). The choice of the technical parameters must take into account the need for adequate detail, the examination time (a majority of patients have large noses (15-20cm)), and the possibility of measuring attenuation values. Different scanning techniques have been used involving contiguous 3mm thick serial slices (Moore et al., 1991), contiguous 5mm serial thick slices (Sackman et al., 1989; Park et al., 1992; Thrall et al., 1992; Schwartz, 1997), contiguous 10mm thick serial slices (Burk, 1992b; Codner et al., 1993), and 1-3mm thick slices with an interval of 5mm (Mathews et al., 1996).

The CT features of nasal aspergillosis have been reported in two studies about chronic nasal disease. Unilateral loss of turbinates, local nasal mucus accumulation and regional mucosal swelling were described (Burk, 1992; Schwartz, 1995). In another study involving 12 dogs with nasal aspergillosis, turbinate destruction and paranasal fluid accumulations were evident. A cavitating lesion was again a significant feature (Mathews et al., 1996).

Differentiation from nasal neoplasia and non-specific rhinitis is based on the type of the process and the appearance of the nasal and frontal bones. Nasal neoplasia shows patchy areas of increased density within a soft tissue opacity (mass-like process), nasal aspergillosis shows an "empty" nasal cavity (cavitated-like process), and non-specific rhinitis shows the absence of destruction of the turbinates with fluid or soft tissue (non-destructive process) (Burk, 1992b). The surrounding bones are usually destroyed with nasal neoplasia, whereas they are unaffected by non-specific rhinitis and they are usually hyperostotic (frontal bone) with nasal aspergillosis due to chronic irritation (Burk, 1992b).

Magnetic resonance (MR)

Recently, magnetic resonance (MR) has been used in the diagnosis of head diseases and principally brain conditions in small animals (Hudson et al., 1995; Vernau et al. 1997; Dennis, 1998; Forrest, 1999). However, the studies that describe the use of MR to evaluate animals with nasal disease are limited (Moore et al., 1991; Voges and Ackerman, 1995; Forrest, 1999). One study documented that MR was better than CT for evaluating intracranial involvement of nasal tumors and for showing anatomic features and secondary pathologies attributed to the mass (Moore et al., 1991). Another study confirmed the usefulness of MR for evaluating intracranial (one dog) or retrobulbar invasion (one cat) of a nasal tumor (Voges and Ackerman, 1995).

Comparison between the imaging techniques

The nasal cavities and paranasal sinuses can be imaged by the use of radiography, CT and MR, each of which has its own merits (Moore et al., 1991; Forrest, 1999). An accurate comparison of these different imaging modalities is not easy because of the numerous commercially available CT and MR scans covering a wide range of imaging capabilities, and because of the rapid and ongoing development of the two techniques. Radiography is universally available and is relatively low in cost, but it requires general anesthesia and it is less reliable than CT and MR for the diagnosis of CND in dogs (Sackman et al., 1989; Thrall et al., 1989, Moore et al., 1991; Park et al., 1992; Codner et al., 1993). CT and MR avoid superimposition, thus decreasing the complexity of the image (Forrest, 1999). CT and MR are available in some academic veterinary hospitals, in some general practices and through access to cooperative human hospitals. The access to MR is more restricted at the current time, however, mainly due to its high cost. CT is generally more expensive than radiography, though in many clinics not significantly (Davidson et al., 2000). Due to its fast acquisition speed, helical CT enables an examination of the nasal cavities and frontal sinuses to be performed under deep sedation instead of general anesthesia. A complete examination of the nasal cavities and frontal sinuses requires 1 to 4 minutes with a helical CT, as compared to 15 to 50 minutes with radiography, axial CT or MR. Consequently, the costs and the risk of anesthesia are reduced with helical CT. There are several theoretical advantages of MR as compared to CT: multiplanar acquisition without repositioning the dog, superior soft tissue contrast, and the use of different pulse sequences to amplify signal intensity differences between normal and pathological tissue, or between soft tissue and fluid (Forrest, 1999; Davidson et al., 2000). However, CT also has certain advantages over MR: reduced cost and acquisition time, better demonstration of bone detail, and better geometrical

delineation of certain anatomic structures for computerized radiotherapy planning (Forrest, 1999).

CULTURE

Before the beginning of rhinoscopy, swabs could be taken for bacterial and fungal culture. However, the nasal cavities of the dog harbor a wide variety of microorganisms that are often present as a mixed bacterial and fungal population (Abramson et al., 1980; Harvey, 1984b; Norris and Laing, 1985). A bacterial culture of the nose or nasal discharge is nearly always positive but unrewarding, and therefore not performed in the clinic. A negative culture for fungal organisms does not rule out a diagnosis of aspergillosis (Sharp et al., 1992). And, even when Aspergillus species are isolated, the result is hard to interpret because 40% of normal dogs, as well as those with nasal neoplasia, will yield these fungi on nasal culture (Lane and Warnock, 1977; Harvey and O'Brien, 1983; Harvey, 1984b; Sharp et al., 1992). Samples may be taken under rhinoscopic guidance for identification of the Aspergillus species and differentiation with Penicillium.

Aspergillus fumigatus is a thermotolerant fungus which grows well in a few days on routine fungal culture media (Sabouraud agar) at temperatures over 40°C. This property is unique to Aspergillus fumigatus among the Aspergillus species. Aspergillus fumigatus can grow at a temperature range of 20 to 50°C. Macroscopically, Aspergillus fumigatus colonies are initially white, turning blue-greenish when the conidia develop, and taking on a smoky gray appearance when old. The colonies are clearly separated, circular and downy or powdery. Microscopically, a culture of Aspergillus fumigatus shows septated hyphae. The conidiophores originate from the supporting hyphae and terminate in a vesicle at the apex. Bottled-shaped phialides cover the entire surface of the vesicles. Conidia or spores develop in the phialides.

RHINOSCOPY

There is an extensive array of instruments available for rhinoscopy. Flexible or rigid endoscopes may be used. The rigid arthroscope, the flexible or rigid cystourethroscope and the flexible pediatric bronchoscope are the three types most commonly employed (McCarthy and McDermaid, 1992; Noone, 2001). Rhinoscopy should be performed under general anesthesia. A significant portion of the nasal cavities can be examined in all dogs. The frontal sinuses can only be viewed and entered in dogs with extensive destructive disease, and for some authors only in large dogs (McCarthy and McDermaid, 1992; Noone, 2001) (Figure 3). The caudal portion of the nasopharynx should be included in the examination (Forbes Lent and Hawkins, 1992). Gentle manipulation within the nasal cavity is needed to minimize the risk of iatrogenic hemorrhage (Hawkins, 1988). The use of saline lavage and suction may facilitate visualization (Norris and Laing, 1985; Venker-Van Haagen et al., 1985).

The lesions associated with nasal aspergillosis are turbinate destruction, abnormal mucosa and secretions, and the presence of irregular, white shiny masses corresponding to fungal colonies (Norris and Laing, 1985; Venker-Van Haagen et al., 1985; Forbes Lent and Hawkins, 1992; McCarthy and McDermaid, 1992; Noone, 2001).

Aspergillosis results in the loss of turbinate structure (Venker-Van Haagen et al., 1985; Sullivan, 1987; Noone, 2001). Early in the disease, the destruction may not be obvious but the examination may seem easier than in a normal dog. With progression, the turbinates take on a crumbled shrunken appearance and appear unsupported as cartilage is lost and hence will seem to float in the fluid stream coming from the endoscope (McCarthy and McDermaid, 1992; Noone, 2001). With further progression of the disease, there is additional loss of turbinate mass. A nearly empty nasal cavity lined with rough, irregular, inflammatory granulation tissue is the endpoint of the destructive process. The destructive process eventually penetrates the septum in one or more locations until it is completely destroyed. At this point, the nasal cavities become one large cavity without turbinate or septal tissue (Sullivan, 1987; McCarthy and McDermaid, 1992; Noone, 2001).

Early mucosal changes include hyperemia, increased vascularity, mucosal thickening with a roughened surface, and friability (McCarthy and McDermaid, 1992; Noone, 2001). As the extent and severity of the disease progresses, a greater portion of the nasal mucosa becomes involved within the granulomatous inflammatory process, until there is complete turbinate destruction and lining of the entire nasal cavity with inflammatory tissue (McCarthy and McDermaid, 1992; Noone, 2001). An increase in mucus secretion or purulent debris is common. The mucosal and content changes are aspecific. The primary indication of the involvement of the frontal sinuses is a concentration of thick mucopurulent exudate dorsally in the caudal or caudolateral aspect of the nasal cavity

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There is an extensive array of instruments available for rhinoscopy. Flexible or rigid endoscopes may be used. The rigid arthroscope, the flexible or rigid cystourethroscope and the flexible pediatric bronchoscope are the three types most commonly employed (McCarthy and McDermaid, 1992; Noone, 2001). Rhinoscopy should be performed under general anesthesia. A significa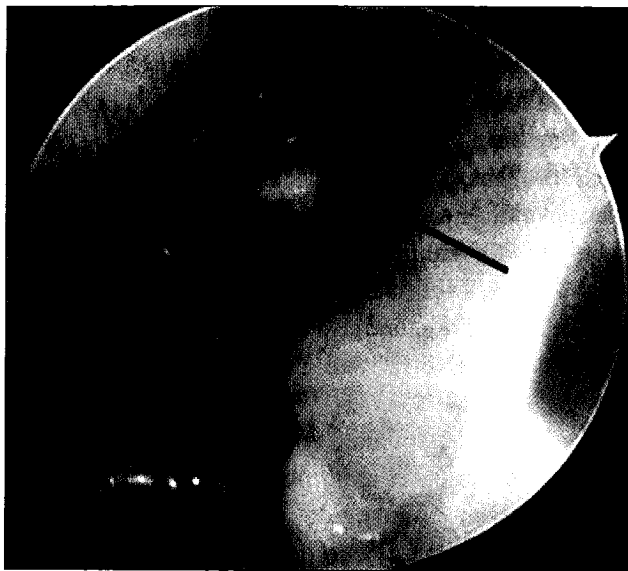nt portion of the nasal cavities can be examined in all dogs. The frontal sinuses can only be viewed and entered in dogs with extensive destructive disease, and for some authors only in large dogs (McCarthy and McDermaid, 1992; Noone, 2001) (Figure 3). The caudal portion of the nasopharynx should be included in the examination (Forbes Lent and Hawkins, 1992). Gentle manipulation within the nasal cavity is needed to minimize the risk of iatrogenic hemorrhage (Hawkins, 1988). The use of saline lavage and suction may facilitate visualization (Norris and Laing, 1985; Venker-Van Haagen et al., 1985).

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Figure 3. Rhinoscopic image of a dog with nasal aspergillosis. Severe destruction of the turbinates allows visualization of the entrance of the frontal sinuses with the use of a 30° angled endoscope. Fungal colonies (arrow) are visible at the entrance of the frontal sinuses.

(McCarthy and McDermaid, 1992) (Figure 3). Focal accumulations of mucus may be confused with fungal plaques to a less experienced eye (Norris and Laing, 1985; Sullivan et al., 1987).

The likelihood of finding fungal colonies increases as the disease progresses (Noone, 2001). Small early fungal colonies are flat, irregular, white dull or shiny masses sitting on the mucosa or, more commonly, on a bed of granulation tissue covered with a layer of mucopurulent exudates (Figure 4A). As fungal colonies enlarge, they become grayish to black and form solid sheets of material covering significant areas of the nasal cavity (McCarthy and McDermaid, 1992; Noone, 2001) (Figure 4B). These larger fungal colonies are dry and hard, and can be felt as a hard rigid structure when contacted with the endoscope or biopsy instrument. They are usually found on the floor of the nasal cavity (McCarthy and McDermaid, 1992; Noone, 2001). Detection of fungal plaques is made in 75-100% of confirmed cases of aspergillosis (Sullivan, 1987; Venker-Van Haagen et al., 1990; Forbes Lent and Hawkins, 1992; Tasker et al., 1999). Fungal colonies may be found in the frontal sinuses when they cannot be seen in the nasal cavities.

Biopsies may result in hemorrhage and should therefore be done after gross examination is completed (Forbes Lent, 1992). However, complications of rhinoscopy and rhinoscopy-assisted biopsy are rare. Protracted hemorrhage was a complication in 2% of the dogs and was fatal in < 1% of dogs undergoing rhi-

noscopic-guided biopsies (Forbes Lent and Hawkins, 1992). Coagulation profile should be considered in the dog's history (Forbes Lent and Hawkins, 1992).

Criteria for differentiation from nasal neoplasia are the presence of suspected tissue (± 10% of the cases) or a mass (± 90% of the cases) which, if large enough, reduces the space for endoscopic examination and causes displacement of adjacent structures. Non-specific rhinitis is characterized by a bilateral moderate to large amount of mucoid to mucopurulent exudates, thickened turbinates that reduce the working space, and the absence of a mass or fungal colonies (Venker-Van Haagen et al., 1990; McCarthy and McDermaid, 1992; Noone, 2001).

CYTOLOGY AND HISTOLOGY

Nasal specimens may be collected either surgically or non-surgically. Non-surgical techniques include superficial and deep nasal swab, nasal flush, cytobrush, traumatic nasal flush, pinch biopsy and core biopsy (McEwen et al., 1977; Withrow, 1977; Withrow et al., 1985; Forbes Lent and Hawkins, 1992; Legendre et al., 1993; Clercx et al., 1996b). The latter three techniques result in deeper tissue that can be examined both with cytology by impression smears and with histology (Rebar et al., 1992).

The limiting factor for nasal cytology is the obtainment of a representative sample (Andreasen et al., 1989; Rebar et al., 1992). Cytologic specimens can be stained with routine Wright's hematologic stains or prepared as wet mounts with 10 to 30% KOH or new methylene blue (Wolf, 1992). Special fungal stains such as periodic acid-Schiff (PAS) or Gomori's methenamine silver (GMS) may improve detection if few organisms are present (Wolf, 1992). Nasal aspergillosis typically causes mixed or predominantly macrophagic reactions but can also be associated with a neutrophilic or a mild eosinophilic inflammation (Andreasen et al., 1989). However, evaluation with inflammatory cells does not enable a definite diagnosis of a type of CND, as most diseases result in a marked superficial inflammation and a secondary bacterial infection that can obscure the underlying disease (Andreasen et al., 1989; Rebar et al., 1992). Cytologic evidence of a fungal rhinitis is based on the visualization of hyphae or microconidia, which are frequently present within dense accumulations of cells and debris (Rebar et al., 1992) (Figure 5A,5B). Both Aspergillus and Penicillium species appear as 2 to 6 μm wide septated, branching hyphae on cytologic preparations. Specific
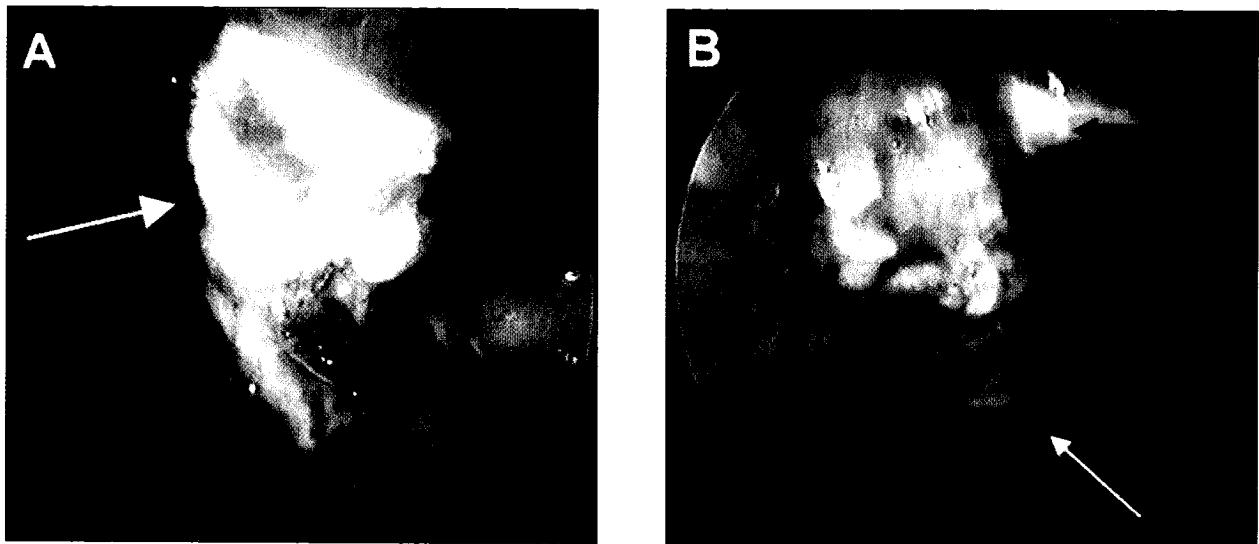
Figure 4. Rhinoscopic images of the nasal cavity of a dog with nasal aspergillosis. (A) Early fungal colonies (arrow) appearing as a white dull or shiny mass. (B) Old-grayish to black fungal plaque on the floor of the nasal cavity (white arrow). A small amount of mucopurulent exudate is present (black arrow).
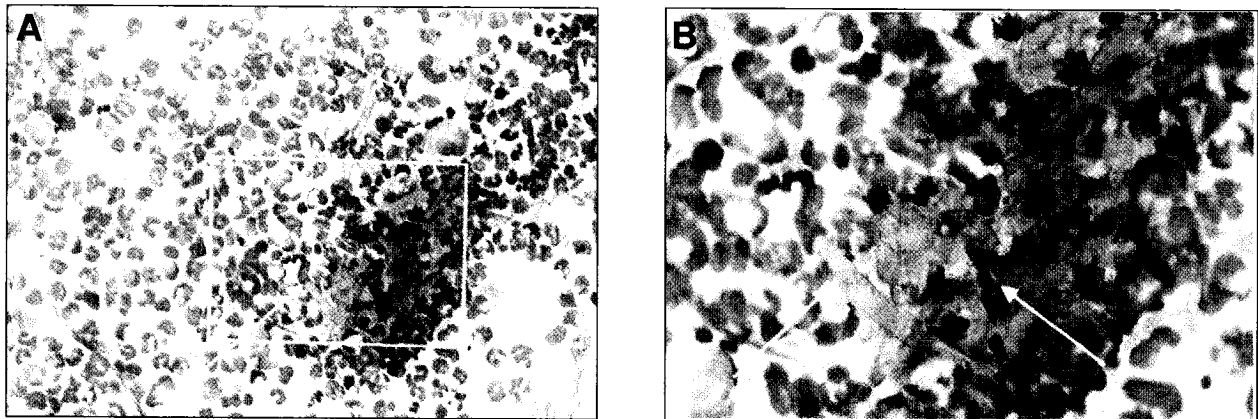

Figure 5. Cytology of nasal aspergillosis (Wright's staining). (A) Fungal hyphae (arrow) can be seen in a background of inflammatory cells. (B) Close-up view of fungal hyphae (arrows).
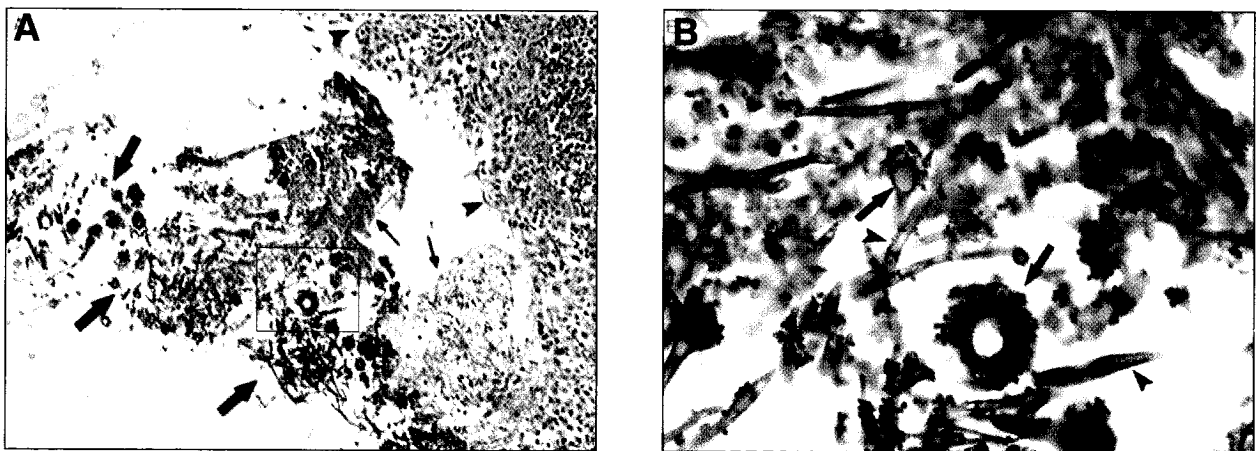

Figure 6. Histologic examination of a dog showing the features of nasal aspergillosis. (A) Necrotic areas of the nasal mucosa (arrows), fibrinopurulent exudates (small arrows) and Aspergillus conidia and hyphae (arrowhead) (PAS X 200). (B) Close-up view of the framed area showing Aspergillus conidia (arrows) and hyphae (arrowheads).

identification requires fungal culture (Sharp, 1990). A diagnosis of nasal neoplasia is based on the presence of neoplastic cells (Andreasen *et al.*, 1989).

The diagnostic success of the non-surgical non-rhinoscopic techniques for histological diagnosis of chronic nasal disease is 50 to 100% (Withrow 1977; Legendre *et al.*, 1983; Withrow *et al.*, 1985; Love *et al.*, 1987, Hawkins, 1992; Tasker *et al.*, 1999). Surgical biopsies (rhinotomy, sinus trephination) may be required when less invasive techniques are not diagnostic (Gartrell *et al.*, 1995). Nasal biopsies of aspergillosis typically reveal extensive necrosis of the nasal epithelium and thick plaques of fibrinopurulent exudates mixed with fungal hyphae (Bright, 1979; Lopez, 2001) (Figure 6A,6B). It has been postulated that the aflatoxins associated with *Aspergillus* may be responsible for the necrosis (Brandwein, 1993). A mixed inflammatory cell infiltrate is also present in all dogs and is sometimes the sole histological finding (Tasker *et al.*, 1999). In the latter case, differentiation between nasal aspergillosis and non-specific rhinitis is not possible. Non-specific rhinitis shows (1) a severe lymphoplasmocytic infiltrate (lymphoplasmocytic rhinitis), (2) a varied inflammatory cell infiltrate of neutrophils, eosinophils and plasma cells without predominant cell type (allergic rhinitis), and (3) hyperplasia with a mixed inflammatory cell infiltrate (hyperplastic rhinitis) of the nasal mucosa and submucosa (Burgener *et al.*, 1986; Tasker *et al.*, 1999). Nasal neoplasia is characterized by the presence of neoplastic cells in a monomorphic population of cells (Confer & De Paoli, 1978).

RHINOTOMY

Exploratory rhinotomy has been used to obtain diagnostic biopsies in dogs in which the other diagnostic procedures have been unrewarding (Gartrell *et al.*, 1995). The technique for rhinotomy and turbinectomy has been described (Birchard, 1986).

DEFINITIVE DIAGNOSIS

The criteria for a definitive diagnosis of aspergillosis are controversial. Some investigators suggest that the definitive diagnosis of aspergillosis can only be made by visualization of fungal hyphae in affected tissues along with isolation of the organism on tissue culture (Barsanti, 1984). Other investigators require macroscopic evidence at surgery or microscopic evidence of fungal elements invading nasal tissue (Harvey, 1984a). However, the same author has stated that in dogs not undergoing surgery, biopsy specimens for microscopic examination are difficult to obtain blindly (Harvey and O'Brien, 1983). Rhinoscopy has progressively replaced surgical visualization (Harvey, 1984a). For an accurate diagnosis, clinical, radiologic, rhinoscopic, serologic and mycologic (histology, cytology, culture) evidence of infection are required (Sharp and Sullivan, 1989). A less stringent diagnosis consists in evidence of fungal invasion of the host (best visualized on rhinoscopy) in conjunction with serologic and either mycologic or radiologic evidence of the disease (Harvey, 1984a; Sharp, 1990; Sharp and Sullivan, 1992).

CONCLUSIONS

Nasal aspergillosis still represents a diagnostic challenge in many dogs. Hematology, biochemistry and fungal culture are not conclusive when used alone. The diagnostic value and reliability of radiography are controversial, while cytology and histology show aspecific features in approximately 50% of the affected dogs. Serology is reliable and easily performed but may be negative in the early stages of the disease. Rhinoscopy is essential, as it allows direct visualization of fungal colonies in 80-100% of the affected dogs and it also has a therapeutic role.

REFERENCES

A complete list of the references referred to in this article is available from the author, J. H. Saunders.