Diagnosis of canine nasal aspergillosis by cytological examination: a comparison of four different collection techniques

OBJECTIVES: To compare the efficacy and diagnostic value of four different sample collection techniques for cytological identification of nasal aspergillosis-penicilliosis in dogs.

METHODS: Fifteen dogs with a history of persistent nasal discharge and clinical and radiographic findings suggestive of aspergillosis were evaluated using four different cytological sampling techniques. These were a direct smear from the nasal discharge, blind swab collection under general anaesthesia, brushing from suspect lesions under direct endoscopic visualisation and a squash technique of mucosal biopsies from suspect lesions obtained under direct endoscopic visualisation.

RESULTS: Direct smear collection and blind swab collection detected fungal hyphae in 13.3 and 20 per cent of examined cases, respectively; brush samples detected fungal hyphae in 93.3 per cent and fungal spores in the 45 per cent of examined cases and squash samples detected fungal hyphae in 100 per cent and fungal spores in 36 per cent of examined cases.

CLINICAL SIGNIFICANCE: This study confirmed the high accuracy of cytology samples in the diagnosis of nasal aspergillosis-penicilliosis when collected under direct endoscopic visualisation and showed the poor value of samples that were collected by blind swabs or prepared from samples of nasal discharge.

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INTRODUCTION

Fungal infections are a relatively common cause of nasal disease in dogs. Aspergillus fumigatus is the species most often isolated from infections in the nasal cavities of these animals (Morellaro and others 1989, Wolf 1992). The first symptoms suggesting nasal mycosis are a painful nose, prolonged sneezing with unilateral or bilateral seropurulent malodorous nasal discharge, epistaxis and severe erosion with depigmentation of the nostrils (Mathews 2004). The most important differential diagnosis for nasal aspergillosis is nasal neoplasia (Sharp 1998) and therefore a thorough diagnostic evaluation should be performed in all patients with these clinical signs.

Several diagnostic techniques can be used to investigate nasal disease, but no single test can be considered in isolation as false-positive and false-negative results can occur. Sharp (1998) stated that the biopsy of fungal plaques under direct visualisation was probably the best way to obtain meaningful samples for mycological examination by cytology, histology and culture on a fungal medium. The aim of the current study was to compare the efficacy and diagnostic value of four different sample collection techniques for cytological identification of nasal aspergillosis-penicilliosis in dogs.

MATERIALS AND METHODS

A total of 15 dogs with clinical (chronic unilateral or bilateral seropurulent discharge) and radiographic (localised, increased radiolucency of nasal chambers) features suggestive of nasal mycosis were selected to participate in this study. All the patients had routine haematology and serum biochemistry profiles performed and nasal and frontal sinus radiographs were taken under inhalatory general anaesthesia. Anterograde rhinoscopy with a rigid endoscope (64018BS; K. Storz; diameter 2-7 mm, length 18 cm, forward oblique 30°) and nasopharyngoscopy with a flexible bronchoscope (60001VL; K. Storz; diameter 5-2 mm, length 85 cm) were also performed during the same anaesthetic episode.
Samples for cytopathological evaluation were taken from each dog using four different methods in the following order: direct smear from nasal discharge, blind endonasal swab, mucosal brushing of suspected lesions under endoscopic guidance, and squash preparation from at least two biopsy samples of suspect lesions under endoscopic guidance. Suspect lesions were considered those which, after lavage of nasal cavities with physiological saline, off-white or greenish, fuzzy plaques were adherent to nasal mucosa.

All the cytological samples were air dried, then stained with May–Grunwald Giemsa in an automatic slide stainer (7100 Aerospray Slide Stainer; Wescor). At least two adequate slides were available from each technique. In all cytological samples the presence of inflammatory cells, bacteria, fungal hyphae, fungal spores and fungal conidial heads was recorded.

From each dog, two biopsy tissue fragments from suspect lesions were gently implanted onto Sabouraud glucose agar (SGA; Difco Laboratories), supplemented with chloramphenicol and then incubated (37°C) and fungal conidial heads was recorded.

Fifteen dogs were included in this study. The breeds of dogs included three English setters, two dobermanns, two Newfoundland, two mixed breed dogs, one Irish setter, one pointer, one bull terrier, one German shepherd dog, one rottweiler, and one beagle (Table 1).

The average age of the dogs was four years and nine months (range eight months to nine years). There were 12 entire males and three females, one of which was spayed.

On the basis of cytological evaluations the following results were found (Fig 1). All four techniques showed neutrophilic inflammation, free bacteria and phagocytosed bacteria in all 15 cases (100 per cent). Using a direct smear from the nasal exudate, fungal hyphae was seen in two cases (13.3 per cent) and no fungal spores or fungal conidial heads were seen at all. A blind endonasal swab showed fungal hyphae in three cases (20 per cent) and fungal spores in one case (6.6 per cent), and fungal conidial heads were not seen using this technique. Endonasal brushing under endoscopic guidance identified fungal hyphae in 14 of the 15 cases (93.3 per cent), fungal spores in four cases (26.6 per cent) and fungal conidial heads in one case (6.6 per cent). Squash preparation from endonasal biopsies under endoscopic guidance showed fungal hyphae in all 15 cases (100 per cent), fungal spores in five cases (33.3 per cent) and fungal conidial heads in one case (6.6 per cent).

The different cytological findings in the 15 dogs for each different collection technique is shown in Fig 1. In all 15 cases, *A. fumigatus* was cultured and identified from the nasal biopsies on Sabouraud glucose agar. In three dogs (cases 1, 6 and 7), a nasal foreign body (grass awn) was found and removed during the endoscopic examination of the nasal cavities.

**RESULTS**

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**DISCUSSION**

*Aspergillus* and *Penicillium* species are ubiquitous saprophytic hyphal fungi regarded as opportunistic pathogens. *A. fumigatus* is the species most commonly isolated from nasal infections in dogs and *Aspergillus niger, Aspergillus nidulans* and *Aspergillus flavus* have also been recovered from canine nasal cavities, but the species of *Penicillium* causing nasal mycoses has not yet been identified (Sharp and others 1991). The term aspergillosis is generally applied to infections with both *Aspergillus* and *Penicillium* species, which are indistinguishable both in cytological and histological samples (Sharp 1989).

The cytomorphological characteristics of these fungal organisms have been well described (Sharp 1998, Andreason and others 1999, Baker and Lumdsen 2000, Burkhard and others 2001): negatively staining or large (4 to 6 μm in diameter), dark blue septate hyphae with parallel sides that branch dichotomously at a 45° angle is the most common finding (Fig 2). As the inflammatory cellular response to *Aspergillus* is primarily neutrophilic, a large number of moderately lytic neutrophils is usually seen and many show bacterial phagocytosis due to secondary bacterial infection.
Fungal spores are round, small (1.5 to 2 μm in diameter), greenish structures, sometimes interspersed among inflammatory cells, necrotic debris and fungal hyphae. Conidial heads, which are the most useful criterion for fungal identification, are seen only rarely in cytological samples from nasal swabs (Fig 3).

The cytomorphological features of Aspergillus and Penicillium are completely different from all other fungal organisms that can cause nasal diseases, such as Cryptococcus, Histoplasma, Blastomyces, Schizosaccharomyces and Alternaria (Wolf 1992, Burkhard and others 2001). As a consequence of this, the presence of fungal structures with the above described morphological features in cytological samples from nasal swabs, in association with clinical history, radiographic and endoscopic findings can be considered pathognomonic for a diagnosis of nasal aspergillosis. A rapid cytological diagnosis is particularly important considering the most recent updates in the therapy for nasal aspergillosis (Davidson and Mathews 2000, Mathews 2004): in fact, if a definitive diagnosis of nasal aspergillosis can be achieved during rhinoscopy, then a non-invasive intranasal infusion of clotrimazole can be performed during the same anaesthetic episode.

As shown in the present study, the collection technique is of critical importance: fungal hyphae from cytological samples were detected in 13.5 per cent of cases with a direct smear of nasal discharge examination, in 20 per cent of cases using a blind swab collection technique, in 93.3 per cent of cases with brushings taken under endoscopic guidance, and in 100 per cent of cases using squash preparations of biopsies taken under endoscopic guidance. Fungal spores were not detected in samples from direct smear collection, but were identified in 6–6 per cent of cases in samples from blind swab collection, in 26–6 per cent of cases with brushings taken under endoscopic guidance, and in 33–3 per cent of cases with squash preparations from biopsies taken under endoscopic guidance. The presence of conidial heads was recorded in only one case (case 11) and only in the samples collected by brushing and squash preparations from biopsies taken under endoscopic guidance.

In all the cases examined and with all sampling techniques, the most common cytological finding was of a neutrophilic (purulent) inflammation with phagocytosed bacteria (both cocci and rods). In the smears from nasal exudates, a misdiagnosis of bacterial rhinitis was made in 86–6 per cent of cases (13 of 15), while in the samples from a blind swab the same misdiagnosis was made in 80 per cent of cases (12 of 15). The same misdiagnosis was only made in a single case in the samples obtained by nasal brushings under endoscopic guidance (6–6 per cent), while the squash technique did not fail to detect the primary cause of nasal disease. This fact is of paramount importance because a cytological diagnosis of bacterial rhinitis can result in prolonged antibiotic therapy that is inappropriate for the primary disease.

In addition, this study shows the importance of an accurate endoscopic evaluation of the nasal cavities when nasal mycosis is suspected. In three of the 15 cases (20 per cent) a grass awn was detected and removed. As the presence of fungal elements does not rule out other underlying problems, such as nasal foreign bodies or neoplasia, an accurate anterograde as well as retrograde rhinoscopy should always be performed. This study also suggests that biopsies should be taken from apparently normal as well as clearly abnormal nasal mucosa.

**Conclusions**

This study has shown that in this group of dogs with confirmed nasal aspergillosis the cytological evaluation of samples collected...
under rhinoscopic inspection by squash preparation of biopsies from suspect lesions is a highly accurate method of detecting fungal organisms. In addition, this study has shown that in the same group of dogs cytological samples collected by direct smear from nasal exudate and from blind nasal swabs detected fungal organisms in only a small number of cases and a correct identification of the primary cause of the nasal disease occurred in only 13.3 and 20 per cent of the evaluated cases, respectively.

References
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