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Whole blood and tissue fungal DNA quantification in the diagnosis of canine sino-nasal aspergillosis

Dominique Peeters [a,*], Iain R. Peters [b], Chris R. Helps [b], Sandrine Dehard [a], Michael J. Day [b], Cécile Clercx [a]

[a] *Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, University of Liège, Sart-Tilman B44, 4000 Liège, Belgium*
[b] *School of Clinical Veterinary Science, University of Bristol, Langford House, Langford, Bristol BS40 5DU, UK*

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Abstract

Various combinations of tests are used to confirm the diagnosis of canine sino-nasal aspergillosis (SNA) because false-positive and false-negative results can occur with each test. Therefore, the aim of this study was to evaluate whether detection of fungal DNA in blood and nasal tissue samples was of value in the clinical diagnosis of this disease.

Four groups were included in the study (dogs with SNA, lymphoplasmacytic rhinitis or nasal neoplasia, and control animals). Real-time PCR assays detecting DNA from all *Penicillium* and *Aspergillus* species (PenAsp assay) or species-specific DNA from *A. fumigatus*, *A. terreus*, *A. flavus* and *A. niger* were applied to whole blood and nasal tissue samples. Results obtained by PCR were compared between the groups. Sensitivity, specificity, positive and negative predictive values (PPV and NPV) for fungal DNA detection were compared with those for alternative diagnostic procedures including histopathology, serology and fungal culture.

Significantly more fungal DNA was detected by the PenAsp assay in tissue biopsies from dogs with SNA than in the three other groups. Sensitivity, specificity, PPV and NPV for this method were 1.00, 0.06, 0.32 and 1.00. *A. fumigatus* DNA was detected in seven tissue biopsies from dogs with SNA and in one biopsy from a dog with a nasal tumour. Sensitivity, specificity, PPV and NPV for this diagnostic test were 0.50, 0.97, 0.87 and 0.82. No significant difference was found between the groups with respect to the amount of DNA detected in blood by the PenAsp assay. Sensitivity, specificity, PPV and NPV for this method were 0.71, 0.24, 0.31 and 0.64. *A. fumigatus* DNA was detected in the blood of three dogs with SNA and sixteen dogs without SNA. Sensitivity, specificity, PPV and NPV for this diagnostic tool were 0.21, 0.45, 0.15 and 0.54. Detection of *A. fumigatus* DNA in nasal tissue had the highest specificity, PPV and NPV but sensitivity of this method was low. Detection of fungal DNA in whole blood was of no value in the diagnosis of SNA.

Keywords: Dog; Canine; Aspergillosis; Rhinitis; Real-time PCR; DNA detection

Abbreviations: Afumi assay, *Aspergillus fumigatus* assay; Ct, threshold cycle; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; gDNA, genomic DNA; LPR, lymphoplasmacytic rhinitis; NPV, negative predictive value; PCR, polymerase chain reaction; PenAsp assay, *Penicillium/Aspergillus* assay; PPV, positive predictive value; SNA, sino-nasal aspergillosis.

* Corresponding author. Tel.: +32 4 366 4200; fax: +32 4 366 4241.
E-mail address: dpeeters@ulg.ac.be (D. Peeters).

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1. Introduction

Sino-nasal aspergillosis (SNA) is a common cause of nasal discharge in the dog and is most often caused by *Aspergillus fumigatus* (Sharp et al., 1991). Various combinations of diagnostic tests, such as radiography, computed tomography, rhinoscopy, histological examination, cytology, fungal culture, and serology are used to confirm the diagnosis of canine SNA (De Lorenzi et al., 2006; Saunders and van Bree, 2003; Sharp et al., 1991). Currently, no single test can be used to make the diagnosis because false-positive and false-negative results can occur with each (De Lorenzi et al., 2006). Therefore, the gold standard for diagnosing this disease is considered to be the direct visualization of fungal plaques by rhinoscopy or the microscopical observation of fungal elements in samples taken for cytological or histopathological examination (De Lorenzi et al., 2006; Johnson et al., 2006; Zonderland et al., 2002).

As the fungus does not invade the mucosa or disseminate throughout the body in canine SNA (Peeters et al., 2005), this disease resembles a form of human fungal sinusitis that occurs in immune competent patients. Although quantification of fungal DNA by real-time polymerase chain reaction (PCR) is widely used in human medicine for the diagnosis and monitoring of therapy of invasive aspergillosis (Ferns, 2006), we are not aware of any report of the use of fungal DNA detection in the diagnosis of human non-invasive fungal sinusitis.

Idiopathic lymphoplasmacytic rhinitis (LPR) is another common cause of nasal discharge in the dog, but in this disease the clinical signs tend to be less severe than in SNA (Windsor et al., 2004). The etiology of this condition in dogs is unknown. In human medicine, airborne fungi have been shown to play a key role in the pathogenesis of disease in some patients with chronic rhinosinusitis (Ponikau et al., 2006; Rao et al., 2006). In a recent study, molecular analysis has shown higher loads of fungal DNA in nasal biopsies from dogs with idiopathic LPR than in nasal biopsies from healthy dogs, suggesting a possible role for fungal organisms in idiopathic LPR of dogs (Windsor et al., 2006).

Therefore, the aims of the present study were (1) to evaluate the potential value of detection of fungal DNA in samples of whole blood and nasal tissue in the diagnosis of canine SNA, and (2) to investigate whether *Aspergillus* may have a role in the pathogenesis of canine LPR.

2. Materials and methods

2.1. Animals

Fourteen dogs with a diagnosis of aspergillosis involving the nasal cavities and frontal sinus were included in the study. Ages ranged from 1 to 10 years (median 4.5). Diagnosis of SNA was based on results of physical examination and observation of typical intranasal or intrasinusal fungal plaques associated with turbinate destruction on rhinoscopy/sinuscopy.

Seven dogs with a diagnosis of LPR were included in the study. Ages ranged from 0.5 to 13 years (median 6). Diagnosis of LPR was made on the basis of clinical signs, rhinoscopic (unilateral or bilateral mild to moderate turbinate lysis with congestion and oedema of the mucosa, mucoid to mucopurulent secretions, and failure to demonstrate a foreign body, neoplasia or fungal plaques in the nasal cavities) and histopathological findings.

Thirteen dogs suffering from neoplasia of the nasal cavity were entered into the study. Ages ranged from 6 to 18 years (median 10). Diagnosis was based on visualisation of a mass during rhinoscopy and histopathological findings.

Eight client-owned dogs, euthanased for various independent reasons (non-metastatic gastric carcinoma in two dogs, brain tumour in two dogs, and uncontrollable aggression, congestive heart failure, pulmonary carcinoma and non-metastatic osteosarcoma in one dog each), and four healthy beagle dogs were sampled as control animals. None of these dogs had clinical history or signs of upper respiratory disease, immune-mediated disease or systemic immunosuppression. Ages of these dogs ranged from 4 to 12 years (median 7.5).

Seven dogs with SNA had received oral antifungal therapy (ketoconazole in four dogs and itraconazole in three dogs) for 8–15 days prior to rhinoscopy. In all other animals, no systemic antifungal therapy had been administered for at least 2 months preceding rhinoscopy.