ORIGINAL ARTICLE

Diagnostic findings in sinonasal aspergillosis in dogs in the United Kingdom: 475 cases (2011–2021)

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OBJECTIVES: To describe the diagnostic tests used and their comparative performance in dogs diagnosed with sinonasal aspergillosis in the United Kingdom. A secondary objective was to describe the signalment, clinical findings and common clinicopathologic abnormalities in sinonasal aspergillosis.

MATERIALS AND METHODS: A multi-centre retrospective survey was performed involving 23 referral centres in the United Kingdom to identify dogs diagnosed with sinonasal aspergillosis from January 2011 to December 2021. Dogs were included if fungal plaques were seen during rhinoscopy or if ancillary testing (*via* histopathology, culture, cytology, serology or PCR) was positive and other differential diagnoses were excluded.

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RESULTS: **A total of 662 cases were entered into the database across the 23 referral centres. Four hundred and seventy-five cases met the study inclusion criteria. Of these, 419 dogs had fungal plaques and compatible clinical signs. Fungal plaques were not seen in 56 dogs with turbinate destruction that had compatible clinical signs and a positive ancillary test result. Ancillary diagnostics were performed in 312 of 419 (74%) dogs with observed fungal plaques permitting calculation of sensitivity of cytology as 67%, fungal culture 59%, histopathology 47% and PCR 71%.**

CLINICAL SIGNIFICANCE: **The sensitivities of ancillary diagnostics in this study were lower than previously reported challenging the clinical utility of such tests in sinonasal aspergillosis. Treatment and management decisions should be based on a combination of diagnostics including imaging findings, visual inspection, and ancillary testing, rather than ancillary tests alone.**

INTRODUCTION

Sinonasal aspergillosis (SNA), most frequently caused by *Aspergillus fumigatus*, is a common cause of chronic nasal disease in dogs. A predisposition in dolichocephalic breeds has been previously established (Sharman et al., 2010). A combination of clinical signs including chronic mucopurulent nasal discharge, nasal pain and nasal planum depigmentation or ulceration can prompt a suspicion of SNA (Peeters & Clercx, 2007; Sykes, 2013).

Diagnosis of SNA is often based on compatible clinical findings, diagnostic imaging or rhinoscopic evidence of turbinate destruction and the identification of fungal plaques (Peeters & Clercx, 2007). Additional diagnostic tests include serological testing for Aspergillus-specific antibodies and the demonstration of fungal elements via cytology, histopathology, culture or PCR. Reported sensitivity for these tests varies between 50% and 88% (Billen, Clercx, et al., 2009b; Billen, Peeters, et al., 2009a; Peeters et al., 2005; Pomrantz et al., 2007).

The performance of these diagnostic modalities, in a wider (multi-centre) population has not been reported. This study aimed to assess the sensitivity of cytology, histopathology, culture and PCR in dogs where a diagnosis of SNA was established in referral practices in the United Kingdom over the last 10 years. Additional objectives of this study were to describe the signalment and clinical findings of dogs diagnosed with SNA in the United Kingdom as well as common clinicopathologic abnormalities.

MATERIALS AND METHODS

Ethical approval for this study was obtained. Electronic medical records from 23 referral centres in the United Kingdom (21 in England and two in Scotland) were searched to identify dogs with a final diagnosis of SNA for the period of January 2011 to December 2021 (dates adapted to reflect years of operation of each centre).

Dogs diagnosed with SNA were entered into an electronic data capture (EDC) system, Castor®. For each case, the following information was retrieved from the clinical records: signalment, clinical signs, physical examination findings on initial presentation at the time of referral and results of clinicopathologic and diagnostic tests. Data from Castor was then collated into an electronic spreadsheet.

To be included for further analysis, cases were required to meet the following criteria: (a) Compatible clinical signs, turbinate destruction or sinus involvement, and fungal plaques observed during rhinoscopy, trephination or sinusotomy, OR (b) compatible clinical signs, presence of turbinate destruction or sinus involvement AND at least one positive result from ancillary diagnostic tests. Dogs that had turbinate destruction, but no fungal plaques observed, and negative ancillary test results, were excluded from further analysis. Dogs were also excluded if other potential destructive disease processes, such as neoplasia or nasal foreign bodies, were documented (possible secondary aspergillosis).

Information regarding fungal culture was collected from 13 different laboratories across the United Kingdom. Information including the incubation temperature, and methodology used for each lab was acquired (Table S1). Case records were reviewed by two separate investigators (CP and FA). Where turbinate damage was recorded, the degree of destruction was retrospectively categorised as mild/moderate/severe.

Statistical analysis

Continuous data were presented as median values with interquartile ranges (IQR). Percentages were calculated based on the final sample of eligible dogs unless otherwise specified or implied. Where the dataset was incomplete, percentages were

based on the total number of responses received. Thus, denominators varied depending on whether the data was available. Where confidence intervals were given for percentages, these were exact 95% confidence intervals but were not calculated for subgroups smaller than 10. As blood tests were conducted at different diagnostic laboratories, values were categorised as below, within or above the respective laboratory reference interval. Dates of onset of clinical signs and of referral were divided into four meteorological seasons [Winter (December to February), Spring (March to May), Summer (June to August) and Autumn (September to November)]. All calculations were undertaken in Minitab 21. Sensitivity of ancillary tests (histopathology, culture, cytology and PCR), and of serology was only calculated for the cases where fungal plaques were visualised.

RESULTS

Patient characteristics and history

Signalment

A total of 662 cases were entered into the database across the 23 referral centres. Three cases were excluded due to incomplete data entry (records were created but no further data inputted). A further 187 cases did not meet the inclusion criteria on review leaving a total of 475 cases (Fig 1). Of these, 419 dogs (88%) had observed fungal plaques, and 56 dogs (12%) had no fungal plaques observed but a positive ancillary test result (Fig 1).

Of these 475 dogs, there were 316 male dogs (67%; 206 neutered, 110 entire) and 159 female dogs (33%; 122 spayed 37 entire). Median age was 6.0 years (IQR 3.1 to 9.3). Fifty different breeds were represented including 80 (17%) golden retrievers, 80 (17%) Labrador retrievers, 70 (15%) cross breeds, 38 (8%) border collies, 23 (5%) Staffordshire bull terriers, 23 (5%) cocker spaniels and 20 (4%) bull terriers. Other breeds are listed in Table S2. The median bodyweight at the time of presentation to the referral centres was 27.7 kg (IQR 20.4 to 33.6). Twenty-one of 279 dogs (8%) for whom travel history had been documented had travelled outside the United Kingdom.

Duration of illness prior to referral

The median duration of clinical signs prior to presentation was 61 days (IQR 31 to 118). Concurrent diseases were reported in 164 of 427 (38%) of dogs with 32 of 164 (20%) presenting with more than one comorbidity at the time of SNA diagnosis. No information was provided for the remaining 48 dogs. The most commonly reported comorbidities were orthopaedic disease in 35 dogs (8%) and atopic dermatitis in 30 dogs (7%).

The date (and hence season) of onset of clinical signs could be calculated for 474 of 475 cases. Onset was roughly uniformly distributed throughout the year, with 114 (24%) cases in winter, 114 (24%) in spring, 122 (26%) in summer and 124 (26%) in autumn. The distribution of dogs to seasons according to the date presented to the referral centre was similarly uniform.

Medications and management strategies prior to referral

Treatments prescribed prior to referral, specifically to manage clinical signs attributable to nasal disease, included systemic antimicrobials in 337 (71%) dogs and anti-inflammatory medications in 162 (34%), including 74 (16%) that had received prednisolone. Treatment for comorbidities (201/475 dogs) included antimicrobials in an additional 28 (6%) dogs, glucocorticoids in 34 (7%) dogs, oclacitinib in 15 (3%) dogs, ciclosporin in three (1%) dogs and lokivetmab in one (<1%) dog.

Clinical signs, physical examination findings and clinical course

Clinical signs and physical examination findings for all dogs are summarised in Table 1. The most common presenting complaints included nasal discharge [406 dogs (87%)], sneezing [367 dogs (77%)] and epistaxis [292 dogs (63%)]. Physical examination findings included nasal pain and nasal planum ulceration and/or depigmentation [188 dogs (40%)], enlarged mandibular lymph nodes ipsilateral to the affected nostril [169 dogs (44%)] and decreased nasal air flow [35 dogs (17%)].

Haematologic and biochemical findings

Haematology results were available for 320 of 475 (67%), manual PCV for 54 of 475 (11%), serum biochemistry for 303 of 475 (64%), coagulation profiles (PT/APTT) for 184 of 475 (39%) and C-reactive protein (CRP) for 28 of 475 (6%) cases. Blood tests were either not performed or not recorded in 142 of 475 (30%) dogs. Haematologic and biochemistry findings are summarised in Table 2.

The most common haematological abnormality was a mild neutrophilia 62 of 304 (20%). The most common biochemical alteration was hypoalbuminaemia 93 of 293 (32%), which was typically mild. CRP was performed in 28 dogs, with 23 including available reference interval information for interpretation. Of these, five of 23 (22%) were increased whilst the remainder were within the reference interval. Coagulation profiles were not performed on all dogs with epistaxis. Where they were performed, PT and PTT were prolonged in three of 143 (2%) and seven of 141 (5%) of dogs respectively; none of 141 (0%) had an increase in both.

Diagnostic imaging modalities performed at the referral centres

Computed tomography (CT) was performed in 420 (88%), magnetic resonance imaging in 30 (6%) and skull radiography in 16 (3%) of dogs. Eleven (2%) cases did not have imaging but had rhinoscopic visualisation of plaques and turbinate destruction.

Identification

Total cases entered into Castor

n=662

Incomplete records

n=3

Screening

Cases with complete records

n=659

Observation of fungal plaques

Eligibility

Fungal plaques seen
n=419

Fungal plaques not seen
n=240

Other differentials (e.g., neoplasia, foreign body, lymphoplasmacytic rhinitis) documented
n=8

No turbinate destruction
&
no evidence/evidence of sinus involvement
n=8/n=2
Turbinate destruction described but compatible clinical signs† not recorded
n=49

Included

Visual confirmation of fungal plaques

n=419

Turbinate destruction
OR
Sinus involvement
AND
Compatible clinical signs†
AND
Positive ancillary diagnostic testing
AND
Other differentials excluded

n=56

Turbinate destruction
OR
sinus involvement
AND
Compatible clinical signs†
AND
Negative ancillary   diagnostic testing
AND
other differentials excluded

n=117

Total included & excluded

n=419

n=56

n=117

Total excluded cases
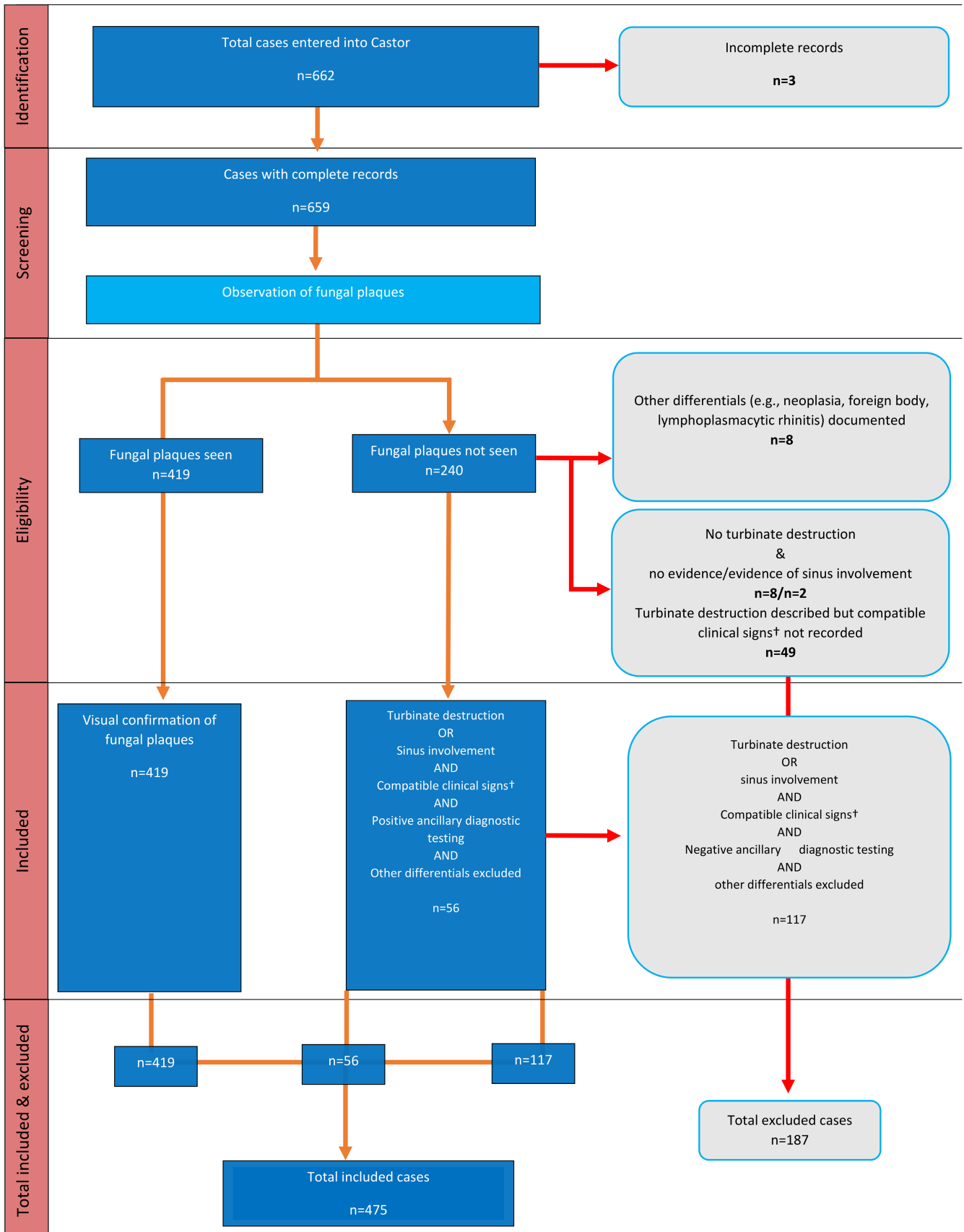n=187

Total included cases

n=475

FIG 1. Flow diagram showing the identification, screening and selection of cases. †Clinical signs mucopurulent/haemorrhagic/serous/serosanguinous nasal discharge, nasal depigmentation or facial pain

Table 1. Summary of clinical signs and physical examination findings at initial visit to the veterinary referral centre in 475 dogs diagnosed with sinonasal aspergillosis

Recorded findings	All cases, *n/N* (%)
Clinical signs	
Nasal discharge	406/464 (87)
Unilateral nasal discharge	268/390 (69)
Bilateral nasal discharge	111/390 (28)
Nature of discharge	
a. Mucopurulent	209/386 (54)
b. Serous	72/386 (19)
c. Serosanguinous	49/386 (13)
d. Haemorrhagic	38/386 (10)
Sneezing	367/475 (77)
Epistaxis	292/461 (63)
Reverse sneezing	76/475 (16)
Snorting	56/475 (12)
Coughing	45/475 (9)
Physical examination	
Upper respiratory tract signs†	456/475 (96)
Lower respiratory tract signs‡	45/475 (9)
Regional lymphadenomegaly	169/380 (44)
Nasal pain and nasal planum ulceration/ depigmentation	188/475 (40)
Depressed mental state	46/475 (10)
Decreased nasal air flow	35/204 (17)
Increased nasal air flow	2/204 (1)
Epiphora	14/475 (3)
Facial deformity	14/400 (3)
Forebrain dysfunction (seizures, head pressing)	3/475 (1)

Denominator included in areas where valid responses were received; *n* denotes the number of samples, and *N* represents the number of samples in the cohort
†Upper respiratory tract signs defined as nasal discharge, sneezing and ocular discharge
‡Lower respiratory tract signs defined as coughing, lower adventitial lung sounds, including wheezing, and crackles

Common CT findings (Table S3) included destruction of the nasal turbinates 414 of 420 (99%), fluid and/or soft tissue opacities in the nasal passages 214 of 420 (51%), nasal septum/vomer destruction 101 of 420 (24%) and cribriform plate lysis two of 420 (1%). Nasal cavity involvement was detected in 402 of 409 (98%) cases of which 242 of 392 (62%) had concurrent frontal sinus involvement. Only one of 392 (<1%) dogs had frontal sinus involvement alone.

Nasal turbinate destruction was also the most common finding in dogs undergoing MRI and was seen in 29 of 30 (97%) dogs, with nasal septum/vomer destruction in seven of 30 (23%) and hyperostosis of nasal frontal bones in two of 30 (7%) of cases. Turbinate destruction was a commonly reported radiographic finding seen in 15 of 16 (94%) dogs, with sinus involvement suspected in eight of 13 (62%) dogs that underwent skull radiography only.

Diagnostic tests performed at referral centres

Ancillary diagnostics were performed in 319 of 419 dogs (76%) in which fungal plaques were observed (Table 3). This included histopathology in 251 dogs (60%), fungal culture in 223 (53%), serology in 56 (13%), cytology in 70 (17%) and PCR in seven (2%). In 224 (53%) dogs, more than one of these diagnostic tests was performed.

Where fungal culture was performed, sample type included fungal plaques 93 (41%), mucosal biopsies 80 (35%) and nasal swabs (unknown if focused or blind) 18 (8%); and sample type was not recorded in 32 (14%) cases. Fungal culture isolated *A. fumigatus* in 132 of 219 (60%) and was negative in 87 of 219 (40%) of the samples giving a test sensitivity of 59%. Positive fungal culture differed between sample type: 67 of 93 (72%) positive for fungal plaques, 11 of 18 (61%) for nasal swabs and 37 of 80 (46%) for mucosal biopsy.

Table 3 describes the comparative performance of each diagnostic test. In some instances, a combination of tests was run in the same patient. Sensitivity of combined testing is displayed in Table 4.

Table 2. Median and interquartile ranges of recorded haematology (*n*=320) and biochemistry (*n*=303) parameters in 475 dogs diagnosed with sinonasal aspergillosis

Outcome	*n*	Median (IQR)	Decreased		Within reference		Increased	
			n (%)	Median (IQR)	*n* (%)	Median (IQR)	*n* (%)	Median (IQR)
Haematology								
Haematocrit (L/L)	317	43.5 (40.2 to 48.0)	43 (14)	33.7 (28.9 to 35.5)	267 (84)	44.3 (41.8 to 48.2)	7 (2)	56.6 (56.0 to 59.2)
Neutrophils (×10⁹/L)	312	8.3 (6.2 to 10.7)	1 (<1)	0.2	241 (79)	7.5 (5.7 to 9.2)	62 (20)	13.5 (12.3 to 16.0)
Platelets (×10⁹/L)	312	316 (253 to 396)	11 (4)	136 (91 to 173)	263 (87)	312 (257 to 377)	29 (10)	535 (488 to 589)
WBC (×10⁹/L)	315	11.0 (8.6 to 14.6)	8 (3)	5.5 (4.9 to 5.8)	252 (82)	10.4 (8.5 to 13.0)	47 (15)	18.3 (16.6 to 21.2)
Biochemistry								
Albumin (g/L)	293	28.3 (25.6 to 31.0)	93 (32)	25.0 (23.0 to 27.2)	196 (67)	30.0 (27.0 to 32.0)	4 (1)	34.6 (29.9 to 53.9)
ALT (U/L)	298	36.3 (25.0 to 57.8)	8 (3)	16.5 (9.5 to 17.8)	228 (77)	34.0 (24.0 to 47.7)	60 (20)	126.5 (41.5 to 212.5)
ALP (U/L)	299	63.0 (40.0 to 114.0)	8 (3)	20.0 (16.2 to 21.0)	212 (71)	51.5 (35.2 to 79.0)	78 (26)	149.0 (99.3 to 417.8)
CRP (mg/L)	28	6.9 (1.7 to 11.9)	0 (0)	–	18 (78)	4.1 (1.0 to 7.0)	5 (22)	14.1 (11.1 to 78.3)
PCV (%)	54	42.5 (36.7 to 47.2)	0 (0)	–	1 (2)	19.0	53 (98)	43.0 (37.5 to 47.5)
PT (secs)	184	8.6 (7.3 to 12.8)	51 (29)	7.0 (6.3 to 7.4)	122 (69)	10.5 (8.2 to 14.5)	3 (2)	12.6 (12.5 to 17.8)
aPTT (secs)	182	14.9 (12.2 to 87.2)	54 (31)	12.5 (11.8 to 13.9)	113 (65)	21.8 (13.2 to 95.4)	7 (5)	27.0 (25.7 to 104.0)

ALT Alanine transaminase, ALP Alkaline phosphatase, CRP C-reactive protein, PCV Packed cell volume, PT Partial thromboplastin time, aPTT Activated partial thromboplastin time
The number and percentage of dogs falling within and outside the reference ranges, where provided, is given
Results captured are a combination of both external and internal blood performed

Table 3. Sensitivities of comparative diagnostic tests

Ancillary test	Positive (n)	Negative (n)	Equivocal (n)†	Sensitivity (%)	95% CI‡
Cytology	47	15	8	67	55 to 78
Direct smears§	7	4	4	47	21 to 73
Blind swabs§	0	2	0	0	–
Brush§	7	2	1	70	35 to 93
Squash§	19	4	1	79	58 to 93
Culture	132	87	3	59	53 to 66
Suspected fungal plaque§	67	26	0	72	62 to 81
Nasal swab§	11	7	0	61	36 to 83
Mucosal biopsy§	37	41	2	46	35 to 58
Serology	29	27	0	52	38 to 65
Histopathology	118	108	24	47	41 to 54
PCR	5	2	0	71	–

†Equivocal cases (final ancillary diagnostic provided not confirmatory but suggestive of SNA) included in the final analysis
‡Confidence interval not calculated for $n < 10$
§Excludes where more than one type of sample was taken

Table 4. Sensitivities of comparative diagnostic tests when used in combination

Histopathology	Fungal culture	Serology	Cytology	PCR	Positive	Negative	Equivocal	Sensitivity based on all results (95% CI)†
✓	✓				80	44	1	64 (55–72)
✓	✓		✓		19	6	0	76 (55–91)
✓	✓	✓			17	5	0	77 (55–92)
✓			✓		7	2	2	64 (31–89)

Combinations are arranged in decreasing order of frequency
†CI calculated when n greater than 10

Rhinoscopy and sinoscopy performed at referral centres

Rhinoscopy was performed in 447 of 475 dogs (94%), and the sinus was accessed via trephination in 90 of 268 (34%). Fungal plaques were seen in the nasal cavity in 385 of 434 dogs (89%) and within the sinus in 43 of 338 cases (13%). In all the latter cases, there were no cases where fungal plaques were documented in the sinuses but not in the nasal passages.

Rhinoscopic findings included destruction of turbinates in 333 of 349 (95%) of cases, which was categorised as mild in 37 of 274 (13%), moderate in 119 of 274 (43%) and severe in 118 of 274 (43%). Of the 37 dogs with mild turbinate destruction: 28 (76%) had histopathology, 23 (62%) fungal culture, five (14%) serology, four (11%) cytology and two (5%) PCR. Only four (11%) were recorded as having none of the above. Where recorded, unilateral destruction was observed in 137 of 194 (71%) of cases and bilateral destruction was observed in 57 of 219 (26%).

DISCUSSION

This multi-centre study reports clinical signs and diagnostic findings for 475 dogs with a diagnosis of SNA. The signalment is similar to that previously reported (Billen, Clercx, et al., 2009b; Billen, Peeters, et al., 2009a; Peeters & Clercx, 2007; Pomrantz et al., 2007; Sharman & Mansfield, 2012). Numerically higher proportions of Golden and Labrador Retrievers were seen in this study. This is however most likely a reflection of the popularity of these breeds in the United Kingdom; and calculations based around breed prevalence across the timeframe and referral clinics included were not performed. Overall, the results of this study further support that middle aged, dolichocephalic breeds are overrepresented in studies of canine SNA (Gatherton, 2020). Male dogs (67%) were also overrepresented, similar to previous reports (Peeters & Clercx, 2007; Sharman et al., 2010; Sharman & Mansfield, 2012).

Although National Health Service surveillance within the United Kingdom, documents seasonality in peak *Aspergillus* spp spore aerosolization over the last 5 to 10 years (Gatherton, 2020), there was no strong evidence of seasonality in the presentation of SNA within the described cohort. This may reflect the United Kingdom's temperate climate, or possibly a considerable time lag between initial infection and subsequent development of clinical signs.

Some cases had a very prolonged duration of clinical signs prior to referral likely reflecting the variable severity of presentation (and variable owner tolerance of these signs) (Ettinger et al., 2017; Lobetti, 2009; Meler et al., 2008; Peeters & Clercx, 2007; Sharman et al., 2010; Sharman & Mansfield, 2012). The presenting and physical examination findings were similar to what has been previously reported (Ettinger et al., 2017; Sykes, 2013). In contrast to previous studies where increased airflow has been documented (Peeters & Clercx, 2007; Sharman & Mansfield, 2012) (coupled with the strong degree of subjectivity surrounding this clinical sign), decreased nasal airflow was more frequently seen in this study across different referral centres and clinicians (decreased airflow recorded in 16 of 23 referral centres). Accumulation of mucopurulent nasal discharge may obstruct airflow and should not decrease the index of suspicion

of SNA. Similarly, subtle clinical signs, such as nasal depigmentation or hyperkeratosis, may not be consistently recorded in the clinical notes, thereby underestimating their true prevalence. Although nasal depigmentation is considered a specific clinical finding, as reported previously (Peeters & Clercx, 2007; Sharman & Mansfield, 2012), less than 50% in this population had depigmentation, which could be attributed to underreporting.

Common CT findings affecting the nasal cavities included turbinate destruction accompanying the accumulation of fluid and soft tissue density material in the nasal passages. The percentage of dogs with destruction/damage to the nasal septum (24%) and sinus involvement (62%) in this cohort was less than the 54% and 80% respectively previously reported (Saunders et al., 2002, 2004; Saunders & van Bree, 2003) (Table S3). This variation in turbinate destruction may reflect pathogenicity differences between *Aspergillus* species and lineages, improved detection with increased availability of advanced imaging, and/or earlier detection of infection in the United Kingdom than that reported elsewhere (particularly in Australia and Europe). Conversely, cribriform plate lysis was recognised in 1% within this study, which is similar to previous reports (Saunders et al., 2002, 2004; Saunders & van Bree, 2003).

SNA is a focal disease process, highlighting the importance of direct assessment measures. However, as the required equipment (CT/MRI and rhinoscopy) may not always be widely available outside of referral practice, there is a need for reliable and accessible adjunctive diagnostic tests. Although a positive association between the ancillary test and a diagnosis was established in dogs with observed plaques, none of the tests evaluated had a sensitivity over 80%. Given that in this study a diagnosis of SNA was made at a referral level, where one might expect increased access to appropriate equipment and a higher experience level, the disappointing sensitivity of some ancillary diagnostics is considered to more likely reflect the challenges of acquiring optimal sample material and the limitations of the diagnostic tests themselves.

The sensitivities of many ancillary diagnostics in dogs with observed plaques were lower than has been reported elsewhere (Billen, Peeters, et al., 2009a; Peeters et al., 2005; Pomrantz et al., 2007; Pomrantz & Johnson, 2010; Sharman & Mansfield, 2012). There were lower yields of brush (70%) and squash (79%) samples for detection of fungal elements in this study, compared to that reported previously (93.3% to 100%) (Peeters et al., 2005). Although, it is worth noting that these samples yielded a higher diagnostic accuracy when compared to the sensitivity of cytology of nasal exudate of 47% and blind endonasal swabs with a sensitivity of 0% and therefore remain the preferred sample type. In contrast to previous reports, fungal mucosal biopsies sensitivity was 46%, which also had a lower diagnostic accuracy when compared to plaques and endonasal swabs for culture.

Dogs with neither observed plaques nor a positive ancillary test result were excluded from the main analysis despite presenting compatible clinical signs, evidence of turbinate destruction and having had other potential diagnoses excluded. All these cases had been managed as SNA at their respective referral institution. It is likely that some (even many) of these dogs had

SNA, but a definitive diagnosis could not be confirmed. This finding further highlights the diagnostic uncertainty associated with this condition. Lymphoplasmacytic rhinitis is considered a diagnosis of exclusion but can also result in focal turbinate destruction (Windsor et al., 2004) and may account for some of these dogs.

Given the nature of this study, as a retrospective case series across multiple centres, some differences in test sensitivity compared to previous studies may be reflective of the heterogenous array of centres from which data were collected, compared to that obtained in prospective and controlled studies where a single (or few) investigator(s) are responsible for sample collection. The retrospective nature of this study also meant that determination of sample technique may be incorrectly recorded. Other factors that were not standardised, include sample handling and transport, and laboratory methodology. Unfortunately, this information, and the level of operator experience and compliance with protocols to ensure sample optimisation, were not available.

Overall, this study indicates that ancillary tests should be used in combination with other findings to support a diagnosis of SNA in a dog with compatible clinical signs. Sensitivity of ancillary testing in this study was lower than has been reported elsewhere. This data highlights the importance of optimal sampling technique (sample collection, handling, submission and interpretation).

This study has limitations inherent to its retrospective nature as already outlined. Furthermore, the lack of a standardised scoring system to describe imaging and rhinoscopy findings including the degree of turbinate destruction may have influenced our findings although the impact is difficult to estimate. Records may have been incomplete, and an absence of reporting may contribute to an underestimation of the frequency of certain physical examination findings. Reliance on visual identification of fungal plaques may also be questioned. Inexperienced clinicians may misdiagnose accumulations of mucopurulent exudates as fungal plaques leading to false positive diagnosis introducing a possible type II error.

Information relating to the type of serological test undertaken at each lab, *i.e.* agar gel double immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA) and counterimmunoelectrophoresis, was not available. Although AGID remains the most commonly used serological test, previously reported sensitivities of these tests have varied between 81% to 100% (Billen, Peeters, et al., 2009a; Johnson et al., 2006; Pomrantz et al., 2007). Information relating to whether recombinant antigen or home-made antigen solutions were used was unknown. Details of the PCR assays (real time or conventional) used and whether they were directed against different *Aspergillus* species or subtypes were not obtained in this study. Further information on the fungal identification based on culture growth, types of targeted PCR and serological tests undertaken may have improved overall diagnostic utility of these tests in this study. Some tests utilised within this cohort may have been inferior than others (due to the methodology employed).

This study provides a national view of the clinical characteristics of SNA in dogs within the United Kingdom and the utility of

commonly employed ancillary diagnostics within referral centres. Overall, the sensitivities of the ancillary diagnostics performed in these dogs with observed fungal plaques were lower than have been reported previously. However, the true diagnostic sensitivity of each test in this study may have been underestimated because plaque visualisation may have resulted in fewer ancillary tests done. Treatment and management decisions should be based on a combination of diagnostics including imaging findings, visual inspection and ancillary testing, rather than ancillary tests alone.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Author contributions

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author (CP).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Details and counts of techniques used to grow sinonasal aspergillosis in the United Kingdom laboratories used in this study.

Table S2. Breeds affected with sinonasal aspergillosis.

Table S3. Computed tomography (CT) image findings from included dogs compared to previous imaging studies.