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A retrospective multi-center study of treatment, outcome, and prognostic factors in 34 dogs with disseminated aspergillosis in Australia

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Abstract

Background: Disseminated aspergillosis (DA) in dogs has a guarded prognosis and there is a lack of a gold standard treatment protocol.

Objective: To retrospectively assess survival times and factors influencing survival times.

Animals: Dogs diagnosed with DA from January 2007 to June 2017.

Methods: Disseminated aspergillosis case data were retrieved from 13 Australian veterinary referral centers, with a diagnosis confirmed with culture or PCR. Factors influencing survival time after diagnosis were quantified using a Cox proportional hazards regression model.

Results: Thirty-four dogs met the study inclusion criteria. Twenty-two dogs were treated with antifungal treatment and 12 dogs received no antifungal treatment. Accounting for censoring of dogs that were either still alive on the date of data collection or were loss to follow-up, dogs treated with itraconazole alone (n = 8) had a

median survival time (MST) of 63 (95% CI: 20−272) days compared to 830 (95% CI: 267-1259) days for the n = 14 dogs that received multimodal antifungal therapy (χ^2 test statistic 8.6; df = 1; P < .01). The daily hazard of death (DHOD) for dogs with abnormally high serum creatinine concentration at the time of diagnosis was 7.4 (95% CI: 1.9-29) times that of dogs with serum creatinine within the reference interval.

Conclusion and Clinical Importance: Serum creatinine concentration at the time of diagnosis is a useful prognostic indicator for survival after a diagnosis of DA. The MST for dogs treated with multimodal antifungal therapy is longer than itraconazole alone and warrant further investigation (P < .01).

KEYWORDS
canine, median survival time, outcome, prognosis

1 | INTRODUCTION

Aspergillus is a ubiquitous fungus that can cause a wide spectrum of diseases in dogs, including sinonasal, bronchopulmonary and systemic syndromes.[1] *Aspergillus terreus* is commonly reported as the causative agent of disseminated aspergillosis (DA) and *A. fumigatus* of sinonasal or bronchopulmonary aspergillosis in dogs.[2-4]

Disseminated aspergillosis in dogs is associated with low rates of clinical cure, high rates of relapse, and a high case fatality rate.[3,5] The largest series of dogs with DA reported to date is a 17-year retrospective study (1990-2007) of 30 cases from a single institution in the United States.[3] In that study, 57% of dogs were euthanized within 7 days of diagnosis.[3] As such, the optimal management strategy for DA is not well defined and treatment is primarily based on the judgment of the attending veterinarian, anecdotal evidence, and the few available retrospective case series.

The most common clinical features of DA are osteomyelitis, discospondylitis, lymphadenopathy, nephritis, splenitis, and meningoencephalitis.[3,6] Over half of the reported cases are German shepherd dogs (GSDs), possibly associated with the breed's documented immunodeficiencies, in particular IgA dysfunction.[3,4,7-9] Furthermore, toll-like receptor polymorphism, which is a risk factor for invasive aspergillosis in humans, is associated with chronic enteropathies in GSDs.[10]

A definitive diagnosis of DA in dogs can be challenging to achieve as fungal cultures might not always be obtained depending on the degree of clinical suspicion for DA.[3] The correct identification of the fungus is central to ensure appropriate treatment, as some fungi are intrinsically resistant to some drugs. For example, *A. terreus* is intrinsically resistant to amphotericin B.[11]

In published reports of DA, single agent itraconazole is the most common treatment administered to dogs with overall poor efficacy and survival beyond 12 months is rare.[3,4,12] One recent small study identified a median survival time (MST) of 241 days with posaconazole in 9 dogs, with 3 dogs lived >1 year after starting treatment.[5] Despite these reported outcomes, it should be noted that newer antifungals, such as poscaonazole, are expensive and there is a lack of prospective, randomized clinical trials data to objectively compare different treatments.

With this background, our hypothesis was that efficacy of treatment of DA has increased with the utilization of multimodal antifungal therapy and the increasing use of newer antifungal agents than itraconazole. The aims of this study were to: (a) review the clinical data of dogs diagnosed with DA in Australia over the last 10 years, (b) document medical therapies used to treat DA cases, and (c) identify factors associated with efficacy of treatment of DA after diagnosis.

2 | MATERIALS AND METHODS

An invitation to participate in the study was distributed to veterinary referral centers across Australia to gather medical records of dogs with DA diagnosed between January 2007 and June 2017. One nominated veterinarian from each participating center was asked to complete an online questionnaire using REDCap.[13] Veterinarians were asked to provide de-identified details of dogs diagnosed with DA at their hospital for the period of 1 January 2007 to 30 June 2017 (inclusive). The following search terms were used to identify eligible cases using each hospital's digital medical record database: "Asperg*" and "dog" with "galactomannan," "galact*," "PCR," or "culture." Clinical records for each identified case were reviewed by the primary author (AL) to confirm that the diagnosis of DA was consistent with the case definition developed for this study. The case definition for DA comprised identification of organisms consistent with filamentous fungal hyphae on cytologic or histologic specimens with concurrent clinical signs and consistent imaging findings along with a positive culture or PCR for *Aspergillus* from blood, urine, or an affected sterile site.

The following information was retrieved from the clinical records for each confirmed case: signalment, date of birth, sex, breed, reproductive status, clinical signs, clinical findings on initial presentation at the time of referral, clinicopathologic and diagnostic findings, date of

commencement of administration, and details of each antifungal treatment prescribed and outcome.

Informed client consent for privacy and personal data use was not obtained as there was no direct contact with owners and no client information was shared with the study investigators.

2.1 | Statistical analyses

Our objectives were to identify clinical signs and physical findings as well as hematologic and biochemical measures recorded at the time of diagnosis that were associated with survival time after a diagnosis of DA. The data were organized in a counting process format where start and end dates for each treatment episode, defined as the date on which a given treatment regimen started and the date on which the same treatment regimen ended (respectively), were recorded for each dog.[14] The outcome of interest was the number of days between the date on which DA was diagnosed and the date on which the dog died, either due to DA or on request of the owners to euthanize the dog for DA-related reasons.

Treatment episodes were coded as censored observations if the dog was alive at the end of the treatment episode or the dog had died for a reason not related to DA. For each treatment episode, a 1 or 0 dichotomous variable was used to indicate the presence or absence of use of a given drug. This approach allowed us to take account of changes to the antifungal treatment regimen of individual dogs over time as well as the concurrent use of >1 antifungal agent.

The selection of variables hypothesized to influence time to DA-related death was based on previous data describing DA.[3,15,16] Nine non-therapeutic explanatory variables were considered: (a) age (in years) at first presentation, (b) administration of prednisolone at any time before presentation, (c) breed (GSD, non-GSD), (d) rectal temperature >39.1°C at first presentation, (e) blood urea concentration greater than the upper limit of the reference interval (RI) at the time of diagnosis, (f) serum creatinine concentration greater than the upper limit of the RI at the time of diagnosis; (g) absence of well concentrated urine (urine specific gravity [USG] <1.030) at the time of diagnosis, (h) serum albumin concentration less than the lower limit of the RI at the time of diagnosis, and (i) a positive culture or PCR of *A. terreus* complex species from tissues sampled at any time from the date of initial presentation to the date of diagnosis.

The following therapeutic explanatory variables were considered: (a) combination antifungal therapy, (b) adjunctive antifungal therapy (treatment used together with primary antifungal therapy) with terbinafine, (c) no therapy, (d) amphotericin B, (e) itraconazole, (f) posaconazole, and (g) voriconazole. To avoid loss of statistical power that would have arisen from removal of records from the data set where data for a given explanatory variable was missing, we used the indicator method to create a separate "missing value" category for dogs with no value recorded for serum creatinine or urea concentration at the time of diagnosis.[16]

Candidate explanatory variables were tested for their association with time to DA-related death using univariable Cox proportional hazard regression models and Kaplan-Meier survival curves.[17,18] Median survival times were calculated as the smallest survival interval for which the Kaplan-Meier survival function was less than or equal to 0.5 with confidence intervals (CIs) estimated (where appropriate) using the method of Peto (1973) and Rothman (1978).[14,19,20] Explanatory variables were selected for multivariable modeling if the P-value from the univariable Cox model was <0.2. A multivariable Cox proportional hazards model was then developed to quantify factors influencing time to DA-related death after diagnosis using a backwards stepwise selection procedure. Explanatory variables were removed from the model 1 at a time in order of decreasing statistical significance and re-fitting the model. The effect of possible confounders was assessed by comparing models with and without the explanatory variable of interest. An explanatory variable was included in the model if its inclusion changed the regression coefficients of 1 or more of the other explanatory variables by more than 20%.

Analyses were carried out to identify the presence of biologically plausible interactions between each of the explanatory variables included in the final model. Interaction terms were included in the final multivariable model if they were statistically significant and if they significantly altered model fit as measured by the likelihood ratio test.

The assumption of proportionality of hazards was assessed both globally and, for each explanatory variable, using the weighted residual method.[21] The proportional hazards assumption was further investigated for each explanatory variable by plotting the scaled Schoenfeld residuals for each dog as a function of DA diagnosis to death interval. The proportional hazards assumption was judged to have not been met if a line of gradient zero could not be drawn between the 95% CIs of a lowest-smoothed line of best fit for each of these plots.[22] Analyses were conducted using the contributed survival package in R.[22,23]

3 | RESULTS

A total of 1454 dogs from 4 veterinary referral centers initially met the search criteria as eligible cases. Of this group, 13 fulfilled the inclusion criteria and 1441 were excluded because they had sinonasal aspergillosis, there were no confirmatory tests, fungal species other than *Aspergillus* were identified on culture or they were not dogs (Figure 1). Nine additional centers identified 21 cases, but the breakdown of screening for each center was not available. Hence, a total of 34 dogs from 3 veterinary teaching hospitals and 10 private referral centers met the criteria to be classified as a confirmed case of DA. The geographical distribution of the veterinary centers was as follows, with the total number of dogs shown in parentheses: 2 centers in Queensland (n = 4), 3 in New South Wales (n = 5), 4 in Victoria (n = 10), 1 in South Australia (n = 5), and 3 in Western Australia (n = 10).

The most common pure-breeds affected were GSD (n = 19, 56%) and vizsla (n = 2, 6%) and there were 4 mixed breed dogs (12%, including 1 GSD crossbred). A total of 19 dogs were female (16 of which were neutered) and 15 were male (9 of which were neutered).

FIGURE 1 Flow diagram for inclusion of cases for analysis
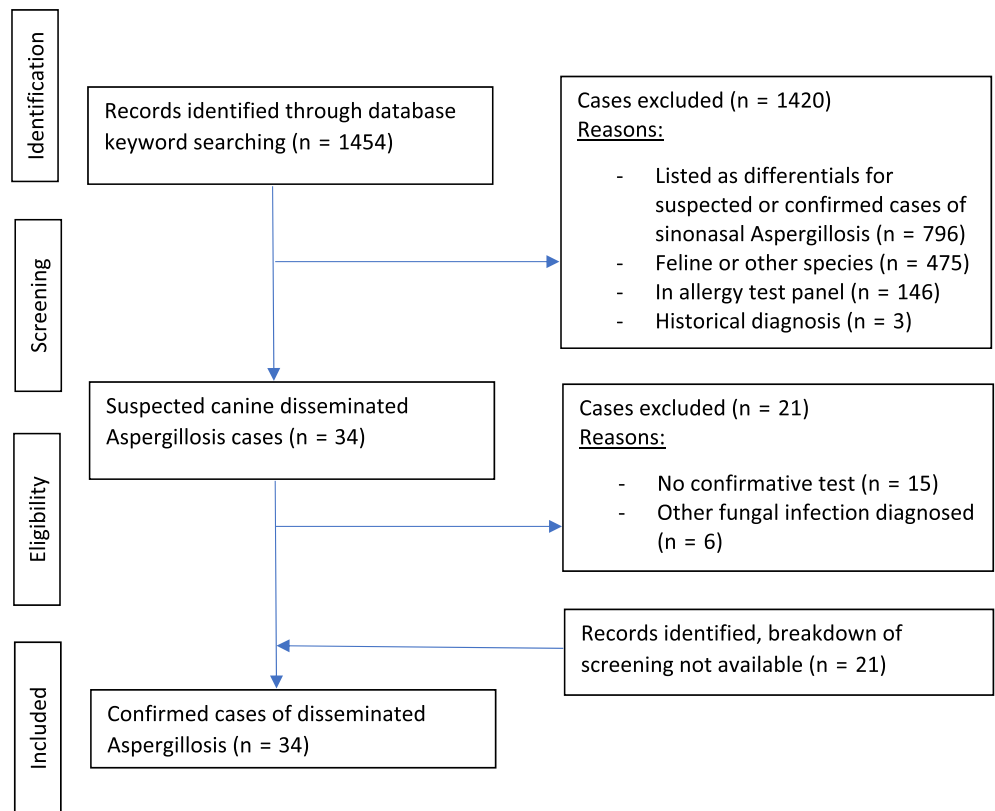
The median age at diagnosis was 3.5 (range, 1-10) years. Median bodyweight at the time of diagnosis was 31 (range, 4.7-48) kg.

the time of first presentation with a median temperature of 39.5°C (range, 39.1-40.2).

3.1 | Clinical findings

The duration of clinical signs before diagnosis was variable, with a median of 40 (range, 4-611) days. Therapeutic intervention before referral was reported for 19 dogs; treatments included antimicrobials (n = 16), glucocorticoids (n = 8), non-steroidal anti-inflammatory drugs (n = 7), and analgesic medications (n = 4).

Clinical signs were reported for all dogs and are summarized in Table 1. The most common clinical signs were lethargy or weakness (n = 20, 59%), musculoskeletal lameness (n = 18, 53%), and weight loss or inappetence (n = 13, 38%). Less frequent clinical signs included signs of urinary disease (polyuria, polydipsia, urinary incontinence, or any or all these signs), signs of gastrointestinal disease (vomiting, diarrhea, or both signs), neurological deficits (paresis, paralysis, ataxia, signs of vestibular disease, or any or all these signs), vision impairment, or signs of respiratory disease (coughing, exercise intolerance or both signs).

Physical examination findings were reported for all dogs and are summarized in Table 1. Physical examination findings were closely aligned with the clinical signs (paresis/paralysis, ataxia, uveitis, respiratory disease and cardiac arrhythmia or murmur) along with musculoskeletal pain (n = 24, 71%) and peripheral lymphadenomegaly (n = 8, 24%). Twelve (35%) dogs had a temperature of 39.1°C or higher at

3.2 | Diagnostic findings

A summary of hematologic (n = 26) and serum biochemical data (n = 28) is presented in Table 2. Twenty dogs had both serum creatinine and USG measured. Eleven of the 20 dogs were non-azotemic. Of the 11 non-azotemic dogs, 2 had well-concentrated urine (USG >1.030), 6 had moderately concentrated urine (USG 1.013-1.029), and 3 were isosthenuric (USG 1.008-1.012). The remaining 9 dogs were azotemic. Five of them had moderately concentrated urine and 2 each were isosthenuric and hyposthenuric (USG < 1.008). Urine sediment was reported for 24 dogs with 16 dogs (67%) having pyuria.

Most dogs (n = 31, 91%) had diagnostic imaging performed, with findings summarized in Table S1. This included thoracic radiographs (n = 10), cervical/spinal radiographs (n = 10), appendicular skeletal radiographs (n = 7), abdominal radiographs (n = 1), abdominal ultrasound (n = 15), computed tomography (n = 10), and magnetic resonance imaging (n = 2).

There were 35 positive cultures for *Aspergillus* spp. obtained from 33 dogs (2 dogs had a positive culture from urine and tissue samples); 20 dogs had a positive culture from the urine and 14 from tissue samples (specimen used for fungal culture was not specified in 1 dog, Table 3). Fourteen dogs had fine needle aspiration and 10 dogs had biopsy of soft tissue or bony lesions submitted, with pyogranulomatous

TABLE 1 Presenting clinical signs and physical findings at the time of initial presentation in 34 dogs diagnosed with disseminated aspergillosis

Observation	n	%
Clinical signs		
Lethargy/weakness	20	59
Musculoskeletal lameness	18	53
Weight loss/inappetence	13	38
Polyuria/polydipsia/urinary incontinence	8	24
Vomiting or diarrhea	4	12
Paresis or paralysis	4	12
Ataxia/signs of vestibular disease	3	9
Vision deficits	2	6
Signs of respiratory disease[a]	2	6
Physical examination		
Musculoskeletal/neck/spinal pain	24	71
Temperature >39.1°C	12	35
Peripheral lymphadenomegaly	8	24
Paresis or paralysis	6	18
Ataxia	7	21
Uveitis	4	12
Signs of respiratory disease[a]	2	6
Cardiac arrhythmia/murmur	1	3

[a]Signs of respiratory disease reported in 1 dog included coughing, reduced exercise intolerance, and mild increase in lower respiratory sounds with no wheezes or crackles. The respiratory signs of the other dog included coughing and panting without abnormality on thoracic auscultation and no evidence of dyspnea.

inflammation and fungal hyphae present in 11 and 7 samples, respectively (3 histopathology reports were unavailable for review). One dog had a negative culture for *Aspergillus* spp., but a positive PCR test for *Aspergillus*. All 3 dogs tested for *Aspergillus* with PCR had a positive result from affected tissues. Five dogs were tested for serum galactomannan and 2 had a positive result.

Fungal species were identified in 31 dogs: 29 from fungal culture and 2 from positive *Aspergillus* PCR tests. *Aspergillus terreus* complex species (n = 24) was the most frequent species identified followed by *A. deflectus* (n = 4) with 1 case each of *A. fumigatus*, *A. puulaauensis*, and *A. alabamensis*.

3.3 | Treatment

Of the 34 dogs included in the study, 22 dogs were treated of which 8 were either still alive on the date of data collection or were lost to follow-up. Twelve dogs received no specific treatment of which 6 were either still alive on the date of data collection or were lost to follow-up. Eleven dogs were prescribed a single antifungal agent at the time of diagnosis, 11 dogs received combination antifungal

therapy with 2 (n = 9) and 3 (n = 2) antifungal agents, respectively (Table 3).

The most commonly used initial antifungal therapeutic was itraconazole (n = 20), which was used as the single agent in 9 dogs at diagnosis and as sole treatment throughout the study for 8 dogs. Terbinafine was used as a second antifungal agent or additional antifungal agent for 11 dogs. The median dose of itraconazole was 6.8 (range, 2.5-16.2) mg/kg/day and terbinafine was 15.4 (range, 10.0-60.6) mg/kg/day. Seven of the 22 treated dogs had additional antifungal agents added to their therapy after commencement of initial antifungal treatment. Reasons for commencement of an additional antifungal agent included adverse effects of the previous treatment (n = 3), disease progression (n = 3), or relapse of the disease (n = 2) with 1 dog having 2 reasons listed. Five dogs had a single agent added (posaconazole in 1 dog, terbinafine in 2 dogs, and voriconazole in 2 dogs), 1 dog had 2 agents added (terbinafine and voriconazole), and 1 dog had 3 agents added (amphotericin B, posaconazole, and terbinafine). Adverse clinicopathologic signs reported after antifungal treatment include inappetence, an abnormally high serum ALT activity and development of targetoid skin lesions. For the 3 dogs with reported adverse drug reactions, 1 had been treated with itraconazole and 2 with a combination of itraconazole and terbinafine. One of the dogs received compounded itraconazole and terbinafine combination.

One non-GSD dog (a vizsla) that was diagnosed with presumptive discospondylitis and pyelonephritis (*A. terreus* complex species) had multi-modal treatment with surgical debridement (disc space and unilateral nephrectomy), intrathecal treatment (amphotericin and voriconazole), and oral antifungal agents (itraconazole, voriconazole, and terbinafine) for 14 months. This treatment resulted in prolonged clinical improvement with a disease-free interval of 4 years. This dog had a clinical relapse, but remission was achieved with reinstitution of oral voriconazole and terbinafine. This dog was alive 6.5 years after the date of initial diagnosis, as recorded at the date of last follow-up.

3.4 | Survival analyses

Figure 2 is a Kaplan-Meier survival curve showing the cumulative proportion of dogs still alive as a function of the number of days since diagnosis. Median survival time was 226 (97 to 1259) days for untreated dogs and 531 (95% CI: 174-898) days for treated dogs (χ^2 test statistic 0.1; df = 1; P = .80). Three dogs were still alive at the time of data collection, 1 died, 19 were euthanized and 11 were lost to follow-up. No cause of death was recorded for the dog that died naturally. Reasons for euthanasia included progression of DA in 13 dogs, poor prognosis in 9 dogs, and presumptive cause unrelated to DA in 1 dog (refractory congestive heart failure secondary to mitral valve disease) with some dogs having several reasons listed.

Median survival time of dogs receiving itraconazole only (n = 8) was 63 (95% CI: 20-272) days compared with 830 (95% CI: 267-1259) days for dogs prescribed other treatment regimens (n = 14). Differences in survival times for these 3 groups were

TABLE 2　Counts (and percentages shown in brackets) of dogs with disseminated Aspergillosis (n = 34) with hematology (n = 26) and biochemistry (n = 28) measured at the time of initial presentation

Outcome	Decreased	Within reference	Increased
Hematology			
Hematocrit (L/L)	1 (4)	23 (88)	2 (8)
Neutrophils ($\times 10^9$/L)	1 (4)	16 (62)	9 (34)
Monocytes ($\times 10^9$/L)	0 (0)	16 (62)	10 (38)
Lymphocytes ($\times 10^9$/L)	0 (0)	19 (73)	7 (27)
Eosinophils ($\times 10^9$/L)	1 (4)	19 (73)	6 (23)
Platelets ($\times 10^9$/L)	3 (12)	22 (84)	1 (4)
Biochemistry			
Globulin (g/L)	0 (0)	14 (50)	14 (50)
Urea (mmol/L)	2 (7)	13 (46)	13 (47)
Creatinine (μmol/L)	0 (0)	18 (64)	10 (36)
Calcium (mmol/L)	2 (7)	23 (82)	3 (11)
Phosphorus (mmol/L)	0 (0)	22 (79)	6 (21)
Albumin (g/L)	3 (11)	23 (82)	2 (7)
ALT (U/L)	2 (7)	24 (86)	2 (7)
ALP (U/L)	2 (7)	24 (86)	2 (7)
Bilirubin (μmol/L)	0 (0)	28 (100)	0 (0)

TABLE 3　Numbers of dogs and antifungal combinations used at the time of diagnosis of disseminated aspergillosis

Antifungal	Number of antifungal agents administered at diagnosis						
	One		Two		Three		
Itraconazole	+		+	+	+	+	
Amphotericin B				+	+	+	+
Posaconazole					+		+
Terbinafine			+			+	+
Voriconazole		+					
Total of dogs	10	1	5	3	1	1	1

statistically significantly different at the alpha level of .05 (χ^2 test statistic 8.6 df = 1; $P < .01$).

Seven of the 16 candidate explanatory variables assessed were associated with survival with a *P*-value of <.2 on univariable analyses. These included age at first presentation, breed, temperature >39.1°C, urea concentration at the time of diagnosis, serum creatinine concentration at the time of diagnosis, treatment with itraconazole, and treatment with terbinafine. Three explanatory variables were retained in the final Cox proportional hazards regression model (Table 4): age at first presentation, serum creatinine concentration at diagnosis and treatment (a categorical variable comprised of 3 levels: no treatment, itraconazole only and other drug combinations). Age at first presentation was retained in the final model (regardless of its statistical significance) because a priori it was believed to confound the association between observations and measurements recorded at the time of diagnosis and survival time.

The daily hazard of death (DHOD) for dogs with abnormally high serum creatinine concentration at the time of diagnosis was 7.4 (95% CI: 1.9-29) times that of dogs with normal serum creatinine concentration (Table 4, Figure 3). The DHOD for dogs treated with itraconazole only was 3.8 (95% CI: 0.9-16) times that of dogs receiving no treatment. The DHOD for dogs receiving other antifungal agents (eg, amphotericin B, posaconazole, terbinafine, voriconazole with or without itraconazole) was 0.7 (95% CI: 0.20-2.3) times that of dogs receiving no treatment. Analyses were repeated removing 2 dogs that had survived longer than 3.5 years after diagnosis. This produced no marked change in the ranking of the estimated hazard ratios providing reassurance that inclusion of these longer-lived dogs did not markedly bias our estimates of the effect of age, serum creatinine concentration at diagnosis, and treatment on survival after diagnosis.
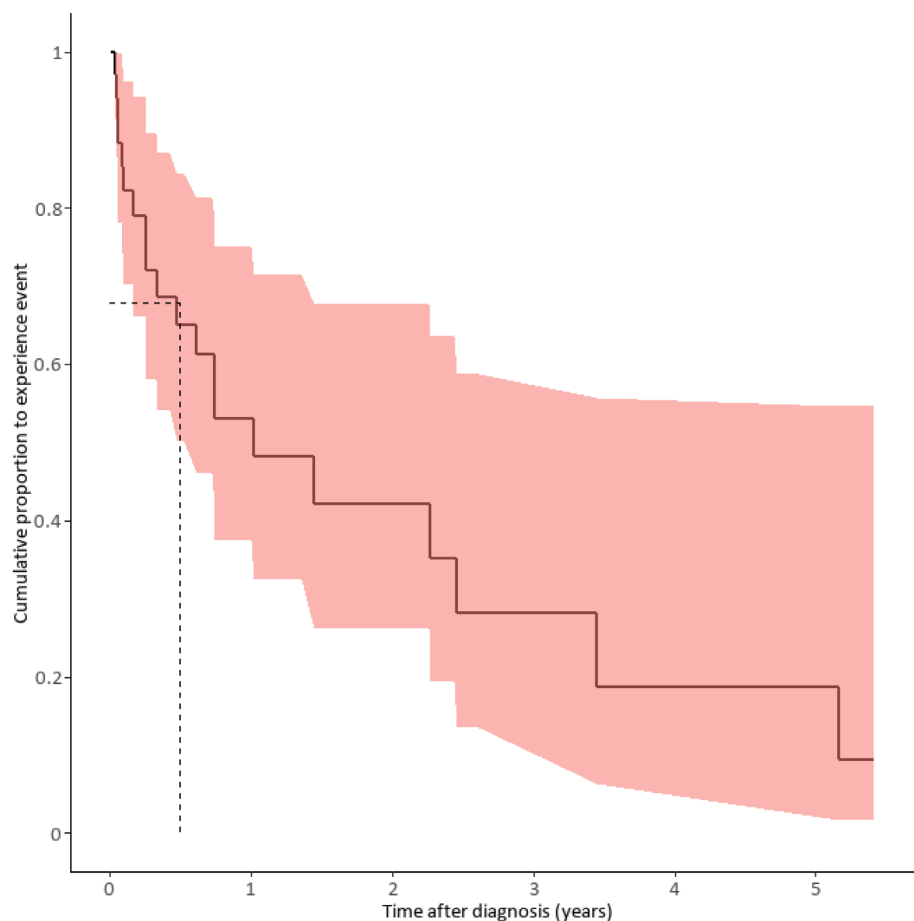
FIGURE 2 Kaplan–Meier survival curve showing the cumulative proportion of dogs that survived as a function of the number of years after diagnosis of disseminated aspergillosis. The shaded area shows the 95% confidence interval around each survival estimate. Across the whole data set, the proportion of dogs surviving 6 months after the date of diagnosis was 0.68 (95% CI: 0.50-0.84), as shown by the dashed vertical line

TABLE 4 Estimated regression coefficients and their SEs from a Cox proportional hazards regression model of factors associated with survival in dogs after a diagnosis of disseminated aspergillosis

| Explanatory variable	At risk	Events	Coefficient (SE)	P	Hazard ratio (95% CI)
Age at diagnosis (years)	34	20	0.0344 (0.1331)	.80	1.0 (0.8-1.3)
Serum creatinine concentration at time of diagnosis					
Normal	17	7	Reference		1.00
Abnormally high	10	8	2.004 (0.7012)	<.01	7.4 (1.9-29)[a]
Missing	7	5	0.6969 (0.6451)	.28	2.0 (0.6-7.1)
Treatment					
No antifungal	12	6	Reference		1.00
Itraconazole only	8	6	1.3459 (0.7149)	.06	3.8 (0.9-16)
Other drug combinations	14	8	−0.3841 (0.6281)	.54	0.7 (0.2-2.3)

Abbreviation: CI, confidence interval.

[a]Interpretation: For dogs with an abnormally high serum creatinine concentration at the time of diagnosis the daily hazard of death was increased by a factor of 7.4 (95% CI: 1.9-29) compared with dogs with normal serum creatinine concentration at the time of diagnosis.

4 | DISCUSSION

This retrospective study reports clinical signs, diagnostic findings, treatment regimens, and survival outcomes for 22 dogs with a confirmed diagnosis of DA. Factors associated with shorter survival times after DA diagnosis included an abnormally high serum creatinine concentration at the time of diagnosis and treatment with itraconazole as a single agent.

Median survival time after diagnosis was 531 days for treated dogs (regardless of treatment) and 8 of these dogs (36%) survived more than 12 months, which is longer than previously reported. Although MSTs were not reported in a previous retrospective study of Californian dogs, 13 dogs out of 30 were treated, 11 dogs were euthanized, or lost to follow-up within 2 to 270 days with only 2 (15%) dogs surviving more than a year, consistent with an MST of

FIGURE 3 Kaplan-Meier survival curves showing the cumulative proportion of dogs that survived as a function of the number of years after diagnosis of disseminated aspergillosis, stratified by serum creatinine concentration at the time of diagnosis
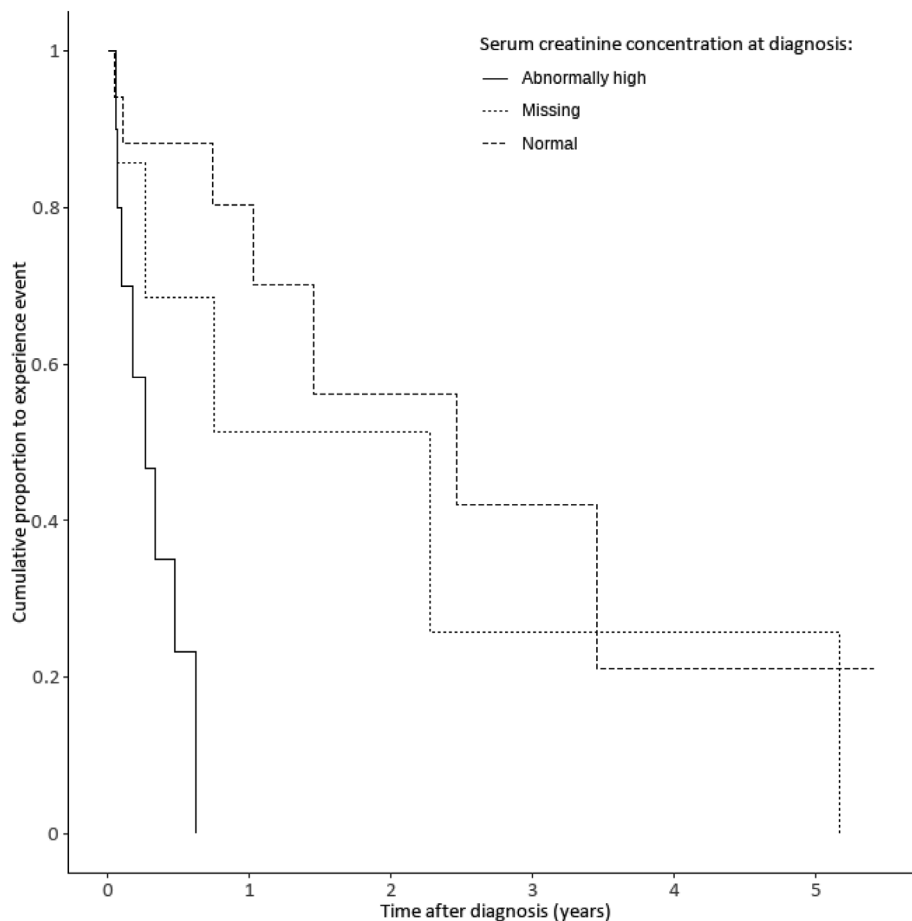
less than 90 days in treated dogs.[3] Seventeen (57%) dogs out of 30 in that study did not receive antifungal treatment.[3] These findings contrast with this study where only 12 dogs (35%) received no specific antifungal treatment of which 6 were either still alive on the date of data collection or were lost to follow-up.

Historically, itraconazole has been the antifungal agent of choice to treat DA in dogs.[3,12] Although the majority of dogs in our current study (20 of 22) were treated with itraconazole, it was used as a single agent treatment in only 8 dogs. Dogs treated with itraconazole only had a significant shorter MST (63 days) than the remaining treated dogs (830 days) and dogs that received no treatment at all (226 days). This shorter survival time for DA dogs treated with itraconazole only compared to dog without treatment is difficult to explain and is unlikely to be entirely due to differences in the stage of disease at the time of diagnosis. The species of *Aspergillus* might also have played a role as all dogs treated with itraconazole alone were infected with *A. terreus* complex whereas half of the untreated dogs were infected with *A. deflectus*, *A. fumigatus*, or had no species identification. Definitive inferences about the effectiveness (or otherwise) of itraconazole as a single therapy for DA should be made with caution given the small number of dogs (n = 8) that comprised this group. Our finding that therapies apart from itraconazole were associated with longer survival times compares favorably with findings of another study where dogs treated with posaconazole had a MST after diagnosis of

241 (range, 44-516) days.[5] While seemingly effective, at the time of writing treatment with posaconazole is likely to be cost-prohibitive for many pet owners, particularly those with large breed dogs.[5] In contrast to previous studies, half of the treated dogs in this study (n = 11) received terbinafine as adjunctive therapy. Terbinafine is an allylamine antifungal that acts via inhibition of squalene epoxidase, an enzyme involved in the synthesis of ergosterol, a component of the fungal cell membrane.[24] Use of antifungal agents with differing mechanisms of action such as terbinafine with azoles or amphotericin B has a potent synergistic interaction and fungicidal activity against *Aspergillus*.[25] The long-term survivors in our study, as well as several cases from other studies, received combination antifungal therapy with terbinafine, either concurrently or sequentially.[5,6]

The use of combination therapy might account for the difference in survival outcomes documented in our study and the study of Schultz et al.[3] Other possible reasons for these differences include differences in specific causative *Aspergillus* species, itraconazole dosage and itraconazole bioavailability (compounded medication or administration fasted/unfasted).[26-28] Evaluation of client compliance was not possible due to the retrospective nature of both studies.

Abnormally high serum creatinine concentration was a strong negative prognostic factor in our study (DHOD of 7.4; 95% CI: 1.9-29). The presence of abnormally high serum creatinine concentration might indicate the presence of fungal pyelonephritis, but definitive

diagnosis of pyelonephritis in these dogs could not be made due to the retrospective nature of our study. Consistent with the finding that abnormally high serum creatinine concentration is a negative prognostic factor in dogs, acute renal failure has been identified as a substantial risk factor for death in people with invasive aspergillosis.[29,30]

Similar to previous studies, *A. terreus* complex species was the most commonly isolated organism in our study samples and *A. terreus* is associated with the highest case fatality in people.[3,5,31] It is hypothesized that *A. terreus* can produce a specialized structure to form spores in the host, resulting in effective dissemination of the disease via hematogenous routes.[1,32] The detailed medical history of the dog with *A. deflectus* in our study was recently reported.[33] Despite the absence of antifungal treatment and the presence of azotemia at the time of diagnosis, this dog survived approximately 38 months from the date of diagnosis. Two additional dogs infected with *A. deflectus* and untreated were still alive at, respectively, 97 and 123 days after diagnosis. This raises the possibility of increased pathogenicity of *A. terreus complex species* compared to other *Aspergillus* species such as *A. deflectus*. Despite including the long-term survivor dog in the current study, serum creatinine concentration remains a strong negative prognostic indicator, meaning that the *Aspergillus* species might play a more substantial role in survival outcome.

As documented in published studies, some dogs in our study continued to have disease progression or experienced relapse despite being treated with antifungals.[3,5] In our study, only 8 out of the 32 positive fungal culture specimens were submitted for antifungal agent susceptibility testing and determination of minimum inhibitory concentrations. Although fungal susceptibility testing and antifungal therapeutic drug monitoring are becoming common in human medicine, to date, the susceptibility of *Aspergillus* species in dogs is not well established.[4,34] It is likely that, with acquisition of additional data in dogs, determining the *Aspergillus* species, antifungal susceptibility, and therapeutic drug monitoring will improve antifungal treatment efficacy and reduce the risk of treatment failures in dogs with DA.

4.1 | Limitations

This was a retrospective analysis of medical records collected across multiple centers and, as a result, this design might have inherently introduced several factors that might have negatively impacted on the external validity of our findings. These include case selection bias. Although the intended method of case screening was outlined and communicated to collaborators in each of the participating centers, it is possible that some cases of DA were not identified correctly using the documented database search terms and, for this reason, they were excluded from the study samples. The expected impact of this bias, if any, on our findings is difficult to estimate.

Reduced follow-up time for some cases and differences in treatments and dosages reduced the number of dogs in treatment groups and therefore limited statistical power to detect differences in survival

times. This limitation precluded gaining more information on the ideal treatment regimen for DA. Hence, the information on the ideal treatment regimen for DA remains limited, but current and previous data support that itraconazole should not be used as single antifungal treatment. Despite these limitations, abnormally high serum creatinine concentration was identified as a strong negative prognostic factor.

As discussed previously, fungal identification and susceptibility testing is likely to result in improved decision making around choice of chemotherapeutic agent and therapeutic drug monitoring. We were not able to assess the effect of susceptibility testing on survival after DA diagnosis due to the small numbers of dogs in which fungus susceptibility testing was carried out.

Dogs included in this study received a variety of treatment protocols with respect to both antifungal agents and dosages. Prospective clinical trials evaluating the effect of antifungal protocols are necessary to further characterize their specific treatment efficacy, but because of the rarity of this condition, multi-center studies would be required to ensure recruitment of adequate case numbers to make objective comparisons of treatment efficacy possible.

Finally, survival outcome included dogs that were euthanized, which might represent a bias because the timing of euthanasia is entirely at the discretion of each dog's owner.[35] Importantly, dogs with disseminated fungal disease might have clinical deterioration before improvement after antifungal treatment resulting from an exuberant host inflammatory response to dying fungi.[36] This consequence can often be interpreted as treatment failure or disease progression. The decision for euthanasia can also be influenced by other factors such as the cost of treatment and emotional decisions of owners, which might not have been documented in medical records. Most of the dogs in our study did not have an autopsy performed and some dogs might have been incorrectly assigned DA as a cause of death. Such errors might have led to misclassification bias. The case definition used in this study and the fact that DA cases were well monitored by their respective veterinarians throughout the course of their illness make the likely impact of this bias on our findings small.

5 | CONCLUSIONS

This study provides a national view of the clinical characteristics and outcomes of DA in Australian dogs. Our results show that abnormally high serum creatinine concentrations and the use of itraconazole alone were negatively associated with survival times after diagnosis, but it should be noted that there were only 8 dogs in the itraconazole-only group with follow-up time data available.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Sykes JE. Aspergillosis. *Canine and Feline Infectious Diseases*. Missouri: Saunders; 2014:633-648.
2. Day MJ, Penhale WJ, Eger CE, et al. Disseminated aspergillosis in dogs. *Aust Vet J*. 1986;63:55-59.
3. Schultz RM, Johnson EG, Wisner ER, et al. Clinicopathologic and diagnostic imaging characteristics of systemic aspergillosis in 30 dogs. *J Vet Intern Med*. 2008;22:851-859.
4. Elad D. Disseminated canine mold infections. *Vet J*. 2019;243:82-90.
5. Corrigan VK, Legendre AM, Wheat LJ, et al. Treatment of disseminated aspergillosis with posaconazole in 10 dogs. *J Vet Intern Med*. 2016;30:167-173.
6. Taylor AR, Young BD, Levine GJ, et al. Clinical features and magnetic resonance imaging findings in 7 dogs with central nervous system aspergillosis. *J Vet Intern Med*. 2015;29:1556-1563.
7. Whitbread TJ, Batt RM, Garthwaite G. Relative deficiency of serum IgA in the german shepherd dog: a breed abnormality. *Res Vet Sci*. 1984;37:350-352.
8. Olsson M, Tengvall K, Frankowiack M, et al. Genome-wide analyses suggest mechanisms involving early B-cell development in canine IgA deficiency. *PLoS One*. 2015;10:e0133844.
9. Day MJ, Penhale WJ. Serum immunoglobulin A concentrations in normal and diseased dogs. *Res Vet Sci*. 1988;45:360-363.
10. Allenspach K, House A, Smith K, et al. Evaluation of mucosal bacteria and histopathology, clinical disease activity and expression of toll-like receptors in German shepherd dogs with chronic enteropathies. *Vet Microbiol*. 2010;146:326-335.
11. Vahedi Shahandashti R, Lass-Florl C. Antifungal resistance in *Aspergillus terreus*: a current scenario. *Fungal Genet Biol*. 2019;131:103247.
12. Kelly SE, Shaw SE, Clark WT. Long-term survival of four dogs with disseminated *Aspergillus terreus* infection treated with itraconazole. *Aust Vet J*. 1995;72:311-313.
13. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377-381.
14. Kleinbaum D, Klein M. *Survival Analysis: A Self Learning Text*. New York, NY: Springer-Verlag; 2012.
15. Garbati MA, Alasmari FA, Al-Tannir MA, et al. The role of combination antifungal therapy in the treatment of invasive aspergillosis: a systematic review. *Int J Infect Dis*. 2012;16:e76-e81.
16. Groenwold RWI, Donders R, Carpenter J, et al. Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. *Can Med Assoc J*. 2012;184:1265-1269.
17. Cox D. Regression models and life-tables. *J Royal Stat Soc*. 1972;34: 187-220.
18. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
19. Rothman KJ. Estimation of confidence limits for the cumulative probability of survival in life table analysis. *J Chronic Dis*. 1978;31: 557-560.
20. Peto R. Experimental survival curves for interval-censored data. *Appl Stat*. 1973;22:86-91.
21. Grambsch P, Therneau T. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika*. 1994;81:515-526.
22. Therneau T, Grambsch P. *Modelling Survival Data: Extending the Cox Model*. New York, NY: Springer-Verlag; 2001.
23. Team RC. *R: A Language and Environment for Statistical Computing. In*. Vienna, Austria: R Foundation for Statistical Computing; 2019.
24. Ryder NS. Terbinafine: mode of action and properties of the squalene epoxidase inhibition. *Br J Dermatol*. 1992;126 (Suppl 39):2-7.
25. Ryder NS, Leitner I. Synergistic interaction of terbinafine with triazoles or amphotericin B against *Aspergillus* species. *Med Mycol*. 2001;39:91-95.
26. Hasbach AE, Langlois DK, Rosser EJ Jr, et al. Pharmacokinetics and relative bioavailability of orally administered innovator-formulated itraconazole capsules and solution in healthy dogs. *J Vet Intern Med*. 2017;31:1163-1169.
27. Renschler J, Albers A, Sinclair-Mackling H, et al. Comparison of compounded, generic, and innovator-formulated itraconazole in dogs and cats. *J Am Anim Hosp Assoc*. 2018;54:195-200.
28. Mawby DI, Whittemore JC, Genger S, et al. Bioequivalence of orally administered generic, compounded, and innovator-formulated itraconazole in healthy dogs. *J Vet Intern Med*. 2014; 28:72-77.
29. Bitar D, Lortholary O, Le Strat Y, et al. Population-based analysis of invasive fungal infections, France, 2001-2010. *Emerg Infect Dis*. 2014; 20:1149-1155.
30. Koehler P, Salmanton-Garcia J, Grafe SK, et al. Baseline predictors influencing the prognosis of invasive aspergillosis in adults. *Mycoses*. 2019;62:651-658.
31. Steinbach WJ, Benjamin DK Jr, Kontoyiannis DP, et al. Infections due to *Aspergillus terreus*: a multicenter retrospective analysis of 83 cases. *Clin Infect Dis*. 2004;39:192-198.
32. Deak E, Wilson SD, White E, et al. *Aspergillus terreus* accessory conidia are unique in surface architecture, cell wall composition and germination kinetics. *PLoS One*. 2009;4:e7673.
33. Bennett PF, Talbot JJ, Martin P, et al. Long term survival of a dog with disseminated *Aspergillus deflectus* infection without definitive treatment. *Med Mycol Case Rep*. 2018;22:1-3.
34. Karthaus M, Lehrnbecher T, Lipp HP, et al. Therapeutic drug monitoring in the treatment of invasive aspergillosis with voriconazole in cancer patients—an evidence-based approach. *Ann Hematol*. 2015;94: 547-556.
35. Hosgood G, Scholl DT. The effects of different methods of accounting for observations from euthanized animals in survival analysis. *Prev Vet Med*. 2001;48:143-154.

36. Segal BH, Herbrecht R, Stevens DA, et al. Defining responses to therapy and study outcomes in clinical trials of invasive fungal diseases: Mycoses Study Group and European Organization for Research and Treatment of Cancer consensus criteria. *Clin Infect Dis.* 2008;47:674-683.

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