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Original article

Clinical features and long-term follow-up of 70 cases of canine idiopathic eosinophilic lung disease.

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The study was carried out at the University of Bristol, University of Edinburgh, University of Liverpool and University of Cambridge.

List of abbreviations used in the manuscript.

BALF – bronchoalveolar lavage fluid

BRON -Cases with bronchial/peribronchial pattern on imaging

CT – computed tomography

Ct –cycle threshold

EB – eosinophilic bronchitis

ELD – eosinophilic lung disease

EBP – eosinophilic bronchopneumopathy

EPG -eosinophilic pulmonary granuloma

INT -Cases with interstitial/alveolar patterns on imaging

IQR – interquartile range

NCI - Cases with no changes on thoracic imaging

PCR – polymerase chain reaction

Vs – versus

Abstract

Background: Canine idiopathic eosinophilic lung disease (ELD) is sparsely documented in the literature.

Methods: Clinical presentation and outcome of dogs diagnosed with ELD(eosinophilic bronchitis or eosinophilic bronchopneumonia) were reviewed. Subgroups were made based on chronicity of clinical signs and findings of thoracic imaging: NCI (no changes in thoracic imaging), BRON (bronchial/peribronchial pattern), INT (Bronchointerstitial/Interstitial/alveolar).

Results: Seventy cases were included. There were more young to adult, crossbreed and female dogs. Compared to the other two groups NCI dogs showed lower BALF eosinophilic pleocytosis and absence of circulating eosinophilia, bronchiectasis or death due to respiratory disease.All dogs responded clinically to corticosteroids. Median treatment duration was 4 months. Remission (no clinical signs after treatment discontinuation for >1 month) and long-term remission (>6 months) was achieved in 60%, and 51% of patients respectively. Relapse occurred in 26% of cases after remission but was rare (3%) after long- term remission. The 1,2, and 4-year survival to death due to respiratory disease was 98%, 97% and 91% respectively.

Conclusion: Prognosis and initial clinical response for ELD was generally good although achievement of long-term remission was only seen in 51% of dogs. Different outcomes based on chronicity of signs, corticosteroid dose, thoracic imaging abnormalities and other clinical variables were not appreciated.

Keywords: Eosinophilic bronchopneumopathy; Eosinophilic bronchitis; Asthma; Endoscopy

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INTRODUCTION:

Eosinophilic lung disease (ELD) is well-recognized in both human and veterinary medicine and is characterized by eosinophilic infiltration into the airways or the pulmonary parenchyma. Sub-classification has previously been suggested for the dog based on the human classification system and is centred around the affected location (airways vs parenchymal) and the underlying aetiology. [1,2,3,4] ELD has been associated in some instances with parasitic pneumonitis, airway foreign bodies, neoplasia or systemic eosinophilic disease. However, in most instances no underlying aetiology is identified, and the disease is termed idiopathic: i.e. idiopathic eosinophilic bronchitis (EB) or idiopathic eosinophilic bronchopneumopathy (EBP). [1,2,5,6,7,8,9]

It is suspected that hypersensitivity plays a role in these cases, but the exact mechanism has not been fully elucidated. [1,5,6,7,8,10] EBP and EB are categorized as separate processes in the human classification [1,3,11], but most of the previous retrospective studies of canine idiopathic ELD have described the disease as EBP and have combined cases with primarily bronchial, peri-bronchial or parenchymal disease into one population. What constitutes EB, as a different entity to EBP, has not been historically well described in canine medicine. [1,2,9, 10, 12,13,14] but a recent retrospective study reported the clinical presentation and findings of ELD dogs with a presumptive diagnosis of EB or EBP based on the severity of their bronchoscopy findings [15]. Studies separating canine ELD cases primarily by their thoracic imaging characteristics have not been reported.

The two most common idiopathic eosinophilic parenchymal lung diseases in humans are idiopathic acute eosinophilic pneumonia which has an acute presentation, shows more severe

alteration in pulmonary function, occurs most commonly in younger male individuals and rarely requires long term treatment; and idiopathic chronic eosinophilic pneumonia characterized by a longer course of presentation, affecting middle-aged to older patients, often female and usually requiring long term therapy. [3,4,11] It is unknown whether similar presentations and associated responses to treatment are observed in canine ELD patients.

Previous EBP studies comprised single centres populations and have evaluated the clinical and diagnostic presentation and the short-term response to therapy [2,5,6,9,12,13,14,15,16]. Corticosteroid therapy is the treatment of choice. A favourable short-term response with variable short-term remission and relapse rates has been reported. [1,2,9,12,14] The optimal dose of oral corticosteroids for canine ELD has not been fully elucidated. Inhaled monotherapy with fluticasone has been shown to improve clinical signs in a small single-centre study, however relapses and ongoing clinical signs were observed in the majority of the cases [14].

The aims of this paper were 1) to retrospectively describe the clinical presentation, therapeutic management, long-term follow-up and remission and survival rates of a large group of patients diagnosed with ELD in a multicentre population within the United Kingdom (UK); 2) to evaluate the differences in diagnosis and treatment between dogs with acute and chronic ELD, and among dogs with three different appearance on thoracic imaging: no changes, bronchial/peribronchial patter or interstial/alveolar patterns. 3) to investigate whether the dose of corticosteroids used (anti-inflammatory versus immunosuppressive) had an effect on outcome.

MATERIALS AND METHODS

Ethical approval was obtained from the ethics board of each participating University. Records of all cases with a final diagnosis of EB or EBP diagnosed by a board-certified internist or cardiologist or a supervised resident at four university referral hospitals in the UK (Cambridge, Liverpool, Edinburgh and Bristol) between 2004 and 2016 were reviewed. Follow-up for each case was acquired through the referral centre or via the referring veterinarian's medical records. Cases were only considered if they had a full medical record available for review, presence of clinical signs suggestive of pulmonary disease (coughing, dyspnoea or tachypnoea), thoracic imaging at diagnosis, complete bronchoscopic examination, bronchoscopy-guided bronchoalveolar lavage fluid (BALF) analysis and eosinophilic pleocytosis on BALF results. Eosinophilic pleocytosis was defined as > 10% of the leukocyte differential comprising of eosinophils, with neutrophils accounting for <10%. If neutrophils were >10% the eosinophilic component had to be substantially higher. For cases to be included, parasitic disease had to have been ruled out by one or a combination of: a negative faecal analysis, absence of parasites on BALF analysis or previous regular veterinary approved anti-parasitic therapy. No case had a history of travel outside the UK. Cases were also excluded if any other underlying cause of ELD such as parasitic pneumonitis was identified.

The following information was retrieved for each case: signalment, weight, presence of circulating eosinophilia, thoracic imaging findings, bronchoscopy report, BALF cytology results, infectious disease screening results, concurrent diseases, clinical signs and their duration prior to diagnosis, treatment prior to diagnosis or referral.

Patients with clinical signs < 1 month were categorized as acute and those with clinical signs > 1 month categorized as chronic, similar to the criteria used for human idiopathic eosinophilic pneumonia (11).

Thoracic imaging was performed by radiography, computed tomography (CT) or both. All images were reviewed by a board-certified specialist in diagnostic imaging or a resident working under his/her supervision, and sub-classified based on the final report as: no pulmonary abnormalities or pulmonary abnormalities (bronchial/peri-bronchial, interstitial, alveolar) (Figure 1, 2 and 3). CT was used in preference to radiography when both modalities were available. Patients without pulmonary abnormalities were grouped as NCI, and those with pulmonary abnormalities as BRON (if a bronchial/peribronchial pattern was present) or INT (if bronchointerstitial/interstitial/alveolar patterns were present).

Bronchoscopy was performed in all patients and reported abnormalities were described as: erythema, oedema, mucus, presence of proliferative changes, bronchiectasis or bronchial collapse (Figure 4,5 and 6). Bronchoscopy findings were subjectively scored based on evaluation of the number of those abnormalities present and graded as: mild (0 - 1 abnormalities), moderate (2-3) or severe (> 4) [2]. BALF was analysed by a board-certified clinical pathologist or a resident under their supervision. BALF results were graded, as previously published [2], based on the degree of eosinophilic pleocytosis: Grade 1-mild- (10-20% eosinophils), grade 2-moderate- (21-50% eosinophils), grade 3-severe-(>50% eosinophils) (Figure 7).

Screening for concurrent infectious bacterial respiratory disease was recorded: BALF bacterial culture, *Bordetella bronchiseptica* PCR, *Mycoplasma spp.* PCR or culture.

Treatment (dose, duration and route administered) was recorded for each patient. Patients receiving oral corticosteroids were dichotomized based on their dose of prednisolone into anti-inflammatory or immunosuppressive groups: patients needing ≤1mg/kg per day or below to control their clinical signs were classified as group A (anti-inflammatory), and patients receiving > 1mg/kg at some point during the treatment were classified as group I (immunosuppressive). Total response was defined as resolution of clinical signs, partial response as improvement of clinical signs and treatment failure as no improvement of clinical signs. Remission and long-term remission were defined as the full absence of respiratory signs following discontinuation of corticosteroid administration for >1 month and >6 months respectively. Relapse was defined as reoccurrence of clinical signs during remission. Time to remission, length of remission and survival were recorded.

Statistical analysis:

Data were entered into a spreadsheet[a] and statistical analyses was performed using two statistical software programs.[b,c] The level of significance was set at 5% for all analyses. Gaussian distribution was assessed graphically and then with the Shapiro-Wilk test. Baseline descriptive statistics were calculated and reported as percentages for categorical data and median and interquartile range for continuous data.

Group comparisons for continuous data were performed with t-test, Mann-Whitney U or Kruskal-Wallis test and chi-square or Fisher's exact test to compare proportions as indicated; no attempt was made to correct for multiple comparisons. Group comparisons were made and are outlined in table 1,2 and 3. When statistical differences were carried out for 3x2 and 3x3 tables further post-hoc analysis was carried out when necessary by looking at the adjusted

standardised residuals and carrying out smaller 2x2 direct comparisons to determine specific P values.

Time to event analysis was carried out for reaching remission and long-term remission. Kaplan-Meier survival curves were constructed to assess time to remission and time to long term remission between groups and the log rank test was used to compare these. Those not achieving remission, or these lost to follow-up were right censored according their follow-up period. Achievement of remission and the probability of achieving remission and long-term remission was further investigated for other numerous variables (table 3).

RESULTS

Population

Of the 95 cases initially evaluated, 70 met the inclusion criteria and were enrolled. The 25 cases excluded were due to parasitic pneumonitis (2), incomplete investigations (4), complete resolution of signs with anthelminthic treatment (1), and incomplete medical histories (18). Patients comprised of the following breeds: Crossbreed (17), Labrador (7); Springer Spaniel (5); Jack Russel Terrier (5); Cocker Spaniel (4); Siberian Husky (4); Border Collie (2); Border terrier (2); Irish Setter (2); Lakeland terrier (2); Shih Tzu (2); Weimaraner (2); Northern Inuit (2); Parsons Russel terrier (2); followed by one each of the following breeds: Toy poodle, Beagle, Bedlington Terrier, Boxer, Chihuahua, Portuguese Podengo, Maltese, Newfoundland, Golden Retriever, Standard Poodle, Italian Greyhound, Rottweiler. Within the cross-breed group 2/13 (12%) were in the IM-group, 6/17 (35%) within the BRON group and 9/17 (53%) within the INT group.

Median age was 4 years (IQR 1-6 years). There were 63% (44/70) females, of these 80% (35/44) were neutered, and 37% (26/70) males, of these 69% (18/26) were neutered. Median weight was 16.4 kg (IQR 9.4-27 kg).

Clinical signs prior to diagnosis were present for a median duration of 2 months (IQR 1-6 months). 48/70 dogs (70%) had clinical signs of > 1-month duration (chronic), and 22/70 dogs had clinical signs lasting < 1 month (acute) (Table 1).

Table 1

	All (n=70)	NCI (n=13)	BRON (n=19)	INT (n=38)	P values[a]	Acute (n=22)	Chronic (n=48)	P values[b]
Age (years)	4 (1-6)	4 (2-6)	4 (2-6)	3 (1-6)	P=0.733	4 (2-6)	2.5 (1-6)	P=0.150
Weight (kg)	16.4 (9.4-27)	15 (9.8-24)	16.5 (9.5-21.5)	16.8 (9.4-29)	P=0.865	14.3 (9.4-28.35)	16.6 (9.8-27)	P=0.810
Circulating eosinophilia	36% (25/70)[c]	0% (0/13)	32% (6/19)	50% (19/38)	**P=0.005**[a,d]	59% (13/22)	25% (12/48)	**P=0.006**[b]
Bronchiectasis	14% (10/70)	0% (0/13)	16% (3/19)	18% (7/38)	P=0.255	18% (4/22)	13%(6/48)	P=0.528
Bronchoscopy score (mild, moderate, severe)	37% (25/68), 54% (37/68), 9% (6/68)[e]	46% (6/13), 54% (7/13), 0% (0/13)	32% (6/19) 53% (10/19) 15% (3/19)	36% (13/38) 56% (20/38) 8% (3/38)	P=0.613	38% (8/21), 52% (11/21), 10% (2/21)	36% (17/47), 55% (26/47), 9% (2/47)	P=0.892
Degree of eosinophilia on BALF (mild, moderate, severe)	10% (7/70), 29% (20/70), 61% (43/70)[f]	23% (3/13), 54% (7/13), 23% (3/13)	5% (1/19) 21% (4/19) 74% (14/19)	8% (3/38), 23% (9/38), 69% (26/38)	**P=0.021**[g]	9% (2/22), 27% (6/22), 64% (14/22)	10% (5/48), 29% (14/48), 60% (29/48)	P=0.965
AI	46% (31/67)	62% (8/13)	53% (9/19)	50% (18/38)	P=0.419	50% (11/22)	52% (25/48)	P=0.871
IM	54% (36/67)	23% (3/13)	47% (9/19)	50% (19/38)	P=0.229	50% (11/22)	42% (20/48)	P=0.515
Oral and inhaled steroids	36% (25/67)	38% (5/13)	42% (8/19)	34% (13/38)	P=0.448	32% (7/22)	37.5 % (18/48	P=0.645

Table 1.

A breakdown of the different clinical parameters by the different subgroups can be found. When statistical significance is reached the P value appears in bold. NCI: Cases with no abnormalities on thoracic imaging. BRON: Cases with bronchial pattern on thoracic imaging. INT: Cases with bronchointerstitial/interstitial and/or alveolar pattern on thoracic imaging. Acute: Cases with presenting clinical signs lasting less than 1 month. Chronic: Cases with presenting clinical signs lasting more than 1 month. AI: Anti-inflammatory corticosteroid. IM: Immunosuppressive corticosteroid. BALF: Bronchoalveolar lavage fluid.

[a]There was no difference (P=0.547) in the percentage of dogs receiving previous corticosteroids within the imaging subgroups (NCI 3/10 23%; BRON 3/19 16%; INT 11/38 28%).

[b] More dogs in the chronic group (15/48; 31%) received steroids prior to referral than in the acute group (2/22; 9%) P=0.045. If these dogs were excluded from the analysis eosinophilia was still significantly different between both groups (P=0.018) and no difference was observed for any of the other studied variables (All P values higher than 0.150).

[c]There was no difference (P=0.228) in the percentage of dogs with eosinophilia for those dogs who had (4/17; 24%) vs had not (21/53; 39%) received previous corticosteroids.

[d]NCI vs BRON **P=0.025**; NCI vs INT **P= 0.001**; NCI vs INT P=0.186

[e]There was no difference (P=0.577) in the severity of bronchoscopy findings severity between dogs receiving (8/17 47%; 8/17 47%; 1/8 6%) or not receiving (17/51 33%; 29/51 57%; 5/51 10%) previous corticosteroids.

[f]There was no difference (P=0.951) in eosinophilic pleocytosis in BAL severity between dogs receiving (2/17 12%; 5/17 29%; 10/17 59%) or not receiving (5/53 9%; 15/53 28%; 33/53 62%). previous corticosteroids.

[g]NCI vs BRON **P=0.016**; NCI vs INT **P=0.017**; BRON vs INT P=0.890

History and physical examination findings

Clinical signs in patients prior to referral included cough (94%; 66/70), exercise intolerance (17%; (12/70), dyspnoea (16%; 12/70), tachypnoea (14%; 9/70), lethargy (13%; 9/72) and nasal/nasopharyngeal signs (10%; 7/70).

A minority of patients (7%;5/70), had not received any medical treatment prior to diagnosis, and the remainder (93%; 65/70) had received various combinations of medical treatment including antibiotics (81%; 57/70), non-steroidal anti-inflammatory drugs (33%; 23/70), corticosteroids (24%; 17/70), mucolytic therapy (3%; 2/70) and anthelminthic treatment (76%; 53/70) prior to referral.

Diagnostic investigations:

Circulating eosinophilia was reported in 36% (25/70) of patients (Table 1). Faecal analysis was performed in 54% (38/70) patients (Baermann technique (24/38), Zinc floatation (10/38), and direct faecal examination (4/38)). *Isopora spp.* was found in one patient, but not considered to be associated with ELD and this patient was therefore included.

All patients had thoracic imaging performed. Thoracic radiographs were obtained from 54% (38/70) patients and thoracic CT from 56% (39/70) patients with both modalities performed in 11% (8/70). Diagnostic imaging reports identified a bronchial/peri-bronchial pattern in 27% of patients (19/70), parenchymal (interstitial or alveolar) changes in 54% (38/70) and absence of

abnormalities in 19% (13/70). Two patients with an interstitial pattern had also significant parenchymal consolidation on thoracic imaging later confirmed to be canine eosinophilic pulmonary granulomatosis (EPG). Bronchiectasis was identified on thoracic imaging in 14% patients (10/70). The entire study population was subdivided in three groups: no abnormalities on thoracic imaging (NCI), bronchial/peribronchial pattern (BRON) or bronchointerstitial/interstitial/alveolar pattern (INT) (Table 1). CT was used in 77% (10/13) cases in the NCI group, in 37% (7/19) cases in group BRON and in 58% (22/38) in group INT.

Full bronchoscopy reports were available for 97% of patients (68/70) with the changes being classified as mild in 38% of cases (25/68), moderate in 54% (37/68) and severe in 9% (6/68) (Table 1).

The BALF results were graded as outlined above and were as follows: grade 1 (10%; 7/70); grade 2 (29%; 20/70); grade 3 (61%; 43/70) (Table 1). Three dogs had concurrent elevation of neutrophils but in these dogs the neutrophil percentage was <20% and the eosinophilic component >50%.

Rhinoscopy was performed in 9% (6/70) of cases and non-infectious chronic rhinitis diagnosed in 5/6 cases. The remaining case had no biopsies taken or diagnosis reached.

Bacterial culture of the BALF was performed in 97% patients (68/70). It was negative in 79% of cases (54/68) and positive in 21% (14/68). *Mycoplasma spp*. was specifically screened for in 50% patients (35/70) and was isolated in 17% of these patients (4/35). In each case when bacterial infection was identified, clinical records indicated the positive culture was considered to be due to contamination or concurrent infection. This was considered because of a scant

(semiquantitatively) growth of an organism and/or no initial response to antibiotics, and a good subsequent response to steroid therapy in the context of a BALF eosinophilic pleocytosis.

Treatment

In some patients, there was an initial treatment trial with fenbendazole (74%; 52/70) or antibiotics (53%; 37/70) prior to the administration of corticosteroids. No patient had resolution of clinical signs with these treatments alone. Corticosteroids were administered to all patients either at the time of diagnosis or when they failed to respond to antibiotic and, or anthelminthic therapy. The breakdown of corticosteroid therapy by dose and route is outlined in Figure 8.

Of the patients initially receiving only oral anti-inflammatory corticosteroids, 5.6% (2/36) subsequently required an increase to an immunosuppressive dose to control their clinical signs. Overall, 24% (6/25) of patients receiving dual therapy had their oral corticosteroid therapy discontinued and were managed solely with inhaled corticosteroid monotherapy (Table 1). Overall treatment duration range was 1-103 months (median 4 months, IQR = 2-17 months).

Follow-up

Duration of follow-up for each case was defined as the time from referral until: the present day/they were lost to follow-up/their death. Follow-up ranged from 1 - 140 months (median 29months, IQR = 11-50 months). During follow-up, 7% (10/70) of patients were euthanized or died. Of these 2/10 were due to progression of their respiratory disease (one patient 3 months' and 38 months' post-diagnosis). The remainder died or were euthanized due to non-respiratory related illnesses.

The 1,2, and 4-year survival to death due to respiratory disease was 98%, 97% and 91% respectively (Table 2).

Table 2

	All (n=70)	NCI (n=13)	BRON (n=19)	INT (n=38)	P values	Acute (n=22)	Chronic (n=48)	P values
Total response (Absence of clinical signs)	93% (65/70)	85% (11/13)	95% (18/19)	95% (2/38)	P=0.442	95% (21/22)	92% (44/48)	P=0.568
Partial response (Improvement of clinical signs)	7% (5/70)	15% (2/13)	5% (1/19)	5% (2/36)	P=0.442	5% (1/22)	8% (4/48)	P=0.568
Remission	60% (42/70)	61% (8/13)	63.2% (12/19)	58% (22/38)	P=0.922	63% (14/22)	58% (28/48)	P=0.674
Long term remission (>6 months)	51% (28/55)	36.4% (4/11)	58% (7/12)	53% (17/32)	P=0.533	56% (10/18)	35% (14/40)	P=0.141
1,2,4 year survival to respiratory disease	98%, 97%, 91%	100%, 100%, 100%	92%, 89%, 88%	97%, 96%, 92%	N/A	94%, 92%, 88%	100%, 100%, 93%	N/A

Table 2.

A break-down of the different therapeutic response by different subgroups can be found. When statistical significance is reached the P value appears in bold. NCI: Cases with no abnormalities on thoracic imaging. BRON: Cases with bronchial/peribronchial pattern on thoracic imaging. INT: Cases with interstitial/alveolar pattern on thoracic imaging. Acute: Cases with presenting

clinical signs lasting less than 1 month. Chronic: Cases with presenting clinical signs lasting more than 1 month. N/A: Non-applicable. The n= represents the total number of cases. For some variables the number may be less than the total patient population if the full information about that case for that variable was not available.

All patients showed a positive clinical response to corticosteroid therapy. Total response was observed in 93% (65/70) of cases and a partial response in 7% (5/70) of cases. Remission was achieved in 60% (42/70) of cases and long-term remission in 51% (28/55) of cases. Treatment duration was 3 months (IQR 2-5 months) and 28.5 months (IQR 16-40 months) for those achieving and not achieving remission respectively. Treatment duration for those achieving long term remission was 3 months (IQR 2-5months). Relapse was reported in 26% (11/42) of cases, 24% (10/42) in the first six-months following discontinuation of corticosteroid treatment and in 3% (1/28) at least six-months after stopping corticosteroid therapy. (Table 2). Group comparisons and the non-parametric log rank test showed that the median time to remission was not different between any sub-groups (table 3).

Table 3

	Remission	P Value	Long term Remission	P Value
Anthelmintic prior to ref. (Yes/No)	63% (35/55) / 47% (7/15)	P=0.234	54% (25/46) / 33% (3/9)	P=0.249
Bronchiectasis (Yes/No)	40% (4/10) / 63% (38/60)	P=0.163	40% (2/5) / 52% (26/50)	P=0.609
Eosinophilia (Yes/No)	64% (16/25) / 57% (26/45)	P=0.611	71% (12/17) / 42% (16/38)	P=0.051
Bronchoscopy score (mild/moderate/severe)	64% (16/25) / 60% (22/37) / 50% (3/6)	P=0.811	57% (12/21) / 51% (15/29) / 0% (0/3)	P=0.179

Eosinophilic grade BALF (mild/moderate/severe)	70% (5/7) / 55% (11/20) / 61% (26/43)	P=0.743	66% (4/6) / 44% (7/16) / 51.5% (17/33)	P=0.628
Corticosteroid dose (AI/IM)	56% (20/36) / 64% (20/31)	P=0.456	41% (11/27) / 61% (10/26)	P=0.130
Corticosteroid dose (AI-IN /AI+IN /IM)	62% (13/21) / 48% (7/15) / 63% (20/31)	P=0.496	50% (7/14) / 31 % (4/13) / 62% (16/26)	P=0.193

Table 3.

Comparison by subgroups of achievement of remission (total response in > 1month) and long-term remission (>6m of remission).

When statistical significance is reached the P value appears in bold. In dichotomous variables the percentages of remission and long-term remission achieved by both categories can be seen. For variables with three categories percentages of the three categories achieving remission or long-term remission are depicted.

BALF: Bronchoalveolar lavage fluid. AI: Anti-inflammatory corticosteroid (all group) IM: immunosuppressive corticosteroid. AI-INH: Anti-inflammatory corticosteroid without inhaled therapy. AI+INH: Anti-inflammatory corticosteroid with inhaled therapy.

DISCUSSION

To the best of the authors' knowledge this is the first and largest multi-centre study of idiopathic canine eosinophilic lung disease evaluating clinical features and long-term outcome. We identified a similar age of onset (most young adult) and sex predisposition (more females) in our study population to those reported in most previous publications [1,2,9,12,13,14]. Similar to a recent publication [15] crossbreed dogs were the most common breed reported. This was different to all previous studies in which crossbreeds were reported only very sporadically

[1,2,9,12,13,14]. The most commonly reported breeds in those studies included Siberian Huskies [1,2], Labradors [9] and Cocker Spaniels [12], all of which were similarly highly represented in our study. The high proportion of crossbreed dogs cannot be explained by the differences in inclusion criteria, as they were present across all imaging subgroups and all dogs in group INT (and also dogs in group BRON in some studies) would have met criteria for inclusion in previous EBP studies. It is possible that the increased representation of crossbreeds may reflect the hospital or regional population characteristics or the increasing awareness of the disease over the past years.

Previously it has been hypothesised that younger dogs with EBP may be more difficult to manage [1,17]. This was not observed in our study where a similar treatment response and remission rate was observed for younger and older dogs.

Cases were also dichotomised into acute and chronic presentation, similar to the human classification [3,4,11]. In contrast to the situation in human medicine, other than acute cases having a higher incidence of circulating eosinophilia, no other significant differences were found with regards to age of presentation, severity of clinical findings or outcome. Therefore, we did not conclude that separating these two groups provided pertinent clinical information for the management and prognosis of ELD in dogs.

Whether different ELD entities with pathophysiological, clinical, therapeutic and/or prognostic differences occur in dogs is currently unknown. In humans, different disease entities with different management and outcome such as pneumonias (acute and chronic), eosinophilic bronchitis, eosinophilic bronchiolitis and asthma are described [11]. In human medicine idiopathic eosinophilic pneumonia is typically associated with pulmonary abnormalities on

353 thoracic imaging and eosinophilic bronchitis is characterized by the absence of pulmonary
354 abnormalities on thoracic imaging. [3,4,11] By dividing our population into three groups in
355 regard to their imaging findings we were able to identify a population of dogs (NCI) with
356 isolated airway disease which would represent EB. At the other side of the spectrum dogs the
357 INT group would be truly airway-parenchymal disease (EBP) and several differences were
358 observed between these two groups of dogs. NCI dogs had less severe eosinophilic pleocytosis
359 on BALF, absence of circulating eosinophilia and absence of bronchiectasis. Additionally, no
360 dogs in this group succumbed to their respiratory disease. Considering the age and time of
361 presentation was similar to the INT cases, it is unlikely that dogs in the NCI group represent an
362 early stage of the disease process and this group likely constitutes true isolated airway disease.
363 Whether the dogs in the BRON group had EB or EBP or whether they constitute a different
364 entity is unknown. In our study they showed similar clinical and laboratory characteristics to the
365 INT group and showed similar differences compared with the NCI group. As the BRON group
366 was the one where CT was used less frequently (37%) it is possible that at least some of these
367 dogs would have shown interstitial changes on CT and indeed belonged to the INT group. A
368 recent paper [15] also attempted to separate ELD into EB and EBP. In this paper however, the
369 definitive criteria to separate both processes were the severity of bronchoscopy findings and
370 dogs could be classified as EB or EBP with a bronchial radiographic pattern: EB had milder
371 bronchoscopic findings and EBP more severe ones. After classification, EB cases in that study
372 ended being mostly cases with absence of radiographic findings, similar to our NCI group, and,
373 similar clinical differences to the findings in our study were observed in regard to blood and
374 BALF eosinophilia and bronchiectasis when compared to the group classified as EBP. No other

paper has attempted to separate bronchial vs parenchymal disease in canine ELD. Previous EBP papers vary in inclusion criteria and in some of them the EBP group would have encompassed just the INT group while in others both the INT and the BRON group would have been considered EBP [1,2,9,12,13,14,18,19]. Overall however, in our study, outcomes for all subgroups were not significantly different: response and remission rates were similar with the only exception that, as mentioned previously, death or euthanasia due to respiratory disease did not occur in the NCI group. However, as death was such a rare event in our study it is difficult to make conclusions in this regard.

A population which may represent a different ELD disease entity are dogs with EPG. In our patient population only two dogs, both in the INT group, were diagnosed with EPG making it challenging to draw meaningful conclusions. Historically however, dogs with EPG have been suspected to represent a more severe form of ELD with a worse prognosis [19,20,21,22]. Consistent with this perception one of the two patients in our population who succumbed to their respiratory disease had a diagnosis of EPG. A recent study [15] suggested a more favourable prognosis for dogs with EPG dogs. However, the diagnosis of EPG dogs in this study was based on a different diagnostic criteria to the usual historical EPG definition of parenchymal lung disease and pulmonary granulomas (consolidation or masses) characterised, when histopathology is performed, by a large population of eosinophils usually with macrophages and epithelioid cells [19,20,21,22]. In this recent study [15], dogs were classified as EPG if they had, on bronchoscopy, airway disease with intraluminal eosinophilic mass lesions (plugging), irrespectively of the presence or absence of parenchymal involvement on thoracic imaging.

Circulating eosinophilia, bronchoscopic findings and the degree of eosinophilic pleocytosis was similar in our study to previous EBP studies. The incidence of bronchiectasis in our population was slightly lower compared to the 26-60% reported in previous studies [2,11,13] and a slightly lower incidence of bronchiectasis persisted when only the INT group was evaluated. Bronchiectasis was not identified in any patient assigned to the NCI group.

Historically the treatment of choice for EBP has been corticosteroids, and as was observed in this study, good initial responses have been reported [1,2,5,9,12,14]. Immunosuppressive therapy has been advocated for the treatment of EBP in the dog [1,2] however, up to 40% of dogs in this study had control of their clinical signs with corticosteroids administered at an anti-inflammatory dose (although 42% of these patients also received concurrent inhaled corticosteroids therapy). Of those patients starting treatment with anti-inflammatory corticosteroids, 5% required an increase dose to immunosuppressive corticosteroids to achieve control of their clinical signs. There was no statistical significance when comparing corticosteroid doses between the different subgroups which would suggest the choice of anti-inflammatory or immunosuppressive dose reflected individual clinician preference and not the type or severity of the ELD.

The overall remission (60%) and long-term remission (51%) rates were modest and similar among the different subgroups; and they were also similar to the remission rates reported in previous EBP publications (30-50%) [2,10]. The relapse rate in our study (26%) was also similar to previous reports (30-72%). Importantly, in our study, it was noted that if relapse occurred, it was most likely to occur during the first 6 months following discontinuation of corticosteroid therapy. Once long-term remission was achieved relapses were very rare.

Similar to previous EBP studies, death or euthanasia due to respiratory disease was a rare event in our population and 1, 2 and 4-year survival to respiratory death exceeded 90%. As death was such a rare event, no comparisons can be made among groups, however as mentioned above none of the NCI population died or were euthanized due to respiratory disease and one of the two patients that died due to respiratory disease had a diagnosis of EPG.

No obvious association was observed between any of the other studied variables indicative of clinical severity (bronchiectasis, eosinophilia, bronchoscopy score, eosinophilic grade) and any of the outcome variables. There was also no difference between dogs receiving anti-inflammatory vs immunosuppressive doses of corticosteroids with regard to likelihood of remission or long-term remission. These results were also similar if the anti-inflammatory group was broken down into those with or without concurrent inhaled therapy. Therefore, a high proportion of dogs appear to achieve remission and long-term remission without using higher immunosuppressive dose which may be associated with increase risk of side effects. However, this is a retrospective paper with several limitations and further specifically designed prospective studies are needed to determine the optimal corticosteroid dose in the management of ELD. It would be of particular interest to investigate whether the remission outcomes observed in this and previous papers could be improved and whether different strategies should be instigated for EB dogs compared to EBP dogs.

There are several limitations of this study. Firstly, it has the intrinsic limitations of a retrospective multi-centre study performed over a 12-year period, including non-standardised treatments and recording of clinical records and a referral population bias. We attempted to minimize this by directly contacting the owner and the referring veterinary surgeon when

441 needed and by collecting a large amount of data, which could counteract some of the errors.
442 Secondly, as in every previous EBP study, response and remission is usually assessed by the
443 owner's perception of resolution of clinical signs and not by the objective documentation of
444 absence of intrapulmonary disease. Treatments were not standardised, and the dose, length
445 and protocols used varied among the different clinicians managing the cases. In our institutions
446 anti-inflammatory doses of prednisolone are usually 0.5 to 1 mg/kg per day and
447 immunosuppressive doses are always above 1.5-2mg/kg but scientific evidence and consensus
448 of what dose causes immunosuppression is lacking [23]. Also, the use of mg/m^2 rather than
449 mg/kg for larger breed dogs has been anecdotally suggested. Unfortunately, we could not
450 retrieve the fluticasone dose accurately from the notes in all cases but in our institutions
451 fluticasone is usually started at 125ug or 250 ug twice a day for dogs <10kg or >10kg
452 respectively and the advice is to administer the dose after placing the mask. Thirdly we carried
453 out many statistical tests, which can lead to type I error, and a higher number of cases would
454 have been preferable to avoid type II error. We elected not to mathematically correct the result
455 to prevent type I error but to report all of them and analyse them critically. In this manner most
456 significant results reported appear robust in our opinion, although caution may be elicited with
457 the higher P values (e.g. between 0.05 and 0.010) observed. Similarly, when subgroups were
458 compared, all the negative results showed, for the most part, very similar populations and
459 although statistical differences might be found with a larger study population we suspect that
460 for the majority of comparisons it is unlikely that these would be of clinical significance.
461 Lastly, despite utilising strict inclusion criteria, inherent limitations remained. Firstly, a
462 consensus of what constitutes eosinophilic pleocytosis in BALF of normal dogs is not clearly

463 defined in the current literature [1,2,10,13, 24,25]. Similar to previous EBP studies
464 [2,7,10,13,14,16,19] we elected to consider eosinophilia >10% in BALF abnormal, however
465 some previous studies have used a marginally higher cut off [7,10,13,14]. Secondly, chronic
466 bacterial respiratory tract infection has been associated with a mixed cell infiltrate on BALF and
467 theoretically could result in higher percentage of eosinophils in BALF [1]. There were however
468 only three included cases with mixed pleocytosis and these cases all had a low proportion of
469 neutrophils (<20%) and high proportion of eosinophils (>50%), thereby meeting the previously
470 suggested diagnostic criteria for ELD in dogs with mixed pleocytosis [26]. Thirdly, parasitic
471 pneumonitis is an important differential for ELD and the ruling out of this aetiology is an
472 essential, yet challenging aspect to the diagnosis. We elected to use the same criteria as in
473 previous studies [1,5,6,9, 13,14] however, absolute certainty in ruling out parasitic disease
474 cannot unfortunately be achieved. Despite this caveat, no statistically significant difference
475 was found between patients that had received adequate anti-parasitic therapy and patients
476 that had not received such treatment. Fourthly, a percentage of dogs received corticosteroids
477 prior to presentation, and this may in theory alter several findings such as the degree of
478 eosinophilia, the bronchoscopy score or the degree of eosinophilic pleocytosis in BAL.
479 However, although we were not able to confirm this in all cases from the notes, it is routine
480 policy in all the hospitals involved in the study to try to stop medications at least one week
481 before seeing the cases. Also, there was no differences in the overall percentages of
482 eosinophilia, bronchoscopy score or degree of eosinophilic pleocytosis when dogs with previous
483 corticosteroids were compared to those without them. The percentage of dogs in which
484 corticosteroids was used prior to the initial appointment was also similar among dogs in the

different imaging groups and although it was significantly higher in the chronic group than in the acute group when cases with previous glucocorticoids were excluded from analysis the overall results for the three studied variables were very similar. For all these reasons we do not believe that previous use of glucocorticoids is likely to have had a significant impact of the study results.

Lastly, there was a proportion of dogs where imaging classification was carried out by radiography rather than CT and this may have led to group misclassification. This was likely less relevant for the NCI group as nearly 80% of them had CT performed and specific characteristics could be observed for this group however, as mentioned above, only 37% in group BRON were diagnosed by CT and we cannot ruled out that some of these cases would have been classified as group INT if CT had been performed.

In conclusion, we report the clinical presentation, therapeutic management and long-term outcome of a large cohort of dogs with ELD in the UK. Dogs in this population were predominantly young, female and crossbreed. The study also showed that dogs without imaging changes, which likely represent EB, have less severe eosinophilic pleocytosis on BALF or circulating eosinophilia than dogs with parenchymal changes suggestive of EBP. Moreover, no dog within this subgroup had bronchiectasis or died due to respiratory disease. However, a noticeable difference however in response to treatment among the different imaging subclassifications or between acute and chronic presentations was not identified. A complete response was achieved in the vast majority of dogs with ELD with the use of corticosteroid therapy, and we found modest remission rates and long-term remission rates of 60% and 51% respectively. Relapses typically occurred during the first 6 months of remission but were rare

after long-term remission. In many patients, total response, remission and long-term remission is achieved with anti-inflammatory doses of corticosteroids, however a small percentage of dogs required dose increases to an immunosuppressive corticosteroid dose. Other factors, such as severity of the clinical findings or age of presentation, were also investigated in this study but found not to be significantly associated with outcome.

Conflicts of interest statement:

No conflicts of interest to declare.

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Footnote

a- Microsoft Office Excel 2007

b- R version 3.0.2, R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

c-SPSS 23, IBM

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Leyends

Figure 1.

Radiography of a dog with eosinophilic lung disease showing bronchial pattern.

Figure 2.

Radiography of a dog with eosinophilic lung disease showing bronchointerstitial pattern.

Figure 3.

CT scan of a dog eosinophilic lung disease showing bronchial thickening, peribronchial infiltrations and a multifocal widespread ground glass (interstitial) pattern.

Figure 4.

Endoscopic image of a dog with eosinophilic lung disease showing severe mucosal erythema and inflammation and mild presence of mucus.

Figure 5.

Endoscopic image of a dog with eosinophilic lung disease showing a large amount of thickened mucus.

Figure 6.

Endoscopic image of a dog with eosinophilic lung disease with evidence of severe bronchiectasis.

Figure 7.

Bronchoalveolar lavage cytology of a dog with severe eosinophilic pleocytosis.

Figure 8.

A breakdown of the corticosteroid treatment administered to the studied eosinophilic lung disease population of the current study. Immunosuppressive therapy was considered when a dose equal or more than 1mg/kg per day of prednisolone was administered. Fluticasone was used in all dogs as inhaled therapy.