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STANDARD ARTICLE

Recurrent bacterial pneumonia in Irish Wolfhounds: Clinical findings and etiological studies

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Background: Increased incidence of bacterial pneumonia (BP) has been reported in Irish Wolfhounds (IWHs), and recurrence of BP is common. The etiology of recurrent pneumonia in IWHs is largely unknown.

Objectives: To describe clinical findings in IWHs with recurrent BP and investigate possible etiologies.

Animals: Eleven affected IWHs, 25 healthy IWHs, 28 healthy dogs of other Sighthound breeds, and 16 healthy dogs of other breeds.

Methods: Prospective cross-sectional observational study. All affected IWHs underwent thorough clinical examinations including thoracic radiographs, thoracic computed tomography, electron microscopic evaluation of ciliary structure, and bronchoscopy and bronchoalveolar lavage fluid (BALF) cytology and culture. Serum and BALF immunoglobulin concentrations were measured using an ELISA method, and peripheral blood lymphocyte subpopulations were analyzed using flow cytometry. Esophageal function was assessed by fluoroscopy (n = 2).

Results: Median age of onset was 5.0 years (range, 0.4-6.5 years), and when presented for study, dogs had experienced a median of 5 previous episodes of BP (range, 2-6). The following predisposing factors to BP were detected: focal bronchiectasis (10/11), unilateral (2/9) and bilateral (1/9) laryngeal paralysis, and esophageal hypomotility (2/2). Local or systemic immunoglobulin deficiencies or primary ciliary defects were not detected.

Conclusions and Clinical Importance: Recurrent BP affects mostly middle-aged and older IWHs without any evident immune deficit or primary ciliary defects. Focal BE was a frequent finding in affected dogs and likely contributed to the development of recurrent respiratory infections. Laryngeal and esophageal dysfunction identified in a minority of dogs may contribute to recurrent BP.

KEYWORDS

canine, dog, pulmonary, respiratory infection

1 | INTRODUCTION

Bacterial pneumonia (BP) is an acquired inflammation of the lower airways and lung parenchyma caused by bacterial infection.[1,2] Physiological protective mechanisms in the lungs are relatively effective, and the development of BP usually requires either a large number of bacteria or impaired pulmonary defenses.[1] Bronchopneumonia most often is caused by opportunistic bacteria belonging to the normal oropharyngeal

Abbreviations: BA, bronchoarterial; BALF, bronchoalveolar lavage fluid; BE, bronchiectasis; BP, bacterial pneumonia; cfu, colony forming unit; CT, computed tomography; ELF, epithelial lining fluid; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; IWH, Irish Wolfhound; LAD, leukocyte adhesion defect; MRSP, methicillin-resistant *Staphylococcus pseudintermedius*; $PaCO_2$, partial pressure of arterial carbon dioxide; PaO_2, partial pressure of arterial oxygen; RBPS, rhinitis and bronchopneumonia syndrome.

flora, which emphasizes the importance of predisposing factors in the development of BP.[3–5] Several predisposing factors to the development of BP have been described in dogs, including infections with respiratory viruses, ciliary defects, an immune deficit, and conditions predisposing to aspiration, such as laryngeal dysfunction, decreased esophageal motility, recent anesthesia, or neurological disease.[3,6–18]

An increased incidence of BP has been reported in the Irish Wolfhound (IWH), and recurrent BP has been identified frequently in this breed.[19–21] A recent questionnaire-based study described at least 1 episode of pneumonia in 37% of IWHs, and the majority of these dogs (53%) experienced recurrent episodes.[19] Bronchopneumonia is also 1 of the most common causes of death in IWHs along with neoplasia, cardiac disease, and musculoskeletal disorders.[19,22–24] Additionally, a significantly shorter life span has been reported in IWHs with a history of pneumonia, indicating that episodes of BP are severe in this breed, and death as a consequence of BP is a notable problem.[19]

The etiology of this breed predisposition is not well established. A retrospective study suggested aspiration as an etiology based on the acute onset of respiratory signs and radiographic changes in the dependent lung lobes.[20] However, a predisposing factor to aspiration was identified only in a minority (16%) of IWHs.[20] Supporting aspiration as a possible etiology, another study reported megaesophagus in a small number (9%) of IWHs with recurrent BP.[19] To our knowledge, prospective studies examining the etiology of recurrent BP in IWHs are lacking.

A unique rhinitis and bronchopneumonia syndrome (RBPS) has been described in young IWHs and is characterized by variable rhinorrhea present mostly from birth, accompanied by recurrent BP.[25–27] The clinical picture of RBPS resembles primary ciliary dyskinesia or primary immune deficiency.[25] These diseases have not been identified in affected dogs, but the possibility of immunoglobulin A (IgA) deficiency has been suggested.[25,28] Pedigree analysis of IWHs with RBPS has identified shared ancestors, suggesting a hereditary component in this disease.[25] Currently, it is still unclear whether recurrent BP in IWHs represents a disease entity distinct from RBPS.

Our aim was to describe the clinical as well as diagnostic imaging features in IWHs with recurrent BP and to investigate possible etiologies.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was conducted as a prospective cross-sectional observational study.

2.2 | Study population

Privately owned IWHs referred for examinations to the Veterinary Teaching Hospital of the University of Helsinki between March 2014 and March 2017 because of recurrent episodes of BP (≥2 episodes) were eligible for inclusion. Dogs were included in the study only between pneumonia episodes when they were clinically healthy and not receiving antimicrobial treatment.

As healthy controls, privately owned healthy IWHs >6 years of age with no history or clinical findings suggestive of previous or current BP, as well as dogs of other Sighthound breeds and dogs of various other breeds, were recruited as healthy controls. These dogs had no clinical signs of illness and had normal physical examination findings as well as normal hematology and serum biochemistry findings. Additionally, 6 healthy purpose-bred laboratory Beagle dogs (normal physical examination findings, blood hematology, serum biochemistry and arterial blood gas analysis results, unremarkable thoracic radiographs and bronchoscopy findings, as well as a negative bacterial culture in bronchoalveolar lavage fluid [BALF]) were included as healthy controls for BALF comparisons.

2.3 | History and follow-up information

Information concerning age of onset, number of previous episodes of BP, and possible clinical signs and medications between BP episodes was gathered from owners of affected IWHs using a questionnaire. Additionally, a separate questionnaire describing clinical signs, diagnostic tests, medications, and treatment responses during each separate episode of BP was completed by the owners. Referring veterinarians were contacted and patient records and radiographs were evaluated retrospectively to verify the diagnosis.

Owners were contacted by phone 1 year after the study end point (April 2018) and information concerning further episodes of BP as well as the time and cause of death if applicable was documented.

2.4 | Diagnostic testing and sample collection

All dogs underwent a full clinical examination, and venous blood samples for hematology, serum biochemistry, immunoglobulin measurements, and lymphocyte differentiation were obtained. Additionally, in IWHs with recurrent BP and in healthy laboratory Beagles, thoracic radiographs (left and right laterolateral and ventrodorsal views) and fecal samples (3 consecutive days) were obtained, and arterial blood gas analysis for partial pressures of oxygen (PaO_2), carbon dioxide ($PaCO_2$), and alveolar-arterial oxygen gradient was performed. Laryngeal evaluation was performed in IWHs with recurrent BP under light anesthesia after IV butorphanol (Butordol 10 mg/mL, Intervet International B.V., Boxmeer, the Netherlands) and propofol (PropoVet Multidose 10 mg/mL, Fresenius Kabi AB, Uppsala, Sweden). Movement of the arytenoid cartilages was observed until either normal movement was observed or the dog was too awake to tolerate the examination.[29] Thoracic computed tomography (CT) was performed under general anesthesia in intubated patients during an expiratory pause with a helical scanner (Somatom Emotion Duo, Siemens Germany, and GE LightSpeed VCT 64, GE Healthcare, Fairfield, Connecticut). The CT examination was performed first in a dorsal recumbency and then in ventral recumbency. After the CT examination, bronchoscopy was performed with the dog in ventral recumbency using a 4.9-mm flexible endoscope (GIF-N180, Olympus Europa SE&Co. KG, Hamburg, Germany), and airway samples for cytology and semiquantitative bacterial culture were obtained by weight adjusted bronchoalveolar lavage (BAL).[30] After BAL, ciliary biopsy specimens were obtained from the distal trachea using a single-use endoscopic biopsy forceps, placed into a buffered glutaraldehyde solution, and shipped to a veterinary diagnostic pathology service (University of Liverpool, Neston, UK) for electron microscopy.

Evaluation of esophageal function was performed at a separate appointment by a fluoroscopic (BV Libra C-arm, Philips Medical Systems, Eindhoven, the Netherlands) swallow study using barium sulfate (Mixobar Colon 1 g/mL, Bracco Imaging S.p.A, Colleretto Giacosa, Italy) mixed in canned food (1:10) for those dogs in which signs suggestive of esophageal dysfunction were identified in the history or during the aforementioned investigations. The study was performed in awake standing animals.

Thoracic radiographs and CT images were assessed by the same radiologist (A.K.L), who was blinded to the patient data. The presence and severity of bronchiectasis (BE) was assessed by using previously established criteria.[31] Each lung lobe was assessed for the presence of bronchiectasis (BE) using transverse images, and bronchoarterial (BA) ratio was measured at several locations including at least 1 normal-appearing central and peripheral airway in each lung lobe as well as all abnormally wide-appearing airways. The largest BA ratio was recorded for each lung lobe.

2.5 | Sample handling and analysis

Hematology, serum biochemistry, arterial blood gas, and fecal analysis as well as with cytological evaluation and bacterial cultures of respiratory samples (semiquantitative aerobic bacterial culture and *Mycoplasma* spp. culture) were performed as previously described.[32] Swab samples were obtained from mucosal membranes (oral mucosa, nares, and perineum) to screen for methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) colonization and were processed as described previously.[33]

Serum and BALF samples obtained for immunoglobulin analysis were frozen immediately and stored at −80°C until analysis.[34] Immunoglobulin A, M, and G (IgA, IgM, and IgG) were measured in serum and in BALF with ELISA kits for canine samples (Bethyl Laboratories Inc., Montgomery, Texas).[35–37] Serum and BALF urea concentrations were measured with a clinical chemistry analyzer (Kone Specific, Thermo Fisher Scientific, Vantaa, Finland) by using an enzymatic method (UREA UV 250, bioMérieux SA, Marcy l'Etoile, France), and the proportion of epithelial lining fluid (ELF) in the BALF was calculated as follows using serum and BALF urea measurements: proportion of ELF = (concentration of urea in BALF / concentration of urea in serum) × 100% as described previously.[30] Epithelial lining fluid immunoglobulin concentrations were calculated by using the known proportion of ELF in BALF.

Fresh EDTA blood samples were stained with monoclonal antibodies to canine lymphocyte cell surface antigens (fluorescent mouse/rat anti-dog CD3, CD4, CD8, CD21, and MHC class II antibodies) as described previously (AbD Serotec, Oxford, United Kingdom).[38,39] Briefly, 100 μL aliquots of fresh EDTA blood were exposed to 3 different combinations of antibodies (tube 1: antiCD3 [FITC], antiCD4 [PE], and antiCD8 [AlexaFluor 647]; tube 2: antiCD3 [FITC] and antiCD21 [PE]; and tube 3: AntiMHC class II [FITC] and antiCD21 [PE]). Five microliter of each antibody was used. A 4th 100 μL aliquot of EDTA blood was not exposed to antibodies. Additionally, aliquots of EDTA blood were stained with each single antibody separately and used as controls. Tubes were incubated for 30 minutes in the dark, and red blood cells were lysed with a commercial erythrocyte lysing buffer (Erythrolyse Red Blood Cell Lysing Buffer, AbD Serotec, Oxford, United Kingdom). Cells were washed with a washing solution (phosphate-buffered saline with 1% bovine serum albumin) and 0.4% paraformaldehyde was used as a cell-fixing solution.[40] Samples were analyzed within 48 hours of staining with a BD FACSAria II flow cytometer (BD Biosciences, San Jose, California) and BD FACSDiva software (BD Biosciences, San Jose, California). Lymphocytes were identified using an electronic gate based on cell size and granularity (forward and side-angle light scatter properties). A minimum of 50 000 events was recorded for each preparation. Absolute concentrations of lymphocyte subpopulations were calculated by hematology analysis results in combination with flow cytometry data.

2.6 | Statistical analysis

Normality testing was performed by the Shapiro-Wilk test of normality and normal Q-Q plots. Differences in response variables (hematology results, serum immunoglobulin measurements, and lymphocyte flow cytometry results) among groups (affected IWHs, healthy IWHs, healthy Sighthounds, and healthy dogs of other breeds) were analyzed separately using analysis of covariance (ANCOVA) models. Different transformations (logarithmic, square root, and rank) were made for the response variables to satisfy the normality assumption of the ANCOVA models. Two separate models were fitted for each response variable: (1) model with age-covariate dog group as fixed factor and an interaction term between age and the dog group, and (2) model with only the main effects of the age-covariate and the dog group. Estimates were calculated for differences between relevant groups for the main effects models.

Differences in BALF parameters and arterial blood gas analysis results between affected IWHs and healthy laboratory Beagles were evaluated by the independent samples Student's *t* test (normally distributed variables) and the Mann-Whitney *U* test (non-normally distributed variables). All statistical analyses were performed by commercial statistical software (PASW Statistics 18, SPSS Inc., Chicago, Illinois; and SAS System for Windows 9.3, SAS Institute Inc., Cary, North Carolina). *P* values <.05 were considered statistically significant.

2.7 | Ethical approval and owner consent

The study was approved by the University of Helsinki, Viikki Campus Research Ethics Committee (Statement 2/2014). Owner consent was obtained from the owners of the dogs before participation. The use of purpose-bred laboratory Beagles (decision ESLH-2008-05403/Ym-23, annex ESAVI-2010-03587/Ym-23) and blood sampling in healthy privately owned dogs (decision ESAVI-9116-04.10.07/2014) were approved by the Board of Animal Experimentation of the Regional State Administrative Agency of Southern Finland. Animals were cared for according to the principles outlined by national laws and regulations on laboratory animals.

3 | RESULTS

3.1 | Dogs

Eleven IWHs with recurrent BP, as well as 25 healthy IWHs without a history of previous BP, 28 healthy dogs of other Sighthound breeds, and 16 healthy dogs of other breeds were included in the study. Age,

body weight, and sex distribution of the dogs are presented in Table 1. Dogs of other Sighthound breeds consisted of 21 Whippets, 2 Greyhounds, 2 Borzois, and 1 Afghan Hound, Hungarian Greyhound, and Polish Greyhound. Dogs of non-Sighthound breeds represented various breeds.

All IWHs with recurrent BP were examined between episodes of BP when they were clinically healthy. At the time of inclusion, 1 dog was receiving prednisolone (0.08 mg/kg PO q24h) and 3 dogs were receiving pain medication for orthopedic problems (carprofen, mavacoxib, and gabapentin).

Affected IWHs had experienced their first BP at a median age of 5.0 years (range, 0.4-6.5 years), and when presented to the study, the dogs had experienced a median of 5 previous episodes of BP (range, 2-6). The dogs had experienced recurrence of BP at a mean interval of 3 months (interquartile range, 1.8-5.5 months; range, 1.3-8 months). Most owners (6/11) considered their dogs to be free of respiratory signs between episodes of BP. Five dogs were reported to cough regularly (1/5, daily; 3/5, weekly; 1/5, monthly). Mild exercise intolerance was reported by 6/11 owners, 3 of whom reported concurrent orthopedic problems. None of the owners reported inspiratory wheezes during exercise. One dog was reported to regurgitate daily and 1 dog was reported to eructate daily and regurgitate occasionally.

Owners of affected IWHs reported altogether 47 episodes of previous BP. All episodes of BP had an acute onset of clinical signs, including tachypnea (47/47), dyspnea (46/47), fever (43/47), cough (37/47), breathing with an extended neck (13/47), unwillingness to lie down (10/47), serous nasal discharge (8/47), purulent nasal discharge (2/47), and sneezing (2/47). In the majority of BP episodes (45/47), rapid recovery was reported after initiation of antimicrobial treatment. In 2 episodes of BP, the owners reported that the first antimicrobial was changed to another before clinical signs were relieved. Owners reported full recovery in 43/47 episodes of BP. In 2 episodes of BP, clinical signs relapsed immediately after antimicrobials were discontinued and in 2 episodes of BP, a mild cough persisted afterward.

3.2 | Review of patient records and radiographs

Patient records and radiographs from referring veterinarians were available from 8/11 dogs for retrospective review. In the remaining 3/11 dogs, the referring veterinarian had established a diagnosis of BP, and the acute onset of clinical signs (fever, tachypnea, dyspnea, and cough) as well as rapid response to antimicrobial treatment were highly supportive of the diagnosis. Radiographs were obtained at the referring veterinarian in 17/47 episodes of previous BP. Especially when BP recurred frequently, the diagnosis was based on typical clinical signs and increased serum C-reactive protein concentration.[32]

3.3 | Clinical findings

Clinical examination findings included normal respiratory rate and character of breathing in all dogs. Lung auscultation was normal in 9/11 dogs and mild crackles were detected ventrally in 2/11 dogs. None of the dogs coughed spontaneously, and a mild cough was provoked by tracheal palpation in 5/11 dogs. Cardiac auscultation, heart rate, and rhythm were normal in all dogs.

The results of blood hematology are presented in Table 1. Arterial blood gas analysis results are presented in Table 2. Fecal analyses were negative for lungworms and intestinal parasites in all Beagles and IWHs with recurrent BP. Three of 11 of the affected IWHs were found to be carriers of MRSP in their mucosal membranes.

TABLE 1 Demographic variables as well as the results of hematology and serum immunoglobulin (Ig) analysis in Irish Wolfhounds (IWHs) with recurrent bacterial pneumonia, in healthy IWHs, in healthy Sighthounds, and in healthy dogs of other breeds

	Median (IQR)			
	IWHs with recurrent bacterial pneumonia n = 11	**Healthy IWHs n = 25**	**Healthy Sighthounds n = 28**	**Healthy dogs of other breeds n = 16**
Age (years)	6.3 (5.7-7.0)	6.6 (6.3-8.9)	6.8 (5.3-8.7)	6.5 (5.7-10.4)
Sex	Male 3/11	Male 7/25	Male 15/28	Male 3/16
	Female 8/11	Female 18/25	Female 13/28	Female 13/16
Body weight (kg)	63.9 (58.6-71.3)	64.2 (56.0-70.0)	19.9 (14.8-33.1)***	29.2 (7.6-36.7)***
Blood hematology				
Leukocyte count (10^9/L)	7.9 (7.2-8.6)	7.2 (5.5-8.2)	5.7 (4.9-6.1)***	7.6 (6.3-9.8)
Segmented neutrophil count (10^9/L)	5.8 (4.5-7.0)	5.1 (3.5-5.9)	3.6 (3.0-4.6)***	4.3 (3.7-6.4)
Lymphocyte count (10^9/L)	1.3 (1.1-1.7)	1.2 (1.0-1.6)	1.2 (1.0-1.7)	2.3 (2.0-2.6)***
Eosinophil count (10^9/L)	0.07 (0.02-0.2)	0.08 (0.03-0.3)	0.2 (0.1-0.5)	0.6 (0.3-0.9)**
Monocyte count (10^9/L)	0.5 (0.3-0.9)	0.5 (0.2-0.9)	0.2 (0.2-0.3)***	0.3 (0.2-0.6)
Basophil count (10^9/L)	0.01 (0.0-0.02)	0.01 (0.01-0.02)	0.01 (0.01-0.01)	0.02 (0.01-0.02)
Serum immunoglobulin concentration				
IgA (mg/dL)	104.8 (76.0-238.6)	137.7 (92.5-168.5)	87.3 (54.5-125.4)*	125.1 (75.9-226.9)
IgG (mg/dL)	1000.4 (858.3-1368.0)	1227.0 (965.9-1482.5)	1349.5 (1042.8-1570.3)	1164.0 (930.8-1266.3)
IgM (mg/dL)	317.4 (251.1-378.3)	296.5 (220.0-404.1)	145.8 (131.9-219.6)***	187.9 (164.8-295.3)*

Abbreviation: IQR, interquartile range.
*Significantly different compared with affected IWHs $P < .05$. **Significantly different compared with affected IWHs $P < .01$. ***Significantly different compared with affected IWHs $P < .001$.

TABLE 2 Arterial blood gas analysis, bronchoalveolar lavage fluid (BALF) cytology, and epithelial lining fluid (ELF) immunoglobulin measurement results in Irish Wolfhounds (IWHs) with recurrent bacterial pneumonia and in healthy laboratory Beagles

	Mean ± SD or median (IQR)		
	IWHs with recurrent bacterial pneumonia n = 11	Healthy Beagle dogs n = 6	*P* value
Arterial blood gas analysis			
Arterial PaO_2 mmHg	95.2 ± 4.7	95.4 ± 4.9	.84
Arterial $PaCO_2$ mmHg	29.6 ± 3.2	33.2 ± 2.2	.04
Alveolar-arterial O_2 gradient	19.8 ± 3.5	16.8 ± 3.6	.16
BALF analysis			
Total cell count (10^9/L)	0.31 (0.17-0.47)	0.26 (0.21-0.32)	.88
Neutrophils (%)	4.4 (1.7-5.7)	2.9 (2.2-3.5)	.40
Eosinophils (%)	1.4 (0.0-2.4)	1.2 (0.6-1.4)	.96
Mast cells (%)	0.7 ± 0.8	1.8 ± 1.1	.05
Lymphocytes (%)	25.1 ± 10.7	18.8 ± 7.9	.13
Macrophages (%)	61.1 ± 16.0	73.9 ± 9.6	.11
Epithelial cells (%)	0.0 (0.0-0.0)	0.6 (0.0-3.8)	.06
Proportion of ELF in respiratory samples (%)	3.2 ± 1.3	2.1 ± 0.9	.10
Respiratory sample immunoglobulin concentration			
ELF immunoglobulin A (mg/dL)	18.6 (9.6-48.5)	20.9 (17.7-42.3)	.46
ELF immunoglobulin G (mg/dL)	57.7 (31.5-131.4)	68.7 (59.6-127.1)	.64
ELF immunoglobulin M (mg/dL)	2.3 (1.4-4.2)	1.3 (0.0-12.1)	.40

Abbreviations: IQR, interquartile range; $PaCO_2$, partial pressure of arterial carbon dioxide; PaO_2, partial pressure of arterial oxygen.

3.4 | Immunoglobulin measurements

Serum immunoglobulin measurements (IgA, IgG, and IgM) were available for all dogs. Immunoglobulin concentrations did not differ significantly between affected IWHs and healthy IWHs (Table 1). Age significantly affected serum IgA concentrations in all dogs when statistically examined as 1 group ($P < .001$): older animals had higher serum IgA concentrations.

Epithelial lining fluid immunoglobulin concentrations did not differ significantly between affected IWHs and healthy laboratory Beagles (Table 2).

3.5 | Lymphocyte subpopulations

Flow cytometry analysis was performed in all affected IWHs, in 23/25 healthy IWHs, in 23/28 healthy Sighthounds, and in all healthy dogs of other breeds. In all dogs (when statistically examined as 1 group), the percentage and the absolute concentration of CD8+ lymphocytes as well as the CD4/CD8 ratio were significantly affected by age: older dogs had significantly more CD8+ lymphocytes ($P = .005$) and a significantly lower CD4/CD8 ratio ($P < .001$). The results of flow cytometry analysis are presented in Table 3.

3.6 | Imaging findings

A mild to moderate bronchial or bronchointerstitial pattern was the most frequent finding in thoracic radiographs in 8/11 affected IWHs. Cranial and ventral lung lobes were affected most commonly. A focal alveolar pattern was detected in 2 dogs (in the right cranial lung lobe in 1 dog and in the caudal segment of the left cranial lung lobe in the other dog). Bronchiectasis was not detected in any of the thoracic radiographs. Abnormal accumulation of air in the esophagus was not detected in any of the dogs. Cardiac size was considered normal in all affected IWHs (vertebral heart scale mean, 9.0; SD, 0.5).[41]

Bronchiectasis was the most common abnormality detected in CT images of 10/11 affected IWHs (Figure 1). The location, distribution, and type of BE are presented in Table 4. Other CT findings consisted of local mildly to moderately increased attenuation and thickened bronchial walls (2/11), parenchymal band (2/11), local mildly increased attenuation (1/11), focal reticular pattern (1/11), locally thickened pleura (1/11), and subpleural band (1/11).

3.7 | Bronchoscopy and respiratory sampling

Bronchoscopic abnormalities were detected in 10/11 affected IWHs (Figure 2) and consisted of mild to moderate bronchial mucosal irregularity (10/11), small amount of bronchial secretions (4/11), mild to moderate BE (4/11), and local bronchomalacia (3/11). Bronchoscopic changes were most prevalent in cranial and ventral lung lobes.

Results of BALF cytology analysis are presented in Table 3. The BALF bacterial cultures were negative in all Beagles and in 9/11 affected IWHs. In 1 dog, *Streptococcus canis* (10^2 colony forming units [cfu]/mL) and *S. pseudintermedius* (10^2 cfu/mL) were detected, and *Klebsiella pneumoniae* (10^2 cfu/mL) was detected in another. The BALF cytology did not support bacterial infection in either of these dogs. The *Mycoplasma* spp. culture was negative in all affected IWHs.

3.8 | Electron microscopy of ciliary biopsies

Changes indicating primary ciliary dyskinesia were not observed in any of the affected IWHs (Figure 3). Small numbers of compound cilia

TABLE 3 Proportions (%) and absolute concentrations (10^9/L) of peripheral blood lymphocytes expressing specific cell surface markers (CD3, CD4, CD8, and CD21) in Irish Wolfhounds (IWHs) with recurrent bacterial pneumonia, healthy IWHs, healthy Sighthounds, and in healthy dogs of other breeds

	IWHs with recurrent bacterial pneumonia n = 11	Healthy IWHs n = 23	Healthy Sighthounds n = 23	Healthy dogs of other breeds n = 16
			Mean ± SD	
Proportion of lymphocytes (%)				
CD3+	66.1 ± 8.7	68.0 ± 10.3	54.6 ± 14.6	67.8 ± 8.2
CD4+	46.5 ± 8.7	43.3 ± 8.3	25.9 ± 8.2***	36.4 ± 6.7**
CD8+	14.9 ± 5.9	18.1 ± 6.3	22.9 ± 10.9*	23.7 ± 7.8*
CD3+CD4-CD8-	4.6 ± 2.2	6.5 ± 2.4**	5.8 ± 4.3	7.6 ± 2.0***
CD21+	15.3 ± 5.7	14.9 ± 4.8	18.0 ± 5.8	14.4 ± 5.8
CD4/CD8 ratio	3.7 ± 1.8	2.7 ± 1.2	1.4 ± 0.7***	1.7 ± 0.7**
			Median (IQR)	
Concentration of lymphocytes (10^9/L)				
CD3+	0.74 (0.69-1.23)	0.93 (0.72-1.09)	0.75 (0.52-1.07)	1.55 (1.20-1.84)***
CD4+	0.58 (0.48-0.75)	0.58 (0.44-0.67)	0.36 (0.26-0.50)***	0.91 (0.66-1.03)
CD8+	0.21 (0.10-0.30)	0.21 (0.17-0.31)	0.27 (0.18-0.55)	0.54 (0.34-0.73)***
CD3+CD4-CD8-	0.05 (0.03-0.11)	0.08 (0.06-0.12)*	0.06 (0.03-0.11)	0.19 (0.11-0.23)***
CD21+	0.19 (0.13-0.27)	0.20 (0.15-0.25)	0.26 (0.17-0.37)	0.27 (0.21-0.50)*

Abbreviation: IQR, interquartile range.
*Significantly different compared with affected IWHs $P < .05$.; **Significantly different compared with affected IWHs $P < .01$.; ***Significantly different compared with affected IWHs $P < .001$.

(<10% of examined cilia) were detected in 10/11 dogs. Additionally, rod-shaped bacteria were detected on the luminal aspect of cells in 1 dog that had negative bacterial culture in BALF.

3.9 | Laryngeal and esophageal function

Laryngeal function was evaluated in 9/11 dogs. Normal function was observed in 6/9 dogs, 2 dogs had unilateral laryngeal paresis (grade 2), and bilateral laryngeal paralysis (grade 3) was diagnosed in 1 dog.[29]

None of the affected IWHs had findings suggestive of megaesophagus in thoracic radiographs. Clinical signs were suggestive of esophageal dysfunction in 2 affected IWHs (regurgitation and eructation), and a fluoroscopic swallow study was performed in these dogs. The pharyngeal phase was normal, but esophageal transit time was longer than normal in both dogs.[42] In a 2.6-year-old intact male IWH with daily regurgitation, esophageal transit time was prolonged because of the food bolus remaining in the cervical esophagus for 10 seconds. In a 5.8-year-old intact female with daily eructation and occasional regurgitation, esophageal transit time was >4 minutes. In this dog, the diameter of the esophagus was estimated as being normal, but peristaltic waves were completely missing and food material accumulated in the thoracic esophagus during the entire study period. Both of these dogs had normal laryngeal function.

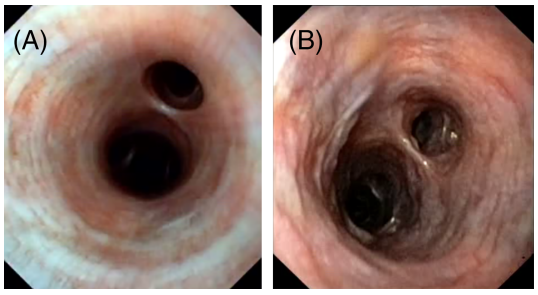

FIGURE 1 Bronchoscopic images of Irish Wolfhounds (IWHs) with recurrent bacterial pneumonia (BP). A, Normal bronchial mucosa in a 0.8-year-old IWH with a history of 2 previous BPs. B, Moderate bronchial mucosal irregularity in a 5.8-year-old IWH with a history of 6 previous BPs

3.10 | Follow-up

Two of the 11 affected IWHs were alive 1 year after the study end point (ages, 9.5 and 4.0 years), and 9/11 dogs had died. The affected dogs had died at a median age of 7.4 years (range, 3.6-8.3), and 4/9 dogs had died because of severe BP (other causes of death were neoplasia [4/9] and hind limb paralysis [1/9]). Recurrent BP had continued after study participation in all dogs except in 1 dog that was euthanized only 2 months after study inclusion because of osteosarcoma. Seven of 11 owners reported a subjective increase in the frequency of BP over time.

4 | DISCUSSION

Recurrent BP recently has been reported as a common disease entity in IWHs in Sweden and the United Kingdom, but detailed description of the disease has been lacking.[19,21] In our study, affected dogs typically were free of respiratory signs until they developed the first episode of BP, which occurred mostly after middle age (median age of onset, 5.0 years). The severity of BP recurrence was indicated by the short intervals between episodes (median, 3 months) and the fact that approximately half of affected dogs eventually died or were euthanized because of BP. However, most of the affected IWHs reached average life expectancy for this breed (reported mean age at death, 6.2-8.2 years) despite suffering from repeated episodes of BP until death.[43,44] A slight female

TABLE 4 The prevalence and characteristics of bronchiectasis (BE) in high-resolution computed tomography studies in Irish Wolfhounds with recurrent bacterial pneumonia. Bronchiectasis was defined as lack of tapering of the bronchial lumen toward the periphery, identification of visible bronchi within 1 cm of the lung margin, or a bronchoarterial (BA) ratio exceeding 2.0. Cylindrical BE was characterized as dilatation of the bronchi without tapering toward the periphery. A saccular BE presented as a focal saccular dilatation or a cyst-like structure, and a varicose BE was described as a focally dilated bronchial segment interposed between normal

	Median (range)			
	Bronchiectasis detected	Distribution of bronchiectasis	BA ratio	Type of bronchiectasis
Right lung				
Cranial lobe	5/11	Focal 5/5	2.2 (1.7-3.0)	Cylindrical 4/5 Saccular 1/5 Varicose 1/5
Middle lobe	10/11	Focal 8/10 Generalized 2/10	2.6 (2.0-3.7)	Cylindrical 10/10
Caudal lobe	0/11			
Accessory lobe	0/11			
Left lung				
Cranial cranial lobe	1/11	Focal 1/1	2.7	Saccular 1/1
Caudal cranial lobe	6/11	Focal 4/6 Generalized 2/6	3.0 (1.6-3.0)	Cylindrical 6/6 Varicose 1/6
Caudal lobe	0/11			

predisposition was noted (8/11 were female), although this predisposition would need to be confirmed in larger studies.

The clinical picture of recurrent BP did not resemble RBPS previously described in young IWHs: dogs with recurrent BP did not have clinical signs from a young age, and nasal discharge was only rarely described during episodes of BP and not at all between episodes.[25] Based on these observations, it is likely that recurrent BP represents a distinct disease entity.

Affected dogs appeared to recover clinically from BP episodes: they were mostly free of clinical signs between episodes, arterial PaO_2 was normal, and alveolar abnormalities on thoracic radiographs resolved in most dogs. Bronchoalveolar lavage fluid bacterial and *Mycoplasma* spp. cultures did not yield clinically relevant bacterial growth in any of the dogs between episodes of BP, which also supports clearance of the infections.[45] However, repeated bacterial cultures during consecutive episodes of BP were not obtained, and therefore it was not determined that each BP was a new infection with different causative organisms.

The late onset of clinical signs suggests an acquired rather than congenital disease. Development of BP generally requires a predisposing factor or event, and in cases of recurrent BP, 1 usually can be identified.[1,2,46] The most common acquired predisposing factors to recurrent respiratory infections in adult dogs are conditions predisposing to aspiration (mainly laryngeal and esophageal dysfunction).[2] Acquired immune deficits can also commonly contribute to development of recurrent infections.[2] Primary ciliary defects or primary immunodeficiency disorders are unlikely when the onset of clinical signs is at an older age. However, it has been reported that humans with primary

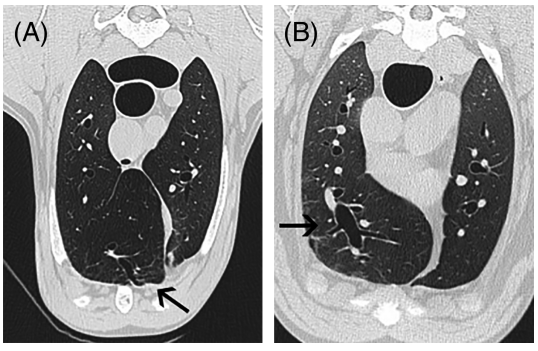

FIGURE 2 Thoracic computed tomographic images of Irish wolfhounds (IWHs) with recurrent bacterial pneumonia (BP). A) Moderate focal bronchiectasis (BE) in the right cranial lung lobe in a 7.0 year old IWH with a history of 4 previous BPs (bronchoarterial ratio 3.0) B) Moderate BE in the right cranial lung lobe in a 2.6 years old IWH with a history of 3 previous BPs (bronchoarterial ratio 2.7). Dogs were scanned in dorsal recumbency.

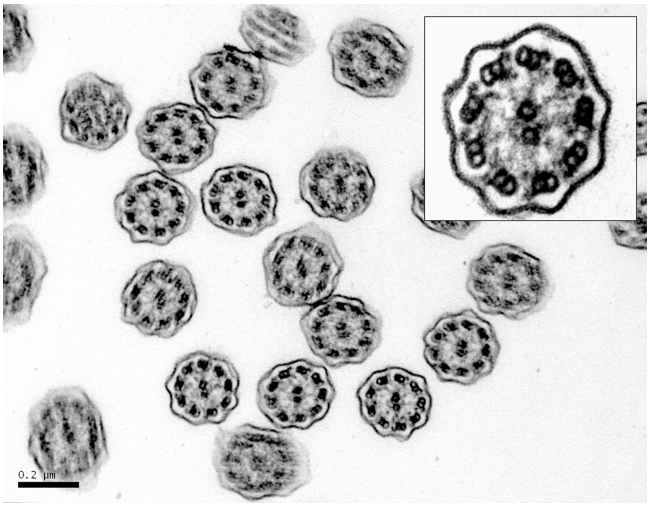

FIGURE 3 Electron microscopy image of normal ciliary ultrastructure from an Irish Wolfhound with recurrent bacterial pneumonia. Cilia exhibit correct number and shape of central and peripheral doublets, spikes and dynein arms

immune deficiency may experience the onset of clinical signs in adulthood, and a case of ciliary dyskinesia has been reported in an aged dog.[47,48] Therefore, these possibilities also were assessed in our study.

Predisposition to aspiration because of laryngeal or esophageal dysfunction was identified in some of the affected IWHs and could have contributed to the development of recurrent BP in these dogs. However, predisposition to aspiration was not a consistent finding in affected dogs and the connection between subclinical laryngeal paralysis and recurrent BP was not fully established; comparison with the prevalence of subclinical laryngeal paralysis in healthy IWHs was not done. Furthermore, it has been shown previously that laryngeal dysfunction also may be detected under anesthesia in asymptomatic dogs.[29] Retention of food in the esophagus was severe in 1 affected IWH and was considered the most likely etiology of recurrent BP in this dog. However, in the other affected IWH with abnormally long esophageal transit time, only transient retention of the food bolus in the proximal esophagus was noted.[42] The connection of this finding to recurrent BP is unclear, and it has been reported that proximal esophageal retention also may occur in healthy dogs.[42] On the other hand, daily regurgitation in this IWH would support the diagnosis of clinically relevant esophageal hypomotility. One limitation of our study was that although laryngeal function was assessed under a light plane of anesthesia, doxapram stimulation was not applied to confirm the diagnosis. Therefore, it is possible that insufficient laryngeal function could have been a result of anesthesia-related suppression rather than true laryngeal paralysis.[49] Another limitation of our study was that esophageal function was assessed only in those dogs in which clinical signs or findings were suggestive of esophageal dysfunction, and therefore subclinical esophageal hypomotility cannot be eliminated in the rest of the dogs. Additionally, the fluoroscopic swallow study was performed using only canned food. Adding other food consistencies to the protocol would have been ideal because significant differences in swallow metrics have been noted among liquid, puree, and kibble meals.[42] However, significant differences in esophageal transit time or the prevalence of food bolus retention have not been noted, and therefore adding liquid or kibble to the protocol was considered unlikely to have changed the assessment.[42]

Defects in the ciliary ultrastructure suggestive of primary ciliary dyskinesia were not detected in any of the affected IWHs. A small number of compound cilia were commonly noted and most likely represent secondary changes caused by repeated BP.[50] However, normal ultrastructure of cilia does not fully eliminate a functional deficit; ciliary dyskinesia without typical ultrastructural changes has been reported rarely in both humans and dogs.[51,52] Ciliary function could be further assessed by using scintigraphic studies and measuring ciliary beat frequency, but such studies were not done in our dogs.[51,52] However, ciliary dyskinesia was considered unlikely in these dogs, because the purulent nasal discharge typical of ciliary dyskinesia was lacking, and accumulation of mucus in the bronchial tree was not noted during bronchoscopy.[6,52–54]

Immunoglobulin deficit has been suggested to underlie RBPS in IWHs and therefore local and systemic immunoglobulin concentrations were evaluated.[25,28] However, deficits in systemic or local IgA, IgG, or IgM were not detected and therefore immunoglobulin deficiency does not explain the recurrent BP.

Flow cytometry analysis of lymphocyte subpopulations did not identify severe deficits of the common B- and T-cell populations in the affected IWHs. A significant decrease was noted in the number of CD4 and CD8 negative T lymphocytes when compared with healthy IWHs. This population of T cells contains small subpopulations including natural killer T lymphocytes and γδ T lymphocytes.[55] Further studies would be needed to evaluate the concentrations of these individual subpopulations in affected IWHs. Nevertheless, because CD4 negative and CD8 negative T cells were not completely lacking in affected IWHs, and the concentrations did not differ when compared with healthy Sighthounds, it was considered unlikely that this finding would explain recurrent infections. An increased CD4/CD8 ratio detected in affected IWHs likely represents immunological activation because of repeated infections.[56,57]

Possible leukocyte adhesion defects (LADs) were not assessed in our dogs and therefore cannot be eliminated. However, persistent leukocytosis has been reported as a uniform feature in LAD patients and because IWHs with recurrent BP all had normal leukocyte counts, LAD was considered unlikely.[58–60]

Focal BE was a common finding and was detected in all except in 1 affected IWH. Bronchiectasis is a permanent abnormal dilatation of airways, which contributes to a loss of normal mucociliary clearance. In humans, BE predisposes to development of bacterial respiratory infections.[61,62] Bronchiectasis in humans develops commonly as a postinfectious consequence of viral or BP but also may be encountered as a congenital defect or develop secondary to aspiration or a bronchial foreign body.[62] In dogs, BE has been described in connection with a variety of infectious and noninfectious respiratory diseases, but the role of BE as a cause or consequence of BP is not fully established.[31,63,64] Local BE was exclusively detected in cranial and ventral lung lobes in affected IWHs, and because the same lung lobes are mostly affected in BP, the development of BE likely represents a postinfectious consequence of BP. This conclusion is further supported by the fact that the only dog in our study without BE was a young dog with only 2 previous episodes of BP. The severity of BE varied from mild (BA ratio, 2.0-2.5) to severe (BA ratio, >3.0), and BE is likely to be an important factor predisposing to further infections.[31] Similarly, as reported previously, thoracic CT examination was more sensitive in detecting BE (10/11) than was thoracic radiography (0/11) or bronchoscopy (4/11).[64] However, bronchoscopy detected only 40% of the BE confirmed by thoracic CT, which is less often than previously reported.[64] This most likely is because the bronchoscope provides a limited view of the most cranial lung areas and cannot access more peripheral airways. In humans, the prevalence of BE varies among ethnic groups, and it is considered likely that genetic factors also contribute to the development of BE.[62] Further studies would be necessary to investigate whether IWHs as a breed are more predisposed to bronchial remodeling and development of BE. Because BE likely develops as a consequence of repeated respiratory infections in affected IWHs, prompt and efficient treatment of acute BP episodes is critical.

The high number of MRSP carriers among affected IWHs suggests that the numerous antimicrobial treatments create a threat of increasing antimicrobial resistance. This problem is not easily solved. Because BP is an acute potentially life-threatening bacterial infection, it needs to be treated with antimicrobials for animal welfare. Because affected IWHs typically respond rapidly to antimicrobial treatment

and appear to recover clinically, owners tend to continue treating BP episodes despite the recurrent nature of the disease. The etiology of recurrent BPs still is largely unknown, and the methods of prevention, therefore, also are limited. Future research efforts could be aimed at identifying possible genetic factors connected with this disease as well as further investigating possible local immune deficits or factors leading to marked bronchial remodeling.

An inherent limitation in our study was that episodes of previous BP were mostly diagnosed and treated by the referring veterinarian and therefore could not be verified using uniform criteria, and patient records were available for retrospective review in only 8/11 affected IWHs. Additionally, healthy IWHs did not undergo all examinations performed in affected dogs and therefore CT, bronchoscopy, and BALF findings could not be compared with those from the healthy IWHs.

To conclude, recurrent BP affects mostly middle-aged and older IWHs, and after onset, the disease typically continues with repeated episodes of BP until death. Focal BE was a frequent finding in affected dogs, and likely contributes to the development of recurrent respiratory infections. Additionally, laryngeal dysfunction and esophageal hypomotility were identified as possible predisposing factors to repeated BP in a minority of dogs. Local or systemic immunoglobulin deficiencies or primary ciliary defects were not detected in affected dogs.

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CONFLICT OF INTEREST DECLARATION

SJV has received research grants from the Finnish Foundation of Veterinary Research and the Finnish Veterinary Foundation. These funding sources did not have any influence on the study design, sample collection, interpretation of results, or preparation of the manuscript.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

The use of purpose-bred laboratory Beagles (decision ESLH-2008-05403/Ym-23, annex ESAVI-2010-03587/Ym-23) and blood sampling in healthy privately owned dogs (decision ESAVI-9116-04.10.07/2014) were approved by the Board of Animal Experimentation of the Regional State Administrative Agency of Southern Finland.

HUMAN ETHICS APPROVAL DECLARATION

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

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REFERENCES

1. Brady CA. Bacterial pneumonia in dogs and cats. In: King LG, ed. *Textbook of Respiratory Disease in Dogs and Cats*. 1st ed. St. Louis, MO: Saunders Elsevier; 2004:412-421.
2. Ford RB. Bacterial pneumonia. In: Bonagura JD, Twedt DC, eds. *Kirk's Current Veterinary Therapy XIV*. Vol 2009. 14th ed. St. Louis, MO: Saunders; 2009:658-662.
3. Viitanen SJ, Lappalainen A, Rajamaki MM. Co-infections with respiratory viruses in dogs with bacterial pneumonia. *J Vet Intern Med*. 2015; 29:544-551.
4. Thayer G, Robinson S. Bacterial bronchopneumonia in the dog-a review of 42 cases. *J Am Anim Hosp Assoc*. 1984;20:731-735.
5. Jameson PH, King LA, Lappin MR, Jones RL. Comparison of clinical signs, diagnostic findings, organisms isolated, and clinical outcome in dogs with bacterial pneumonia: 93 cases (1986-1991). *J Am Vet Med Assoc*. 1995;206:206-209.
6. Watson PJ, Herrtage ME, Peacock MA, Sargan DR. Primary ciliary dyskinesia in Newfoundland dogs. *Vet Rec*. 1999;144:718-725.
7. Merveille AC, Battaille G, Billen F, et al. Clinical findings and prevalence of the mutation associated with primary ciliary dyskinesia in Old English Sheepdogs. *J Vet Intern Med*. 2014;28:771-778.
8. Dhein CR, Prieur DJ, Riggs MW, Potter KA, Widders PR. Suspected ciliary dysfunction in Chinese Shar Pei pups with pneumonia. *Am J Vet Res*. 1990;51:439-446.
9. Blum JR, Cork LC, Morris JM, Olson JL, Winkelstein JA. The clinical manifestations of a genetically determined deficiency of the third component of complement in the dog. *Clin Immunol Immunopathol*. 1985;34:304-315.
10. Breitschwerdt EB, Brown TT, De Buysscher EV, et al. Rhinitis, pneumonia, and defective neutrophil function in the Doberman Pinscher. *Am J Vet Res*. 1987;48:1054-1062.
11. MacPhail CM, Monnet E. Outcome of and postoperative complications in dogs undergoing surgical treatment of laryngeal paralysis: 140 cases (1985-1998). *J Am Vet Med Assoc*. 2001;218:1949-1956.
12. Mercurio A. Complications of upper airway surgery in companion animals. *Vet Clin North Am Small Anim Pract*. 2011;41:969-980, vi-vii.
13. Bahr KL, Howe L, Jessen C, Goodrich Z. Outcome of 45 dogs with laryngeal paralysis treated by unilateral arytenoid lateralization or bilateral ventriculocordectomy. *J Am Anim Hosp Assoc*. 2014;50: 264-272.
14. Dewey CW, Bailey CS, Shelton GD, Kass PH, Cardinet GH 3rd. Clinical forms of acquired myasthenia gravis in dogs: 25 cases (1988-1995). *J Vet Intern Med*. 1997;11:50-57.
15. McBrearty AR, Ramsey IK, Courcier EA, Mellor DJ, Bell R. Clinical factors associated with death before discharge and overall survival time in dogs with generalized megaesophagus. *J Am Vet Med Assoc*. 2011; 238:1622-1628.
16. Ovbey DH, Wilson DV, Bednarski RM, et al. Prevalence and risk factors for canine post-anesthetic aspiration pneumonia (1999-2009): a multicenter study. *Vet Anaesth Analg*. 2014;41:127-136.
17. Fransson BA, Bagley RS, Gay JM, et al. Pneumonia after intracranial surgery in dogs. *Vet Surg*. 2001;30:432-439.
18. Java MA, Drobatz KJ, Gilley RS, Long SN, Kushner LI, King LG. Incidence of and risk factors for postoperative pneumonia in dogs

anesthetized for diagnosis or treatment of intervertebral disk disease. *J Am Vet Med Assoc*. 2009;235:281-287.
19. Orleifson L, Ljungvall I, Hoglund K, Haggstrom J. Occurrence of cardiorespiratory diseases and impact on lifespan in Swedish Irish Wolfhounds: a retrospective questionnaire-based study. *Acta Vet Scand*. 2017;59:53.
20. Greenwell CM, Brain PH. Aspiration pneumonia in the Irish Wolfhound: a possible breed predisposition. *J Small Anim Pract*. 2014;55:515-520.
21. Bodey A. A retrospective questionnaire-based study of pneumonia in the Irish Wolfhound. In: Proceedings of the 25th British Small Animal Veterinary Congress; April 5-8, 2015; Birmingham, UK: BSAVA; 2015.
22. Tyrrell WD, Abbott J, Green H, et al. An update on cardiac disease in the Irish Wolfhound: The North American experience. In: Proceedings of the 2015 ACVIM Forum; June 3-6, 2015; Indianapolis, IN: Wiley; 2015.
23. Vollmar AC, Fox PR. Long-term outcome of Irish Wolfhound dogs with preclinical cardiomyopathy, atrial fibrillation, or both treated with pimobendan, benazepril hydrochloride, or methyldigoxin monotherapy. *J Vet Intern Med*. 2016;30:553-559.
24. Fleming JM, Creevy KE, Promislow DE. Mortality in North American dogs from 1984 to 2004: an investigation into age-, size-, and breed-related causes of death. *J Vet Intern Med*. 2011;25:187-198.
25. Clercx C, Reichler I, Peeters D, et al. Rhinitis/bronchopneumonia syndrome in Irish Wolfhounds. *J Vet Intern Med*. 2003;17:843-849.
26. Leisewitz AL, Spencer JA, Jacobson LS, Schroeder H. Suspected primary immunodeficiency syndrome in three related Irish Wolfhounds. *J Small Anim Pract*. 1997;38:209-212.
27. Wilkinson GT. Some observations on the Irish Wolfhound rhinitis syndrome. *J Small Anim Pract*. 1969;10:5-8.
28. Day MJ. Immunodeficiency disease. In: Day MJ, ed. *Clinical Immunology of the Dog and Cat*. 1st ed. Ames, IA: Iowa State University Press; 1999:197-215.
29. Broome C, Burbidge HM, Pfeiffer DU. Prevalence of laryngeal paresis in dogs undergoing general anaesthesia. *Aust Vet J*. 2000;78:769-772.
30. Melamies MA, Jarvinen AK, Seppala KM, Rita HJ, Rajamaki MM. Comparison of results for weight-adjusted and fixed-amount bronchoalveolar lavage techniques in healthy Beagles. *Am J Vet Res*. 2011;72:694-698.
31. Mesquita L, Lam R, Lamb CR, McConnell JF. Computed tomographic findings in 15 dogs with eosinophilic bronchopneumopathy. *Vet Radiol Ultrasound*. 2015;56:33-39.
32. Viitanen SJ, Laurila HP, Lilja-Maula LI, Melamies MA, Rantala M, Rajamäki MM. Serum C-reactive protein as a diagnostic biomarker in dogs with bacterial respiratory diseases. *J Vet Intern Med*. 2014;28:84-91.
33. Gronthal T, Moodley A, Nykasenoja S, et al. Large outbreak caused by methicillin resistant *Staphylococcus pseudintermedius* ST71 in a Finnish veterinary teaching hospital—from outbreak control to outbreak prevention. *PLoS One*. 2014;9:e110084.
34. Brinc D, Chan MK, Venner AA, et al. Long-term stability of biochemical markers in pediatric serum specimens stored at −80 degrees C: a CALIPER substudy. *Clin Biochem*. 2012;45:816-826.
35. Stuyven E, Verdonck F, Van Hoek I, et al. Oral administration of beta-1,3/1,6-glucan to dogs temporally changes total and antigen-specific IgA and IgM. *Clin Vaccine Immunol*. 2010;17:281-285.
36. German AJ, Hall EJ, Day MJ. Measurement of IgG, IgM and IgA concentrations in canine serum, saliva, tears and bile. *Vet Immunol Immunopathol*. 1998;64:107-121.
37. Vilson A, Hedhammar A, Reynolds A, et al. Immunoglobulins in dogs: correspondence and maturation in 15 litters of German Shepherd dogs and their dams. *Vet Rec Open*. 2016;3:e000173-2016-000173 eCollection 2016.
38. Villaescusa A, Garcia-Sancho M, Delgado AM, et al. Immunophenotypic evaluation of working Labrador Retrievers and German Shepherd dogs living in the same environment. *Vet J*. 2012;193:602-605.
39. Villaescusa A, Garcia-Sancho M, Rodriguez-Franco F, Sainz A. Early-life longitudinal survey of peripheral blood lymphocyte subsets in Beagle dogs. *Vet Immunol Immunopathol*. 2012;149:126-131.
40. Lanier LL, Warner NL. Paraformaldehyde fixation of hematopoietic cells for quantitative flow cytometry (FACS) analysis. *J Immunol Methods*. 1981;47:25-30.
41. Buchanan JW, Bucheler J. Vertebral scale system to measure canine heart size in radiographs. *J Am Vet Med Assoc*. 1995;206:194-199.
42. Harris RA, Grobman ME, Allen MJ, et al. Standardization of a videofluoroscopic swallow study protocol to investigate dysphagia in dogs. *J Vet Intern Med*. 2017;31:383-393.
43. Urfer SR, Gaillard C, Steiger A. Lifespan and disease predispositions in the Irish Wolfhound: a review. *Vet Q*. 2007;29:102-111.
44. Michell AR. Longevity of British breeds of dog and its relationships with sex, size, cardiovascular variables and disease. *Vet Rec*. 1999;145:625-629.
45. Peeters DE, McKiernan BC, Weisiger RM, Schaeffer DJ, Clercx C. Quantitative bacterial cultures and cytological examination of bronchoalveolar lavage specimens in dogs. *J Vet Intern Med*. 2000;14:534-541.
46. Dear JD. Bacterial pneumonia in dogs and cats. *Vet Clin North Am Small Anim Pract*. 2014;44:143-159.
47. Killingsworth CR, Slocombe RF, Wilsman NJ. Immotile cilia syndrome in an aged dog. *J Am Vet Med Assoc*. 1987;190:1567-1571.
48. Rosenberg E, Dent PB, Denburg JA. Primary immune deficiencies in the adult: a previously underrecognized common condition. *J Allergy Clin Immunol Pract*. 2016;4:1101-1107.
49. Radkey DI, Hardie RJ, Smith LJ. Comparison of the effects of alfaxalone and propofol with acepromazine, butorphanol and/or doxapram on laryngeal motion and quality of examination in dogs. *Vet Anaesth Analg*. 2018;45:241-249.
50. McAuley JR, Anand VK. Clinical significance of compound cilia. *Otolaryngol Head Neck Surg*. 1998;118:685-687.
51. Bush A, Chodhari R, Collins N, et al. Primary ciliary dyskinesia: current state of the art. *Arch Dis Child*. 2007;92:1136-1140.
52. Edwards DF, Kennedy JR, Toal RL, Maddux JM, Barnhill MA, Daniel GB. Kartagener's syndrome in a Chow Chow dog with normal ciliary ultrastructure. *Vet Pathol*. 1989;26:338-340.
53. Morrison WB, Wilsman NJ, Fox LE, Farnum CE. Primary ciliary dyskinesia in the dog. *J Vet Intern Med*. 1987;1:67-74.
54. Edwards DF, Patton CS, Kennedy JR. Primary ciliary dyskinesia in the dog. *Probl Vet Med*. 1992;4:291-319.
55. Day MJ. Basic immunology. In: Day MJ, ed. *Clinical Immunology of the Dog and Cat*. Iowa City, Iowa, USA: Iowa State University Press; 1999:9-46.
56. Bruno G, Saracino A, Monno L, Angarano G. The revival of an "old" marker: CD4/CD8 ratio. *AIDS Rev*. 2017;19:81-88.
57. Day MJ. Ageing, immunosenescence and inflammageing in the dog and cat. *J Comp Pathol*. 2010;142(Suppl 1):S60-S69.
58. Hugo TB, Heading KL. Leucocyte adhesion deficiency III in a mixed-breed dog. *Aust Vet J*. 2014;92:299-302.
59. Zimmerman KL, McMillan K, Monroe WE, et al. Leukocyte adhesion deficiency type I in a mixed-breed dog. *J Vet Diagn Invest*. 2013;25:291-296.
60. Trowald-Wigh G, Ekman S, Hansson K, Hedhammar A, Hard af Segerstad C. Clinical, radiological and pathological features of 12 Irish setters with canine leucocyte adhesion deficiency. *J Small Anim Pract*. 2000;41:211-217.
61. Hill AT, Pasteur M, Cornford C, Welham S, Bilton D. Primary care summary of the British Thoracic Society Guideline on the management of non-cystic fibrosis bronchiectasis. *Prim Care Respir J*. 2011;20:135-140.
62. Stafler P, Carr SB. Non-cystic fibrosis bronchiectasis: its diagnosis and management. *Arch Dis Child Educ Pract Ed*. 2010;95:73-82.
63. Nerhagen S, Shiel RE. Chronic pneumonia and focal bronchiectasis in a Siberian Husky dog. *Veterinary Record Case Reports*. 2018;6:e000543.
64. Johnson LR, Johnson EG, Vernau W, Kass PH, Byrne BA. Bronchoscopy, imaging, and concurrent diseases in dogs with bronchiectasis: (2003-2014). *J Vet Intern Med*. 2016;30:247-254.

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