

## Rhinitis/Bronchopneumonia Syndrome in Irish Wolfhounds

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This study describes the clinical, immunologic, genetic, and pathologic features of Irish Wolfhounds with rhinitis/bronchopneumonia syndrome. The dogs examined were from Belgium, The Netherlands, UK, Canada, Germany, and Switzerland. Signs included transient to persistent mucoid or mucopurulent rhinorrhea, cough, and respiratory dyspnea. Radiographic, rhinoscopic, and bronchoscopic findings were variable. Analysis of ciliary ultrastructure was performed in 5 affected dogs, but no characteristic primary ciliary defects (primary ciliary dyskinesia) were detected. Serum and bronchoalveolar lavage fluid (BALF) concentrations of IgA, IgG, and IgM were determined in some affected dogs and clinically normal Irish Wolfhounds. Serum IgA concentration was below the reference range in 5 of 8 affected dogs tested, whereas BALF IgA concentration was above the normal range in 2 affected adult dogs. The CD4 to CD8 lymphocyte subset ratio (CD4:CD8) in peripheral blood was tested in 3 affected dogs and was within the normal range. BALF CD4:CD8 was tested in 1 affected dog and was higher than the normal range. Decreased neutrophil phagocytosis was observed in 1 of the 4 dogs tested. Analysis of pedigrees of the Belgian, Canadian, German, and Swiss dogs revealed common ancestry, suggesting a heritable syndrome.

**Key words:** Dog; Immunoglobulins; Mucociliary function; Rhinitis.

**R**hinitis/bronchopneumonia syndrome (RBPS) in the Irish Wolfhound was 1st described in 1969,<sup>1</sup> but very few reports of the disease have been published since that time.<sup>2,3</sup> RBPS is characterized by respiratory signs that range in severity from slight transient rhinorrhea present from birth to continuous intractable purulent nasal discharge with recurrent bronchopneumonia.<sup>1–3</sup> A primary immunodeficiency is suspected to underlie this condition.<sup>2,3</sup> Affected dogs have been reported to have decreased serum concentration of immunoglobulin A (IgA)<sup>3</sup>; however, serum concentrations of IgA do not necessarily correlate with mucosal production of this immunoglobulin.<sup>4</sup>

Other possible causes of this syndrome include a viral etiology<sup>1</sup> or a primary congenital ciliary defect, but these hypotheses have not been examined. The clinical features of RBPS in the Irish Wolfhound are compatible with those reported in humans<sup>5</sup> or dogs<sup>6</sup> with primary ciliary defect. Canine primary ciliary dyskinesia is a rare disease that has been reported in 16 breeds,<sup>6,7</sup> but not in the Irish Wolfhound. The ultrastructural ciliary abnormalities that occur in primary ciliary dyskinesia result in ineffective and uncoordinated ciliary motion.<sup>6,8</sup>

We present data from a series of Irish Wolfhounds with RBPS that originated from Belgium, The Netherlands, UK,

Germany, Switzerland, and Canada. The 1st aim of this study was to review the clinical, radiographic, and bronchoscopic features of RBPS in this breed. The 2nd aim was to investigate the pathogenesis of the disease by laboratory investigation of systemic and respiratory immunologic function in some affected and control Irish Wolfhounds. Histopathologic and ultrastructural studies are also reported for a limited number of affected dogs. The final aim of this study was to investigate whether RBPS could have an inherited basis in dogs of the Irish Wolfhound breed.

### Materials and Methods

#### Case Material and Sample Collection

Affected Irish Wolfhounds included 4 from Belgium and The Netherlands, 6 from the UK, 10 from Canada, 6 from Germany, and 2 from Switzerland. Clinical history and results of physical examination were available for all dogs. Results of radiography, rhinoscopy, bronchoscopy, or a combination of diagnostic procedures were available in 10 dogs. Samples were also collected for bacteriologic culture (10 dogs), bronchoalveolar lavage fluid (BALF) cytology (10 dogs) and immunology (3 dogs), nasal/bronchial mucosal histopathology (7 dogs), and ultrastructural studies (5 dogs). Results from blood samples for routine CBC analysis and immunologic studies were available for 14 and 8 dogs, respectively.

In addition to the 28 affected dogs, a control population of 38 apparently healthy Irish Wolfhounds, including directly related ( $n = 12$ ) or unrelated ( $n = 26$ ) dogs was also studied. These dogs originated from Belgium (5 unrelated adults 2–5 years old and 21 two-month-old puppies), from the UK (one 4-month-old littermate of affected Irish Wolfhounds, 2 adult 2–4-year-old littermates of affected Irish Wolfhounds, and two 2–4-year-old dams of litters in which there were affected puppies), and from Canada (one 14-month-old littermate and the 4-year-old sire of an affected Irish Wolfhound). Other dogs originated from Germany (two 5-month-old littermates and two 2- and 4-year-old dams of litters in which there were affected puppies) and from Switzerland (one 7-month-old littermate and the 3-year-old dam of affected puppies). Blood samples were obtained from all dogs from Belgium, UK, The Netherlands, and Canada and served as controls for studies of serum immunoglobulin concentrations. The control dogs providing these samples were divided into 3 subgroups: healthy adult unrelated Irish Wolfhounds (HAU,  $n = 5$ ), healthy adult related Irish Wolfhounds (HAR,  $n = 6$ ), or healthy young related or unrelated Irish Wolfhounds ( $n = 22$ ). Radiography and bronchoscopy were performed on the related control dogs from Germany and Switzerland, including

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the collection of samples for bacteriologic culture and bronchial cytology. Ultrastructural analysis of cilia was also performed on samples obtained from one of these dogs.

### **Bronchoscopy**

Bronchoscopic examination was performed under anesthesia with a video endoscope (outer diameter 4.8 mm<sup>a</sup>) that allowed video recording of the bronchi. For the collection of BALF, 2–4 (generally 3) 20-mL aliquots of 0.9% warmed sterile saline solution were used. The 1st 2 aliquots were instilled successively into the same bronchus. The bronchus selected corresponded to the most (or to one of the most) affected area as determined by radiography and by the gross bronchoscopic findings. The 3rd aliquot was instilled into another bronchus in the opposite lung. Each aliquot was instilled through a 3-way stopcock into the biopsy channel and directly retrieved into a sterile container by low-power pump aspiration. Part of the lavage fluid retrieved was sent for aerobic bacterial and mycological cultures. Two milliliters of BALF was placed into a tube containing edetic acid for determination of total nucleated cell count. The lavage fluid was centrifuged, and smears were prepared from the pelleted cells and stained with Wright-Giemsa stain. Alternatively, cytologic preparations were made from brush samples.

### **Histopathology and Ultrastructural Analysis**

Endoscopic biopsies of nasal or bronchial mucosa were placed into 10% neutral buffered formalin and processed before embedding in paraffin wax. Tissue sections were prepared and stained with hematoxylin and eosin for routine light microscopic examination. Tracheal and nasal mucosal biopsies were also processed for ultrastructural analysis of cilia according to previously described techniques.<sup>8–10</sup> Biopsies were fixed in 3% glutaraldehyde<sup>b</sup> in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 hours, dehydrated through graded ethanol, embedded in epoxide resin, cut on a LKB ultramicrotome, and finally stained with uranyl acetate and lead citrate for viewing with a Philips 201 electron microscope.<sup>c</sup> The percentage of transverse sections of cilia showing abnormalities was determined from each biopsy on the basis of the examination of 121–249 cilia.

### **Serum and BALF Immunoglobulin Determinations**

Serum immunoglobulin (Ig) concentrations (IgG, IgM, and IgA) were measured by single radial immunodiffusion.<sup>d,11</sup> The sensitivity of this assay was 0.1 mg/mL. Capture enzyme-linked immunosorbent assays (ELISAs) for quantification of canine IgG, IgM, and IgA were used to analyze immunoglobulin concentrations in BALF according to a technique previously described.<sup>4,12</sup> Results were expressed in ELISA units (EU), in which the concentration of a standard sample was defined as 100 EU/mL.

### **Neutrophil Phagocytosis Assay**

The phagocytic function of peripheral blood neutrophils was evaluated by coculture of neutrophils isolated from blood with fluorescently labeled *Escherichia coli* bacteria by a modified Boyden technique.<sup>13</sup> A blood sample from a clinically normal control dog was tested on each occasion. For each sample, a phagocytic score was calculated by determining the percentage of neutrophils that had phagocytosed 3 or more fluorescent bacteria.

### **Characterization of Lymphocyte Subsets in Peripheral Blood and BALF**

The relative percentage of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes was determined in samples of peripheral blood and BALF. Mononuclear cells were isolated from blood and BALF as described elsewhere.<sup>12</sup> Briefly, cells were separated by differential centrifugation on Ficoll-Hypaque<sup>d</sup>

(density 1.077 g/mL). Cells remaining at the interface were collected and washed in phosphate-buffered saline (PBS) before freezing at –196°C in culture medium containing 10% dimethyl sulfoxide with 5% fetal calf serum (FCS). On the day of analysis, frozen cells were thawed rapidly by incubation at 37°C in a water bath. The cells were then incubated with rat monoclonal antibody raised against canine CD4<sup>e</sup> or CD8<sup>f</sup> for 30 minutes at 4°C. After washing, the cells were incubated with oregon green–conjugated goat anti-rat IgG<sup>g</sup> for 30 minutes at 4°C. After washing, the cells were resuspended in PBS containing 5 µg of propidium iodide (PI). Flow cytometric analysis was performed with a Becton-Dickinson fluorescence-activated cell sorter<sup>h</sup> equipped with an argon laser. Debris was excluded from the analysis by the conventional scatter gating method and exclusion of PI-positive cells. Fluorescein isothiocyanate and PI emission signals were collected with the use of appropriate filters at 530 nm (band-pass 30 nm) and 575 nm (band-pass 26 nm), respectively. In most cases, 10,000 events per sample were collected in list mode fashion, stored, and analyzed by Cell-Quest software.<sup>i</sup> The threshold of positivity for fluorescence intensity was arbitrarily set on the basis of the negative control sample.

### **Pedigree Analysis**

Complete pedigrees were available from the dogs from Belgium, The Netherlands, Canada, Germany, and Switzerland, and only limited information was available on the immediate relationship of the UK dogs. The pedigrees were examined to establish relationships among the animals. The owners and breeders of the dogs were questioned about any other affected dogs that they might have encountered.

### **Statistical Analysis**

A 1-way analysis of variance<sup>j</sup> was used to compare the percentage of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes within the peripheral blood and BALF of 3 affected Irish Wolfhounds with those obtained from a control group of 15 young-adult healthy dogs of various breeds. The reference values derived from these control dogs have been used for previous studies.<sup>12</sup> The level of significance was  $P = .01$ .

Because immunoglobulin values followed a normal distribution, they were not log transformed for analysis. A 1-way analysis of variance was used to compare the serum and BALF immunoglobulin concentrations obtained from the 5 young HAU Irish Wolfhounds and those obtained from 6 HAR Irish Wolfhounds. Similarly, the serum and BALF immunoglobulin concentrations obtained from a control group of 15 young-adult healthy dogs of various breeds (data obtained from previous studies<sup>12</sup>) were compared with those obtained from the HAU and HAR groups. Because no differences were noted, data from all 3 groups were combined to create a single large control group of 26 adult dogs (HA group). The level of significance was chosen as  $P = .01$ .

In the 2nd stage of analysis, affected Irish Wolfhounds were divided into 2 groups. The 1st group comprised 4 dogs younger than 14 months (dog 1, from which 3 samples were obtained at 2–3-week intervals; dog 2, from which 2 samples were obtained at 2–3-week intervals; dog 7; and dog 20); the 2nd group comprised 4 adult dogs 14 months and older (dog 4, from which 2 samples were obtained at 2–3-week intervals; dog 5; dog 6; and dog 8). Values from the 2nd group were compared with values in the large control group of adult dogs described above (HA group). Values obtained in puppies and young dogs were compared with those obtained in another control group that comprised 22 Irish Wolfhound puppies.

## **Results**

### **Patient Signalment, History, Clinical, and Gross Pathology Findings**

Dogs 1–3 were a 5-year-old female, a 4.5-year-old male, and a 2-year-old female originating from Belgium; dog 4

was a 4-month-old male from The Netherlands. Dogs 5–10, originating from the UK, comprised 2 males and 4 females; dogs 5 and 6 were 13-week-old littermates, and dogs 7–10 were aged 1 year 4 months, 5 months, 5 years, and 3.5 years, respectively.

Dogs 11 and 12 were 5-month-old littermates (1 male, 1 female) originating from Germany. Dogs 13–15 were 7-month-old littermates (2 males and 1 female) originating from Switzerland, whereas dogs 16–18 were 7-week-old littermates (2 males, 1 female) originating from Germany. Dogs 19–28 all originated from Canada. Dogs 19–22 were littermates but were examined at different ages (from 6 to 14 months). Dogs 23 and 24 were 6-month-old littermates, and dogs 25–28 were 3-day-old littermates. The most complete clinical histories were available from those dogs referred to the University of Liège in Belgium (dogs 1–4), and as an example of the general clinical presentation of these dogs, details of dog 1 are presented below.

Dog 1, a 5 year-old female (70 kg), presented with bilateral nasal discharge and sneezing that had been present since birth. She came from a litter of 5 puppies, and another male littermate that was not available for this study was also affected. The discharge was initially intermittent but became persistent. The discharge was purulent in nature but became serous while the dog was receiving treatment. The dog had been regularly treated with antibiotic therapy and intranasal mucolytic drugs. The dog developed an episodic cough and pyrexia at age 1.5 years. This condition also had been successfully treated with antibiotic therapy. Acute episodes of bronchopneumonia recurred approximately once each year in the winter. A previous cardiac evaluation, including echocardiographic-doppler measurements, did not detect cardiac disease.

On presentation to the University of Liège, the clinical examination was unremarkable, except for copious serous nasal discharge. Thoracic radiography showed a diffuse and severe bronchointerstitial pattern with thickening of the right pleura. CBC examination indicated only leukocytosis ( $21.9 \times 10^3$  WBC/mL, reference range  $3\text{--}15 \times 10^3$  WBC/mL;  $17.5 \times 10^3$  neutrophils/mL, range  $3\text{--}11.5 \times 10^3$  neutrophils/mL). Serum biochemistry indicated high urea (12.3 mg/L, range 1.3–5.1 mg/L) and creatinine (35 mg/L, range 5–15 mg/L). Urinalysis showed hyposthenuric urine (specific gravity 1.007) with a urinary protein:creatinine ratio of 2.32.

Two weeks later, rhinoscopy and bronchoscopy were performed under general anesthesia. The owner reported a worsened general condition, although the clinical examination was unchanged. Gross rhinoscopic findings included abundant, thick, mucopurulent material and turbinate atrophy. These changes were bilateral. *Klebsiella pneumoniae* was cultured from the nasal cavity. Gross bronchoscopy was unremarkable except for the presence of some mucus in the small airways of both left and right sides. Repeat CBC examination indicated slight anemia ( $3.87 \times 10^9$  RBC/mL, reference range  $5.5\text{--}8.5 \times 10^9$  RBC/mL;  $10.2 \times 10^3$  WBC/mL;  $8.4 \times 10^3$  neutrophils/mL). Bacteriologic and mycologic culture from BALF was negative. Histopathologic analysis of bronchial biopsies showed evidence of epithelial degeneration, with edema and focal microhemorrhage of the underlying lamina propria. There was a

mild neutrophilic infiltration into the tissue. The nasal biopsies had a range of appearance from normal to inflamed. Most were covered by an intact respiratory epithelium with prominent cilia and goblet cells. The underlying lamina propria contained aggregates of lymphocytes and plasma cells that were, at least in part, probably a reflection of normal mucosal lymphoid tissue. Focally, there was marked edema of the lamina, with a moderate to marked neutrophilic infiltration. This included evidence of vascular margination, and there was active exocytosis of neutrophils through the epithelium.

There was an initial response to a 6-week course of antibiotic therapy, but there were subsequent relapses of disease, and the dog was euthanized 5 months later without postmortem examination.

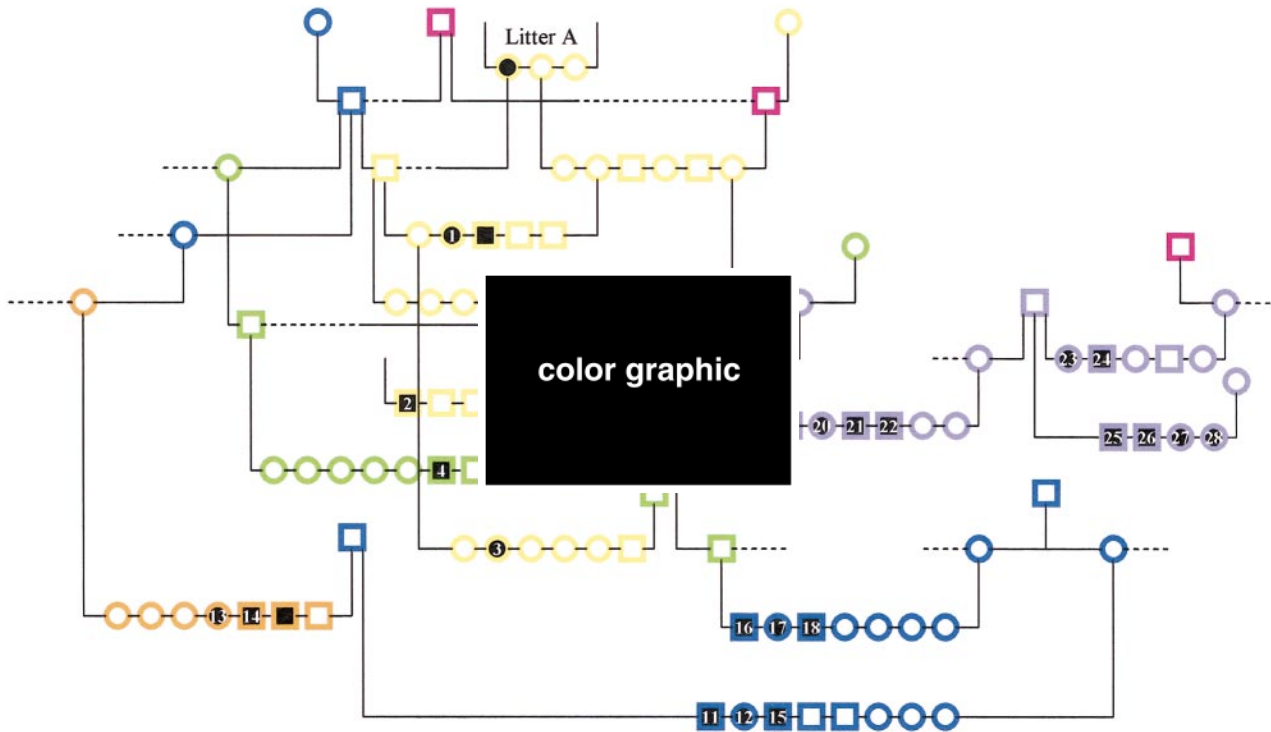
**Affected Dogs from Belgium and the Netherlands.** The other dogs investigated in Liège had had similar signs, with the exception of dog 2, in which signs were obvious only at the age of 4 years and included both nasal and bronchial involvement. In each dog, the course of the disease was similar to that described for dog 1. There was some positive response to antibiotic therapy, but all dogs required regular periodic or continuous treatment.

**Affected Dogs from the UK.** Most affected dogs from the UK had rhinorrhea since birth, which tended in many dogs to become purulent. Not all dogs presented with the same severity of signs. Only 2 dogs from the UK had bronchopneumonia at the time of diagnosis.

**Affected Dogs from Canada.** The 10 affected dogs from Canada were generally more severely affected. These dogs were all puppies from 3 litters (Fig 1), all of which shared the same dog as sire or grandsire. In the 1st litter, 4 of 8 puppies were affected; in the 2nd litter, 2 of 5 puppies were affected; in the 3rd litter, 4 of 11 puppies were affected. All of these dogs had rhinorrhea since birth. Typically, in the affected dogs from the two 1st litters, the nasal discharge became thick and green after 1 or 2 weeks, and the dogs then had bouts of bronchitis. These signs were treated with antibiotic therapy. Four of these dogs died from severe pneumonia at the ages of 5, 6, 8, and 14 months. Another was alive at 38 months with sporadic copious green nasal discharge, whereas no follow-up was available for the last dog. In the 3rd litter, 4 of 11 puppies had green nasal discharge at the age of 3 days and were euthanized without postmortem examination.

**Affected Dogs from Germany and Switzerland.** All affected Irish Wolfhounds from Germany and Switzerland had exhibited bilateral nasal discharge since birth. The 5- and 7-month-old dogs (dogs 11–15) had recurrent purulent rhinorrhea and periods of fever, which were responsive to antibiotic treatment. In addition, 2 dogs (dogs 12 and 17) had concomitant otitis. Only 1 of the affected dogs (dog 11) is still alive (8 years old); he is under sustained antibiotic treatment and inhalation therapy. All of these dogs had an echocardiographic-Doppler examination that failed to detect any cardiac abnormalities.

Thoracic radiographs showed findings compatible with bronchitis, bacterial bronchopneumonia, or both, such as slight to severe diffuse bronchointerstitial pattern (8 dogs), bronchial pattern (3 dogs), alveolar pattern (5 dogs), and evidence of bronchiectasis (2 dogs—1 and 14). CBC ex-



**Fig 1.** Familial relationships in Irish Wolfhound dogs with rhinitis/bronchopneumonia syndrome from Belgium, Canada, Germany, and Switzerland. Pedigrees from affected Irish Wolfhounds from Belgium, The Netherlands, Canada, Germany, and Switzerland. Colors indicate the country of origin of the dogs. Females are indicated by circles, males by squares. Black numbered symbols refer to individual dogs. Black symbols without numbers refer to affected dogs that were not clinically evaluated. Horizontal dotted lines indicate a lack of information about the relationship of the dogs. Vertical dotted lines indicate a close relationship between 2 dogs (1st or 2nd degree). Blood lines: yellow, Belgium; red, United Kingdom; green, The Netherlands; purple, Canada; blue, Germany; and orange, Switzerland.

amination most commonly indicated moderate absolute leukocytosis because of absolute neutrophilia. Leukocyte and neutrophil counts were increased in 8 dogs, ranging between  $17.2$  and  $23.6 \times 10^3$  leukocytes/mL and from  $14.6$  to  $17.7 \times 10^3$  neutrophils/mL, whereas blood leukocyte counts were within the normal range in 4 other dogs.

Rhinoscopy and bronchoscopy indicated variable findings, from absence of obvious macroscopic abnormalities to congested mucosa and the presence of variable amounts of mucopurulent material. Bacteriologic examination of the BALF was negative in 3 dogs, a single agent was cultured in 1 dog, and mixed cultures were observed in 5 dogs. Bacteria retrieved was mainly *E. coli*, *Pasteurella*, *Streptococcus*, and *Staphylococcus* species. Mycoplasma was found in the BALF of 1 of 8 samples tested. Fungal cultures were negative from both nasal and BALF samples ( $n = 3$  tested).

Cytologic examinations were compatible with severe acute bronchopneumonia. The bronchial and nasal biopsies in dog 2 showed similar findings to those described for dog 1, including evidence of vascular margination and active exocytosis of neutrophils through the epithelium.

Results of postmortem examination were available for 3 dogs (dogs 12–14) and included macroscopic evidence of hydrocephalus, rhinitis, chronic bronchitis, pneumonia (both purulent and focal), and, in 1 dog, bronchiectasis.

In the healthy relatives of affected dogs from Germany and Switzerland, thoracic radiography and bronchoscopy,

including the collection of samples for bacteriologic culture and bronchial cytology, failed to detect any abnormalities.

### Ultrastructural Analysis

Ultrastructural analysis was performed on biopsies of the nasal or bronchial mucosae, or both, in dogs 1, 2, 4, 13, and 16, as well as in a healthy littermate of dog 16. No primary ciliary abnormalities were detected. In dog 13, absence of the central pair was observed in 3 of 63 transverse sections only. Secondary ciliary abnormalities (membrane alterations and peripheral microtubular defects) were observed in 4.4–33.9% of the transverse sections in the affected Irish Wolfhounds, whereas in the healthy littermate, no abnormalities were detected. Membrane alterations were observed in 33, 23.1, 11.9, 4.0, and 25% of the transverse sections in dogs 1, 2 (bronchial and nasal), 4, and 16, respectively. Peripheral microtubular pair abnormalities were observed in 0.9, 6.6, 0, 0.4, 11, and 16% of cilia in transverse sections in dogs 1, 2 (bronchial and nasal), 4, 13, and 16, respectively.

### Serum and BALF Immunoglobulin Determinations

Serum IgG concentration was significantly higher in affected adult Irish Wolfhounds than in the group of all healthy adult dogs (HA group,  $n = 26$ ; Table 1). Serum IgG concentration was also significantly higher in young



**Table 1.** Immunoglobulin concentrations (mean  $\pm$  SE and range) from Irish Wolfhounds with rhinitis/bronchopneumonia syndrome (RBPS) and control dogs.

| Dogs   | Serum <sup>a</sup> (mg/mL)               |  |   | BALF <sup>b</sup> (EU/mL)  |                                       |  |
|--|--|--|---|----------------------------|---------------------------------------|--|
|  | IgG                                      | IgM                                      | IgA                                       | IgG                        | IgM                                   | IgA                                      |
| Adult affected Irish Wolfhounds <sup>c</sup> | 16.8 $\pm$ 1.2 <sup>g</sup><br>12.5–25.0 | 0.85 $\pm$ 0.17<br><0.1–2.35             | 0.15 $\pm$ 0.06<br><0.1–0.32              | 12.6 $\pm$ 3.5<br>4.1–21.1 | 2.8 $\pm$ 0.7 <sup>g</sup><br>0.8–4.9 | 12.9 $\pm$ 1.4 <sup>g</sup><br>42.9–43.0 |
| Young affected Irish Wolfhounds <sup>d</sup> | 5.7 $\pm$ 1.4 <sup>h</sup><br>2.3–9.0    | 1.69 $\pm$ 0.21 <sup>h</sup><br>0.85–2.4 | 0.18 $\pm$ 0.07 <sup>h</sup><br><0.1–0.49 | 1.28 <sup>i</sup>          | 0.18 <sup>i</sup>                     | 0.68 <sup>i</sup>                        |
| Adult control dogs <sup>e</sup>              | 9.2 $\pm$ 0.6<br>3.2–18.0                | 0.65 $\pm$ 0.1<br><0.1–2.35              | 0.15 $\pm$ 0.03<br><0.1–0.26              | 4.6 $\pm$ 4.1<br>0.7–16.8  | 0.46 $\pm$ 0.8<br>0.02–3.0            | 2.06 $\pm$ 2.07<br>0.3–7.3               |
| Healthy Irish Wolfhound puppies <sup>f</sup> | 1.5 $\pm$ 0.7 <sup>j</sup><br><0.1–3.2   | 0.60 $\pm$ 0.1<br><0.1–0.85              | 0.001 $\pm$ 0.03 <sup>j</sup><br><0.1     | ND                         | ND                                    | ND                                       |

<sup>a</sup> Measured by single radial immunodiffusion.

<sup>b</sup> Measured by capture enzyme-linked immunosorbent assay.

<sup>c</sup> Measurements in 4 adult affected Irish Wolfhounds 14 months to 5 years old.

<sup>d</sup> Measurements in 4 young affected Irish Wolfhounds 7 weeks to 10 months old.

<sup>e</sup> Reference measurements in all healthy adults (15 young adult Belgian healthy dogs [5 Beagles and 10 random source dogs of other breeds] + 5 young-adult unrelated Irish Wolfhounds + 6 young-adult related Irish Wolfhounds).

<sup>f</sup> Reference measurements in 22 healthy Irish Wolfhound puppies (<4 months old).

<sup>g</sup> Value in adult affected Irish Wolfhounds significantly different from value in adult control dogs.

<sup>h</sup> Value in young affected Irish Wolfhounds significantly different from value in healthy Irish Wolfhound puppies.

<sup>i</sup> From a single value.

<sup>j</sup> Value in healthy Irish Wolfhound puppies significantly different from value in all healthy adults.

affected Irish Wolfhounds compared with the group of 22 healthy Irish Wolfhound puppies.

Although serum IgA and IgM concentrations were low in some individual affected dogs tested, the mean IgM and IgA concentrations were not significantly different when affected adult Irish Wolfhounds were compared to the HA group. In young affected Irish Wolfhounds, serum IgM was significantly higher than in healthy Irish Wolfhound puppies. Moreover, in the healthy Irish Wolfhound puppies, serum IgG and IgA concentrations were significantly lower than in all other groups of dogs. In 2 dogs (1 and 2), in which serum Ig determinations were repeated, serum IgG, IgM, and IgA decreased during an acute episode of bronchopneumonia.

BALF fluid IgM and IgA concentrations were significantly higher in the 2 affected adult dogs tested compared with the HA group. No difference was detected for IgG concentrations in BALF.

#### Neutrophil Phagocytosis Assay

Neutrophil phagocytosis was determined in 4 affected dogs (dogs 1–4), and 7 controls (3 adults, including 1 Irish Wolfhound, 2 mixed breed dogs, and four 3-month-old Irish Wolfhound puppies). In dog 4, 35% of the neutrophils had phagocytosed  $\geq 3$  bacteria per cell, whereas in the control puppies, 55–79% of neutrophils had phagocytosed this number of bacteria. In dogs 1–3 (adult dogs), 68–87% of neutrophils had phagocytosed  $> 3$  bacteria per cell, whereas in the 3 healthy adults, the same score ranged from 58 to 71%.

#### Characterization of Lymphocyte Subsets in Peripheral Blood and BALF

The proportion of CD4 and CD8 lymphocyte subsets in the peripheral blood of 3 affected dogs (dogs 1, 2, and 4)

and in the BALF of dog 1 was determined. In the peripheral blood, the percentages of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes were significantly greater than in a control population of 15 healthy dogs (data from previous studies,<sup>12</sup> mean  $\pm$  SD; 41.0  $\pm$  2.8% versus 21.5  $\pm$  8.1% for CD4<sup>+</sup>; 18.5  $\pm$  2.2% versus 8.8  $\pm$  3.8% for CD8<sup>+</sup>, respectively). The CD4<sup>+</sup> to CD8<sup>+</sup> ratios of affected and clinically normal dogs were not significantly different (2.4  $\pm$  0.4 versus 2.6  $\pm$  0.7, respectively).

In the BALF from dog 1, the percentage of CD4<sup>+</sup> lymphocytes was greater than in the control group (data from previous studies<sup>12</sup>; 53.2  $\pm$  5.0 versus 30.5  $\pm$  1.4, respectively), whereas the percentage of CD8<sup>+</sup> lymphocytes was lower (3.6  $\pm$  4.7 versus 13.4  $\pm$  1.2, respectively). As a consequence, the CD4:CD8 ratio was higher than in the same control group (14.9  $\pm$  1.4 versus 2.6  $\pm$  0.4, respectively).

#### Pedigree Analysis

Pedigrees were obtained for dogs from Belgium, The Netherlands, Canada, Germany, and Switzerland (Fig 1). All of these dogs belong to 6 blood lines: 1 Belgian, 2 Dutch, 1 Canadian, 1 German, and 1 Swiss. In addition, encoded pedigrees were made available from some dogs from the UK, but the breeders did not release the specific pedigree names of the dogs. However, the affected Canadian, Belgian, and Dutch Irish Wolfhounds had shared British ancestor dogs, and the affected Swiss and German Irish Wolfhounds also had distant British ancestry.

A single dog was identified as a grandsire of at least 1 of the 3 affected Canadian litters, a Belgian litter (which included dog 3), and a German litter (which included dogs 16–18). The sire of this dog had fathered at least 1 other litter that included affected animals (dog 1 and another dog with compatible clinical history). Unfortunately, no infor-

mation could be obtained concerning the ancestry of litter A (Fig 1, top), which includes 1 presumably affected animal and 11 study dogs (dogs 1–4, 16–18, and 19–22).

We had the opportunity to examine the pedigrees of 3 more affected Irish Wolfhounds, 2 from the UK and 1 from Pennsylvania. Both share several common ancestors with the dogs described in this study.

## Discussion

The clinical features of the dogs reported in this study are similar to those described in the limited literature on this syndrome in the Irish wolfhound. RBPS in this breed is characterized by respiratory signs that range in severity from slight transient rhinorrhea from birth to continuous, intractable, purulent discharge with bouts of recurrent bronchopneumonia. Rhinorrhea from birth was present in all dogs of the present series, with the exception of dog 2, in which the signs were delayed in onset. The disease appears to be episodic in nature, but this likely reflects the repeated administration of antibiotic therapy to affected dogs.

The 2 most likely underlying causes of RBPS in the Irish Wolfhound would be a primary ciliary dyskinesia or an underlying primary immunodeficiency, and each of these possibilities was investigated in this study. The clinical signs of RBPS (rhinosinusitis, bronchitis, bronchopneumonia) are very similar to those reported in dogs with primary ciliary dyskinesia.<sup>6</sup> In general, dogs affected by primary ciliary dyskinesia or RBPS have an early onset of clinical signs (rhinorrhea), but some dogs with either disorder can remain asymptomatic until they are several years of age. Dogs affected by primary ciliary dyskinesia can also display bronchiectasis as a probable consequence of chronic mucus plugging and inflammation of small airways. Bronchiectasis has also been recognized in some of the more chronically affected Irish Wolfhounds in this study.

Dogs with primary ciliary dyskinesia typically present with respiratory signs; however, clinical signs related to lesions in other tissues with ciliated epithelia or microtubules can occur (eg, otitis media, infertility, hydrocephalus, and renal fibrosis or dilation of renal tubules). In this regard, renal insufficiency was detected in dog 1, although this was not investigated further. Hydrocephalus was identified in dogs 12–14 and otitis in dogs 12 and 17.

Primary ciliary dyskinesia in humans is caused by a genetic defect leading to immotility, dysmotility, or absence of cilia.<sup>5</sup> Primary ciliary dyskinesia is a heterogeneous disease; more than a dozen different specific ciliary defects have been recognized.<sup>5</sup> The excessive mucus production and retention that occurs in this disorder is a secondary consequence of the failure of the normal function of respiratory cilia. Ultrastructurally, primary ciliary dyskinesia in both humans and dogs is associated with a number of specific abnormalities, whereas acquired secondary ciliary dyskinesia is mostly associated with other ultrastructural abnormalities. In the present study, there was no ultrastructural evidence of primary ciliary abnormality, whereas secondary ciliary abnormalities were frequently observed. However, an overlap between the abnormalities of either type of dyskinesia has been recognized, so the ultrastructural distinction between primary ciliary defect and sec-

ondary ciliary defect is difficult. Absence of the central pair of microtubules, as observed in 3 of 63 transverse sections of cilia, has been recognized as a primary defect, but in humans and dogs with primary ciliary defect, it is found in a much higher percentage of cilia.<sup>7,8</sup>

The possibility that a primary immunodeficiency disorder was the underlying cause for RBPS in the Irish Wolfhounds was also partially addressed in this study. Measurement of serum immunoglobulin concentrations is the most widely available means of assessing immune competence in the dog. A likely candidate defect would be IgA deficiency because affected individuals can have weakened mucosal defence, thus predisposing them to infection.<sup>3</sup> Five of the 8 affected Irish Wolfhounds tested in this study had low concentrations of serum IgA, although it is not easy to interpret serum IgA concentrations because normal values vary according to age and breed, as well as from 1 country to another.<sup>12,14</sup> Moreover, because it can be assumed that serum Ig concentrations are low in newborn puppies and then gradually increase during the 1st weeks or months after birth, it was not possible to compare serum concentrations from adult affected dogs with concentrations from healthy puppies or very young animals. Therefore, we only made such comparisons between groups of dogs older than 14 months of age. This limit was arbitrarily chosen to reflect an age at which the dogs would have attainment of a fully developed adult immune system.

Some unaffected relatives also had low serum IgA, suggesting that other predisposing factors might be required for expression of the disease. It has been documented recently that serum IgA concentrations in the dog are not an adequate reflection of mucosal immunity, as shown by analysis of various secretions.<sup>4</sup> For this reason, immunoglobulin concentrations were also quantified in the BALF obtained from the affected Irish Wolfhounds in this study. The BALF IgA concentration in affected Irish Wolfhounds was higher than in a group of control dogs. This finding corroborates the suggestion that serum IgA concentration is a poor indicator of mucosal secretion of IgA in the dog.<sup>4</sup> In contrast, the high IgA concentration in the BALF of affected dogs could in fact reflect a heightened immune response at the respiratory mucosal surface.

It was interesting to note that, in the only 2 dogs in which CBC was repeated (dogs 1 and 2), both WBC and RBC were lower at the time of the acute episode when compared to the analysis performed 3 weeks earlier when the dogs were asymptomatic. Similarly, serum IgG, IgM, and IgA also were lower during the acute episodes in these dogs. This could be a consequence of early infection from sudden excessive tissue demand, destruction, or consumption but also could suggest a cyclical defect in WBC or immunoglobulin concentration, although the small number of dogs studied makes any interpretation difficult.

A range of other immunologic parameters was also assessed in affected dogs of this study. Enhanced susceptibility to infection also can occur in dogs with neutrophil dysfunction. Abnormalities in neutrophil function include defective neutrophil adherence, decreased responsiveness to chemotactic stimuli, altered phagocytic capability, or decreased bactericidal capability.<sup>15</sup> A group of related Doberman Pinschers with rhinitis and pneumonia had defective

neutrophil bactericidal function,<sup>16</sup> and defective migration of neutrophils into tissue occurs in Irish Setters with canine leukocyte adhesion deficiency.<sup>17</sup>

The only test of leukocyte function readily available for application to samples from the Irish Wolfhounds in this study was determination of the phagocytic function of blood-derived neutrophils, and no clear abnormality of this function was documented. Moreover, tissue biopsies from the affected Irish Wolfhounds demonstrated clear evidence of vascular margination and egress into tissues of neutrophils, which would be inconsistent with the canine leukocyte adhesion deficiency mutation.

The final immunologic parameter that was assessed in a limited number of affected Irish Wolfhounds in this study was the relative proportions of lymphocyte subsets within blood or BALF. Examination of the ratio of BALF CD4:CD8 T lymphocytes has been informative in other canine respiratory diseases.<sup>12</sup> In the Irish Wolfhounds tested, the blood CD4:CD8 ratio was not different from that in normal control dogs, whereas in BALF, only 1 value could be obtained. These findings might be interpreted to mean that there was adequate reactivity of blood CD4<sup>+</sup> T lymphocytes in response to the infectious stimulus and suggest that there is no simple defect in the number of T lymphocyte cells, at least within the blood.

The final aspect of this study was a preliminary analysis of the relatedness of Irish Wolfhounds affected by RBPS, which was addressed by examining the pedigrees of affected dogs from individual geographic areas and, where possible, by comparing the ancestry of affected dogs in different geographic locations. Unfortunately, pedigrees were incomplete, so the mode of inheritance of this syndrome could not be determined. Such canine pedigree data are always difficult to interpret, and we had anecdotal information that some Irish Wolfhound breeders might have provided inaccurate information for studbook purposes.

Despite these problems, examination of the available pedigrees did suggest that the affected dogs were closely related, leading us to propose an inherited basis for RBPS.

The etiology of RBPS in dogs of the Irish Wolfhound breed remains unclear. A simple ciliary defect or primary immunodeficiency was not identified in the dogs of this study, and finding normal concentrations of respiratory IgA suggests that IgA deficiency per se is unlikely to have a role in the disease pathogenesis. More extensive studies of a range of other immunologic and physical parameters (eg, assessment of respiratory mucus) are required. Our preliminary observations provide support for the hypothesis that this syndrome has a hereditary component. The long-term goal of future research would be to identify the genetic basis of the disease and to develop a means of detecting and eliminating carrier dogs from the Irish Wolfhound population.

<sup>e</sup> CD4 rat YKIX302.9 (IgG2a) purified FITC, Serotec, Grimbergen, Belgium

<sup>f</sup> CD8a rat, YCATE 55.9 IgG1 purified RPE, Serotec, Grimbergen, Belgium

<sup>g</sup> 0.6382 Oregon green 488 goat anti-rat IgG (H+L) conjugate, Molecular Probes, France

<sup>h</sup> Dacstar Plus, Becton-Dickinson, Franklin Lakes, NJ

<sup>i</sup> Cell-Quest software, Becton-Dickinson, Franklin Lakes, NJ

<sup>j</sup> Proc Univariate, Statistical Analysis Systems Institute, Cary, NC

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## Footnotes

<sup>a</sup> Fujinon EB-4105, Onys s.a., Brussels, Belgium

<sup>b</sup> Glutaraldehyde, Fluka, Buchs, Switzerland

<sup>c</sup> Philips 201, Eindhoven, The Netherlands

<sup>d</sup> Pharmacia, Roosendaal, The Netherlands