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Pathology in Practice

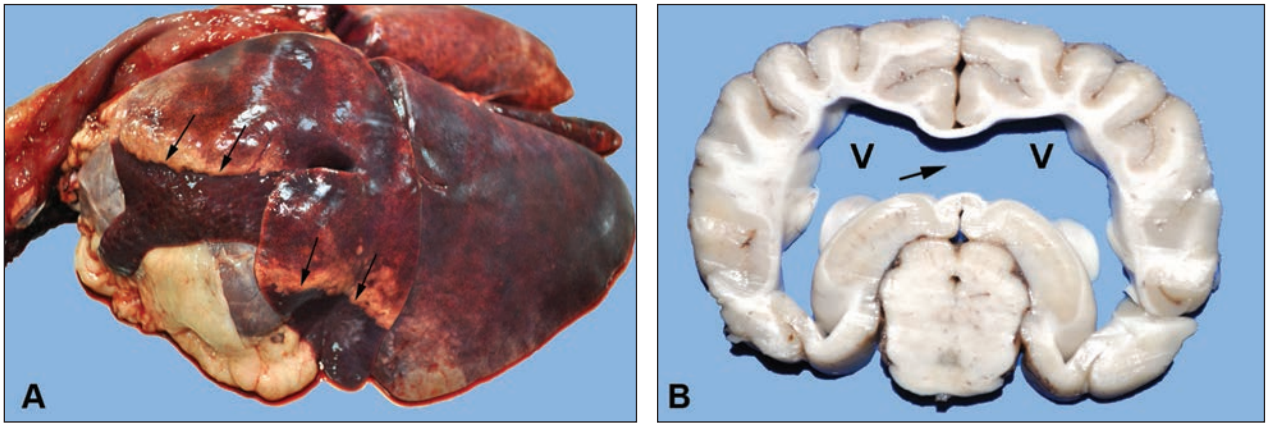

Figure 1—Gross photographs of the lungs (A) and a cut section of the formalin-fixed brain (B) of an 8-month-old Alaskan Malamute that was evaluated because of persistent mucopurulent nasal discharge. Owing to deteriorating quality of life, the dog was euthanized. In panel A, notice that the cranioventral portions of the lungs are depressed, dark red, and sharply demarcated from the remaining lung tissue (arrows show the line of demarcation). These regions of the lungs were firm and slightly nodular when palpated, and a mucoid exudate could be expressed from them when cut. Although less striking, the caudodorsal lung portions are congested (dark red) with some evidence of edema (rib imprints). Although neurologic signs were absent prior to euthanasia, the lateral ventricles are severely dilated (V), the periventricular white matter is markedly thinned, and the septum pellucidum is absent (arrow).

History and Clinical Findings

An 8-month-old sexually intact female Alaskan Malamute was examined because of persistent, left-sided purulent nasal discharge since 3 months of age. At 6 weeks of age, the dog was adopted from a breeder. At that time, the dog was described as sniffly with a clear nasal discharge. Neither the puppy's siblings nor parents had similar clinical signs. The nasal discharge was suspected to be either a viral infection or environmental irritation. However, this clear nasal discharge did not resolve as expected but rather progressed to a predominantly left-sided purulent nasal discharge. Once the nasal discharge became purulent, the dog was prescribed amoxicillin-clavulanate potassium for 10 weeks at a standard dosage (13.75 mg/kg [6.25 mg/lb], PO, q 12 h) that was adjusted on the basis of growing body size. Unfortunately, the treatment resulted in no improvement in the dog's condition. Incidentally, the puppy ingested a toy a few weeks later, creating the need for diagnostic imaging. Radiographically, the puppy had evidence of lung consolidation, which was interpreted as pneumonia. A sample of the nasal discharge was collected, but microbial culture yielded no growth; microbial culture of a biopsy sample of lung tissue also failed to yield a causative agent for the pulmonary disease. No additional treatment was prescribed at this time. Although the puppy continued to be bright and alert, it was reported to have a persistent cough, sparking the owner's desire for further diagnostic workup. Rhinoscopy was performed, and no foreign bodies, anatomic defects, or potentially causative lesions were identified. After several inconclusive attempts at definitive diagnosis and treatment followed by a steady decline in quality of life, the dog was euthanized.

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Gross Findings

At necropsy, copious mucopurulent discharge originating from the left nostril and a mucoid exudate in the frontal sinus were noted. The cranioventral portions of the right and left cranial and middle lung lobes were depressed, dark red, and sharply demarcated from the remaining lung (**Figure 1**). These regions of the lung were firm and finely nodular on palpation. When the affected lung regions were sectioned, the nodules were found to be airways and a mucoid exudate could be expressed from them. The lateral ventricles of the brain contained abundant CSF and collapsed slightly when punctured. No obstructions of the interventricular foramina were observed during evaluation of the brain. Sectioning of the formalin-fixed brain revealed that the lateral ventricles were severely dilated, and there was severe thinning of the periventricular white matter with loss of the septum pellucidum.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

Histopathologic Findings

Sections of formalin-fixed nasal turbinates, lungs, brain, liver, kidneys, ovaries, gastrointestinal tract, trachea, heart, eyes, and pituitary, adrenal, and thyroid glands were examined histologically. Large quantities of mucus admixed with neutrophils covered the mucosal surface of the nasal turbinates, which were lined by markedly hyperplastic epithelium. The submucosa was edematous and diffusely infiltrated with moderate to large numbers of lymphocytes and plasma cells, multifocally admixed with neutrophils. No bacteria were observed. The changes in the lung tissue were centered on the bronchi and bronchioles, many of which were dilated and contained large amounts of mucus admixed with mostly degenerated neutrophils (**Figure 2**). The ciliated airway epithelium ranged from normal in appearance to hyperplastic and ulcerated, and the surrounding lymphoid nodules were enlarged and prominent. Few macrophages were seen. The mucopurulent exudate extended into the adjacent alveoli. In sections from the cranioventral lung regions, the parenchyma was markedly collapsed (atelectatic), explaining why these regions were dark red and depressed grossly.

At necropsy, small pieces of oviduct were fixed in a routine electron microscopy fixative (2% paraformaldehyde, 2% glutaraldehyde, and 0.2% picric acid in 0.1M cacodylate-HCl buffer; pH, 7.2) and processed for transmission electron microscopy. Areas with cilia were identified in 1-µm sections stained with toluidine blue stain, and these areas were sectioned at 50 to 60 nm, poststained with uranyl acetate and lead citrate, and examined with a transmission electron microscope.[a] Twenty cilia were examined in cross section, and 90% of these had severe and multiple abnormalities. The microtubules of most examined cilia had a 9+0 arrangement rather than the normal 9+2 arrangement. In this abnormal arrangement, the 2 central microtubules were absent (interpreted as central microtubular agenesis) and 1 peripheral doublet microtubule was often displaced to this open central position (interpreted as transposition; **Figure 3**). This abnormal change resulted in 8 microtubules on the periphery, with the ninth transposed centrally. All of these peripheral microtubules had further abnormalities; they lacked inner and outer dynein arms and were disorganized and slightly rotated so that they were no longer parallel to the plasma membrane. In some of the cilia, this 9+0 arrangement varied further as some of the peripheral microtubules were singlets rather than doublets. Even the few cilia with 9+2 arrangements of microtubules were not structurally normal; abnormalities such as splitting and contraction were found in the basal bodies.

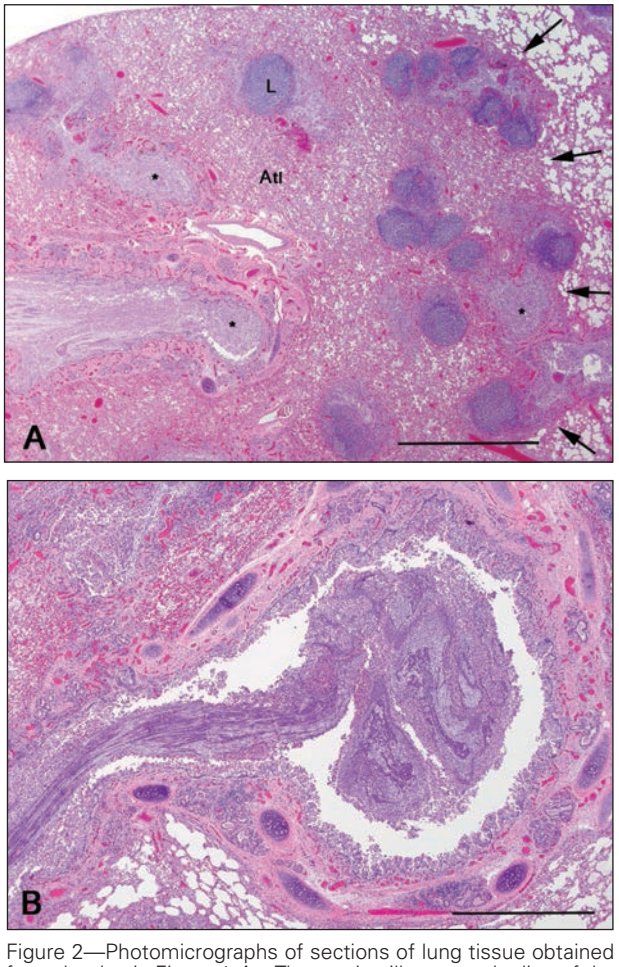

Figure 2—Photomicrographs of sections of lung tissue obtained from the dog in Figure 1. A—The section illustrates the line of demarcation noted grossly within the cranioventral portions of the lungs. To the left of the arrows, the lung parenchyma is atelectatic (Atl), multiple bronchi and bronchioles are dilated and filled with mucus and neutrophils (asterisk), and bronchus-associated lymphoid tissue (L) is hyperplastic. H&E stain; bar = 500 µm. B—Section of a typical airway in lung tissue. The airway is dilated and filled with mucus and neutrophils consistent with bronchiectasis, which is a characteristic finding in primary ciliary dyskinesia. H&E stain; bar = 1 mm.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis: severe, subacute, diffuse, mucopurulent rhinitis and sinusitis; severe subacute, diffuse mucopurulent bronchitis-bronchiolitis with mild multifocal bronchiectasis, bronchus-associated lymphoid tissue hyperplasia, and marked multifocal atelectasis; and severe hydrocephalus of the lateral ventricles.

Case summary: primary ciliary dyskinesia (PCD) with associated bronchopneumonia, bronchiectasis, and hydrocephalus in a dog.

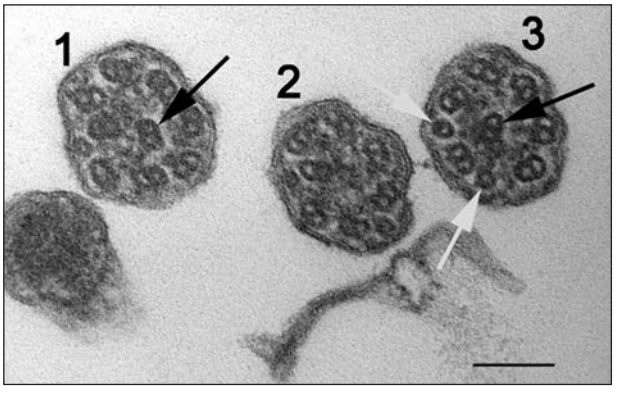

Figure 3—Transmission electron micrograph of oviduct cilia from the dog in Figure 1. In normal cilia, there is a 9+2 arrangement of microtubules with 2 single microtubules in the central position surrounded by a peripheral ring composed of 9 pairs of microtubules. The 3 cilia (1, 2, and 3) present in this preparation are abnormal. Notice that there are only 8 peripheral microtubules, and some of these are singlets rather than doublets (white arrows). Additionally, the 2 microtubules that are normally in the central position are often absent and instead replaced by a peripheral doublet microtubule (black arrow). Both inner and outer dynein arms are missing from the peripheral microtubules. Uranyl acetate and lead citrate stain; bar = 100 nm.

Comments

Ultrastructural features of the cilia in the dog of the present report were consistent with PCD, which explains the clinical findings and pathological changes in the nasal cavity, lungs, and brain. This dog had chronic respiratory tract disease that remained undiagnosed despite appropriate diagnostic testing and failed to resolve despite treatment. Unresolved chronic rhinitis from an early age is considered the most common clinical finding in dogs with PCD[1]; other clinical findings associated with PCD can vary. Bronchiectasis is a common finding in humans with PCD; because of dysfunctional mucociliary clearance, affected individuals develop persistent airway inflammation as a means to fight infections and protect the airways.[2] In the dog of the present report, mild bronchiectasis was evident and some airways were found to be dilated on histologic evaluation.

Primary cilia dyskinesia affects multiple species, including humans and dogs.[3] Although the causative mechanism behind PCD has not been fully elucidated in every case, PCD is believed to have an autosomal-recessive inheritance pattern.[3] Interestingly, in the case described in the present report, neither the littermates nor the parents of the affected puppy had any PCD-related clinical signs.

As observed in the dog of the present report, hydrocephalus is a reported finding in both dogs and people with PCD.[1,4] Although not proven, hydrocephalus may be attributable to abnormally immotile ependymal cilia that are rendered unable to facilitate the normal drainage of CSF.[5,6] Although not observed in the case described in the present report, some dogs with PCD also have situs inversus totalis, a condition in which the internal organs are located in a mirrored position.[7] The true prevalence of situs inversus totalis in dogs is still unknown, but it has been detected in 50% of humans with PCD.[2]

In a practice setting, diagnostic testing for PCD should be completed in any young dog with chronic, recurrent respiratory tract signs that are not responsive to appropriate treatment. Radiographic findings of PCD may be consistent with bronchitis, bronchiectasis, or consolidating pneumonia,[8] depending on the progression and stage of disease. Computed tomography[9] and MRI[10] can be used to assess for the presence of hydrocephalus, although not all dogs with PCD will have this finding. If clinical findings are consistent with PCD, nuclear scintigraphy can be used to evaluate the tracheobronchial mucosal transit time, which is prolonged or absent in these patients.[11]

Although findings of these diagnostic examinations can be highly suggestive of PCD, a definitive diagnosis requires evaluation of cilia by means of electron microscopy.[8] For electron microscopy, a biopsy specimen of the tracheal epithelium is of greater diagnostic value than a specimen of the nasal epithelium; however, collection of the former requires anesthesia of the patient.[7] Other sources of cilia for examination include a sample of spermatozoa, a biopsy specimen from the middle ear, or a section of an oviduct.[7] These alternative sources of cilia are important to keep in mind because they represent sites that would not be complicated by secondary changes associated with inflammation as would be expected in the nasal or tracheal epithelia of an affected dog. In any of these tissues, the ciliary changes in animals with PCD can vary widely in severity, including exceptional cases in which the cilia are ultrastructurally normal.[12] In the dog of the present report, the changes were severe, including singlet peripheral microtubules (instead of the normal doublet structures), microtubules that lacked inner and outer dynein arms, splitting and contraction of basal bodies, and central microtubule agenesis with subsequent transposition of a peripheral microtubule into the vacant central position.

On finding such ciliary defects, it is critical to consider secondary ciliary dyskinesia (SCD) as well as PCD. Secondary ciliary dyskinesia reflects ciliary defects that are sequelae of chronic respiratory infections, inflammation, or toxic injuries rather than a primary condition.[11] Whereas the defects in dogs with PCD or SCD may be similar, the damage to the cilia of dogs with SCD is reversible and less diffuse.[11] Further complicating the diagnosis, dogs with PCD can concurrently have SCD as a result of chronic respiratory infections.[11] If a diagnosis remains unclear, ciliogenesis (a technique commonly applied in humans) can be used in dogs to determine whether ciliary defects are congenital (ie, PCD) instead of acquired (ie, SCD).[11]

In dogs with PCD, the goal of treatment is to control infections and facilitate respiratory secretions.[8] Given the dysfunction of the cilia of the mucociliary elevator, respiratory secretions serve as the main defense against pathogens and method of clearance of microorganisms for the respiratory system; thus, cough suppressants are contraindicated.[8] With regard to prognosis, it is extremely important to make a diagnosis early in the disease course so that precautions can be made to prevent any permanent airway damage.[12] There is some question as to whether these patients are able to maintain a reasonable quality of life.[12] For these reasons, the prognosis for dogs with PCD is often considered guarded but variable depending on the quality of care available and individual response to treatment.

a. JEM 1210, Jeol USA Inc, Peabody, Mass.

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