Title

Avian pulmonary proteinosis: six cases and a review of the literature

Journal

Journal of Veterinary Diagnostic Investigation, 31(3)

ISSN

1040-6387

Authors

Goldsmith, Dayna A
Mete, Aslı
Pesavento, Joseph B
et al.

Publication Date

2019-05-01

DOI

10.1177/1040638719830753

Peer reviewed

Journal of Veterinary Diagnostic Investigation
2019, Vol. 31(3) 378–381

DOI: 10.1177/1040638719830753
jvdi.sagepub.com

Avian pulmonary proteinosis: six cases and a review of the literature

Dayna A. Goldsmith,[1] Aslı Mete, Joseph B. Pesavento, John M. Adaska

Abstract. Pulmonary alveolar proteinosis (PAP) is a disease of surfactant clearance in which functional abnormalities in alveolar macrophages lead to accumulation of surfactant within alveoli in mammals. Histologic examination of 6 avian autopsies, including 4 chickens, a turkey, and a cockatiel, revealed accumulation of hypereosinophilic densely arrayed lamellar material in the lungs that was magenta by periodic acid–Schiff stain and diastase resistant. Transmission electron microscopy of the proteinaceous material in 2 cases demonstrated alternating electron-dense and electron-lucent lamellae that formed whorls and had a regular periodicity of 6–14 nm, consistent with pulmonary surfactant. Given the anatomic differences between avian and mammalian lungs, we designated the presented condition "pulmonary proteinosis," which can be observed as both an incidental finding or, when severe, may be a contributing factor to death through respiratory failure.

Key words: avian; poultry; pulmonary alveolar proteinosis; pulmonary proteinosis; surfactant.

A 1.5-y-old backyard chicken was submitted to the Tulare branch of the California Animal Health and Food Safety (CAHFS) laboratory for postmortem examination following 1 wk of weakness and lethargy (Table 1, case 1). The only significant postmortem finding was moderate dilation of the right ventricle. Histologic examination of the lungs revealed accumulation of dense lamellar eosinophilic material in the majority of the air spaces (Fig. 1A). In less-affected areas, this material was restricted to the parabronchi; in more severely affected areas, it extended into the atria, infundibulum, and rarely into air capillaries. Some affected atria and infundibula were lined by cuboidal epithelium characterized by abundant foamy cytoplasm, resembling mammalian type II pneumocytes (Fig. 1B). There was minimal inflammation and no disruption of the pulmonary architecture. The accumulated material was periodic acid–Schiff (PAS) positive and diastase resistant. Based on the histologic appearance and staining characteristics of the accumulated material, a condition involving abnormal accumulation of pulmonary surfactant was suspected. Additional histopathologic findings in this case included lesions resembling the inflammatory phase of Marek's disease in the nerves, lungs, and intestines, and early ovarian carcinoma. Death in this bird was attributed to hypoxia secondary to the observed pulmonary changes, and possible early right-sided heart failure secondary to pulmonary hypertension.

To confirm the surfactant origin of the accumulated material in our case, transmission electron microscopy (TEM) was performed. Sections of formalin-fixed lung were immersed in modified Karnovsky fixative (50% strength) and postfixed in 1% osmium tetroxide. After osmification, the tissue was rinsed in 0.1 M sodium cacodylate, dehydrated through a graded ethanol series, transitioned through propylene oxide, and infiltrated and embedded in epoxy formulation (Eponate-12; Ted Pella, Redding, CA). Thick sections were cut, mounted on glass slides, stained with toluidine blue, and examined by light microscopy. Thin sections were mounted on 200-mesh copper grids and stained in 4% uranyl acetate in 75% ethanol, followed by post-staining in lead citrate. The grids were examined in a transmission electron microscope at 80 kV accelerating voltage (JEOL 1400+; JEOL USA, Peabody, MA).

The accumulated material appeared as osmiophilic lamellar arrays with a periodicity of 6–14 nm. Lamellae frequently formed whorls centered on electron-dense bodies or clear vesicles (Fig. 1C). Similar accumulations were observed within the cytoplasm of type II pneumocytes and occasionally within luminal macrophages (Fig. 1D). The findings are consistent with the reported ultrastructural appearance of mammalian surfactant, which appears as alternating electron-dense and electron-lucent bands, representing lipid bilayers, that self-arrange into unit membrane structures with a periodicity of 5–30 nm.[2] These resemble lamellar bodies, the secretory organelle of type II pneumocytes.[2,9]

A review of cases within the CAHFS laboratory system identified 5 additional cases of pulmonary proteinosis in multiple avian species, including 3 chickens, a turkey, and a cockatiel (Table 1, cases 2–6). All 5 cases had similar microscopic pulmonary proteinaceous deposits, and electron

California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, University of California, Davis, CA.

[1]Corresponding author: Dayna A. Goldsmith, 18760 Road 112, Tulare, CA 93274. dagoldsm@ucdavis.edu

Table 1. Summary of 6 cases of avian pulmonary proteinosis.

Case	Species	Age (y)	Sex	Severity of PP	Cause of death	Additional lesions
1	Chicken	1.5	F	Severe	PP	Right ventricular dilation; Marek's disease; ovarian carcinoma
2	Chicken	4.0	M	Severe	PP suspected	Mild lymphocytic endocarditis
3	Chicken	1.0	F	Mild	Euthanasia	Chronic hepatopathy; ulcerative ingluvitis
4	Chicken	1.0	F	Severe	Euthanasia	Ascites; endocardiosis
5	Turkey	5.0	M	Moderate	Undetermined	Granulomatous airsacculitis and pneumonia; focal air sac aspergillosis; mild lymphocytic endocarditis
6	Cockatiel	>1.0	U	Mild	Undetermined	None

F = female; M = male; PP = pulmonary proteinosis; U = unknown.

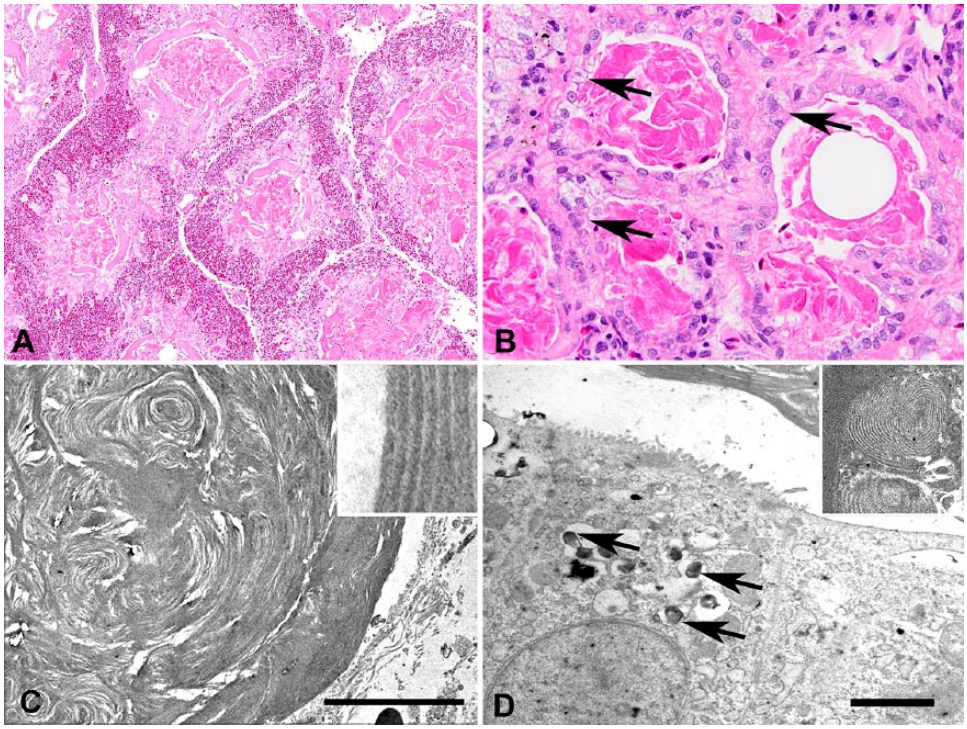

Figure 1. Pulmonary proteinosis (PP) in a 1-y-old female chicken (case 4). **A.** Parabronchi and atria are filled with lamellar eosinophilic surfactant. Overall pulmonary architecture is preserved, and inflammation is minimal. H&E. **B.** Higher magnification showing atria and infundibula lined by foamy type II pneumocytes (arrows) surrounding luminal surfactant. H&E. **C.** Transmission electron micrograph of surfactant within a parabronchus showing the whirling pattern and regular periodicity of 6–14 nm (inset). Bar = 10 µm. **D.** Transmission electron micrograph of type II pneumocyte containing cytoplasmic electron-dense lamellar bodies (arrows), the secretory organelle for surfactant; higher magnification in inset. Bar = 2 µm.

microscopy performed on one of the cases (case 4) confirmed the material as surfactant. All additional cases were in adult birds (≥1-y-old), and both males and females were affected. At autopsy, 3 of these birds had bilaterally red and edematous lungs, and one bird had endocardiosis and ascites, indicative of heart failure. Microscopically, the 3 birds with pulmonary edema also had myxomatous valvular degeneration of the left atrioventricular valves and mild, multifocal, lymphocytic-to-lymphoheterophilic endocarditis. Pulmonary surfactant accumulation was considered the probable cause of death in 1 of the 5 cases (case 2) and likely contributed to the decision to euthanize in a second case in which lung involvement was extensive (case 4). In the remaining 3 cases, surfactant accumulation was mild-to-moderate and was considered an incidental finding.

Surfactant plays several roles within the mammalian lung including the reduction of surface tension, and immune functions including opsonization and killing of microbial pathogens.[1] Mammalian surfactant is composed of 90% lipid and 10% protein including 4 major surfactant proteins A–D.[8] The tubular unidirectional airflow design of the avian lung results in much lower levels of negative pressure compared to the

mammalian tidal flow system. As such, the requirement for reduced surface tension is much lower in birds, resulting in structural and chemical differences in avian surfactant. Studies of avian surfactant have shown that birds lack 1 or more of the 4 surfactant proteins observed in mammals, and avian surfactant has a reduced ability to lower surface tension, but may be better at promoting air flow.[12] In mammals, surfactant is produced by type II pneumocytes lining alveoli, where it is stored in the form of cytoplasmic lamellar bodies before being released into the alveolar lumen.[10] In contrast, developmental studies of the avian lung have found that type II pneumocytes, also known as granular pneumocytes in birds, are most abundant within the atria, parabronchi, and air sacs, and are absent within pulmonary capillaries.[11] Old surfactant is taken up and recycled or catabolized by both type II pneumocytes and alveolar macrophages.[10] The major regulator of surfactant homeostasis is granulocyte–macrophage colony-stimulating factor (GM-CSF). GM-CSF is required for the stimulation of surfactant catabolism, terminal differentiation of alveolar macrophages, and mediation of the immune functions of alveolar macrophages.[1]

Diseases of surfactant homeostasis in mammals are divided into problems of surfactant clearance, known as pulmonary alveolar proteinosis (PAP), and problems of surfactant production, known as PAP-like diseases.[8] In general, PAP involves a problem with alveolar macrophages that leads to reduced clearance and increased accumulation of normal surfactant within air spaces. In contrast, PAP-like diseases lead to either a deficiency in surfactant or the accumulation of abnormal surfactant that cannot be cleared through normal mechanisms.[1] PAP can be primary or secondary. *Primary PAP* includes congenital and autoimmune PAP (previously known as idiopathic PAP). Autoimmune PAP is the most common form in humans, accounting for ~90% of cases and involves the development of autoantibodies against GM-CSF leading to reduced levels of GM-CSF in the blood and reduced clearance of surfactant by alveolar macrophages.[9] Autoantibodies to GM-CSF can be detected in bronchoalveolar lavage fluid and blood from these patients, and therapies include removal of antibodies by plasmapheresis and GM-CSF supplementation.[1] Congenital forms of PAP are much less common and usually involve mutations in the receptor for GM-CSF.[8] *Secondary PAP* occurs when alveolar macrophages are reduced in function or number secondary to another condition such as hematologic disorders, immunological disease, infections, and toxin and/or irritant inhalation.[1,7,9] PAP-like diseases involve both spontaneous or hereditary mutations in the genes coding for surfactant proteins, or other proteins involved in surfactant production and release. Unlike PAP, PAP-like diseases result in abnormal surfactant function and, therefore, are more likely to result in damage and architectural changes to the pulmonary parenchyma.

Significant advances in the understanding of PAP were made when GM-CSF–deficient mice ($Csf2^{-/-}$) were found to develop pulmonary lesions resembling human primary PAP.[7] Since then, PAP has also been described in several other laboratory animal species including rats, hamsters, guinea pigs, and moustached tamarins.[5,6] Rare cases of PAP have also been reported in domestic species including a single report in a cat and 3 in dogs.[3,4,6,8] All 4 of these cases involved young animals, and a definitive etiology was not established in any case. Based on the young age of the affected individuals, congenital PAP was considered in 2 of the cases.[3,8] In one of the dog cases, the animal recovered after its third whole-lung lavage therapy.[6] Whole-lung lavage remains a standard treatment for this condition in animals and people. In the remaining cases, euthanasia was elected because of respiratory distress. At postmortem examination, one of the dogs had evidence of right-sided heart failure secondary to pulmonary hypertension.[3] Respiratory failure is the most common cause of death in human patients with PAP.[7,9] Additional deaths occur as a result of secondary infections that result from loss of the normal immunity roles of surfactant, as well as abnormalities in macrophage function that are frequently associated with PAP. No deaths attributed to secondary infections have been reported in the veterinary cases of PAP, to our knowledge. PAP in production animals is less commonly reported; however, a secondary form of PAP is believed to occur in goats with the pulmonary form of caprine arthritis-encephalitis virus infection.[6] This likely occurs secondary to the targeting of macrophages, including alveolar macrophages, by this virus.

In our cases, the ultrastructural characteristics of the accumulated material, as well as the histologic appearance and staining characteristics, confirmed it as surfactant. Given the minimal associated inflammation or parenchymal damage, a condition resembling true PAP was considered more likely than a PAP-like condition. Given the absence of alveoli in birds, we chose to refer to this condition as avian pulmonary proteinosis (PP). More specific classification of the form of surfactant clearance problem, following the classification system for PAP, is difficult in these cases. Given the presence of additional lesions in multiple birds including a hematologic disorder in 1 (Marek's disease), and cardiac lesions in 4, secondary PP is a possibility; however, primary PP cannot be ruled out. Given the adult ages of the birds, we consider congenital PP to be less likely. To our knowledge, disrupted surfactant homeostasis has not been reported previously in an avian species. Further determination of the etiology of PP would require investigation into the presence of GM-CSF antibodies, or mutations in genes encoding the GM-CSF receptor or surfactant proteins.

Review of our cases identified several differences between mammalian PAP and avian PP. Pulmonary surfactant in birds accumulates first within the parabronchi, not within the more terminal air capillaries. This likely reflects the differences in distribution of type II pneumocytes in birds versus mammals, which was discussed previously. There are differences in the histologic and ultrastructural appearance of PP versus PAP.

Histologically, mammalian surfactant appears eosinophilic and finely granular, whereas avian surfactant appears more densely eosinophilic and lamellar. Rare areas of more granular surfactant were observed in some cases of PP and may represent a more acute form of the condition. The dense granular material apparent in mammalian PAP was also lacking in the 2 cases of avian PP that we studied by TEM. In addition, when hydrated, mammalian surfactant will rearrange into a loose net-like pattern called tubular myelin. No tubular myelin was observed in our cases. This is consistent with previous ultrastructural studies on avian surfactant that failed to identify tubular myelin.[11,12] Such differences between PP and PAP likely reflect differences in the functional requirements for surfactant between birds and mammals.

Acknowledgments

We thank Kevin Keel and the University of California, Davis Virtual Microscopy Laboratory, for help with slide scanning and image capture and editing.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Carey B, Trapnell BC. The molecular basis of pulmonary alveolar proteinosis. Clin Immunol 2010;135:223–235.
2. Costello JF, et al. Diagnosis and management of alveolar proteinosis: the role of electron microscopy. Thorax 1975;30: 121–132.
3. Cummings AC, et al. Imaging diagnosis—pulmonary alveolar proteinosis in a dog. Vet Radiol Ultrasound 2013;54:634–637.
4. Jefferies AR, et al. Pulmonary alveolar proteinosis (phospholipoproteinosis) in a dog. J Small Anim Pract 1987;28:203–214.
5. Michaud CR, et al. Spontaneous pulmonary alveolar proteinosis in captive “moustached tamarins” (*Saguinus mystax*). Vet Pathol 2012;49:629–635.
6. Silverstein D, et al. Pulmonary alveolar proteinosis in a dog. J Vet Intern Med 2000;14:546–551.
7. Suzuki T, Trapnell BC. Pulmonary alveolar proteinosis syndrome. Clin Chest Med 2016;37:431–440.
8. Szatmári V, et al. Pulmonary alveolar proteinosis in a cat. Vet Res 2015;11:302:1–8.
9. Trapnell BC, et al. Pulmonary alveolar proteinosis. N Engl J Med 2003;349:2527–2539.
10. Whitsett JA, et al. Diseases of pulmonary surfactant homeostasis. Annu Rev Pathol Mech Dis 2015;10:371–393.
11. Wolfgang B, et al. Pulmonary surfactant in birds: coping with surface tension in a tubular lung. Am J Physiol Regul Integr Comp Physiol 2001;281:R327–R337.
12. Zhang W, et al. Developmental regulation of chicken surfactant protein A and its localization in lung. Dev Comp Immunol 2016;61:80–87.