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NEOPLASTIC DISEASE

Pleomorphic Malignant Mesothelioma in a Broiler Breeder Infected with Avian Leucosis Virus Subgroup J

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Summary

Avian leucosis virus (ALV) is an oncogenic retrovirus that induces tumours including lymphoid leucosis and myeloid leucosis. Pleomorphic malignant mesothelioma and myelocytoma, which were thought to be induced by ALV subgroup J (ALV-J) infection, were identified in a 432-day-old broiler breeder. The bird showed no clinical signs; however, at necropsy examination there were multiple nodules in the alimentary tract. Microscopical analysis showed that these consisted of pleomorphic cells and myelocyte-like cells. Immunohistochemistry revealed that the pleomorphic cells were atypical and expressed cytokeratin, vimentin, c-kit, calretinin and ALV. The myelocyte-like cells were also positive for ALV. Retroviral type C particles were observed by electron microscopy. ALV-E and ALV-J nucleotide sequences were detected in DNA extracted from formalin-fixed and paraffin wax-embedded small intestinal tissue. Based on these results, the tumours were diagnosed as pleomorphic malignant mesothelioma and myelocytoma and were thought to have been induced by ALV-J infection. This is the first report of malignant mesothelioma associated with naturally acquired ALV-J infection.

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Avian leucosis viruses (ALVs) belonging to the family *Retroviridae*, subfamily *Orthoretrovirinae* and genus *Alpharetrovirus* are classified into subgroups A, B, C, D, E and J (ALV-A, -B, -C, -D, -E and -J) in chickens (Fadly and Nair, 2008). ALV is tumourigenic and induces various tumours including lymphocytic leucosis and myeloid leucosis (ML). ALV-J is a new subtype of ALV that induces a variety of tumours including ML (Payne *et al.*, 1991). Experimental infections with ALV-J strain HPRS-103 have been shown to induce mesotheliomas (Payne *et al.*, 1992); however, there are no reports of spontaneously arising cases of mesothelioma associated with naturally acquired ALV infection. Moreover, mesotheliomas are rare in birds (Reece, 2008). To the best of our knowledge, there are no previous reports of malignant mesothelioma in chickens, regardless of the presence or absence of ALV infection.

In 2015, a 432-day-old female gamecock, which was reared as a broiler breeder at a livestock research institute in Japan, was found dead. There had been no clinical signs. At necropsy examination, multiple white masses of various size were observed in the alimentary tract between the gizzard and rectum. The alimentary tract was relatively free of content. The skin over the head was pale and there was considerable ascitic fluid. There were no other significant lesions.

The intestinal masses were collected for histopathological and immunohistochemical examination. Formalin-fixed and paraffin wax-embedded (FFPE) samples were sectioned (2 μm) and stained with

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haematoxylin and eosin (HE). Microscopically, multinodular masses were observed in the intestinal wall and mesentery. These contained a mix of myelocyte-like and pleomorphic cells and showed infiltrative growth with proliferation of the connective tissue (Supplementary Fig. 1). The myelocyte-like cells consisted of various cells including metamyelocyte-like, myelocyte-like and myeloblast-like cells. The myelocyte-like cells were spread diffusely or multifocally within the masses and some invaded the lamina propria and mesentery (Supplementary Fig. 2). Numerous myelocyte-like cells were also seen in the blood vessels. Immunohistochemistry (IHC) showed that the myelocyte-like cells were strongly positive for c-kit and Wilms tumour WT-1 (Table 1 and Supplementary Fig. 3).

The pleomorphic cells had eosinophilic cytoplasm and one or more nuclei (Fig. 1). These cells varied in size and giant cells were often observed. The nuclei of the pleomorphic cells showed striking anisokaryosis and atypia. The pleomorphic cells spread in a cobblestone pattern, in bundles or as simple epithelium with multifocal necrosis (Supplementary Fig. 4a). The cytoplasm of the pleomorphic cells often had one or more vacuoles of various sizes and 'signet-ring' cells with large vacuoles were observed occasionally. Some pleomorphic cells had basophilic granular cytoplasmic inclusions (Supplementary Fig. 4b). Basophilic granular or filament-like material was occasionally found in the interstitium. IHC showed that the pleomorphic cells were moderately to strongly positive for cytokeratin, vimentin, c-kit and calretinin and weakly positive for α-smooth muscle actin (SMA), Wilms tumour WT-1 and progesterone receptor (PR) (Table 1 and Supplementary Fig. 5).

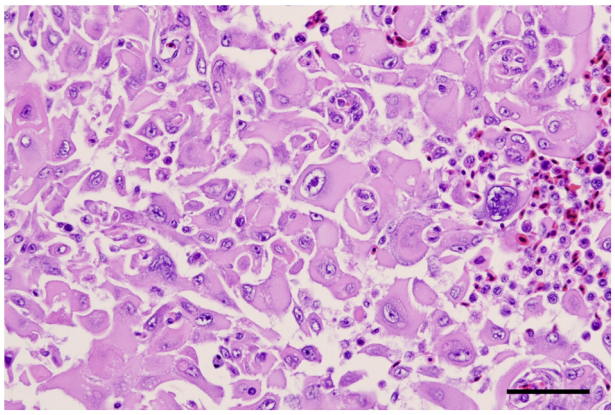

Fig. 1. Histological features of atypical pleomorphic cells in the intestinal mass. HE. Bar, 50 μm.

Based on the histological and immunohistochemical features, this case was diagnosed as myelocytoma and malignant mesothelioma. In man, diffuse malignant mesothelioma is classified into four major histological subtypes: epithelioid, sarcomatoid, biphasic

Table 1
Primary antibodies and results of immunohistochemistry

Antibody	*Clone*	*Dilution*	*Antigen retrieval*	*Source*	*Expression by tumour cells*	
					Pleomorphic cells	*Myelocytes*
Cytokeratin	AE1/AE3	1 in 50	Autoclave	Dako, Kyoto, Japan	4+	−
Vimentin	Polyclonal	1 in 200	Autoclave	Santa Cruz Biotechnology, Santa Cruz, California, USA	3+	−
α-SMA	Polyclonal	1 in 200	Autoclave	Abcam, Tokyo, Japan	1+	−
Desmin	D33	Ready to use	−	Dako	−	−
Iba-1	Polyclonal	1 in 300	−	Wako, Osaka, Japan	−	−
S100	Polyclonal	Ready to use	Autoclave	Dako	−	−
NSE	BBS/NC/VI-H14	Ready to use	Autoclave	Dako	−	−
c-kit	Polyclonal	1 in 50	Autoclave	Biorbyt, Cambridge, UK	3+	4+
Calretinin	Polyclonal	1 in 40	Autoclave	LSBio, Seattle, Washington, USA	4+	−
WT-1	Polyclonal	1 in 100	Autoclave	Santa Cruz	1+	4+
OR	Polyclonal	1 in 100	Autoclave		−	−
PR	hPRa 2	1 in 100	Autoclave	Thermo Fisher Scientific, Fremont, California, USA	1+	−
ALV	Antiserum	1 in 40,000	Autoclave	Tsukamoto *et al*., 1991	4+	4+

The specificity of antibodies was assessed by evaluating a normal area of tissue in the same specimen or normal abdominal tissues of the same breed of chicken without ALV infection.
α-SMA, α-smooth muscle actin; NSE, neuron specific enolase; WT-1, Wilms tumour-1; OR, oestrogen receptor; PR, progesterone receptor.
Immunoreactivity score was graded according to the percentage of reactive cells: −, <1%; 1+, 1–25%; 2+, 26–50%; 3+, 51–75%; 4+, 76–100%.

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