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J. Comp. Path. 2022, Vol. 195, 7–11

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INFECTIOUS DISEASE

Marek's Disease in an Indian Peafowl (*Pavo cristatus*) with Clinical Ocular Disease and Paraparesis

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Summary

Marek's disease (MD) is caused by virulent strains of *Gallid alphaherpesvirus* type 2 (MD virus serotype 1; MDV 1) and frequently causes a lymphoproliferative disorder in poultry and other galliform birds worldwide. However, within the peafowl (Phasianinae) subfamily, there are only rare confirmed reports of MD. Here we report MD in an Indian peafowl (*Pavo cristatus*), which clinically presented with hindlimb paraparesis and intraocular swelling of the right eye. Soft, off-white to tan masses within the right eye, sciatic nerves and coelomic cavity were identified at post-mortem examination which effaced the cranial pole of the kidneys and diffusely effaced the testes. Lymphoid neoplasia was identified histologically at all of these sites and there was extensive hepatic lymphoid cell infiltration, which had not been grossly evident. The T-cell origin of the lymphoid cells was confirmed by immunohistochemistry for CD3 antigen. A virulent strain of MDV 1 was detected by real-time polymerase chain reaction in DNA samples extracted from the kidney and testes. As MD is rare in peafowl it should be considered as a differential diagnosis for intraocular and coelomic masses with associated clinical signs.

Keywords: Marek's disease; ocular lesions; paraparesis; peafowl

Marek's disease (MD) is caused by the double stranded DNA virus, *Gallid alphaherpesvirus* 2 (MD virus serotype 1; MDV 1), of the *Mardivirus* genus. Typical lesions in poultry include soft tissue masses resulting from lymphoid neoplasia, classically leading to hindlimb paralysis due to compromise of the sciatic nerves (Osterrieder *et al*, 2006). MD has been detected worldwide (Dunn and Gimeno, 2013) and results in significant economic losses in commercial poultry operations (Morrow and Fehler, 2004). Definitive diagnosis usually requires molecular methods including polymerase chain reaction (PCR) amplification of target sequences of the MDV 1 genome or identification of tumour-related antigens using immunohistochemistry (IHC; Kennedy *et al*, 2017; Nair, 2018). MD virus serotypes 2 (*Gallid alphaherpesvirus 3*) and 3 (*Meleagrid alphaherpesvirus 1*), both of which are naturally non-pathogenic, are also frequently detected in poultry (Davison *et al*, 2009; Baigent, unpublished data). *Gallid alphaherpesvirus 3* circulates naturally in chicken flocks, while *Meleagrid alphaherpesvirus 1* is used as an MD vaccine in chickens. Current outbreaks of MD in commercial flocks are due to

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0021-9975/$ - see front matter
https://doi.org/10.1016/j.jcpa.2022.04.003

increasingly virulent strains of MDV 1 (including virulent, vMDV; very virulent, vvMDV; very virulent plus, vv + MDV; Witter, 1997).

Viral entry into the host bird usually occurs via inhalation of MDV-infected dander (Baaten *et al*, 2009) with dissemination by macrophages to the regional lymph nodes (Calnek, 2001). Subsequent viral replication in lymphocytes leads to lymphoid proliferation and neoplasia of predominantly T-cell origin (Mwangi *et al*, 2011). T-cell infection with MDV can result in latency and eventual recrudescence of the virus (Nair, 2013). Viral transformation of T cells is promoted by various transforming factors acting as oncogenes including Meq (Jones *et al*, 1992; Liu *et al*, 1999) and a viral homologue of telomerase (Osterrieder *et al*, 2006).

While MD is most commonly reported in commercial chickens, it it has been also reported in a range of other species including turkeys (Hauck *et al*, 2020), pheasants (Seimon *et al*, 2012) and quail (Pennycott *et al*, 2003). Although detection of MDV in non-galliform species is less frequent (Halliwell, 1971; Lian *et al*, 2018), anseriforms, including geese and ducks, have been suggested as possible significant reservoirs of MDV (Murata *et al*, 2012).

Although peafowl (subfamily Phasianinae) are galliform birds, MD has only rarely been reported in peafowl, and only in single birds. In two of these reports, a white peafowl (*Pavo cristatus*) in Brazil (Blume *et al*, 2016) and a green peafowl (*Pavo muticus*) in Iran (Ranjbar and Khordadmehr, 2018) presented with non-specific clinical signs including lethargy and anorexia. A third unpublished case from North America (Joint Pathology Center, 2019) is the only known example of hindlimb immobility in a peafowl with MD. MD disease in all three cases was characterized grossly by organ enlargement and/or evident neoplastic tissue and histologically by lymphoid cell infiltration of multiple visceral organs and/or the coelomic cavity. Considering that MD has only rarely been identified in peafowl, they are not routinely vaccinated against this disease, which may result in maintenance of disease susceptibility in an unvaccinated population in contrast to commercial poultry, which are typically routinely vaccinated. In addition to MDV, a novel herpesvirus (*Phasianid herpesvirus*) has been reported in peafowl species, but was predominantly associated with hepatic necrosis rather than lymphoproliferation in the affected birds (Seimon *et al*, 2012).

A 6-year-old male Indian peafowl was presented to the Dick Vet Rabbit and Exotic Practice at the Royal (Dick) School of Veterinary Studies, University of Edinburgh. The bird had been housed with three other peafowl and no previous health concerns had been reported in the affected or in-contact birds. The owner reported a 3-week duration of right eyelid closure, which was associated with periocular swelling, and intermittent periods of sternal recumbency. Over the course of several days prior to presentation, the bird had become anorexic and lethargic. On clinical examination, it was found to be underweight and significantly ataxic. There was moderate periorbital swelling with pale yellow material evident in the ventral anterior chamber. Hypopyon was initially suspected as a differential diagnosis prior to additional diagnostic tests.

Due to the peafowl's clinical condition, it was hospitalized and supportive care instigated, which included intravenous fluid therapy and crop feeding. Antibiosis was initiated on the basis of a presumed diagnosis of hypopyon/systemic infection. Automated haematology identified a marked leucocytosis (56×10^9/l; reference interval $4.3–21.0 \times 10^9$/l) and lymphocytosis (47.6×19^9/l; reference interval $1.2–27.7 \times 10^9$/l). Blood smear evaluation identified a mild polychromasia of erythrocytes, marked lymphocytosis and mild monocytosis. All other haematology and biochemistry values were within the reference intervals. As there was no clinical improvement, the animal was euthanized on welfare grounds.

At post-mortem examination, there was exophthalmos of the right eye. The anterior chamber of this eye contained a large amount of indistinct pale tan material (Fig. 1) with a small amount of irregular, soft, pale yellow to tan material in the retrobulbar space. Bilaterally, at the cranial poles of the kidneys and adrenal glands, there were soft to semi-firm, off-white to pale tan, smooth masses ($9 \times 6 \times 4.5$ cm), which effaced and replaced the normal tissue (Fig. 1). On cut section, these soft tissue masses were homogeneous, pale tan to off-white, with no discernible normal tissue structures. The renal parenchyma contained multifocal deposits of white crystalline material consistent with urates. Extending caudally from the kidneys there were bilateral, multilobular masses of similar, variably well-demarcated tissue, which extended dorsally to the vertebral column and partially engulfed the nerve roots of the sciatic nerves. The sciatic nerves were otherwise grossly normal. The other visceral organs had no gross evidence of underlying pathology but were variably congested.

Samples of representative tissues were collected into 10% neutral buffered formalin, processed routinely and stained with haematoxylin and eosin (HE). Histologically, effacing and replacing most of the normal renal tissue were sheets of neoplastic round cells supported by a scant fibrovascular stroma

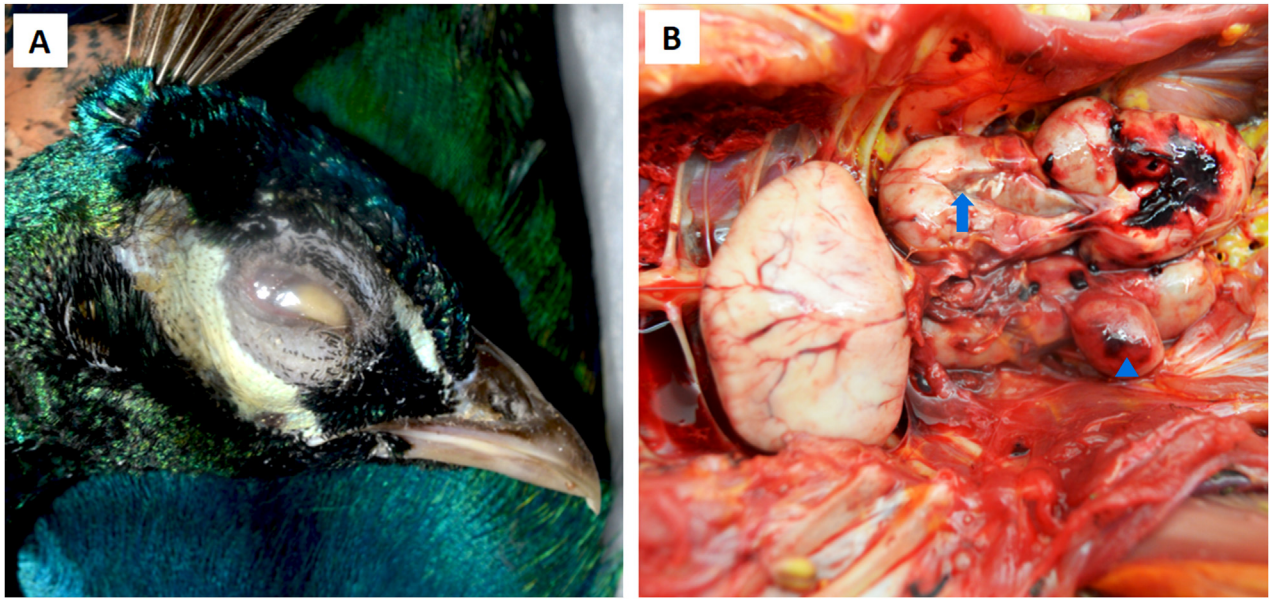

Fig. 1. Marek's disease, peafowl. (A) Mild exophthalmos and periorbital swelling of the right eye with pale tan material in anterior chamber. (B) Kidneys (arrow) and testes (arrowhead) effaced and expanded by multilobular, off white to pale tan, soft to semi-firm neoplastic tissue.

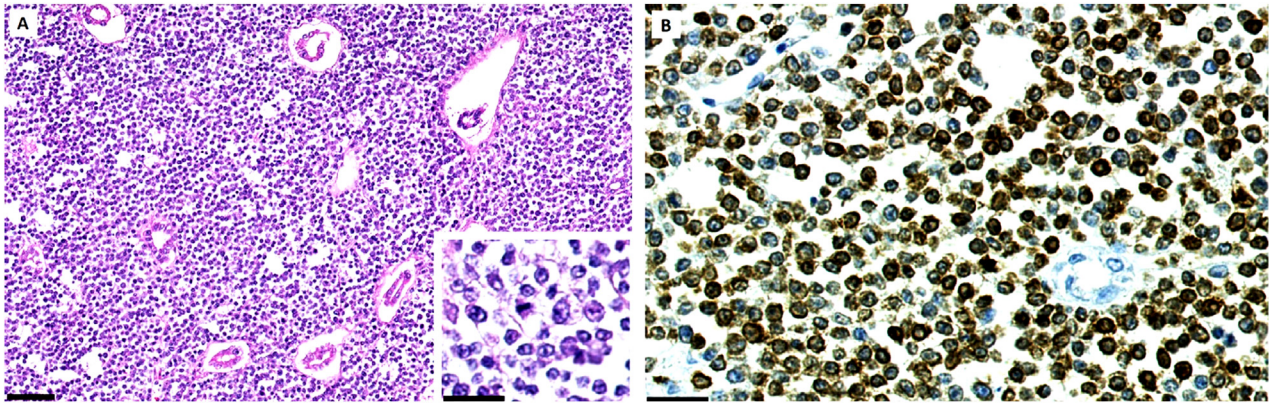

Fig. 2. Marek's disease, kidney, peafowl. (A) The normal renal parenchyma is almost entirely effaced by sheets of atypical round cells. Several degenerate renal tubules are engulfed by atypical round cells. HE. Bar, 50 µm. Inset: detail of proliferated round cells. HE. Bar, 20 µm. (B) Diffuse cytoplasmic to perimembranous immunolabelling of CD3 in proliferated round cells. IHC. Bar, 50 µm.

(Fig. 2). Areas of necrosis were present and some renal tubules had evidence of regeneration and degeneration. Individual cells had distinct cell borders and either no discernible or scant, pale eosinophilic cytoplasm. The nuclei were round and euchromatic with stippled chromatin; single prominent nucleoli were sometimes present. On average, neoplastic cells were up to approximately 10um diameter. The cells predominantly resembled small lymphocytes but occasional blast-type cells had larger nuclei. There was moderate anisocytosis and anisokaryosis and up to 17 mitotic figures per high-power field (×400 magnification/0.237 mm^2). Bizarre mitotic figures were also present.

Sections taken from a coelomic mass in the region of the testes contained similar sheets of neoplastic round cells with almost no normal testicular architecture remaining. In the liver, predominantly in periportal and centrilobular areas, the normal hepatic architecture was multifocally expanded and replaced by large aggregates of neoplastic round cells. In the proximal sciatic nerve, there was multifocal infiltration of the endoneurium by rows of moderately sized aggregates of neoplastic round cells. Rarely, myelin sheaths were dilated and contained axonal debris. In the right eye, the anterior chamber was partially filled by large numbers of neoplastic round cells, while the retina was detached with extensive atrophy.

IHC was performed on histological sections from the eye and kidney to confirm suspected neoplastic proliferation of T cells, which are the typical proliferating lymphoid cell in MD in galliforms (Mwangi *et al*, 2011). Sections were pretreated with 0.01 M citrate buffer at 110°C for 5 min followed by a 30 min incubation with a mouse monoclonal anti-CD3 antibody (NCL-L-CD3-565, 1:200; Leica Biosystems, www.leicabiosystems.com) at room temperature. In the absence of species-matched controls, spleen from a chicken was used as positive control tissue. IHC revealed proliferation of homogeneous round cells, which replaced the kidney parenchyma. In the eye, there was diffuse, strong, cytoplasmic to perimembranous CD3 immunolabelling, indicative of T-cell neoplasia (Fig. 2).

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) kidney and testis tissues. Kidney and testis were selected because these were the tissues most effaced by the lymphoproliferation and were therefore most likely to contain the largest amount of representative DNA. DNA extraction was performed at the Virus Surveillance Unit, Moredun Research Institute, Penicuik, UK. The DNA extracted from the FFPE tissues was subjected to quantitative real-time PCR specific to virulent field strains of MDV 1 using published primers and probe (Baigent *et al*, 2016) in a 40-cycle protocol. This PCR test detects all virulent strains of MDV-1 (vMDV, vvMDV and vv+MDV) but does not distinguish between these types. Viral DNA was detected in both kidney and testis tissues.

We have confirmed MD in a male Indian peafowl for which there are only rare reports of MDV infection causing overt disease. CD3 IHC was used to confirm the neoplastic proliferation of T cells and MDV infection was subsequently confirmed using real-time PCR. In addition to the clinically evident paraparesis and lethargy, there was obvious ante-mortem ocular involvement, which has not been previously reported in peafowl. While there was no confirmed exposure to similarly affected birds, this peafowl was from an area of the UK in which there are multiple poultry units and small 'backyard' flocks, which may have been a potential source of viral infection for this case.

At post-mortem examination, the most striking gross finding was the presence of multiple, large coelomic masses, which replaced large areas of the renal and testicular tissues. There was also extensive, microscopic hepatic involvement. Despite these lesions, the bird's main clinical signs were exophthalmos and anterior chamber infiltration (with retrobulbar involvement detected at post-mortem examination) and marked paraparesis (due to infiltration of the bilateral sciatic nerve roots). In addition to the clinical paraparesis and recumbency in this bird, the development of visceral masses, with anorexia and lethargy, is more typical of MD in poultry.

Histologically, the neoplastic tissues comprised sheets of round cells. It is possible that the marked lymphocytosis detected ante-mortem was related to the lymphoid proliferation. Without access to detection of MDV by PCR, as used in this investigation, an alternative diagnosis of lymphoid leukosis might have been considered. However, lymphoid leukosis does not typically present with severe neural involvement. Previously reported cases of MD in peafowl had visceral masses similar to those in this case (Blume *et al*, 2016; Joint Pathology Center, 2019), suggesting that this finding may be a typical presentation in these species and may aid ante-mortem diagnosis if diagnostic imaging were to be utilized. MDV PCR on feather pulp samples is also used for ante-mortem diagnosis of MD in chickens. This method could also be investigated as a diagnostic approach in peafowl, although additional work would need to be undertaken to determine if MDV replicates in peafowl skin as in chickens.

In conclusion, this case highlights the need for consideration of MD as a differential diagnosis when assessing non-commercial galliform species that develop intraocular swelling, coelomic masses, paraparesis or lethargy.

Acknowledgments

We thank the staff at the Virus Surveillance Unit, Moredun Research Institute, Pentland Science Park, Penicuik, UK, for extracting the DNA from the formalin-fixed, paraffin-embedded tissues in this study. We are also very thankful to the histopathology section of the Royal (Dick) School of Veterinary Studies, University of Edinburgh, for production of the histological slides and for performing the immunohistochemistry.

Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research, authorship or publication of this article. All authors have read and agreed to the published version of the manuscript.

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[*Received, January 17th, 2022*
Accepted, April 18th, 2022]