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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Positive Immunolabelling for Feline Infectious Peritonitis in an African Lion (*Panthera leo*) with Bilateral Panuveitis

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Summary

A 15-year-old male African lion (*Panthera leo*) was presented with blindness due to bilateral panuveitis with retinal detachment. Feline coronavirus (FCoV) antigen was identified immunohistochemically in ocular macrophages, consistent with a diagnosis of feline infectious peritonitis (FIP) infection. This is the first report of FIP in an African lion and the first report of ocular FIP in a non-domestic felid.

Keywords: African lion (*Panthera leo*); feline infectious peritonitis; immunohistochemistry; panuveitis

A 15-year-old, 210 kg male African lion (*Panthera leo*) kept in the Munda Wanga Zoo, Lusaka, Zambia, was presented to the Veterinary Clinic of the University of Zambia (UNZA) with suspected visual impairment that had appeared to worsen in the preceding months. The lion had normal gait and posture, but it collided with objects in its immediate surroundings. A motion detection test performed by tossing objects across the lion's field of view revealed no responses and the animal failed to visually track the moving objects. The animal was anaesthetized for basic examination.

Physical examination revealed no wounds or swelling that might indicate trauma, but the face had scratches. Ophthalmological examination revealed both corneas to be clear. Several attempts at medical treatments in the zoo had not led to improvement. The lion was taken to UNZA for radiography and haematology. An X-ray of the cranium ruled out any space-occupying lesions. Haematological examination revealed anisocytosis, hypochromasia and Heinz bodies, but no blood parasites. Packed cell volume (PCV) was 31% and total plasma protein (TPP) was 7.8 g/dl. The animal was humanely destroyed because of the poor prognosis.

At necropsy examination, there were no significant gross findings. Samples of the brain and eyes with optic nerves were collected for histopathological examination and fixed in 10% neutral buffered formalin.

Fixed tissues were processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE). Selected sections of brain were also stained with Azan, elastic van Gieson, Berlin blue and Luxol fast blue with HE (LFB-HE). Additional sections were also subjected to immunohistochemistry (IHC). Primary antibodies were specific for Iba-1 (1 in 250 dilution; Wako Pure Chemical Industries, Osaka, Japan), glial fibrillary acidic protein (GFAP; prediluted; Nichirei Biosciences, Tokyo, Japan) and feline coronavirus (FCoV; a mixture of monoclonal antibodies against each nucleocapsid, integral membrane and spike of FCoV; 1 in 10 dilution, kindly supplied by Dr T. Hohdatsu, Kitasato University; Hohdatsu *et al.*, 1991). Labelled antigens (Iba-1 and GFAP) were detected with a Histofine Simple Stain MAX-PO (MULTI) kit (Nichirei). Each antibody was

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visualized using 3, 3′ diaminobenzidine (Nichirei). FCoV antigen was detected with a Histofine Simple Stain AP kit (Nichirei). All sections were counterstained with Mayer's haematoxylin.

Grossly, the eyes were characterized by an irregular pupil margin, uveal oedema, small white collars on the iris and a gelatinous white membrane covering the retina (Fig. 1). Histopathologically, the ocular lesions were characterized by bilateral panuveitis with retinal detachment (Fig. 2). The predominant infiltrating cells were lymphocytes and plasma cells with scattered macrophages. There were also localized areas of plasma cell-rich infiltration. These cells infiltrated diffusely or sometimes aggregated around small veins. The pigmented layer of the iris was lost and depigmentation with scattered melanophages was observed in the thickened iris and retina. Eosinophilic proteinaceous exudate had accumulated in the anterior chamber. IHC revealed FCoV antigen in macrophages (Fig. 3).

The brain showed dilated perivascular spaces mainly in the white matter. There were no hypertensive vascular changes in arteries or arterioles. Iba-1-positive macrophages had accumulated in some perivascular spaces associated with fibrosis (Fig. 4), but there were no FCoV-positive macrophages in the brain. There was diffuse GFAP labelling of astrocytes within the white matter, both of the cytoplasm and swollen processes of these cells.

Feline infectious peritonitis (FIP) is classified into two forms on the basis of its clinical and pathological presentation: a non-effusive or dry form and an effusive or wet form. In the non-effusive form of FIP, the inflammatory reaction can be restricted to individual organs, such as the brain or eyes (Drechsler *et al.*, 2011). Among the organs, the brain was the second and the eyes were the third most affected organs in cats with FIP and the eyes were always involved when there were brain lesions (Kipar and Meli, 2014).

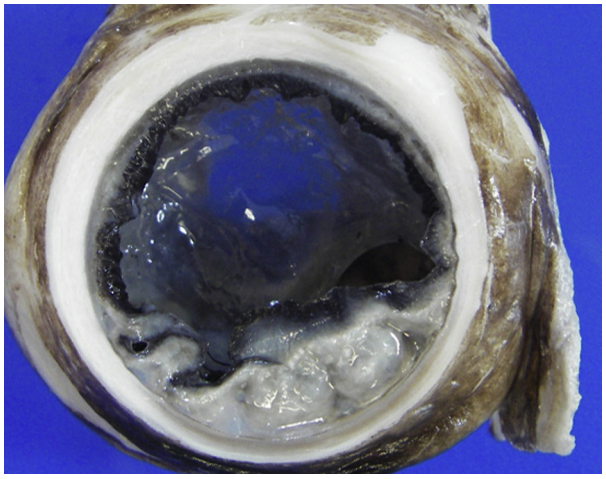

Fig. 1. Anterior aspect of the frontal plane of the fixed left eye (with lens removed), trimmed at the level of the iris. There is oedema of the iris and a gelatinous membrane covers the retina.

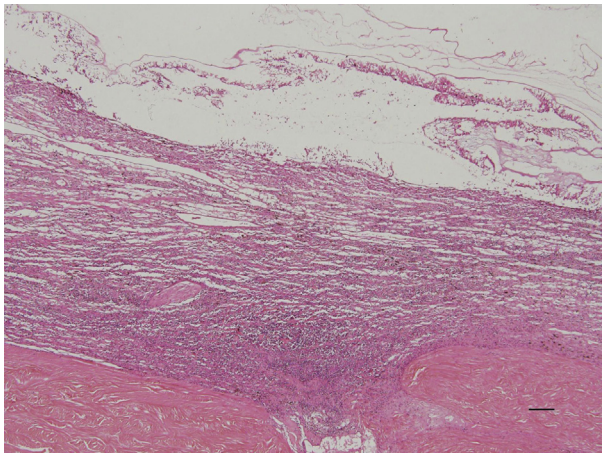

Fig. 2. Section from left eye showing panuveitis with retinal detachment. HE. Bar, 50 μm.

A similar but separate classification of FIP divides it into three main variants: effusive or wet form, non-effusive or dry form and neuro-ocular FIP (Dubielzig *et al.*, 2010). Neuro-ocular FIP can be seen in the absence of other systemic manifestations or in combination with the dry form of FIP. Ocular disease can occur in combination with neurological involvement, or either manifestation can occur in isolation (Dubielzig *et al.*, 2010).

The ocular manifestations of FIP were first reported as bilateral endogenous granulomatous uveitis (Doherty, 1971). Clinically, anterior uveitis and

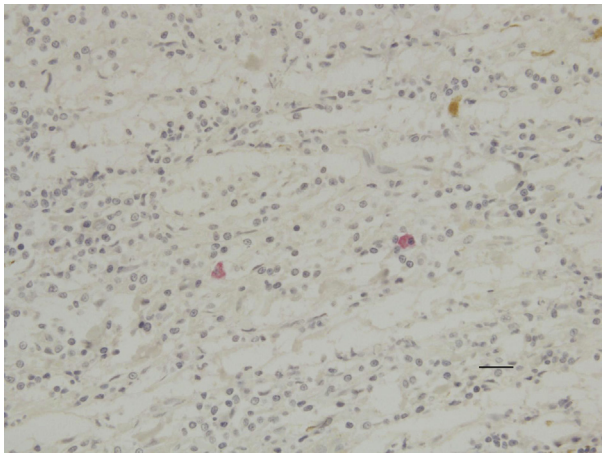

Fig. 3. Section of retina of the left eye showing FCoV antigen in macrophages. IHC. Bar, 20 μm.

Fig. 4. Section of the right occipital lobe labelled for GFAP expression. There are multiple dilated perivascular spaces in the white matter. IHC. Bar, 500 µm. Inset shows Ib-a 1-positive macrophages in a perivascular space. IHC. Bar, 100 µm.

retinitis are common in neurological FIP as ophthalmic lesions (Foley and Leutenegger, 2001). However, histopathological diagnosis of ocular FIP presents a challenge because of multiple morphological patterns (Dubielzig *et al*., 2010).

The gold standard and definitive diagnostic test available for FIP is histopathological examination and IHC for the detection of FCoV antigen in macrophages (Dubielzig *et al*., 2010; Drechsler *et al*., 2011; Giori *et al*., 2011; Bauer *et al*., 2013; Kipar and Meli, 2014). However, there is disagreement about the diagnostic significance of detection of coronavirus antigen by IHC in ocular pathology, with the reliability of the test being questioned (Dubielzig *et al*., 2010).

In the present case, the presence of FCoV-positive macrophages confirmed the diagnosis of FIP. The brain lesions were similar to lacunar lesions in man (Bailey *et al*., 2012) and unrelated to FIP. They were not compatible with feline hypertensive encephalopathy because there were no characteristic hypertensive vascular changes or arteriolosclerosis (Vandevelde *et al*., 2012).

FIP is a fatal, systemic disease caused by a mutant form of feline enteric coronavirus and has been reported to occur worldwide in domestic cats and numerous wild feline species (McReynolds and Macy, 1997). However, there have been few confirmed reports of FIP in non-domestic felids (Stephenson *et al*., 2013). There has been some confusion in the diagnosis of FIP in African lions. Clinical FIP was reported in two lions in a zoo in the USA and was the first indication that FIP may occur in feline species other than domestic felids (Colby, 1970; Colby and Low, 1970). FIP was also reported in three lions in a zoo in South Africa (Robison *et al*., 1971). However, although these reports used the common name "lion", the scientific name was not mentioned. Therefore, we cannot confirm whether these cases were African lions. There have been no confirmed reports in African lions to date.

In non-domestic felids, there may be species differences in the susceptibility to FIP. Cheetahs (*Acinonyx jubatus*) are known to be highly susceptible to FIP infection (Stephenson *et al*., 2013). The present lion was housed with three other healthy lions in one enclosure. Apart from these, the only other non-domestic felid in the zoo was a male cheetah. The cheetah's enclosure was located about 150 m away from the lion enclosure and the cheetah had died from feline infectious anaemia about 6 months before the present case showed ocular symptoms.

FCoV is transmitted by the faecal–oral route (Drechsler *et al*., 2011). The design of the lion enclosure made contact with domestic cats and their faeces possible. Therefore, a faecal–oral infection from virus-carrying domestic cats was suspected in the present case. FCoV was detected in southern African non-domestic felids (Kennedy *et al*., 2003), but there are no data for either domestic or non-domestic felids in Zambia.

In conclusion, FIP should be taken into consideration for the differential diagnosis of blindness in non-domestic felids. Confirming ocular lesions as FIP in non-domestic felids, including African lions, faces many challenges, not least in finding suitable cases for investigation, especially in free-ranging lions. It is hoped that further investigations will add to the info8rmation about the susceptibility to FIP and the ocular manifestation of FIP in this species. As far as we know, this is the first confirmed case of FIP in an African lion and of ocular FIP in a non-domestic felid.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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