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SHORT CONTRIBUTION

Acute hepatic necrosis and death in a subadult southern white rhinoceros (*Ceratotherium simum*) associated with exposure to sterigmatocystin in forage contaminated with *Aspergillus nidulans*

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A young male southern white rhinoceros (*Ceratotherium simum*), which was resident in a zoo as part of a multi-rhinoceros group, died suddenly. Necropsy and histopathological findings supported a diagnosis of death from acute hepatic necrosis. The microscopic distribution of liver lesions was suggestive of hepatotoxicosis. Further investigation revealed potential exposure to a mycotoxin, sterigmatocystin, present in spoiled lucerne hay contaminated with *Aspergillus nidulans*. It was concluded that mycotoxicosis was the likely cause of the hepatic necrosis and death in this animal.

Keywords *Aspergillus nidulans*; hepatic necrosis; hepatotoxin; mycotoxin; sterigmatocystin; white rhinoceros

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The white rhinoceros (*Ceratotherium simum*) is one of two extant species of African Rhinocerotidae, both of which are under intense pressure from poaching in their range states. White rhinoceros are held and managed *ex situ* in Australasian zoos in a regional cooperative conservation breeding program.

A 3-year-old weaned male white rhinoceros resident in an Australian zoo was found dead in his enclosure. No premonitory signs had been observed. At the time of the animal's death there were six animals in the white rhinoceros facility. These included two adult females and a male calf, which were cohabiting with an adult male. A second adult male was held in an adjacent, separate paddock. The dead animal had recently been removed from the female/calf group and was being held in a separate yard, to facilitate introduction of the adult male for breeding. The rhinoceros were predominantly maintained on a diet of oaten hay with access to some pasture. All except the calf were given an additional 2 kg of lucerne hay daily, which was used as the vehicle for administration of a commercially available equine vitamin and mineral supplement.

Necropsy examination revealed the animal to be in good body condition. There was generalised icterus, with the sclerae, subcutaneous tissues and serosal surfaces all noted to be jaundiced. The liver was swollen and oozed bile from its cut surface. Serum harvested from heart blood sampled at necropsy and submitted for laboratory examination confirmed hyperbilirubinaemia with total bilirubin being 88 μmol/L (reference range, 1.1–8.4 μmol/L). All other biochemical tests, including liver enzymes, were within the normal reference ranges. Urine sampled from the bladder was strongly positive for bilirubin on urine dipstick. Tissue samples were collected from numerous organs and fixed in 10% buffered formalin then sectioned and stained with haematoxylin and eosin for histopathological examination.

Histopathological changes included extensive, severe subacute hepatic necrosis (Figure 1). In two sections the periacinar hepatocytes were hypereosinophilic with pyknotic or karyolytic nuclei and surrounded by abundant neutrophils. Elsewhere, periacinar hepatocytes were pale and vacuolated compared with their periportal counterparts. There was multisystemic haemosiderosis, including in the liver where Kupffer cells were noted to be laden with a golden-brown pigment. Perl's stain confirmed this to be iron. Samples were collected from lung, liver, spleen and kidney for microbiology. *Escherichia coli* was cultured from all these tissues except the liver, which was culture negative for bacteria. The presence of *E. coli* was attributed to postmortem contamination of tissues and deemed coincidental to the death of the animal.

The diagnosis was death from acute hepatic necrosis. The pathological process was likely of several hours duration (i.e. long enough for neutrophil infiltration into lesions, but not long enough for increases in serum liver enzymes to occur). The presence of iron in tissues is a common coincidental finding in grazing animals housed at this particular zoo and has been attributed to relatively high regional soil levels of iron oxide. In the case described here, haemosiderosis was thought to be coincidental as it was not associated with histopathology typical of haemochromatosis. A periacinar distribution of hepatocellular damage is a typical histopathological response to a toxic hepatic insult.[1] Accordingly, ongoing disease investigation in this case focussed on identifying a potential hepatotoxin to which the animal had been exposed. Environmental inspection excluded agricultural and industrial toxins. Pasture and forage inspection excluded hepatotoxic plants. Water, which was reticulated from a common source to the entire rhino group and other zoo animals nearby, was free of cyanobacteria on visual inspection. There had been no history of recent administration of any medication derived from equine blood products that might have initiated the hepatic necrosis typical of Theiler's disease.

Inspection of lucerne hay being fed to the rhino group revealed spoilage. Hay concealed within the individual 'biscuits' comprising the rectangular bales was extensively affected by a black–grey mould. Samples of affected hay were submitted for fungal culture and

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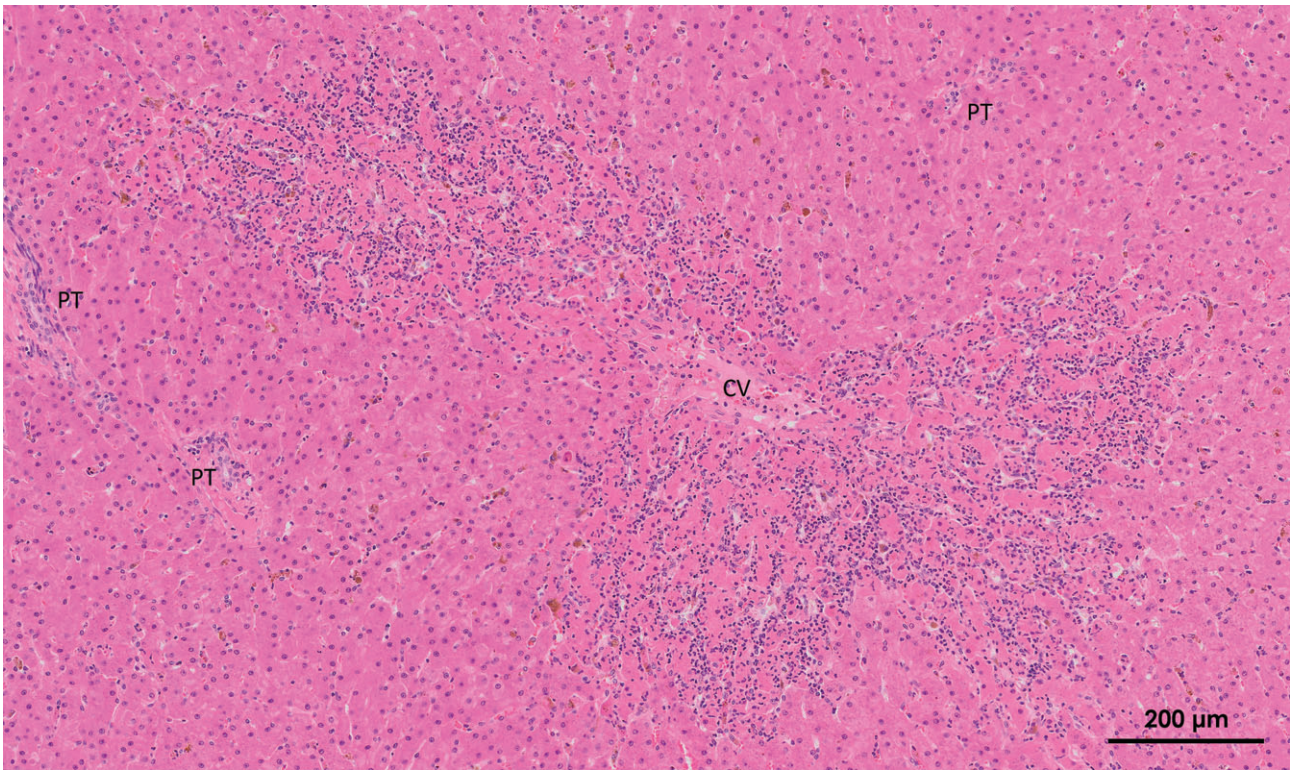

Figure 1. **Section of liver from a white rhinoceros that was found dead, showing periacinar necrosis and neutrophil infiltration. CV, central vein; PT, portal triad. (H&E; scale bar = 200 µm.)**

Aspergillus nidulans was isolated. Additional samples of affected hay were submitted for liquid chromatography–mass spectrometry analysis for mycotoxin screening, which revealed the presence of 1.56 ppb of sterigmatocystin.

Sterigmatocystin is a polyketide secondary metabolite produced by many species of *Aspergillus* and several other genera of fungi.[2] It is closely related to the aflatoxins, with which it shares biosynthetic pathways, and has been reported in grains, nuts, green coffee beans, spices, beer and cheese, as well as in hay and silage.[3] Sterigmatocystin is an uncommon mycotoxin that has teratogenic, mutagenic and carcinogenic potential. It has been shown experimentally to be acutely hepatotoxic to rats, mice, monkeys and guinea pigs.[4] Ingestion of feed contaminated with 7.75 mg/kg sterigmatocystin resulted in bloody diarrhoea and death of some members of a dairy cattle herd.[5]

Acute hepatic mycotoxicosis, which has not been previously reported in the rhinoceros, is relatively uncommon in domestic perissodactylids. This has been ascribed to the horse's particular disinclination to eat unpalatable, mouldy feed.[6] Young animals generally tend to be less fastidious, however, and are regarded to be more likely to ingest spoiled foodstuffs. This is likely also the case with zoo-based rhinoceros. Additionally, experimental evidence suggests that sterigmatocystin is intrinsically more toxic to younger animals.[3] The acute oral toxicity of sterigmatocystin is relatively low in the small number of species for which it has been experimentally ascertained, with LD_{50} values ranging from 120 to 166 mg/kg body weight. The LD_{50} of sterigmatocystin for the white rhinoceros is unknown. Although the levels of sterigmatocystin in the hay samples selected for analysis in this case were relatively low, the distribution of mycotoxin in agricultural products can be markedly heterogeneous, with high concentrations occurring in 'hot spots' in the forage.[3] Accordingly, it is feasible that the young rhinoceros in this case ingested sufficient sterigmatocystin to cause death by liver failure attributable to hepatic mycotoxicosis.

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