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Cyanobacterium *Cylindrospermopsis raciborskii* as a probable cause of death in cattle in northern Queensland

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Livestock deaths due to cyanobacterial (blue-green algal) poisoning have mainly been caused by toxic strains of *Anabaena circinalis, A spiroides, Aphanizomenon flos-aquae, Microcystis aeruginosa* and *Nodularia spumigena.*[1-5] In Australia, cattle and sheep have since 1878 succumbed to cyanobacterial poisoning caused by *A circinalis, M aeruginosa* and *N spumigena.*[1,6-8]

The possibility of another cyanobacterium being a cause of poisoning in man and animals in Australia was raised by Hayman in 1992.[9] He suggested that the signs of the illness known as Barcoo fever, which has been known in northern Australia since the 1880s, were reminiscent of those caused by the tropical cyanobacterium *Cylindrospermopsis raciborskii.* The severity of illness, although it only manifested in the mildest form of nausea and fever, was related to the amount of water ingested from contaminated water supplies.[9] During the same time, there have been numerous anecdotes about losses of cattle and sheep in northern and western Queensland after drinking from dams and waterholes contaminated with scum or 'paint slicks' indicative of cyanobacteria. In 1992, stock poisoning due to *C raciborskii* was suspected in Western Australia.[10]

This article reports the death of three cows and ten calves due to suspected poisoning by cyanobacterium *C raciborskii* on a property in the McKinley Shire of northern Queensland.

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Clinical history

A herd of 300 cattle (150 cows and 150 calves 4 to 5 months of age) were drinking from a dam located on the property and showing a heavy growth of blue-green algae. One cow and three calves were found dead around the dam. The owner proceeded to muster the cattle and remove them from the area. It was noticed at the end of the muster that one calf was unable to keep up with the rest of the herd. Within 24 h, the calf had deteriorated markedly and it was showing signs of staggering and weakness before it died. On necropsy, the gross findings were severe abdominal and thoracic haemorrhagic effusion, hyperaemic mesentery, pale and swollen liver, and extremely distended gall bladder containing dark yellow bile. Samples were sent to the Oonoonba Veterinary Laboratory: several organ samples in 10% buffered neutral formalin for histopathological examination, samples and swabs of thoracic, abdominal and intestinal contents for bacteriological testing and a sample of the algae for identification.

During the next 3 weeks, a further two cows and six calves from this herd died, but no necropsies were performed.

Laboratory findings

Histopathological examination of the liver revealed extensive fibrosis and bile duct proliferation with isolated groups of hepatocytes remaining (Figure 1). The heart had extensive epicardial haemorrhages and the small intestine and omentum had extensive subserosal haemorrhages. The brain, lungs, spleen and kidney exhibited no abnormality. *Escherichia coli* was cultured from the thoracic, abdominal and intestinal samples.

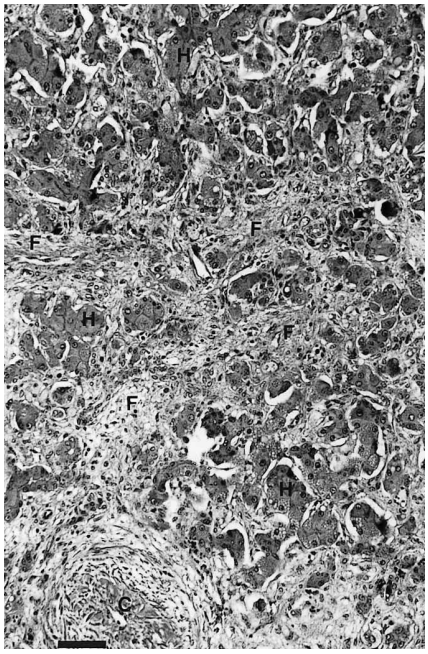

Figure 1. A section of liver from the calf showing extensive fibrosis (F), especially around the central vein (C). Isolated groups of hepatocytes (H) remain. Haematoxylin and eosin, bar = 27 µm.

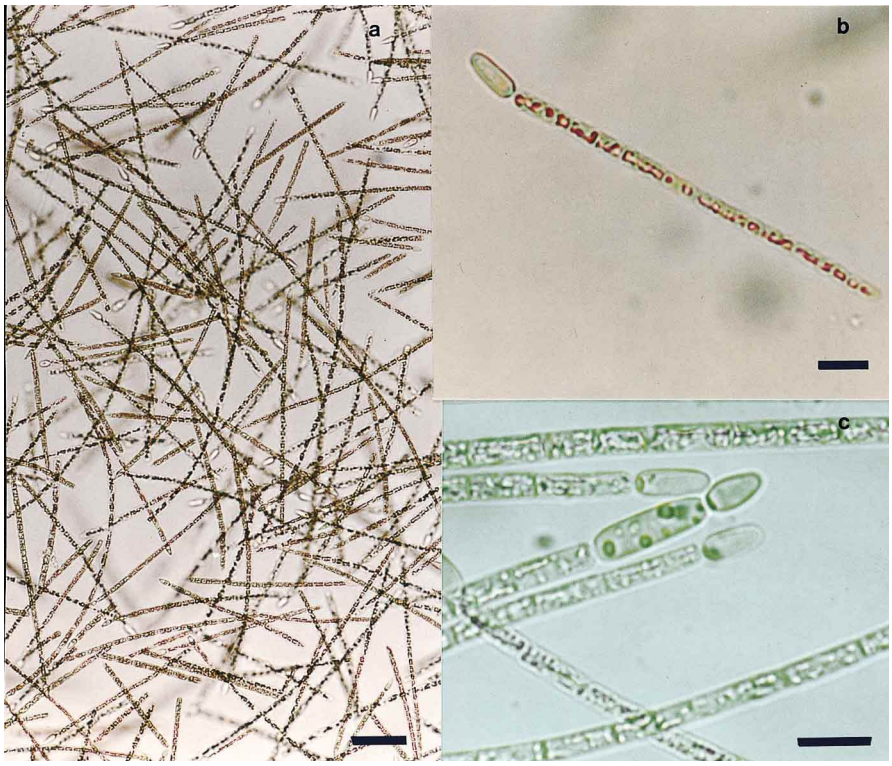

Figure 2. *Cylindrospermopsis raciborskii* isolated from a private dam in the McKinley Shire. a) Culture of cyanobacterium, bar = 34 µm. b) A single trichome with typical oval heterocyst, bar = 10 µm. c) A trichome with an akinete associated with the terminal heterocyst, bar = 10 µm.

The water sample contained predominantly a large, straight-chain strain of *C raciborskii*, showing the typical pointed terminal heterocysts at one or both ends of the trichome (Figure 2). Akinetes, when present, were oval to barrel-shaped and generally adjacent to one of the heterocysts either as a single cell or in series. The septa were indistinct and the cells were gas-vacuolated. The isolate was grown in pure culture in an algal selective medium (ASM) during a 3 week period at 25°C under a light intensity of 50 µmol/m^2/s. The resulting packed cells were freeze-dried and stored until a mouse bioassay could be performed.

For the mouse bioassay, the technique of Baker and Humpage[11] was used. Doses of 1.0 mL, 0.5 mL and 0.1 mL were inoculated by intraperitoneal injection into each of two mice of known weights. Two control animals were given sterile saline by the same route. The two mice receiving the 1.0 mL dose died within 7 to 8 h after inoculation. Their livers were reddened and swollen and were 12.6% and 13% of the total body weight. The other six mice were sacrificed at 7 days but showed no sign of any toxic effect. The control mice had livers that were 5.7% and 7.7% of their body weight. Histological examination was performed on the livers of all mice. Extensive necrosis and pooling of blood were seen in the liver of the mice receiving the 1.0 mL dose, but the livers of all the other mice showed no significant lesions.

The toxic dose of *C raciborskii* was calculated to be 153 mg/kg mouse (range 144 to 165) at 7 to 8 h.

Discussion

C raciborskii is regarded as a tropical or subtropical cyanobacterium[12] although it has been isolated from warmer, temperate regions. Blooms are rare in the latter climates.[11] The organism itself can vary in size, shape and morphological features. It has appeared as a small, coiled trichome in some areas such as the Fitzroy River in central Queensland[13] and as a large, straight-chain trichome as seen at Lake Julius near Mt Isa.[14] *C raciborskii* has an optimum temperature for growth at > 25°C and therefore tends to bloom in the warmer months.[13] Other factors that enhance bloom formation are surface irradiation of > 2200 µmol/m^2/s and pH 8.4 to 9.0.[13] Toxic strains produce a cytotoxic alkaloid called cylindrospermopsin which is known to damage most organs, especially the liver, in chronic cases.[11] Cylindrospermopsin is a hepatotoxin, the effect of which, in cattle, is weakness, anorexia, pallor of the mucous membranes, coldness of the extremities, diarrhoea and, with larger doses, death within 2 h to several days.[15,16] The route of entry is by oral ingestion of toxic cyanobacterial cells and exposure is a result of drinking from an area of a dam or waterhole where prevailing winds have concentrated the bloom.[17,18]

The toxin found in the present case has a similar toxicity to the strain of *C raciborskii* associated with 149 human cases (139 children and 10 adults) of cyanobacterial poisoning on Palm Island in the mid-1980s.[19] Other workers using the mouse bioassay showed toxic doses of *C raciborskii* isolates to be 168 mg/kg

mouse at 6 to 9 h[11] and 52 to 569 mg/kg mouse at 24 h.[11,19-21] Studies have indicated that massive doses or early deaths in mice at 6 to 9 h are associated with centrilobular or massive hepatocyte necrosis,[19] accompanied by a reddened, swollen liver, usually associated with increased weight gain of 60 to 100%.[5,17,19] The findings in the mice in our case were consistent with this pattern.

Deaths from smaller doses, which usually occur after 24 h, are associated with pale livers, a result of fatty degeneration affecting hepatocytes.[15] Work done by these authors suggests that only the liver is affected in mice[15] although other authors have noted changes in other organs.[12]

In the present case cyanobacterial poisoning by a toxic strain of *C raciborskii* was the probable cause of death in 1 calf and possibly 12 other cattle (including 9 calves) drinking from a dam on a Queensland property. The organism was the major alga in the dam water, it was toxic for mice at 153 mg/kg, and the histopathological examination of liver and other organs of one of the calves was indicative of blue-green algal hepatotoxic poisoning.

To reduce losses on properties, owners will have to monitor dams and waterholes for the presence of algal scum and keep animals from drinking the water by supplying an alternative water supply where possible. Smaller watering areas could be aerated to destratify the water column.[22,23] The addition of barley straw has been shown, in some cases, to reduce the numbers of algae present and thus lessen the chance of bloom formation.[24]

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