CYANOBACTERIAL (BLUE-GREEN ALGAE) POISONING OF LIVESTOCK IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA

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ABSTRACT

Three outbreaks of cyanobacterial (blue-green algae) poisoning involving cattle and sheep are described. In 2 of these acute mortality was followed by photodermatitis in some of the surviving animals. In all 3 outbreaks the hepatotoxicity of the water collected from the dams where the animals had been drinking was confirmed following the intraperitoneal administration to mice. Nodularia spumigena was the dominant cyanobacterium in the first 2 outbreaks, and Microcystis aeruginosa in the third. The presence of the heptapeptide toxin microcystin-LR in the third outbreak was demonstrated by high pressure liquid chromatography.

Key words: Cyanobacterial toxicosis, Microcystis sp., Nodularia sp., cattle, sheep, hepatotoxicosis, photosensitisation.


INTRODUCTION

Toxicity problems associated with cyanobacteria (previously referred to as blue-green algae) have existed for at least a century. The first authenticated description of scum on a lake in Wales dates back to the 12th century. There are 150 cyanobacterial genera and approximately 2000 species, of which 40 have toxic properties. Excessive growth of cyanobacteria in the aquatic environment is supported by increased concentrations of nitrogen and phosphorus and promoted by various combinations of environmental conditions such as low hydraulic flows, high temperatures and pH and calm weather. Blooms may consist of one or more species of cyanobacteria, and the genera commonly involved include Microcystis, Anabaena, Aphaniotoxon, Oscillatoria and Nodularia, all of which have previously been implicated in cases of poisoning. The amounts and proportions of toxins produced vary greatly, but blooms and especially wind-blown scum may result in massive concentration of the toxins and may result in poisoning. The toxins are predominantly intracellular in healthy blooms, but are released once they mature, senesce and die. Mortalities associated with these toxins have been described worldwide in a variety of mammals, birds and fish. A considerable amount of research has also been undertaken to study the effects of exposure (including chronic exposure) in humans. Cyanobacteria produce a number of classes of secondary toxic metabolites, including hepatotoxins, neurotoxins, saxitoxins, lipopolysaccharide endotoxins and a variety of other, as yet undefined, toxic compounds. The hepatotoxins, the largest and most common class of cyanobacterial toxins, have been known by several names (i.e. cyanoginosins, cyanoviridin). However, these hepatotoxins are now referred to as microcystins. At present more than 40 structural variants of microcystin are known, of which microcystin-LR is the most common. These toxins are very stable and highly toxic compounds, and the intraperitoneal LD₅₀ for mice is in the order of 50 μg kg⁻¹ body mass. Toxicity can unfortunately not be predicted, as there is no correlation between the density of the bloom and toxin production, and toxin production is also highly variable. In South Africa the majority of poisonings have been associated with Microcystis aeruginosa, and as far as could be ascertained cases of poisoning have only been described from the previous Transvaal and Orange Free State provinces, even though cyanobacteria occur throughout South Africa.

HISTORY

Outbreak 1

Twenty 4-5 month-old Friesian calves were introduced into a lucerne camp on a farm in the Malmesbury district during mid-December 1993. Approximately half of the animals developed signs of photosensitivity within 3 days. The calves were
immediately removed, and although the unpigmented skin of affected animals sloughed, they all recovered.

Towards the end of December 1993 a group of approximately 70 lactating cows was introduced into the same camp. The following day one of the cows was found dead and another 2 died over the next 2 d, whereafter this group was also removed from the camp. No clinical signs were observed prior to death. An autopsy was performed on one cow by a private veterinarian, and revealed severe, acute liver haemorrhage and necrosis. Unfortunately no organ specimens were collected. A heavy windblown algal scum was evident in the dam on the side where the cows had been drinking. A water sample was collected and submitted to the Regional Veterinary Laboratory, Stellenbosch.

Outbreak 2
During January 1994, 5 out of a group of 21 adult cattle were found dead in an old stubble-land camp in the Malmesbury district, where they had been grazing for the preceding 2 months. Unfortunately the carcasses were too decomposed for autopsy due to the prevailing hot summer weather. The remaining cattle were immediately removed from the camp. A group of 249 sheep was introduced into the same camp in early March 1994. At the end of March 27 sheep were found dead on one day and a further 2 died the following day. An autopsy was performed on one of these sheep. The farmer had observed that the flock had, until the deaths occurred, been drinking from water troughs at one end of the camp, but a day or two before the outbreak they had started drinking from a vlei on the opposite side. Heavy windblown algal scum was evident in this vlei, and samples were collected and submitted to the Regional Veterinary Laboratory, Stellenbosch.

Outbreak 3
In the Paarl district a group of 130 ewes with their lambs had been in an old lucerne camp for approximately 2 months. At the end of May 1994 11 sheep were found dead without any premonitory clinical signs. Unfortunately no autopsies were performed, but the farmer submitted a liver sample from one of the sheep because of the frank haemorrhage present. This was submitted to the Regional Veterinary Laboratory. The farm was visited on the following day and water samples were collected from the earth dam, where heavy windblown algal scum was observed on the side of the dam where the sheep had been drinking. Photosensitisation and icterus were evident in approximately 30 of the surviving sheep.

MATERIALS AND METHODS

Mouse Bioassay
Fifty ml of the water samples (n = 3) containing cyanobacteria were frozen and thawed to rupture cell walls, which were then filtered through Whatman no. 4 filter paper. The filtrate (0.5 ml) was injected intraperitoneally into white mice (n = 3) (approximate mass 30g). An equivalent volume of sterile water was injected intraperitoneally into control mice (n = 3) in each trial. Clinical signs were observed and time to death noted. The control mice were euthanased after 3 h. Livers of both the control and test mice were weighed and fixed in 10% formalin.

Histopathology
The available organ samples from affected animals were fixed in 10% buffered formalin and routinely processed and stained with haematoxylin and eosin (HE) for histopathological examination.

Blood chemistry (outbreaks 2 and 3 only)
During each of outbreaks 2 and 3 a blood sample was collected from the jugular vein of a sheep exhibiting signs of photosensitivity. Bilirubin-concentration and gamma-glutamyl transferase (GGT) and aspartate transaminase (AST) activities were determined by spectrophotometry.

Cyanobacteria identification
The samples (n = 3) were examined fresh and after preservation with Lugol's iodine using a Zeiss Axiocvert 35 inverted- photomicroscope over a magnification range of 200 to 1000x.

Toxin assay (outbreak 3 only)
The suspected microcystin toxins were extracted from a pre-weighed aliquot of freeze-dried algal scum by adding 25 ml of methanol, vortex mixing the sample for 3 min and allowing to stand for 1 h at room temperature. The sample was then centrifuged at 3 500x g for 20 min at 10°C. The supernatant was retained and the pellet extracted twice more using the same procedure. The supernatants were pooled, rotary evaporated to dryness and made up in methanol containing 0.1% trifluoroacetic acid to a final volume of 1 ml.

The sample extract was quantitatively analysed using reverse-phase photodiode-array (PDA) high pressure liquid chromatography
The liver sample submitted by the farmer revealed severe centrilobular necrosis with haemorrhages and fatty degeneration of periportal hepatocytes.

**Blood chemistry**

*Outbreak 2:* The blood sample collected from a surviving sheep showed markedly elevated bilirubin concentration, GGT and AST activities.

*Outbreak 3:* The blood sample collected from a sheep with signs of photosensitivity and icterus showed a very elevated bilirubin concentration.

**Cyanobacteria identification**

The dominant cyanobacterium in outbreaks 1 and 2 was positively identified on the basis of colony and/or cell morphology according to Komárek et al. as *Nodularia spumigena* Mertens (Fig. 1)\(^9\). This cyanobacterium was conclusively linked to the presence of the toxin nodularin and the death of a dog suspectedly due to this in Zeekoeivlei, Cape Town during March 1994\(^1\).

In outbreak 3 the dominant cyanobacterium present was positively identified as *Microcystis aeruginosa* Kutz, alternatively classified by Komárek\(^4\) as *Microcystis aeruginosa* forma *aeruginosa*, i.e. having the classical lattice-colony formation (Fig. 2) typical of the toxic variety of this species\(^4\ ^{15}\).\(^2\)

**Toxin assay**

HPLC analysis of the freeze-dried material revealed 2 peaks exhibiting characteristic microcystin spectra (Fig. 3)\(^7\). The larger of these was identified as microcystin-LR, while the second, unidentified peak was regarded as an LR-equivalent due to its spectral similarity and proximity of elution. In total these 2 peaks were equivalent to 1900 µg microcystin-LR per gram of freeze-dried material.

**DISCUSSION**

The liver damage evident in the sheep in outbreaks 2 and 3 is consistent with the liver pathology described in other confirmed cases of cyanobacterial toxicosis\(^9\ ^{10}\). The microcystin toxins disrupt the hepatocyte cytoskeleton, possibly through alteration of actin filaments, resulting in the loss of lobular architecture\(^1\ ^{12}\). The acute deaths without premonitory clinical signs correlate with those described in

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**RESULTS**

**Mouse Bioassay**

*Outbreak 1:* The mice injected intraperitoneally with the water/cyanobacteria filtrate showed extreme discomfort within 20 min, terminal convulsions and died within 65; 60 and 45 min respectively for outbreak 1, 2 and 3. The control mice remained normal and were euthanased after 3 h.

In all 3 outbreaks the livers of the test mice were grossly enlarged and congested, and average weight of the test mice livers was 83% greater than that of the controls.

**Histopathology**

Microscopical examination of the test mice livers revealed severe vacuolation of hepatocytes, with dissociation and total loss of lobular architecture as well as massive haemorrhage and congestion. The livers of the control mice revealed no abnormalities.

The liver sample collected from the sheep during the second outbreak revealed severe centrilobular coagulative necrosis with severe haemorrhage. In the kidney there was moderate congestion and tubular degeneration. No significant pathology was observed in the other organs.

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Fig. 2: *Microcystis aeruginosa* colony isolated from outbreak no. 2, x 100

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(Hewlett-Packard Model 1050 autosampler quaternary pump and PDA linked to a ChemStation) fitted with a Shandon-Hypersil-BDS 25 cm column and using a water-acetonitrile gradient\(^7\). The results were expressed as µg microcystin-LR per gram of freeze-dried material. Pure microcystin standards were obtained from Calbiochem (Switzerland).
Fig. 3: Chromatogram from outbreak no. 3. Two peaks at 18:15 and 21.43 minutes typical of microcystin-LR. Concentrations of peaks: Peak A (LR) = 1340 µg microcystin per gram of freeze-dried serum; Peak B (mc) = 585 µg/g.

...heifers by Fitzgerald et al.". Secondary hepatogenous photosensitisation was also observed in surviving cattle and sheep in outbreaks 2 and 3, which is consistent with previous reports. The results of the mouse bioassay agree with those described by Fitzgerald et al."

Cyanobacterial poisoning is usually associated with warm, windy weather, and in outbreaks 1 and 2 poisoning had indeed been preceded by hot, windy conditions. During outbreak 3, however, the prevailing weather had been cool and very windy. In outbreaks 1 and 3, the tracks indicated that the animals had been drinking only from the area where the bloom had accumulated; and it appears that the cyanobacteria, despite their often unpleasant stench, may be palatable or even inviting to livestock. Only *Microcystis aeruginosa* has previously been implicated in cases of cyanobacterial poisoning in South Africa, and outbreaks 1 and 2 are therefore the first reported incidents of poisoning associated with *Nodularia* spp. in South Africa. The occurrence of *N. spumigena* is unusual for the Western Cape Province, and this species was implicated not only in the first two outbreaks reported here, but also linked to the first ever case of cyanobacterial poisoning reported for the hypertrophic lake Zeekoeivlei, near Cape Town. These are the first outbreaks of cyanobacterial toxin-related stock deaths recorded in the Western Cape Province. The toxic cyanobacterial species have a widespread distribution throughout South Africa and, as is the case elsewhere in the world, poisoning may have been overlooked in the past. It is therefore important that veterinarians and stock farmers take note that acute deaths associated with hepatic damage and possibly photosensitisation may occur when stock consume water with cyanobacterial blooms. It is important that fresh material (liver, rumen contents, water and scum) be submitted to confirm the diagnosis. The stock losses incurred in outbreaks 1 and 2, where stock were reintroduced into camps where deaths had occurred previously, illustrate the necessity for early confirmation of a diagnosis to prevent further losses.

Similarly, stock farmers should be made aware of the warning signs indicating the potential for an outbreak (greenness of water; algal accumulations along the shoreline). These signs are generally easily discernible, but may require regular examination of downwind shorelines, especially during periods of warm, dry weather. Where no alternative stock watering supplies exist, the animals should be restricted to drinking from the upwind areas. In all cases where cyanobacterial scums occur, there is a high (60-70%) probability of toxins being present. The safest management practice is, therefore, to consider all scums toxic unless proven otherwise. In addition, the awareness of the need to reduce nutrient (particularly phosphorus) runoff to dams should be developed wherever possible.

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