AN UNUSUAL FUNGAL DISEASE OF CROCODILES

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INTRODUCTION

Fungi commonly occur in the soil and can readily adapt to cause diseases in both man and animals, multiplying rapidly in whatever tissue they are feeding on[22]. They enter the body through cuts, abrasions or bite wounds and are particularly a problem in reptiles during the first stages of growth from the egg, especially in hot, humid conditions. Some types of fungi can also pass across the egg membrane of reptiles just after the eggs are laid, causing death or malformation of the embryo[23,24,25,26].

There are not many published reports of fungal infections in crocodiles. Most of those reported show lung infection and sores in alligators[27,28] and crocodiles[29,30,31].

Contamination and skin sores in reptiles are usually caused by the fungal genera *Fusarium* and *Paecilomyces* or by keratin-loving fungi such as *Trichophyton* spp.[32] and *Chrysosporium* spp.[33,34]. Keratin is a widespread animal protein found in horn, feathers, hair and hoofs. It is difficult to confirm the disease-producing status of the *Chrysosporium* genus because it is commonly found on the skin of healthy, normal-looking animals[35,36].

During a recent Oonoonba Veterinary Laboratory trial on crocodiles (C. *porosus*) from five to 16 weeks of age, we came across unusual skin sores which suggested a keratin-loving fungus. This article describes how the infection affected the crocodiles and the control measures used to eliminate the problem.

MATERIALS AND METHODS

Animals

Two hundred and forty-seven crocodiles were received from six clutches: clutches 89, 90, 96 and 99 transported on May 11th; clutches 102 and 103 transported on 24th May. On arrival, they were kept in separate tanks in their own clutches and allowed to acclimatise for four weeks with a water temperature of 32°C and an air temperature of 30°C before being tagged and measured. After a further eight weeks spent controlling a fungal infection which occurred in one tank, the crocodiles were weighed, measured and re-sorted by weight into six groups containing crocodiles from clutches 89, 90 and 103 and five groups

containing crocodiles from clutches 96, 99 and 102. These reptiles were placed into tanks with duplicated water temperatures ranging from 26°C to 34°C. A second outbreak of fungal infection occurred during this temperature trial in one replicate (statistical trial) of the cooler 26°C water treatment.

Sample Collection

Specimens were collected during the two fungal outbreaks. Plaque (plate-like) wounds were recovered for bacterial and fungal examination. The dead crocodiles were autopsied and their internal organs, including the liver, heart and kidney, were examined as well as any skin sores.

Isolation and Identification

The specimens collected were cultured for fungi using Sabourad's Dextrose Agar (SDA) supplemented with chloramphenicol and cyclohexamide. Plates were incubated in plastic boxes at 28°C for 14 - 21 days and read daily. The growths which formed were examined under a microscope to identify the species.

Bacterial culture was performed on blood agar and MacConkey agar plates also grown at 28°C. Organisms isolated were purified and identified using Microbact 24E and API 20NE kit systems where appropriate.

RESULTS

Examinations

When the animals were first weighed on June 10, a creamy cheese-like mass was seen around, and in some cases under, the scales of the head, back and feet of several of the 34 crocodiles from clutch 89. Four animals were swabbed to get culture specimens. Within three days, the animals appeared to be more severely affected with a plaque, spreading to the snout and under the jaw, and the disease had spread to virtually all animals in the tank - although not to the other tank in the same room. These plaque layers could be peeled off and a number were removed to be grown out in the laboratory for identification. Often, the skin was reddened in the area where the plaque was removed. In all, 12 animals were tested for bacterial and/or fungal diseases and eight crocodiles died between June 23 and 26. After a series of treatments, no further problems were noticed and the remaining 26 crocodiles in the clutch were distributed and mixed with two other clutches for the temperature trial, which started on July 11. The skins of the survivors healed completely, leaving no sign of the infection.

One month after the temperature trial started, animals in one of the 26°C water temperature tanks began to show the same plate-like sores. The 18 animals in this tank were from clutches 96, 99 and 102. The infections were more severe and 13 animals died between August 8 and 31 before the infection was brought under control. The temperature trial was terminated on August 22, and all of the tanks were brought to a water temperature of 34°C. These deaths were also associated with blood poisoning caused by the bacterium *Aeromonas hydrophila*. For several weeks, the healed patches of infection appeared as darker coloured areas, but this disappeared after several months.

Treatments

The treatments included adding the disinfectant and preservative formaldehyde to the infected tank water, a salt water bath for seven minutes and the use of the iodine antiseptic solution called betodine where the plaque layers were swabbed away. The animals were dipped or washed with betodine and left to dry for several hours before replacing in the tank.

The amount of formalin was increased from 0.003 per cent per tank to 0.013 per cent per tank, twice a day, by the end of the infection control.

Cultures

When the skins of 21 of the infected crocodiles were cultured, a number of soil and water bacteria known to cause disease in animals were isolated. These included *Aeromonas hydrophila, Pseudomonas aeruginosa* and *Serratia marcescens*. Fungal species, including *Fusarium* spp and *Paecilomyces lilacinus*, were cultured from a number of the infected crocodiles, and also from crocodiles in a neighbouring tank which did not have the infection.

The plaque was found to be the result of a fungal infection caused by *Chrysosporium tropicum*, after a series of tests carried out by the Mycology Unit at the Children's Hospital in Adelaide ruled out a strain of ringworm fungus *Trichophyton mentagrophytes* found in animals and man, which it closely resembled.

Histopathology

Tissue analysis of the reddened skin areas showed fungal infection in the underlying layers of skin.

DISCUSSION

C. tropicum is a keratin-loving fungus[37], often found on normal appearing skin[38,36] and is common in both Papua New Guinea and mainland Australian soils, especially in animal pastures and areas used by birds[39]. It has been isolated from the scales of reptiles and, in an Australian survey, was found in 11.1 per cent of mammals and reptiles, 21.8 per cent of native birds and 84.8 per cent of domestic birds. None of these animals or reptiles showed signs of infection[32,40,41].

The species has been reported to cause sores in iguanas[33], mice[42], poultry combs[43], and human infections[44] including the bone inflammation (osteomyelitis)[45] and swelling of the heart (endocarditis)[46].

Evidence that this fungus was the cause of the plaque-like skin patches in the crocodiles at Oonoonba lay in the fact that was only isolated from these sores. Other fungi such as *Fusarium* spp. and *Paecilomyces lilacinus* were also present but mainly from the back of the crocodiles, and also from crocodiles in other tanks unaffected by the infection.

The fungal infection occurred on two separate occasions. The first was only associated with clutch 89. It is possible that *Chrysosporium tropicum* was in the soils of the nesting material and contaminated the outside of the eggs but whether the hatchlings were infected by the fungus crossing the egg membrane, as has occurred with *Fusarium* spp. in reptiles[24,25,26] or, as they discarded their shell, is not known.

Transport of the two day old crocodiles to Oonoonba may have triggered the infection. Trapping, handling, temperature changes, overcrowding and transport are just some of the stresses of crocodiles that can allow disease-carrying bacteria and fungi to take hold[47].

The second outbreak was more severe and occurred in one of the two tanks held at 26°C. Fungal infections can be triggered by lower than ideal temperatures and these often involve the skin[47]. *Chrysosporium tropicum* grows well at 26 - 28°C but, in this case, it was not the only cause of the 13 mortalities. Blood poisoning caused by *Aeromonas hydrophila*, bacteria which are often linked to crocodile deaths, was also identified [31,48].

Since the second outbreak occurred in a different tank from the first and also with crocodiles from different clutches than the first, it is interesting to speculate on the origin of the infection.

All five animals in clutch 96 died, as did all six in clutch 99 and five of the seven animals from clutch 102 died.

Clutches 96 and 99 were transported to Oonoonba along with clutch 89, so it is possible that cross-contamination occurred on route. The temperature drop to 26°C may have caused the outbreak. Two of five crocodiles from clutch 102, which had no earlier contact with reptiles from clutch 89, died from bacterial blood poisoning, caused by *A. hydrophila*. The other three animals were not sampled, however histopathology indicated no sign of infection. No *Chrysosporium tropicum* was isolated from the five clutch 102 crocodiles, and the duplicate tank of 26°C water temperature located in a separate room had no problems with crocodiles of similar weight and size.

The salt water wash did not work and caused a 'stinging' reaction with the cuts and abrasions of some of the crocodiles. The combination of formaldehyde in the water and the betodine scrub cleared the infection in time. Tetracycline injections controlled the *Aeromonas* infection.

This was an unusual case of fungal infection in crocodiles caused by the soil fungus *Chrysosporium tropicum*.