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A bacterial disease of the Darwin's frog *(Rhinoderma darwini)*

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Summary

Ulceration and necrosis of the rostrum in Darwin's frogs was investigated microbiologically and histopathologically. The condition was attributed to infection with *Aeromonas liquefaciens* and, possibly, an *Acinetobacter* sp.

A number of important diseases have been recognised in captive amphibians (Reichenbach-Klinke & Elkan, 1965), amongst them infection with bacteria of the family Enterobacteriaceae. Such diseases can prove troublesome in amphibians kept for research as well as those in zoological and private collections. In this paper we report a localised bacterial infection in the South American Darwin's frog, *Rhinoderma darwini*.

History and clinical features

The frogs were a group of 33 imported from Chile on 6 January 1977. The species is found along stream banks in forests where the climate is cool (5°C in July to 21°C in January) and wet. Accordingly, they were housed in 3 groups of 11 in polypropylene tanks containing trays of leafmould, with tree bark to form hiding places and running spring water moving through the tank to provide cool, clean water. The air temperature in the tanks was approximately 21°C. Food consisted of small house crickets (*Acheta domestica*) which were readily taken. Between 6 and 13 January 1 of the male frogs produced a total of 9 very small young frogs from the vocal sac in which the tadpoles of this unusual species develop. These young frogs were kept in a similar environment to the adults.

In addition to the reproductive phenomenon, a particular feature of these frogs is a pronounced forward-pointing rostrum on the front of the skull, giving the head a triangular appearance.

Clinical signs of disease were first seen on 30 January 1977 when a number of frogs were noted to have greyish coloured lesions on their rostra. In the majority of cases the rostrum became ulcerated and necrotic and in some instances disappeared; a few such animals recovered, apparently uneventfully, but the majority died.

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On 16 February, 6 frogs were received for investigation. One was considered normal and showed no clinical signs of disease. Another was alive but had a severely ulcerated rostrum and was killed in extremis, after swabs had been taken. 4 others, 3 with ulcerated rostra, were dead on arrival and were used primarily for histopathological examination.

Pathological findings

Gross pathological findings were minimal. The 4 with rostral lesions showed different degrees of ulceration; the remains of the rostrum were red and swollen. All the frogs examined were in reasonable body condition with internal fat bodies, and 3 females contained considerable quantities of spawn.

Histopathological results

Tissues were fixed in 10% formol-saline, embedded in paraffin wax, and sectioned. Stains used were Cole's haematoxylin and eosin, PAS, Gram and Gram-MGPLG (Sowter & McGee, 1976). Histologically the rostral lesions (Figs 1 and 2) showed disruption and damage to the lining epithelium but the overlying keratin was partly intact. The mucous glands were destroyed or damaged and contained cellular debris. Moderate to marked oedema was present. There was a marked absence of cellular reaction but Gram stains showed masses of Gram-negative bacteria in the damaged tissues.

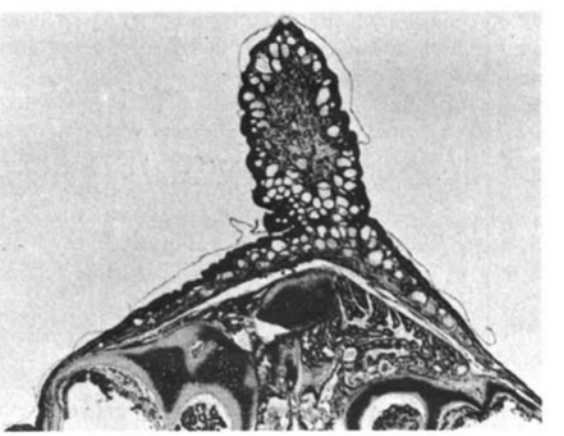

Fig. 1. Normal rostrum showing intact epithelium and underlying tissues.

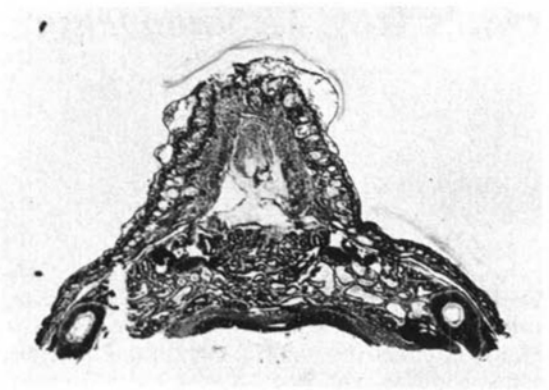

Fig. 2. Affected rostrum: note swelling of base, truncated tip and necrotic epithelium.

Gram-MGPLG stains confirmed that these were Gram-negative cocci and bacilli. Other organs showed a variety of lesions which were relatively minor; other than in a frog which had a severe pneumonia.

Microbiology

Direct smears of infected lesions from unfixed animals showed many Gram-negative bacteria, mainly rod-shaped, and desquamated cells but no protozoa, fungi or other organisms. Bacteriological and mycological examination of the swabs from the infected rostral lesion of one of the clinically affected frogs were carried out using a variety of media and subsequent culture at 2 temperatures. 2 swabs were taken from the rostrum using 'Calgi-swabs' (ref 65-150-15; A. R. Horwell, 2 Grangeway, Kilburn High Road, London, NW6 2BP), the first for mycological examination and the other for bacteriological examination. The first swab was plated on to 2 Sabouraud dextrose-agar plates, one of these being incubated at 37°C, the other at 22°C, for 7 days with inspection for growth every 48 h. The other swab was inoculated on to 2 blood-agar plates and was then placed in Robertson's cooked meat medium. 1 plate was incubated at 22°C and both other media at 37°C for 18 h. Following this, subcultures of the cooked meat medium were made on to 2 additional blood-agar plates; 1 plate being incubated anaerobically at 37°C, the other aerobically. After incubation any colonies of bacteria were identified according to Cowan & Steel (1966).

Microbiological results

3 species of bacteria were isolated; *Aeromonas liquefaciens*, *Citrobacter freundii*, and an *Acinetobacter* sp. The organisms showed different growth characteristics. The *Aeromonas* sp. and *Acinetobacter* sp. could be isolated from all media used, whereas *Citrobacter freundii* was only recovered from the blood agar subcultures of the cooked meat medium. Interestingly, it was noted that the *Acinetobacter* sp. was recovered in its coccal form. After 7 days no growth was observed on either of the Sabouraud dextrose-agar plates.

Discussion

Bacteriological examination was limited to a single frog, but nevertheless interesting results were obtained. The isolation of the coccal form of *Acinetobacter* sp. was not expected and its role in the infection cannot be judged. However the Gram-MGPLG stains did demonstrate the presence of Gram-negative cocci in the other frogs and it may therefore have been involved.

Although precise quantitative studies were not carried out on the swab it was noted that the *Aeromonas* sp. was recovered in greater quantities than the *Acinetobacter* sp. Further, very few colonies of *Citrobacter freundii* were obtained after passage in cooked meat and hence *Aeromonas* sp. was regarded as the dominant organism.

The condition described, based on the bacteriological results, appears to be a localised lesion associated with *Aeromonas liquefaciens* and, possibly, an *Acinetobacter* sp. It seems probable, though not yet proved, that a predisposing factor in the disease was damage to the prominent rostrum, permitting entry and multiplication of the organisms. Although bacteriology was only performed on 1 animal, the histological demonstration of Gram-negative bacteria in the tissues of the other 4 frogs adds weight to a bacterial aetiology for this condition. The Gram-MGPLG stain proved very useful in this respect and should be considered whenever a Gram-negative infection is suspected. Death was probably due to secondary infection such as pneumonia, or dehydration associated with ulceration.

Aeromonas spp. are recognised pathogens in amphibians and as Reichenbach-Klinke & Elkan (1965) point out, can be responsible for devastating epizootics and a high mortality rate. Under such circumstances the disease is usually termed 'red leg', although haemorrhages are commonly found in tissues other than the limbs. In the cases described in this paper the disease showed a rather different clinico-pathological picture and it is on account of this, coupled with the relative rarity and unusual biology of the host, that the outbreak is reported here.

Acknowledgements

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References

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