Aeromonas hydrophila-associated skin lesions and septicaemia in a Nile crocodile (*Crocodylus niloticus*)

H Turutoglu[a*], S Ercelik[a] and M Corlu[b]

ABSTRACT

Aeromonas hydrophila is one of the most common bacteria associated with the aquatic environment. There are , however, limited data on *A. hydrophila* infection in crocodilians. The aim of this report is to describe a case of skin lesions and septicaemia associated with *A. hydrophila* in a Nile crocodile (*Crocodylus niloticus*). A captive male crocodile in the Zoological Park of Antalya (Turkey) was found dead without showing signs of any disease. Gross examination showed brown or red-spotted skin lesions of varying size. These lesions were mostly scattered over the abdomen and occasionally on the tail and feet. At necropsy, numerous white, multifocal and randomly distributed areas were seen on the liver. Gram-stained smears from skin and liver lesions showed Gram-negative bacilli arranged in clusters. Pure cultures of *A. hydrophila* were recovered from skin, internal organs and blood. Isolates were found to be susceptible to ceftiofur, amoxicillin + clavulanic acid, oxytetracycline, enrofloxacin, danofloxacin, neomycin, gentamicin, and lincomycin + neomycin. A pathogenicity test was performed using this isolate on 4 male 2-year-old New Zealand white rabbits. Local abscesses formed in 2 rabbits injected subcutaneously and the 2 that were injected intraperitoneally died as a result of septicaemia. In conclusion, this report has shown that *A. hydrophila* may cause skin lesions and even death due to septicaemia in crocodiles.

Key words: *Aeromonas hydrophila, Crocodylus niloticus,* Nile crocodile, septicaemia, skin lesions.

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INTRODUCTION

Aquatic animals live in an environment where they often are surrounded by potential pathogens such as *Aeromonas hydrophila*[1], one of the most common bacteria in aquatic environments[6]. Diseases caused by *A. hydrophila* have been observed worldwide in numerous species of freshwater fish, and occasionally in marine fish, amphibians, reptiles, cattle and humans. This bacterium is widely distributed in freshwater and sediments of ponds, and is also found in the intestinal tract. It can be transmitted *via* discharge of the intestinal tract and external skin lesions[1]. It is therefore surprising how little data are available on *A. hydrophila* infection in crocodilians[3,12,13,15]. The aim of this report is to describe a case of skin lesions and septicaemia associated with *A. hydrophila* in a Nile crocodile (*Crocodylus niloticus*).

CASE HISTORY

A captive male Nile crocodile that died in the Zoological Park of Antalya, Turkey, on 6 March 2003 was brought on the same day to Burdur Veterinary Faculty for necropsy. It was 5 years old and had been imported from Nigeria about 1 year ago. This crocodile became immobilised by cold and was found dead without showing signs of disease. Gross examination revealed brown- or red-spotted skin lesions of varying size. These lesions were mostly scattered over the abdomen and occasionally on the tail and feet (Fig. 1a). The scale on the lesions was easily separated, and lesions progressed to erosion of the epidermis (Fig. 1b). At necropsy, numerous white, multifocal, randomly distributed areas were seen on the liver. No other macroscopic lesions were observed in the other organs.

Impression smears were made from the liver and skin, and they were stained by the Gram and Ziehl-Neelsen methods. No acid-fast bacilli were seen on slides after Ziehl-Neelsen staining. Gram-stained smears from the skin and liver showed Gram-negative bacilli arranged in clusters. Samples were collected from the liver, lung, kidney, spleen, heart blood and skin. Each sample, except blood, was separately ground with a sterile mortar and pestle and 1 mℓ of sterile phosphate buffered saline was added. At least 3 drops of each suspension and heart blood were then spread on four 10 % sheep blood agar plates (Oxoid Ltd, Hampshire, UK), spread on 2 MacConkey agar (Oxoid) and on 1 Sabouraud dextrose agar (Oxoid) plates. The blood agar and MacConkey agar plates were incubated at 25 °C and 37°C, respectively, in air for 1–4 days.

The other blood agar plates were incubated at 37 °C for 1–4 days under anaerobic conditions. The Sabouraud dextrose agar plate for fungus and yeast isolation was incubated at 25 °C in air for 7 days. After incubation, bacteria were identified on the basis of cultural, morphological and biochemical characteristics[8,10]. Pure cultures of *A. hydrophila* were recovered from heart blood, liver, lung, kidney, spleen and skin. Growth was observed on MacConkey agar and the colonies were beta-haemolytic on blood agar. Gram-stained smears from cultures showed Gram-negative single and paired rods. Characteristics that differentiated *A. hydrophila* from other species of *Aeromonas* and other Gram-negative bacteria are given in Table 1. No fungi were isolated from the samples.

A pathogenicity test with isolates of *A. hydrophila* from the liver and skin was performed on four 2-year-old male New Zealand white rabbits. The experimental protocol was approved by the Animal Use Committee, and the animals were treated in accordance with national and local animal welfare legislation based on European Council Directives. Two rabbits were injected intraperitoneally and 2 subcutaneously with 0.5 mℓ broth culture ($\pm 10^9$ bacteria/mℓ) of *A. hydrophila* isolated from the crocodile samples. Local abscesses formed in 2 subcutaneously injected rabbits (Fig. 2c,d) and the 2 that

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were injected intraperitoneally died 18–22 hours after inoculation as a result of septicaemia. At necropsy, haemorrhages on the surface of the intestine (Fig. 2a) and necrotic foci on the liver were observed (Fig. 2b). Pure cultures of *A. hydrophila* were isolated from heart blood and the liver, lung, kidney and spleen of these rabbits.

Antibiotic susceptibilities of 2 isolates obtained from the skin and liver were determined by the disk diffusion method on a Mueller Hinton agar (Oxoid) plate[2]. The commercial antibiotic disks used in this test were as follows: ampicillin (Oxoid, 10 μg), amoxicillin + clavulanic acid (Oxoid, 30 μg), ampicillin + sulbactam (Oxoid, 20 μg), ceftiofur (Neo-Sensitabs, 30 μg, Rosco, Denmark), oxytetracycline (Oxoid, 30 μg), trimethoprim + sulphamethoxazole (Oxoid, 25 μg), enrofloxacin (Oxoid, 5 μg), danofloxacin (Mast Diagnostics, 5 μg, Mast Group Ltd, Merseyside, U.K.), lincomycin + neomycin (Oxoid, 75 μg), gentamicin (Oxoid, 10 μg), neomycin (Oxoid, 30 μg) and erythromycin (Oxoid, 15 μg). Isolates were found to be susceptible to ceftiofur, amoxicillin + clavulanic acid, oxytetracycline, enrofloxacin, danofloxacin, neomycin, gentamicin, and lincomycin + neomycin, and to be resistant to ampicillin, ampicillin + sulbactam, erythromycin, and trimethoprim + sulfamethoxazole.

DISCUSSION

Bacterial diseases are common and seen in all aquatic animals[11]. It has been reported that *A. hydrophila* acts as a primary pathogen in fish, but isolates differ greatly in their pathogenicity, with some strains being highly virulent and others non-virulent[1]. Death associated with *A. hydrophila* and *Plesiomonas shigelloides* have been documented in an American alligator (*Alligator mississippiensis*) in a eutrophic inland lake in Florida[15].

Crocodiles are very sensitive to stress[9]. Poor adaptation to their captive environment is considered as a predisposing factor[11]. The condition of the reptile's immune system, like that of any other animal, is important in the development of disease. In the absence of sound management, captive reptiles commonly develop dysfunction of the immune system, which leaves them susceptible to many infectious conditions. Most bacterial infections involve Gram-negative bacteria, many of which are found in reptiles as part of their normal commensal microflora. These agents can remain dormant until the reptile becomes immunosuppressed[9]. This crocodile was in a captive environment for nearly a year and became immobilised by cold when he died.

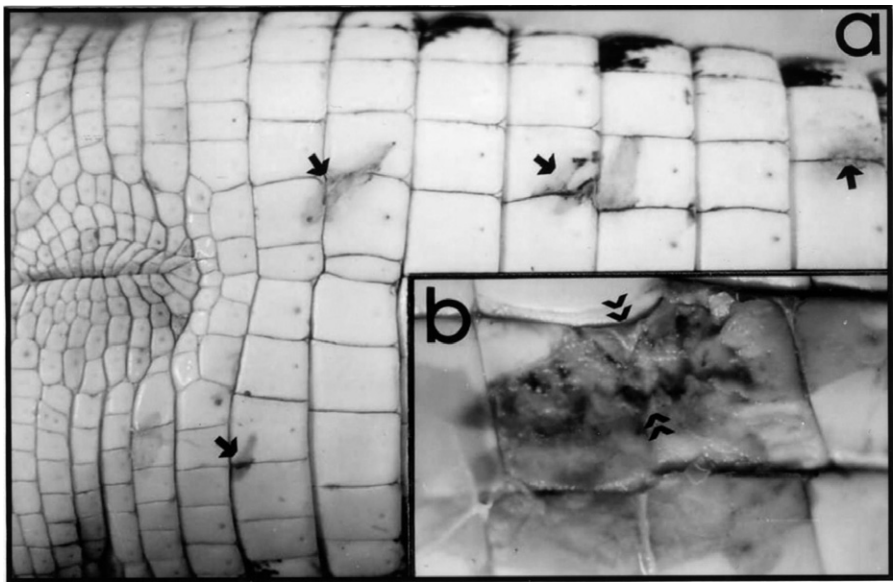

Fig. 1: **Gross appearance of the crocodile with spot lesions on skin;** a: **lesions are mostly restricted to abdominal wall;** b: **epidermal erosion under a separated scale.**

Under cold conditions, the blood supply to the skin is reduced. As the immune system is largely impeded by cold, small skin scratches do not heal and bacterial and fungal infections from a soiled environment persist, causing small erosions between scales which are covered with yellow or brown crusts. These lesions are called 'winter sores' because of their high prevalence in winter[9]. Faecal bacteria and fungi are usually isolated from winter sores[4,5]. In this case, *A. hydrophila* was cultured.

Septicaemic diseases have long been a problem of both free-living and captive reptiles. Signs of the generalised infections are variable and non-specific. In many instances, animals may be found dead without showing signs of disease[11]. According to the zoological park employees this crocodile had also not shown signs of disease before death. Systemic disease may be preceded by traumatic injury, local abscessation, parasitism or environmental stress[11]. It has been stated that bacteria in water (*Aeromonas* sp., *Pseu-*

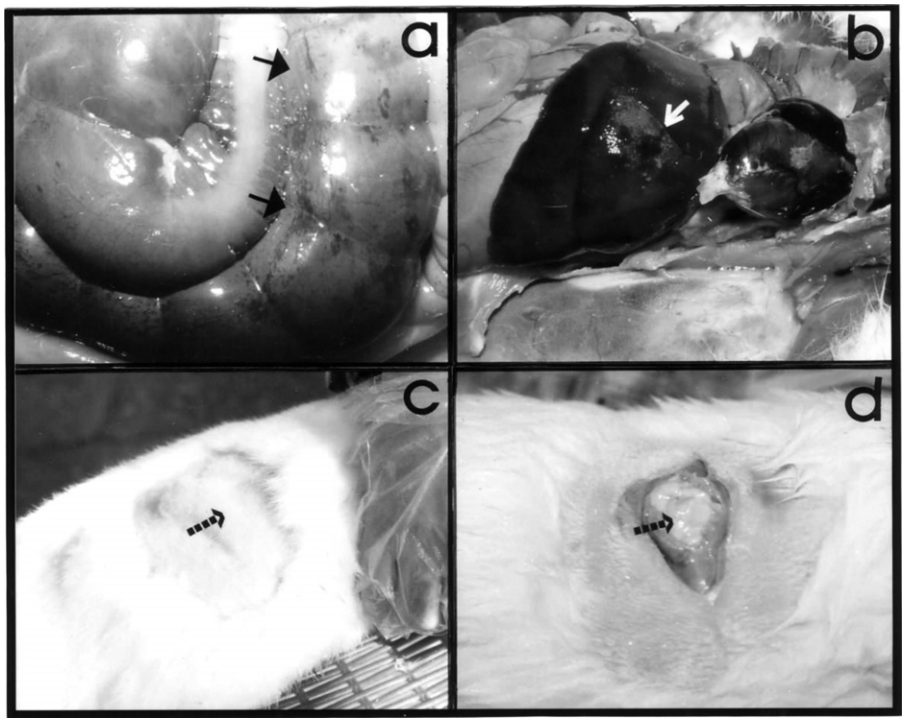

Fig. 2: **Macroscopical appearance of the rabbits inoculated intraperitoneally** (a, b) **and subcutaneously** (c, d) **with *Aeromonas hydrophila.* Note the haemorrhages on serosal surface of colon** (a, black arrows)**, necrosis foci on the surface of the liver** (b, white arrow)**, local abscess formation** (c, broken arrow) **and pus in inoculation area** (d, broken arrow)**.**

domonas sp.) can constitute the major threat to aquatic reptiles[6]. Under severe stress, some bacteria appear to be able to penetrate the mucosal barrier of the intestine and cause septicaemia[9]. It is considered that the death of the crocodile may relate to septicaemia, because the same bacteria were isolated from skin lesions, internal organs and blood of the crocodile. The death of the rabbits inoculated intraperitoneally with *A. hydrophila* indicates that this was a pathogenic strain. However, it is not known whether the septicaemia associated with *A. hydrophila* in the reported crocodile originated from the skin lesions or from the intestine.

Septicaemic infections and skin conditions are of increasing importance in crocodiles. Also, enteric bacteria isolated from crocodiles show a high degree of resistance to antibiotics[7]. Therefore, we performed the antibiotic susceptibility test to determine the susceptibility of *A. hydrophila* isolates to different antibiotics that are used in veterinary medicine. It has been noted that almost all strains of *A. hydrophila* have intrinsic resistance to ampicillin[1]. Beta-lactamase activity was recognised in the isolates. They were also resistant to beta-lactam antibiotics such as ampicillin and ampicillin + sulbactam. Some enteric bacteria may also produce extended-spectrum beta-lactamases (ESBLs) that mediate resistance to cephalosporins, but it can easily differentiated from intrinsic resistance because it is reversed by clavulanate[14]. In the present study, *A. hydrophila* isolates were susceptible to ceftiofur and amoxicillin + clavulanic acid, indicating that the isolates were beta-lactamase producers, and that they could not produce ESBL.

In conclusion, this report has shown that *A. hydrophila* may cause skin lesions and even death due to septicaemia in crocodiles. As bacterial infections generally occur when crocodiles are physiologically stressed during the cold months, they should be protected from cold by housing them inside where there is adequate warmth. As important for the prevention of infections is adequate hygiene, consisting of regular water changes, scrubbing and disinfection of ponds.

Table 1: **Differential characteristics of *Aeromonas hydrophila* isolates.**

Characteristic	Response	Characteristic	Response
Cell morphology	Single and paired rod	Lactose	–
Gram stain	–	Glucose, acid	+
Aerobic growth	+	Glucose, gas	–
Microaerophilic growth	+	Mannitol, acid	+
Anaerobic growth	–	Lysine decarboxylase	–
Beta-haemolysis	+	ONPG	+
Motility	+	Beta-lactamase	+
Indole production	+	Growth at:	
Methyl red	+	45 °C	–
Voges-Proskauer	+	37 °C	+
H_2S	–	28 °C	+
Esculin hydrolysis	+	25 °C	+
Casein hydrolysis	+	Growth on:	
Urea hydrolysis	–	Blood agar	+
Nitrate reduction	+	MacConkey agar	+
Oxidase	+	Tryptone soya agar	+
Catalase	+	Mueller-Hinton agar	+
DNAse	+	6 % NaCl, growth	–

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