Normal intestinal flora of wild Nile crocodiles (*Crocodylus niloticus*) in the Okavango Delta, Botswana

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ABSTRACT

Bacterial and fungal cultures were performed from cloacal swabs collected from 29 wild Nile crocodiles, captured in the Okavango Delta, Botswana. Sixteen species of bacteria and 6 fungal species were cultured. Individual crocodiles yielded 1–4 bacterial species, and 0–2 fungal species. The most commonly isolated bacteria were *Microbacterium, Enterococcus faecalis, Aeromonas hydrophila,* and *Escherichia coli.* No salmonellae were cultured. The most commonly occurring fungus was *Cladosporium.* Several of the bacterial and fungal species isolated have been implicated in cases of septicaemia in crocodilians. Knowledge of the normal intestinal flora will contribute towards the development of a crocodile-specific probiotic for use in farmed crocodiles.

Key words: *Crocodylus niloticus,* intestinal flora, Nile crocodile, Okavango Delta, *Salmonella.*

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INTRODUCTION

Owing to the difficulty in obtaining biological specimens from wild crocodilians, very little is known about their normal intestinal flora. The intestinal tract flora isolated from wild-caught African dwarf crocodiles (*Osteolaemus tetraspis*) has been reported[11].

Salmonellae isolated from wild Nile crocodiles from Lake Kariba, and from wild American alligators (*Alligator mississippiensis*) have also been documented[14,21]. Other studies have dealt with captive crocodilians, including the normal intestinal flora of captive gharials (*Gavialis gangeticus*), and the prevalence of salmonellae in healthy captive crocodilians[15,16,18].

Farmed crocodile hatchlings often fail to develop a normal mixed intestinal flora[10]. In other species, the rapid establishment of bacterial communities of normal flora in the gastrointestinal tract (GIT) is thought to be essential for GIT homeostasis and the prevention of colonization by pathogenic bacteria[4,17]. A deficient intestinal flora is likely to be one of the factors predisposing farmed crocodiles to enteritis. Enteritis, and associated septicaemia, is one of the main causes of mortality in farmed crocodilians[1,6,7]. Determining the normal intestinal tract flora of wild Nile crocodiles is the 1st step towards developing a probiotic for use in farmed Nile crocodiles.

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MATERIALS AND METHODS

Sampling

Crocodiles were captured in the Panhandle of the Okavango Delta during summer (February 2005). Capture was done at night, using a 4.8 m flat bottomed aluminium boat propelled by a 60 hp engine. Crocodiles were located using a powerful spot-light which, when shone into the crocodile's eyes, reflected back a red glow due to the presence of a retinal tapetum lucidum. Once spotted, the beam of light was kept focused on the crocodile's eyes, making it possible to approach the animal with the boat. Crocodiles estimated to be smaller than 1.2 m total length (TL) were captured by hand. Crocodiles between 1.2 m and 2.3 m were captured using a swivelling noose (Animal Handling Co., USA) which was placed over the snout and pulled tight in the neck region. Crocodiles were then brought onto the boat, jaws were taped shut and the animals were physically restrained.

Twenty-nine animals were randomly selected for cloacal swab collection. Each crocodile was blindfolded and restrained in dorsal recumbency. A cloacal swab was taken by inserting a sterile cotton swab (Transwab, Medical Wire & Equipment Co. Ltd., UK) into the cloaca to a depth of 50–100 mm, rotating the swab, withdrawing it and placing it directly into the sterile transport medium supplied.

Isolation and identification procedures

Cloacal swabs were stored at –10 °C in a domestic gas freezer for up to 1 month. On return from the study site, the swabs were submitted to Golden Vet Lab, Johannesburg. An aerobic bacterial culture and a fungal culture were performed as follows. Each cloacal swab was inoculated onto culture plates containing 5 % bovine blood and MacConkey agar no. 1 (Diagnostic Media Products, South Africa) and also onto thiosulphate citrate bile salt sucrose (TCBS) agar, Mycosel agar, cornmeal agar and Rappaport Vassiliadis (RV) broth (the latter 4 from Selectamedia, South Africa). Anaerobic culture could not be attempted, as many anaerobes would not have survived the storage process. All the agar plates were aerobically cultured at 25 °C, but the RV broth was cultured at 37 °C to improve selectivity for *Salmonella* isolation.

The bacterial cultures were incubated for 72 hours before discarding, and the fungal cultures for 28 days before discarding. The RV broths were subcultured twice, after 24 hours and after 6 days of incubation, onto xylose lactose sodium desoxycholate (XLD) agar (Selectamedia). The XLD agars were cultured at 37 °C for 24 hours each time, and examined for the presence of colonies resembling *Salmonella.* Each bacterial and fungal isolate was identified according to standard methods[2,3,12,20,22].

RESULTS

The bacteria and fungi isolated from each wild specimen are presented in Table 1. The bacteria and fungi are given in the order of frequency in which they were isolated from each cloacal swab, with the 1st-named being the most frequent isolate.

Bacteria were cultured from all 29 specimens. There were a total of 79 isolations, and 16 different species. The number of species cultured per specimen varied from 1 to 4, with only 2 (6.9 %) specimens

Table 1: **Bacterial and fungal isolates from each cloacal swab from 29 wild Nile crocodiles that were sampled.**

Animal no.	Bacterial isolate	Fungal isolate
1	*Enterococcus faecalis, Escherichia coli, Microbacterium, Proteus vulgaris*	None
2	*Aeromonas hydrophila, Microbacterium*	None
3	*Cytophaga succinicans, Flavobacterium aquatile, Microbacterium*	None
4	*Enterococcus faecalis, Pseudomonas stutzeri, Microbacterium*	*Penicillium*
5	*Enterococcus faecalis, Microbacterium, Staphylococcus epidermidis, Flavobacterium aquatile*	*Chrysosporium*
6	*Escherichia coli, Enterococcus faecalis, Microbacterium*	*Cladosporium*
7	*Escherichia coli, Enterococcus faecalis, Microbacterium*	None
8	*Escherichia coli, Staphylococcus epidermidis, Microbacterium*	None
9	*Escherichia coli, Enterococcus faecalis, Microbacterium, Enterobacter intermedium*	None
10	*Enterobacter intermedium, Enterococcus faecalis, Microbacterium*	None
11	*Aeromonas hydrophila, Flavobacterium aquatile, Cytophaga heparina*	*Cladosporium*
12	*Enterococcus faecalis, Microbacterium, Cytophaga heparina*	*Cladosporium*
13	*Aeromonas hydrophila*	*Cladosporium*
14	*Escherichia coli, Microbacterium*	*Cladosporium, Penicillium*
15	*Escherichia coli, Enterococcus faecalis, Aeromonas hydrophila*	*Cladosporium*
16	*Microbacterium, Citrobacter koseri, Enterococcus faecalis*	*Cladosporium*
17	*Microbacterium, Enterococcus faecalis, Escherichia coli*	*Trichoderma*
18	*Microbacterium, Aeromonas hydrophila*	*Cladosporium*
19	*Citrobacter koseri, Enterococcus faecalis, Microbacterium, Aeromonas hydrophila*	*Chrysosporium*
20	*Aeromonas hydrophila, Microbacterium*	None
21	*Enterococcus faecalis, Aeromonas caviae*	None
22	*Proteus mirabilis, Microbacterium, Enterococcus faecalis*	*Trichoderma*
23	*Aeromonas hydrophila, Microbacterium, Flavobacterium aquatile*	None
24	*Microbacterium, Citrobacter koseri*	None
25	*Bacillus subtilus, Aeromonas hydrophila, Cytophaga heparina*	*Exophiala, Saprolegnia*
26	*Flavobacterium aquatile, Microbacterium, Escherichia coli*	None
27	*Enterococcus avium*	None
28	*Aeromonas hydrophila, Cytophaga heparina*	None
29	*Cytophaga heparina, Citrobacter koseri*	None

yielding a single species that could be cultured under the incubation conditions described. Eight crocodiles (27.6 %) had 2 isolates and 15 (51.7 %) yielded 3 isolates. Four isolates were obtained from 4 crocodiles (13.8 %). The mean number of isolations per crocodile was 2.7.

Table 2 shows the number of isolates of each bacterium and the percentage of crocodiles carrying each species. The most commonly isolated species was *Microbacterium,* found in 21 of the crocodiles (72.4 %), followed by *Enterococcus faecalis* (14 isolates), *Aeromonas hydrophila* (10 isolates), and *Escherichia coli* (9 isolates). No salmonellae were cultured.

Fungi were isolated from 14 of the 29 crocodiles (48.3 %). There were 16 isolations, of 6 different species. Twelve crocodiles (41.4 %) yielded a single species, while just 2 crocodiles (6.9 %) yielded 2 species.

Table 3 shows the number of isolates of each fungus, and the percentage of crocodiles carrying each fungus. The most commonly isolated species was *Cladosporium,* found in 8 crocodiles (27.6 %).

Table 2: **Number of isolates of each bacterium, and percentage of wild crocodiles carrying each species.**

Bacterium	No. of isolates	Percentage of crocodiles
Aeromonas caviae	2	6.9
Aeromonas hydrophila	10	34.5
Bacillus subtilus	1	3.4
Citrobacter koseri	4	13.8
Cytophaga heparina	4	13.8
Cytophaga succinicans	1	3.4
Enterobacter intermedium	2	6.9
Enterococcus avium	1	3.4
Enterococcus faecalis	14	48.3
Escherichia coli	9	31.0
Flavobacterium aquatile	5	17.2
Microbacterium	21	72.4
Proteus mirabilis	1	3.4
Proteus vulgaris	1	3.4
Pseudomonas stutzeri	1	3.4
Staphylococcus epidermidis	2	6.9

Table 3: **Number of isolates of each fungus, and percentage of crocodiles carrying each fungus.**

Fungus	No. of isolates	Percentage of crocodiles
Chrysosporium	2	6.9
Cladosporium	8	27.6
Exophiala	1	3.4
Penicillium	2	6.9
Saprolegnia	1	3.4
Trichoderma	2	6.9

DISCUSSION

The bacterial species most commonly isolated from the wild Nile crocodiles, *Microbacterium,* is a common soil inhabitant. Neither *Microbacterium* nor *E. faecalis,* the 2nd-most frequently isolated species,

are associated with bacterial septicaemia in crocodiles. Misra *et al.* did not isolate *Microbacterium* or *Enterococcus* from cloacal swabs from 23 gharials, nor was *Microbacterium* isolated from the intestinal contents of 29 African dwarf crocodiles[11,16]. *Enterococcus faecalis* was, however, isolated from 1 of the dwarf crocodiles, and other *Enterococcus* species were isolated from a further 21 of the 29 dwarf crocodiles.

Escherichia coli appears to be a common component in crocodile intestinal tract flora, having also been isolated from 9 of the gharials and 8 of the dwarf crocodiles. Nevertheless, *E. coli* has been recorded as a cause of septicaemia in crocodilians, including the Nile crocodile. One study found 47 of 409 (11.5 %) bacterial infections to be caused by *E. coli*[7].

Aeromonas hydrophila was isolated from 34.5 % of the samples. *Aeromonas hydrophila* is frequently found associated with mortality caused by enteritis and septicaemia. In Zimbabwe it was the 2nd-most frequent isolate, after *Salmonella,* from septicaemic Nile crocodiles[7]. It is also an important cause of septicaemia in *Crocodylus porosus, Crocodylus johnsoni* and *Crocodylus novaeguineae*[1,13]. Besides *E. coli* and *A. hydrophila,* another 5 of the genera isolated (*Bacillus, Citrobacter, Proteus, Pseudomonas* and *Staphylococcus*) are known causes of septicaemia in crocodiles[10]. This supports the view that many bacterial septicaemias are caused by normal intestinal tract inhabitants which act as opportunistic pathogens in an immunosupressed host.

No *Salmonella* were cultured from the wild Nile crocodiles. This is interesting in the light of previous findings. Of 67 wild Nile crocodiles in Lake Kariba, 18 (26.9 %) yielded *Salmonella*[14]. Three of 29 (10.3 %) wild caught African dwarf crocodiles yielded *Salmonella,* while *Salmonella* was found in 2 of 71 (2.8 %) wild *A. mississippiensis*[11,21].

In farmed crocodilians, *Salmonella* has frequently been isolated: Obwolo and Zwart found *Salmonella* in 8 of 50 healthy, 3-year-old, farmed Nile crocodiles, and concluded that *Salmonella* may represent normal flora in the intestinal tract of crocodiles[18]. By contrast, no *Salmonella* were found in cloacal swabs from 23 captive gharials[16]. Healthy farmed *C. porosus* and *C. johnstoni* were found to carry *Salmonella*[15]. On 1 farm 20.0 % of *C. porosus* and 27.8 % of *C. johnstoni* were carriers, while on another farm the prevalence rate was 81 % and 5 % for the 2 species, respectively. In farmed alligators, *Salmonella* was isolated from 4 of 29 specimens (14 %)[21].

In contrast to their frequent occurrence as resident flora, *Salmonella* was cultured from 202 of 409 farmed Nile crocodiles found to have died from bacterial infections in Zimbabwe[7]. In South Africa, 145 *Salmonella* isolates, and a wide range of serovars, were reported from farmed Nile crocodiles submitted for necropsy over a 10-year period[24].

It is clear that while *Salmonella* can be normal intestinal tract flora in healthy farmed crocodilians, they can also be important pathogens. The role of *Salmonella* as normal intestinal tract flora in wild crocodilians is unclear. Several factors may account for the apparent absence of *Salmonella* in this study. The composition of intestinal flora is dependent on ingested food, both the type of food and the amount. Shedding of *Salmonella* is not necessarily constant. It has been shown that *Salmonella* could suddenly be excreted from turtles after a period of 6 months with no excretion[5]. Furthermore, cloacal swabbing may underestimate the prevalence of *Salmonella* compared with faecal swabbing[15]. Logically there will be less efficient horizontal transfer of *Salmonella* in a natural environment than under intensive conditions. Nevertheless, vertical transmission could occur with equal ease in either environment. Recent findings from *C. porosus* eggs tend to support the possibility of vertical transmission of *Salmonella. Salmonella* was cultured in eggs from 12 of 13 clutches on 1 farm. Interestingly, the serotypes isolated were clutch specific[19].

The fungi isolated from the wild Nile crocodiles are considered environmental. Nevertheless, *Cladosporium, Penicillium* and *Trichoderma* have been found in diseased crocodilians[1,8,9,23]. These 3 fungi have also been isolated from the shells of *C. porosus* eggs, but were not present in the egg yolk[19].

Fungi were isolated from the intestinal tract of 24 of 29 African Dwarf crocodiles, a far higher occurrence than the present study[11].

From the limited studies to date, it appears that crocodilian intestinal flora is dynamic and varies according to both crocodilian species and environmental conditions. More studies will be required to improve our understanding of crocodilian intestinal flora, leading to the development of a crocodile-specific probiotic.

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