Aerobic intestinal flora of wild-caught African dwarf crocodiles *Osteolaemus tetraspis*

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ABSTRACT

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Intestinal contents were collected from wild-caught African dwarf crocodiles (*Osteolaemus tetraspis*) in 1993 and 1995 which were slaughtered at urban markets in the Congo Republic. The samples were kept frozen and brought back to Onderstepoort for aerobic culture. Out of 29 specimens, 33 species of bacteria and 20 species of fungi were isolated. The bacteria included three isolates of *Salmonella* and eight isolates of *Escherichia coli*, most of the latter being rough strains. The flora of individual specimens contained 1-5 bacterial and 0-5 fungal species. Neither *Aeromonas hydrophila* nor *Edwardsiella tarda* were isolated from any of the samples.

Keywords: African dwarf crocodile, *Escherichia coli*, intestinal flora, *Salmonella*

INTRODUCTION

Enteritis, often associated with septicaemia, is a major problem in young farmed crocodiles and salmonellae are frequently involved (Foggin 1987; 1992a; 1992b; Huchzermeyer 1991; Manolis, Webb, Pinch, Melville & Hollis 1991; Obwolo & Zwart 1993; Van der Walt, Huchzermeyer & Steyn 1997). There is increasing evidence in favour of the protective action of a normal intestinal flora, partially reviewed by Huchzermeyer (1994). Misra, Kumar, Patnaik, Raman & Sinha (1993) reported briefly on the normal gut flora of captive gharials (*Gavialis gangeticus*). Further knowledge of the normal intestinal flora of wild crocodiles could lead to the development of suitable probiotics for use on crocodile farms and would also be useful when assessing the significance of isolates made from diseased animals.

Because of the protected status of crocodiles (Ross 1998) as well of the remoteness of most wild crocodile populations, it is extremely difficult to obtain any biological specimens from them. However, live wild-caught African dwarf crocodiles (*Osteolaemus tetraspis*) are regularly brought to urban markets in the Congo Republic to be slaughtered for their meat (Behra 1990). Within the framework of a wider project concerning pathology, parasitology and biology of these reptiles, intestinal content samples were collected at the markets with the objective of gaining information on the aerobic intestinal flora of this species.

MATERIALS AND METHODS

Samples

The internal organs of freshly slaughtered African dwarf crocodiles were purchased at markets in Brazzaville during May 1993 and in Brazzaville and

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Impfondo, Congo Republic during April 1995 (Huchzermeyer & Agnagna 1994). Intestinal contents were collected from these organs, placed into sterile plastic tubes and kept frozen in freezing compartments of domestic electric or gas refrigerators (at the time there was no public electricity supply in Impfondo) and in an insulated box with ice packs during transport, until they reached the laboratory on the return of the senior author to South Africa.

Isolation and identification procedures

Each sample of faeces or intestinal contents was inoculated onto plates containing blood tryptose agar medium (Oxoid, Basingstoke, United Kingdom) incorporating bovine blood, MacConkey agar no. 1 (Biolab, Box 1998, Halfway House, South Africa), thiosulphate citrate bile salt sucrose (TCBS) agar (Biolab) and potato dextrose agar (Biolab) with and without chloramphenicol (Centaur, Johannesburg, South Africa) and cyclohexamide (BDH, Poole, United Kingdom), as well as into selenite broth (Sigma, St Louis, USA).

All the agar plates were incubated aerobically at 25 °C and the selenite broth at 42 °C to improve selectivity for *Salmonella* spp. The plates containing blood tryptose agar, MacConkey agar and TCBS agar were incubated for 72 h before discarding, and those containing the two types of potato agar for 28 days before discarding. The selenite broth cultures were subcultured onto xylose lactose sodium desoxycholate (XLD) agar (Oxoid) after 24 h of culture. The XLD agars were cultured for 24 h at 37 °C, and examined for colonies resembling *Salmonella*. Each bacterial and fungal isolate was identified according to standard methods (Cowan 1974; Campbell & Stewart 1980; Krieg & Holt 1984; Sneath, Mair, Sharpe & Holt 1986; Rippon 1988).

RESULTS

The aerobic bacteria and fungi isolated from 21 samples in 1993 and from eight samples in 1995 are presented in Tables 1 and 2, respectively. Bacteria were isolated from all 29 samples, the number of isolates per sample ranging from 1–5 and only one crocodile (specimen 10/93) had a single-species isolate of *Enterococcus faecium*. Of the 101 bacterial isolates, 59 were Gram positive and 42 Gram negative, the two most common being *Enterococcus* species with 22 isolations and *Bacillus* spp. with 18 isolations. Most isolates of *Escherichia coli* were rough, and only one was typable: 051:K23. The four isolates of *Klebsiella oxytoca* were each of a different capsule type: 42, 44, 58 and 60. Details of the three *Salmonella* isolates have been published in a previous paper (Van der Walt *et al.* 1997). They were *S. wangata, S. yoruba* and an unnamed group IIIb *Salmonella* 30:k:enx.

TABLE 1 Aerobic bacteria isolated from intestinal contents of African dwarf crocodiles

Genus	Species	*n* 1993	*n* 1995
Alcaligenes	*faecalis*		2
Bacillus	*alvei*		1
	cereus	11	4
	circulans	1	
	coagulans	1	
Citrobacter	*amalonaticus*	1	
	freundii	3	
Dermacoccus	*nishinomyaensis*		1
Enterobacter	*agglomerans*	1	1
	cloacae	7	
	gergoviae		1
Enterococcus	*caecorum*		1
	durans		1
	faaecalis		1
	faecium	10	1
	pseudoavium		7
	solitarius		1
Escherichia	*coli*	8	
Flavobacterium	*balustinum*	1	
	odoratum		1
Klebsiella	*oxytoca*	2	2
Kocuria	*varians*		1
Kurthia	*gibsonii*		3
Lactobacillus	sp.	1	
Micrococcus	*luteus*	4	
Proteus	*mirabilis*	6	2
Salmonella	susp.	3	
Serratia	*odorifera*	1	
Staphylococcus	*chromogenes*		4
	epidermidis		1
	xylosus		2
Streptococcus	*salivarius*		1
Streptomyces	sp.	1	

n = number of isolates

TABLE 2 Fungal isolates from intestinal contents of African dwarf crocodiles

Genus	Species	*n* 1993	*n* 1995
Acremonium	sp.	1	
Arthrinium	sp.	1	
Aspergillus	*clavatus*	5	
	flavus	2	
	niger	2	
Beauveria	sp.	3	
Candida	*guillermondii*	2	2
	krusei	1	
Chrysosporium	sp.	3	
Cryptococcus	*lipolytica*	3	3
	luteolus	1	
Curvularia	sp.	1	
Fusarium	sp.		1
Geotrichum	*candidum*		4
Paecilomyces	sp.	2	
Penicillium	sp.	7	2
Phoma	sp.	2	
Trichoderma	sp.		1
Trichosporon	*beigelii*	2	
	capitatum	1	

n = number of isolates

Enterobacteriaceae usually occurred as a heavy growth on culture media, whilst Gram negative non-fermenters, such as *Alcaligenes* spp. and *Flavobacterium* spp. were only present in small numbers. *Enterococcus* spp., *Micrococcus* spp. and *Kurthia* spp. were present in heavy growth in only about half the cases; the others occurring in small numbers. *Bacillus* spp., *Lactobacillus* spp. and *Streptomyces* spp. were only present in small numbers in each case where they were isolated.

Fungi were isolated from 24 of the 29 samples and the number of isolates per sample ranged from 0–5, with 12 samples yielding a single species each. Specimen 10/93 also had one fungal species, *Curvularia* sp., only. Most fungi were only present in low numbers and *Aspergillus* spp. and *Penicillium* spp. were the most frequent isolates.

DISCUSSION

It is possible that the results were affected by the long enforced preslaughter fast to which the animals had been subjected, with an estimated average time lapse between capture and slaughter of 30 days (Huchzermeyer & Agnagna 1994). After such a long fast the intestinal contents were of minimal quantity.

In addition, some market slaughterers routinely discarded the very short rectum (rectocolon) during evisceration because of the evil smelling contents of mixed urine and faeces, while the small intestines are sold as edible. Consequently, some of the intestinal content samples had to be taken from the small intestines and two crocodiles yielded no intestinal content sample whatsoever. It is to be expected that the small intestine would have a less diverse flora than the rectum. Preslaughter cloacal swabbing would have been ideal, but was impracticable as the market slaughterers were suspicious of our intentions and tended to be uncooperative or to demand extra remuneration for any slight concession given.

The bacteria and fungi isolated are similar to the normal intestinal flora of other animal species. Most were present in low numbers, probably as a result of a lack of ingesta. The only genera that were usually present in heavy growth were members of the family Enterobacteriaceae, but Enterobacteriaceae were only isolated from 15 of the 29 cases.

Surprisingly few isolates of *E. coli* (8) and of salmonellae (3) were obtained. Misra *et al.* (1993) did not find any salmonellae in cloacal swabs from 23 gharials, but had nine isolates of *E. coli*, while Obwolo & Zwart (1993) obtained eight *Salmonella* isolates from cloacal swabs of 50 healthy farmed Nile crocodiles in Zimbabwe. In contrast, 49,4 % of all bacterial isolates from crocodiles submitted for post mortem examination in Zimbabwe over a four-year period were *Salmonella* spp. (Foggin 1992b). On two farms in Australia 20 % and 81 % of slaughtered *Crocodylus porosus* and 27,8 % and 55 % of *C. johnsoni* were found to harbour salmonellae (Manolis *et al.* 1991). Eleven *Salmonella* isolates were reported by Hibberd, Pierce, Hill & Kelly (1996) from 62 diseased farmed juvenile *C. porosus*. The large number and range of *Salmonella* isolates from farmed crocodiles submitted for necropsy examination in South Africa have been reported by Van der Walt *et al.* (1997), but our wild-caught animals only yielded three isolates: *S. wangata* in heavy growth and *S. yoruba* and *Salmonella* IIIb in small numbers.

Edwardsiella tarda which is frequently found associated with mortality caused by enteritis and septicaemia in farmed Nile crocodile hatchlings (Foggin 1992a; Huchzermeyer & Henton unpublished data), as well as in farmed American alligators (Gorden, Hazen, Esch & Fliermans 1979), was not isolated from any of our specimens. However, Misra *et al.* (1993) reported its isolation from three out of 23 gharials. Similarly, *Aeromonas hydrophila* which has been found associated with bacterial disease in farmed and wild American alligators (Shotts, Gaines, Martin & Prestwood 1972; Gorden *et al.* 1979; Peters & Cardeilhac 1988) as well as in farmed Nile crocodiles (Foggin 1987), was not present in any of our samples.

Hibberd *et al.* (1996) reported *Fusarium* spp. isolations from 24 of 62 diseased farmed juvenile *C. porosus*, as well as *Penicillium* spp. from nine and *Aspergillus* spp. from eight cases. In this study, *Aspergillus* spp. and *Penicillium* spp. were commonly found, albeit in low numbers, but *Fusarium* sp. was only isolated from one sample. The yeasts, *Candida* spp., *Cryptococcus* spp., *Trichosporon* spp. and *Geotrichum* spp. made up 19 of the 52 fungal isolates. They are all considered to be part of the normal flora of mucous membranes.

This paper presents the results of the most comprehensive study of the intestinal flora of any crocodilian species published so far and it is hoped that this is only the beginning. More studies of the gut flora of wild and farmed individuals of the major crocodilian species are required for a better understanding of the function of this flora and the eventual elaboration of crocodile-specific probiotics.

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