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. Salmonella isolated from wild Nile crocodiles from Lake Kariba, and from wild American alligators (Alligator mississippiensis) have also been documented. Other studies have dealt with captive crocodilians, including the normal intestinal flora of captive gharials (Gavialis gangeticus), and the prevalence of salmonella in healthy captive crocodilians. Farmed crocodile hatchlings often fail to develop a normal mixed intestinal flora. 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. 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 morbidity and mortality in farmed crocodilians. Determining the normal intestinal tract flora of wild Nile crocodiles is the 1st step towards developing a probiotic for use in farmed Nile crocodiles.

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 spotlight 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 around 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 thiostrepton 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 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.

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.
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 each species. The most commonly isolated species was Microbacterium, found in 15 of the crocodiles (51.7 %), followed by Enterococcus faecalis (14 isolates), Aeromonas hydrophila (10 isolates), and Escherichia coli (9 isolates). No salmonellae were cultured.

Fungi were isolated from 15 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 %).

**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. 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. 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. It is also an important cause of septicaemia in Crocodylus porosus, Crocodylus johnstoni and Crocodylus novaeguineae. 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. This supports the view that many bacterial septicaemics are caused by normal intestinal flora in healthy farmed crocodilians, which 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. Furthermore, cloacal swabbing may underestimate the prevalence of Salmonella compared with faecal swabbing. 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 cluch specific.

Fungi isolated from the wild Nile crocodiles were considered environmental. Nevertheless, Cladosporium, Penicillium, and Trichoderma have been found in diseased crocodilians. These fungi have also been isolated from the shells of C. porosus eggs, but were not present in the egg yolk. Fungi were isolated from the intestinal tract of 24 of 29 African Dwarf crocodiles, a far higher occurrence than the present study.

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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