Providencia rettgeri septicaemia in farmed crocodiles

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Bacterial septicaemia is a major cause of morbidity and mortality in farmed saltwater crocodiles (Crocodylus porosus) in the Northern Territory. Providencia rettgeri is the most common aetiological agent. Efficacy of antibiotic treatment is dubious and there are high levels of resistance to antibiotics commonly used by farms, underlining the need for exploration of new approaches to managing the disease.

Saltwater crocodile farming is a growing industry in Australia, with an annual gross value of over $50 million, the main product being high quality skins for the luxury leather market. In the Northern Territory, there are several farms, the largest having approximately 40,000 crocodiles. Berrimah Veterinary Laboratories (BVL) is situated with in 30 km of the four largest farms, facilitating a close collaborative relationship. Each year, BVL receives from 50 farms, the largest having approximately 40,000 crocodiles, for exploration of new approaches to managing the disease.
diagnostic submissions, the vast majority concerning hatchling to juvenile crocodiles (2–8 months of age). In this age group, a very common cause of death is bacterial septicaemia, with *Providencia rettgeri* predominating (Figure 1).

Grossly, affected crocodiles may exhibit regional subcutaneous and serosal vascular congestion and oedema (Figure 2a, b). Despite young crocodiles being offered food every 2–3 days, septicaemic crocodiles invariably have no stomach content at necropsy, indicating that affected crocodiles are inappetent. Histological findings include fibrinous pyogranulomatous cellulitis and polyserositis, intravascular coagula of fibrin, macrophages and degranulating heterophils, and acute multifocal splenic necrosis and heterophil infiltration (Figure 2c).

Definitive diagnosis in cases of suspected septicaemia is achieved by bacterial culture of at least two, aseptically sampled, blood filtering organs (lung, liver, spleen or lung). Tissues are homogenised aseptically in physiological saline and a swab soaked in homogenised samples is inoculated on tryptic soy agar with sheep’s blood and MacConkey agar for aerobic culture. The plates are incubated at 35°C and are examined for bacterial growth after overnight and 48 hours incubation. The predominant colony type is then selected for biochemical testing. In cases of *P. rettgeri* septicaemia, the bacterium is typically present as a moderate to heavy growth in pure culture.

*Providencia rettgeri* appears non-haemolytic on sheep’s blood agar and is an aerobic, Gram-negative bacillus, oxidase negative and catalase positive (Figure 3). A commercial kit, Microbact 24E (Oxoid Ltd), is routinely used for biochemical identification and the test results after overnight incubation at 35°C are as follows: negative reactions for lysine decarboxylase, ornithine decarboxylase, H2S production, ONPG, acetoin production, gelatin liquefaction, malonate inhibition, arginine dihydrolase and fermentation of xylose, sorbitol, sucrose, lactose, arabinose and raffinose; positive reactions

Figure 1. Predominant isolates in farmed crocodiles with bacterial septicaemia. Numbers are percentages of a total of 220 isolates from 159 cases that occurred during 2010–2015. *P. rettgeri* was the cause of septicaemia in significantly more cases than other bacterial species (*P* < 0.0001, Chi-squared = 427, df = 5).

Figure 2. Pathology of *P. rettgeri* septicaemia in a hatchling. (a) Crocodile in dorsal recumbency with ventral skin removed to show subcutaneous erythema and oedema (arrow), Bar = 1 cm. (b) Thoracic cavity of same crocodile opened revealing severe serosal oedema with accumulation of watery, slightly cloudy fluid (arrow) and injection of serosal vessels overlying heart (arrowhead). (c) Histological image of spleen showing deposition of pink material (fibrin), infiltration with degranulating heterophils, and macrophages containing phagocytised bacterial rods (arrowheads). Haematoxylin and eosin stain; bar = 10 μm.
for indole, urease, citrate utilisation, TDA and fermentation of glucose, mannitol, adonitol and inositol; mostly positive but occasional negative reactions for fermentation of rhamnose and salicin (octal codes: 06331212, 06331012, 06331210 or 06331010).

The in vitro antimicrobial susceptibility testing on *P. rettgeri* is performed by disc diffusion method using the Clinical and Laboratory Standards Institute guidelines. The three antibiotic treatments, namely sulphafurazole, tetracycline and sulphamethoxazole with trimethoprim, are routinely tested at BVL on crocodilian bacterial isolates as requested by the local crocodile farmers, since these are the antibiotics added to food for treatment. A total of 139 *P. rettgeri* isolates were tested between 2010 and 2015, of which 44% were sensitive to all three antibiotic treatments mentioned above, 21% were resistant to all three antibiotic treatments, 21% were resistant only to tetracycline and 14% were resistant only to sulphonamides. Antimicrobial resistance in crocodilian bacterial isolates may be due to development either of resistance in response to antibiotics used at the farm, or selective pressure for innately resistant bacteria in the environment. The high level of antibiotic resistance, and the fact that antibiotics are being used in food to treat septicaemic crocodiles that are likely not eating, are clear indications of the need for an alternative approach to the use of antibiotics to overcome this problem.

Bacteria belonging to the genus *Providencia*, family *Enterobacteriaceae*, are opportunistic pathogens that have been isolated from a range of environments and hosts including humans. *P. rettgeri*
has been associated with a variety of infections in humans including, travellers’ diarrhoea, urinary tract infection, especially in certain types of immunocompromised patients, hospital-acquired and community-acquired neuroinfection, ocular infections and pertussis. Although *P. rettgeri* is a relatively uncommon isolate in septicaemic reptiles, it has been found to be a frequent isolate from some reptilian environments, either as a part of their normal flora or in opportunistic infections. The bacterium has also been noted to cause granulomatous pneumonia and hepatitis in a crocodile monitor lizard, respiratory tract infection in a ball python, septicaemia and meningitis in American alligators, and meningitis in hatchling saltwater crocodiles.

The occurrence of bacterial septicaemia in farmed crocodiles in the Northern Territory seems to be influenced by age and climate. The infection is rare in crocodiles older than one year of age, suggesting that adaptive immunity likely plays a role in resistance to infection. In young crocodiles, after hatching in the late wet season, the majority of infections do not occur until the onset of the dry season, in which there are relatively low atmospheric and/or water temperatures (average daily maximum temperature is 33°C during both seasons, but minimum temperature averages 18°C during the dry season compared to 24°C during the wet season) (Figure 4). The bactericidal function of crocodilian complement, an important component of the innate immune system, significantly decreases at temperatures below 15°C and above 30°C. The experimental bacterial killing assay on crocodile plasma revealed that juvenile crocodiles have a more established innate ability to neutralise *Escherichia coli* compared with *P. rettgeri*. Avenues for further investigation include characterisation of *P. rettgeri* virulence factors, determination of environmental factors on crocodile farms that may be promoting its presence and ability to cause infection, and efficacy of alternative methods to antibiotics to decrease the environmental and intestinal load and impact of *P. rettgeri* in crocodiles, such as alternative pen cleaning and water treatment methods and/or use of probiotics or vaccination.

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**References**


**Biographies**

**Suresh Benedict** completed his doctoral degree on *Leptospira* and Leptospirosis from the University of Madras, India. He has worked as bacteriologist at BVL since 2001 and his interest comprises working on various tropical veterinary bacterial isolates including *Burkholderia pseudomallei*.

**Catherine M Shilton** received her veterinary training at the University of Guelph, in Canada, including a graduate degree in zoo and wildlife medicine and pathology. She has worked as a veterinary pathologist at BVL since 2002, enjoying the challenge of the variety of species submitted and pathologies present.