BACTERIAL AND FUNGAL CONTAMINATION OF CROCODILE EGGS

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During the past few years, all crocodile eggs that have been submitted to the Oonoonba Veterinary Laboratory have been examined for bacterial and fungal contamination of both the internal contents as well as the external shell. The number of eggs submitted has not been large but a similar pattern of contamination has been observed from farm to farm.

The following is a short note to indicate the types of bacteria and fungi present in crocodile eggs sent in from four different farms in northern Queensland.

All the eggs examined in this survey were a mixture of those that were clearly infertile (no banding) and those that showed, by candling, that a band had originated but had not progressed.

Eggs were handled as little as possible and, in the laboratory, gloves were worn to limit any contamination whilst processing. Processing was performed in a biological safety cabinet. Each egg was placed in a sterile bag with 10 ml of buffered peptone water and massaged gently to wash off organisms that were not irreversibly attached to the egg shell. This liquid was then used to inoculate an appropriate set of bacterial and fungal media. The washed egg was removed from the bag and aseptically cleaned by dipping and washing in amyl alcohol before being allowed to dry. Sterile scissors and forceps were used to crack and cut open the egg so that the contents could be collected and tested for internal microbial contamination.

Samples were examined for the presence of *Salmonella* spp. and other enteric organisms, for soil and water bacteria such as *Pseudomonas* spp., *Aeromonas* spp., etc., and for the presence of fungi. Blood agar, MacConkey agar, LMG (lysine-mannitol-glycerol) agar, bismuth sulphite agar, Rappaport-Vassiliadis broth, Sabourad agar and Mycological agar were used. The bacterial plates were read after 24-48 hours at 37°C and any suspect colonies replated for further study and identification. The fungal plates were incubated at 28°C for 7-14 days. Cultures were examined and identified by morphological characteristics. Where nesting material was available, these too were sampled and examined.

The following tables give an indication of the microorganisms found in and around crocodile eggs. Table 1 shows the results for bacteria isolated from outside and inside the submitted eggs from four different farms while Table 2 shows the results of bacterial isolations from nesting material from three of the four farms. Tables 3 and 4 show results for fungi isolated from eggs and nesting material respectively.

Table 1 **Bacteria isolated from the inside and outside of crocodile eggs collected from four farms in northern Queensland. These eggs were either infertile or were cases of non-progression of banding**

Bacterium	Farm 1		Farm 2		Farm 3		Farm 4		All Farms	
	Inside (21)*	Outside (13)	Inside (7)	Outside (4)	Inside (5)	Outside (5)	Inside (51)	Outside (32)	Inside (84)	Outside (54)
Achromobacter spp.	1	0	0	0	0	0	0	0	1	0
Acinetobacter spp.	1	1	0	0	0	0	1	2	2	3
Aeromonas hydrophila	0	0	0	0	0	0	4	6	4	6
Aeromonas caviae/sobriae	0	0	1	0	0	0	4	1	5	1
Alcaligenes spp.	0	3	1	1	1	1	1	5	3	10
Bacillus spp.	11	10	1	4	0	0	2	24	14	38
Citrobacter spp.	3	2	2	0	0	1	6	5	11	8
Enterobacter aerogenes/agglomerans	4	0	0	0	0	1	0	4	4	5
Enterobacter cloacae	0	0	0	1	4	4	11	13	15	18
Escherichia coli	0	0	0	1	0	0	0	0	0	1
Klebsiella oxytoca/ pneumoniae	0	0	0	0	0	0	0	4	0	4
Proteus spp.	4	0	0	0	0	0	0	0	4	0
Pseudomonas aeruginosa	0	0	1	1	0	1	7	4	8	6
Pseudomonas fluorescens	1	0	0	1	0	1	3	9	4	11
Pseudomonas putida	2	0	0	0	0	0	3	4	5	4
Pseudomonas stutzeri	1	3	0	2	3	4	9	6	13	15
Salmonella spp.	1	1	0	0	0	0	4	4	5	5
Serratia liquifaciens/marcescens	0	2	0	0	0	0	0	0	0	2

* These are the total numbers of samples tested

Table 2 **Bacteria isolated from nesting material received from three farms**

Bacterium	Nesting Material Farm 1 (6)	Nesting Material Farm 2 (2)	Nesting Material Farm 4 (1)	Total (9)
Acinetobacter spp.	1	0	0	1
Aeromonas spp.	2	0	0	2
Bacillus spp.	6	0	0	6
Citrobacter spp.	1	0	0	1
Enterobacter spp.	4	0	0	4
Klebsiella spp.	1	0	0	1
Pseudomonas aeruginosa	4	2	1	7
Pseudomonas fluorescens	3	0	1	4
Pseudomonas putida	2	0	0	2
Pseudomonas stutzeri	4	0	1	5
Pseudomonas spp. (others)	0	2	0	2
Salmonella spp.	0	0	1	1

Table 3 **Fungi isolated from the inside and outside of crocodile eggs collected from four farms in northern Queensland**

Fungus	Farm 1		Farm 2		Farm 3		Farm 4		All Farms	
	Inside (13)	Outside (13)	Inside (4)	Outside (4)	Inside (5)	Outside (5)	Inside (41)	Outside (32)	Inside (63)	Outside (54)
Acremonium spp.	0	3	0	0	0	0	0	0	0	3
Alternaria spp.	0	0	0	0	0	0	0	1	0	1
Aspergillus flavus	0	4	0	0	0	0	0	26	0	30
Aspergillus fumigatus	0	4	0	0	0	0	1	9	1	13
Aspergillus glaucus	0	0	0	0	0	0	0	9	0	9
Aspergillus niger	0	1	0	0	0	2	0	25	0	28
Aspergillus terreus	0	0	0	0	0	0	0	4	0	4
Aspergillus spp.(others)	0	0	0	4	0	0	0	3	0	7
Cladosporium spp.	0	1	0	0	0	0	0	3	0	4
Cunninghamella spp.	0	0	0	0	0	0	0	0	0	0
Curvularia spp.	0	0	0	1	0	0	0	3	0	4
Fusarium spp.	9	12	0	1	4	4	31	6	44	23
Mucor spp.	0	0	0	0	0	0	0	0	0	0
Nigrospora spp.	0	0	0	0	0	0	0	2	0	2
Paecilomyces lilacinus	1	1	0	0	0	0	0	0	1	1
Paecilomyces spp.	0	0	0	0	0	0	1	4	1	4
Penicillium spp.	0	4	0	2	0	3	0	20	0	29
Rhizopus spp.	0	0	0	0	0	0	0	14	0	14
Scedosporium spp.	0	0	0	0	0	0	1	1	1	1
Scopulariopsis spp.	0	0	0	0	0	0	1	5	1	5
Syncephalastrum spp.	0	0	0	1	0	1	0	3	0	5

Table 4 **Fungi isolated from nesting material collected from three farms**

Fungus	Nesting Material Farm 1 (6)	Nesting Material Farm 2 (2)	Nesting Material Farm 4(1)	Total (9)
Aspergillus flavus	0	0	1	1
Aspergillus fumigatus	1	0	0	1
Aspergillus niger	2	1	1	4
Aspergillus terreus	0	0	0	0
Aspergillus spp. (others)	1	0	0	1
Cladosporium spp.	2	0	0	2
Cunninghamella spp.	0	2	0	2
Fusarium spp.	6	0	1	7
Malbranchea spp.	1	0	0	1
Paecilomyces lilacinus	6	0	0	6
Paecilomyces spp.	0	0	1	1
Penicillium spp.	5	2	1	8
Rhizopus spp.	1	1	1	3
Scopulariopsis spp.	1	0	0	1
Stemphyliopsis spp.	1	0	0	1
Trichoderma spp.	6	0	0	6
Veronaea botryosa	1	0	0	1
Verticillium spp.	1	0	0	1

The results are not unexpected from eggs laid into a nest made of local herbage, soil and water especially if the eggs were not washed before transfer to the incubators. The presence of faecal contamination is also expected.

The most common bacterial isolates from the outsides of the eggs were environmental organisms such as *Bacillus* spp., *Alcaligenes* spp. and *Pseudomonas* spp., especially *P. aeruginosa, P. fluorescens* and *P. stutzeri.* The major faecal contaminants included *Enterobacter cloacae, Citrobacter* spp. and *Salmonella* spp.

From the egg contents, a similar picture appeared with *Bacillus* spp., *Citrobacter* spp., *Enterobacter* spp., *Pseudomonas* spp. and *Salmonella* spp. being consistently isolated. With the isolations from the egg contents, it is possible that there may have been contamination from the external shell even with the precautions taken, however, a number of the 'dead' eggs (initial banding seen) were of a custard-like consistency inside.

The nesting material showed presence of the same bacteria.

Where fungi were found on and in the eggs and in the nesting material, a number of ubiquitous fungi were isolated. It appeared, however, that only one of the genera had the ability to easily penetrate the shell and that was *Fusarium* spp., which has recently been incriminated in embryonic deaths of crocodiles in one farm in central Queensland. Other fungi commonly isolated from the outside of eggs and from the nesting material included *Aspergillus* spp., especially *A. flavus* and *A. niger, Penicillium* spp. and *Rhizopus* spp.

As more eggs are submitted for culture, it will be interesting to keep building up the picture of the normal flora as well as determining causes for embryonic death.