

Chloramphenicol cures chytridiomycosis

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Introduction:

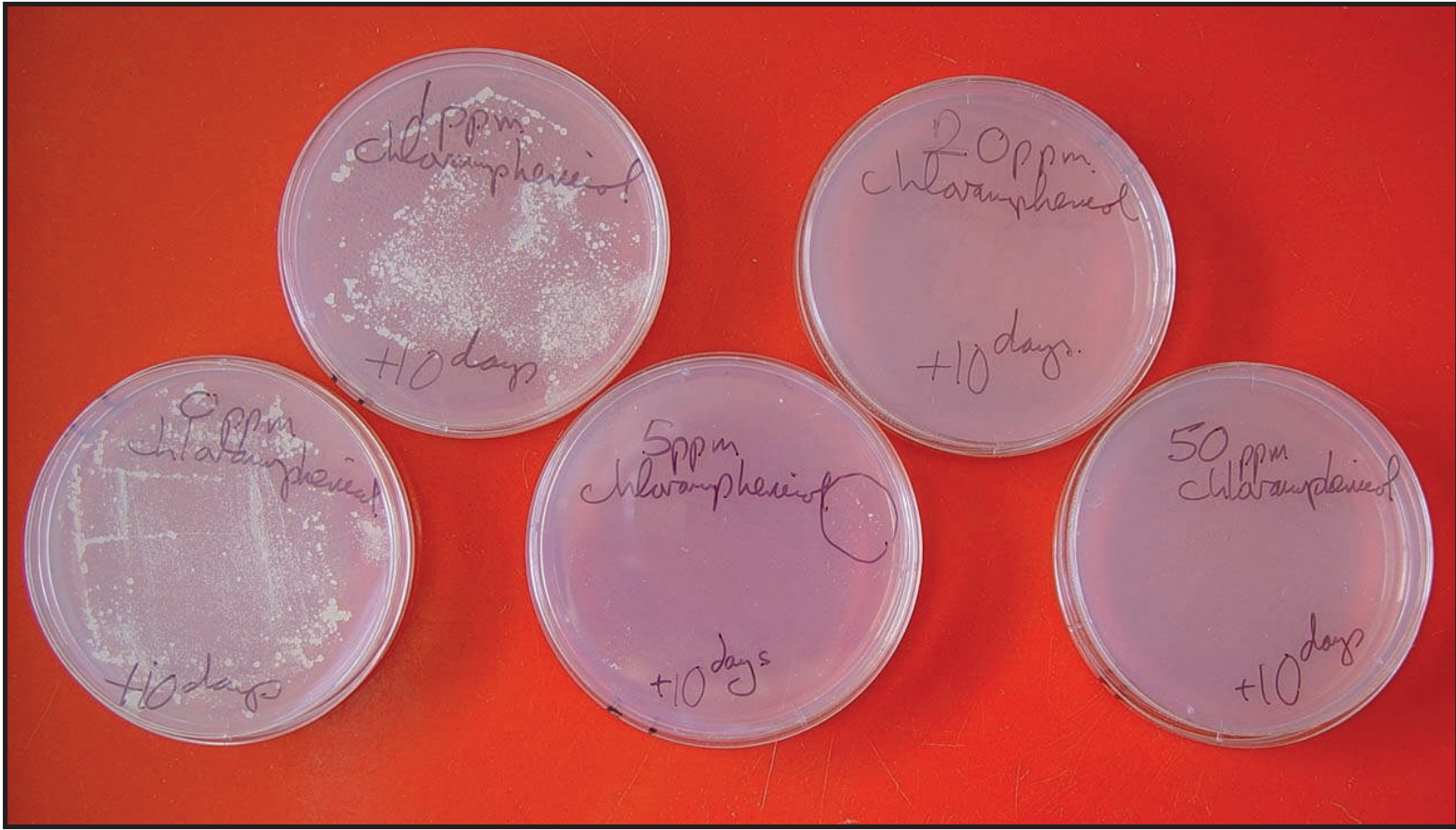
We have developed a safe and effective method of treating captive frogs infected with *Batrachochytrium*. Potentially this method could be extended to treat wild amphibian populations that are infected. Captive breeding programmes, planned as the only way to preserve threatened species until the chytrid can be beaten, remain vulnerable to the introduction of *Batrachochytrium*. A safe and effective protocol to treat infected captive frogs would be an asset in these breeding programmes, including for situations where potentially infected animals are to be relocated to new environments.



Litoria ewingii infected with *Batrachochytrium dendrobatidis*, showing lack of righting reflex.

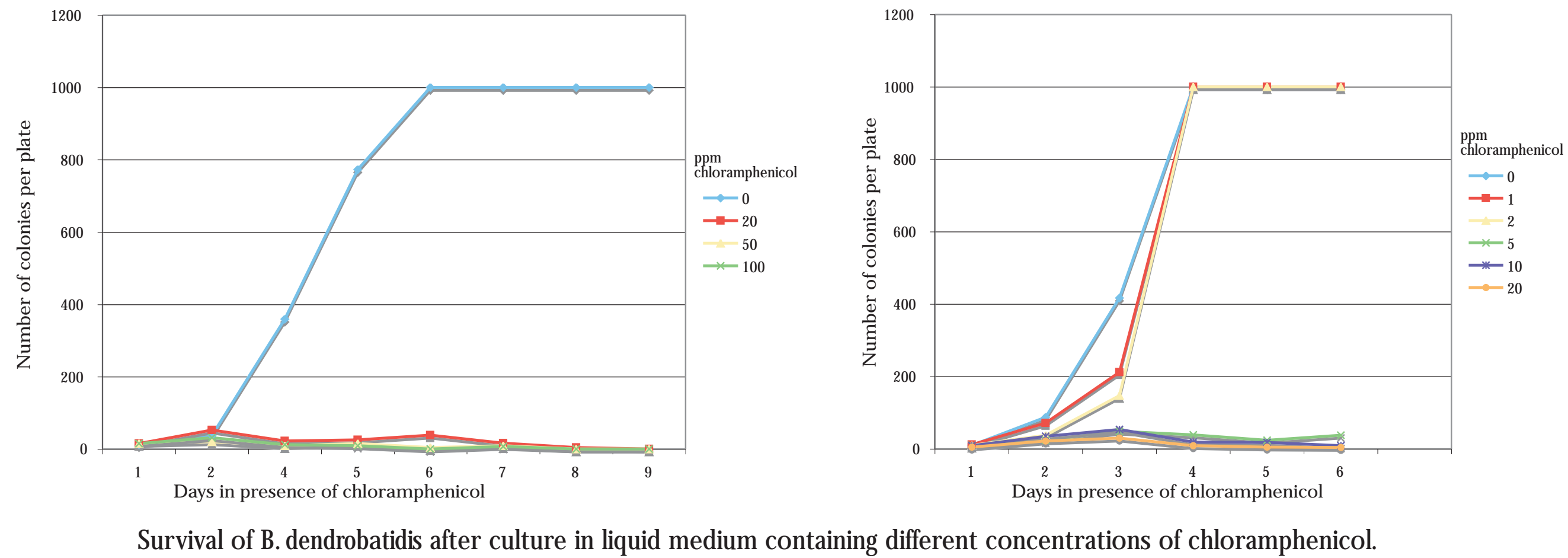
In vitro studies:

We tested the sensitivity of *Batrachochytrium* to chloramphenicol in both liquid cultures and on agar-solidified mTGhL medium.



B. dendrobatidis growth after 12 days when plated on mTGhL medium containing 0ppm (mg/L), 1ppm, 5ppm, 20ppm, 50ppm chloramphenicol.

To test solidified media actively growing *Batrachochytrium* cultures were plated onto mTGhL medium containing different amounts of chloramphenicol and kept at 22°C for 12 days



To test growth in liquid media *Batrachochytrium* was inoculated into 10mL of media in 50mL falcon tubes and incubated for 6-10 days with shaking. To provide a quantitative assessment of growth of *Batrachochytrium* at a range of chloramphenicol concentrations we plated a sample of each culture onto chloramphenicol-free medium at daily intervals. The number of *Batrachochytrium* colonies present 10 days after plating was used as a measure of the viable cell count from each culture sampled.

Frog studies:

25 *L. ewingii* tadpoles were fully immersed in 20ppm chloramphenicol in individual 200mL plastic flasks containing 75mL of solution. A further 25 were immersed in frog water as a control group. The tadpoles metamorphosed into froglets within 3-4 weeks. The froglets were housed individually in 25mm deep petri dishes containing ~5-7mL of either 20ppm chloramphenicol solution or frog water (controls). The chloramphenicol (or water) was changed daily. At the end of this 7-week period the froglets were weighed. There were no significant differences in survival (92%), time to metamorphosis ($P=0.1313$, 2-tailed t-test) or weight ($P=0.3902$, 2-tailed t-test) between the chloramphenicol treated *L. ewingii* tadpoles and the controls. The experiment was extended with the result that the treated tadpoles/froglets spent more than 3 months in a 20ppm solution of chloramphenicol with no apparent ill effects.



Litoria raniformis (Southern Bell Frog)

Litoria ewingii (Brown Tree Frog)

Infection Trials:

Frogs were experimentally infected by bathing them in a suspension of 100,000 *Batrachochytrium* zoospores in a weak salt solution (DS; Boyle et al., 2004) for 4-8h.

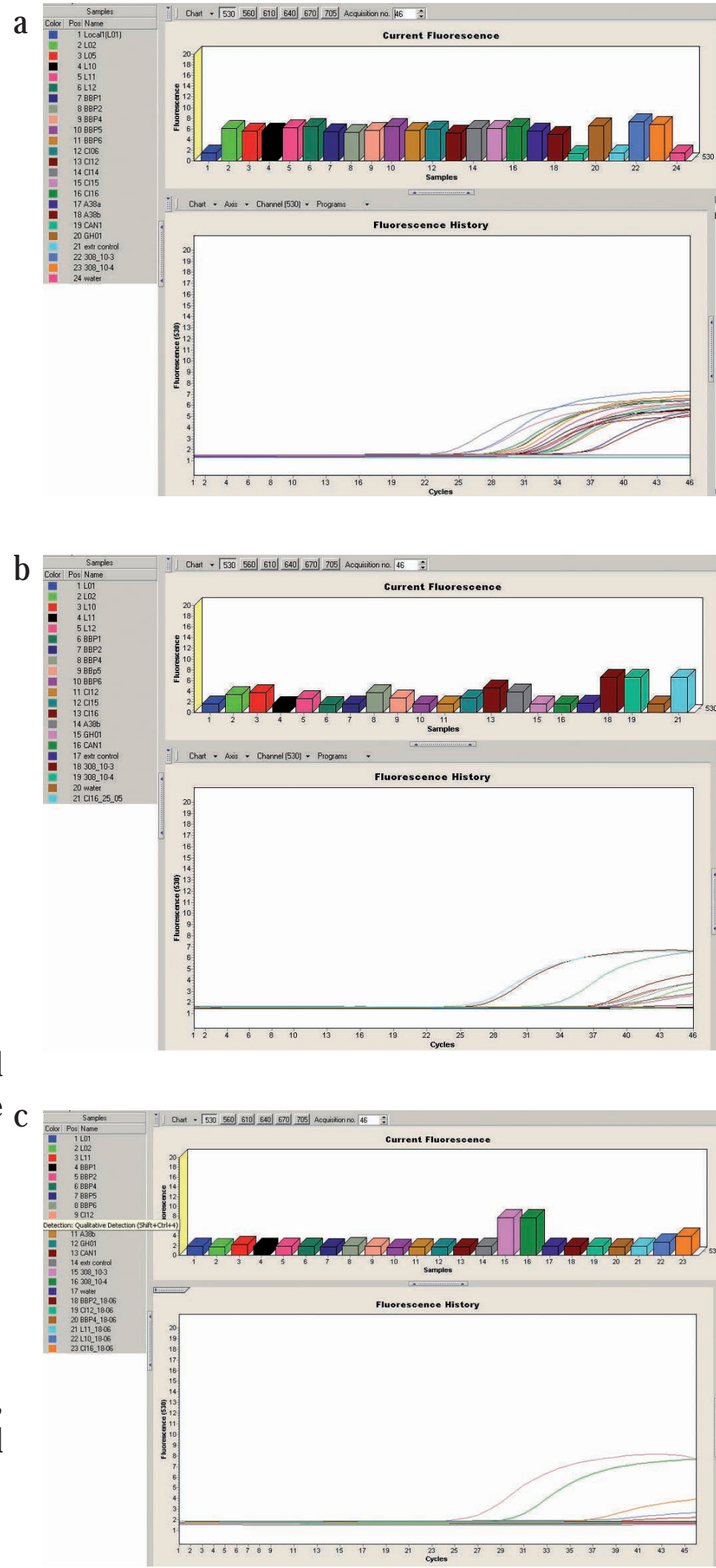
After 7 days, the frogs were placed into clean containers. Frogs were then swabbed in order to determine their infection status via PCR assay. Infected frogs were randomly assigned into the treatment protocol or to non-treatment maintenance.

The levels of infection with *Batrachochytrium* in the frogs were determined by quantitative real-time PCR as per the method of Boyle et al. (2004).

The treatment protocol consisted of adding 20mL (*L. ewingii*) or 100mL (*L. raniformis*) of freshly prepared chloramphenicol solution to the frog containers. The chloramphenicol solution was replaced each day.

Two separate trials were conducted; each trial used frogs from both species. Trial 1: 30 *L. ewingii*, 31 *L. raniformis*; Trial 2: 20 *L. ewingii*, 21 *L. raniformis*.

The results indicate that the frogs of both species were cleared of their infections after treatment with 20ppm chloramphenicol



Real-time PCR amplification curves from infection Trial 2 using *L. ewingii*. 308_10-3 and 308_10-4 are equivalent to 1,000 and 100 zoospore genomes, respectively (by reference c to the AAHL standards).

- a) *L. ewingii* swabbed before treatment began (14 days after infection). L01 and CAN1 were uninfected.
- b) *L. ewingii* swabbed during treatment trial. Frogs treated for 36 days: L02, L11, BBP1, BBP4, CI12, CI15, A38b, GH01.
- L. ewingii* initially untreated, then treated for 18 days: L10, L12 (died), BBP2, BBP5, BBP6, CI16. The result from one of these frogs (CI16) swabbed on an earlier date is included on the right.
- c) *L. ewingii* after another 2 weeks of treatment (total of either 50 days or 32 days). All except L11 clear of infection; results from 6 of these frogs swabbed on an earlier date are included on the right.

Table 1. Treatment trial 1 with Litoria ewingii - estimated number of Bde zoospores per swab													
L. ewingii	Trial 1	Day 7	Day 14	Day 19	Day 26	Day 34	Day 45	Day 65	Day 86	Day 97	Day 103	Day 111	
		10ppm	10ppm	10ppm	10ppm	10ppm	10ppm	10ppm	10ppm	10ppm	10ppm	10ppm	
Treatment began 1 week after infection (Day 7)													
# C108	0	11220	0	0	0	0	0	0	0	0	0	0	
# C119	2451	9600	4170	1883	0	0	0	258	1888	0	0	0	
C120	11010	15000	10410	5700	540	0	0	177	0	0	0	0	
C121	25800	4000	2000	0	201	0	0	0	0	0	0	0	
C122	9510	3090	5700	8710	35	0	0	0	0	0	0	0	
C123	117100	3000	54000	10070	231	0	0	0	0	0	0	0	
C124	15110	21900	3000	1993	393	123	0	0	0	0	0	0	
# C126	24320	0	4000	5580	96	0	0	0	0	0	0	0	
# C127	40800	13200	13740	207	96	0	0	0	0	0	0	0	
# C128	18240	2370	6150	204	181	0	0	0	0	0	0	0	
# C131	1440	3030	2175	1290	0	0	0	0	0	0	0	0	
# C132	3810	11370	4170	798	0	0	0	0	0	0	0	0	
C134	8840	3000	2350	1371	1002	0	0	0	0	0	0	0	
# C136	0	1053	0	0	0	0	0	0	0	0	0	0	
C137	25100	2100	2280	240	0	0	0	0	0	0	0	0	
# C138	7530	8010	10080	0	0	0	0	0	0	0	0	0	
C139	11140	20010	4140	844	2010	0	0	0	0	0	0	0	
C140	16400	13070	0	2550	3450	1008	0	0	0	0	0	0	
C141	1357	6810	0	1398	0	0	0	0	0	0	0	0	
# C145	10070	1320	10080	720	0	0	0	0	0	0	0	0	
Treatment began on Day 34													
C17	18070	86700	73800	140700	50700	36300	8070	618	2070	432	0	0	
C12	4840	18700	10100	200700	14540	71000	0	0	0	0	0	0	
C19	3000	21300	10000	40700	4410	0	0	0	0	0	0	0	
C20	0	0	0	0	0	0	0	0	0	0	0	0	
C23	5870	61700	30000	60000	21000	17410	285	0	0	0	0	0	
C25	30000	43800	227000	97100	149000	53700	0	0	0	0	0	0	
C41	11820	17200	37800	11400	107000	0	0	0	0	0	0	0	
C42	27000	274700	261300	414000	66000	402000	390	0	0	0	0	0	
C43	24100	10030	103000	91700	109000	0	0	0	0	0	0	0	
C46	400	3750	100000	27000	2001	11820	0	0	0	0	0	0	

= no treatment Day 50-52

* = frog died

Summary:

We have performed an extensive screen of available antibiotics and antifungals for their activity against *B. dendrobatidis* in vitro. Amongst these compounds is one (chloramphenicol) that is lethal to chytrids in vitro (after 6 days) and apparently non-toxic to amphibians. We have shown that this protocol cures experimentally infected *Litoria ewingii* (Brown Tree frog) and *L. raniformis* (Southern Bell frog, IUCN status is endangered); both are species introduced to New Zealand from Australia.

Further work:

The observation that chloramphenicol kills chytrids was unexpected because it is an anti-bacterial rather than an anti-fungal compound. We would like to understand this unexpected result.

The two species tested are both members of the genus *Litoria*. It would be useful to know if this protocol is generally applicable, in particular its safety for diverse amphibians needs to be determined. In collaboration with Dr Peter Harlow at Taronga Zoo, Sydney, we are at present evaluating the protocol with six further species of frogs, including the critically endangered *Pseudophryne corroboree* (Southern corroboree frog).

Pseudophryne corroboree (Southern corroboree frog)

