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Alternative Treatments to Koalas with Chlamydia

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An Alternative to Antibiotics for Koalas with Chlamydia

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Koalas on a Decline

Phascolarctos cinereus

- Endemic to Australia
- Habitat specialists
- Diet consists of eucalyptus leaves, they are restricted to certain areas of Australia where this food source can be readily available (Phillips, 2000).

Population Declines

- Dropped 80% from 1996-2014, which has led to the species being listed as **vulnerable** in 2004 (Rhodes, et al. 2015) and later was listed as **threatened** in 2012.

Major causes of population decline:

- infectious diseases (chlamydia and koala retrovirus)
- Vehicles
- Dogs
- Urbanization/deforestation
- Climate-related issues (Craig et al., 2014).

Chlamydia:

- Sexually transmitted disease
- Occurs in humans and a wide variety of animals
- Single-celled, gram-negative bacterium
- *C. pecorum* is most dominant species infecting koalas (Polkinghorne, et al. 2013) and has highest infection rate (Jackson, et al., 1999).
- Mode of transmission: sexual contact and from mother to joey during pap feeding and prolonged close contact (Nyari et al., 2017)

Negative Effects of Chlamydia

- Keratoconjunctivitis
- Blindness
- Damage reproductive tract
- Infertility in both males and females (Polkinghorne, et al. 2013).
- Population decline

Goal

Outline major causes of koala population declines, with emphasis on chlamydia infection.
Explore alternatives to the harsh effects of antibiotic treatment.

Antibiotics for Treatment

Generalities of Antibiotics

- Antibiotics: treatment of bacterial infections since the 1930s ("The History of Antibiotics," 2020).
- Antibiotics disturb natural state of the G.I. microbiota and cause dysbiosis
- After antibiotics, the natural state of the gut microbiome does not fully recover (Francino, 2016).
- **Doxycycline:** 97% cure rate and a reduced risk of causing dysbiosis (Booth and Nyari, 2020)

Issues with Use of Antibiotics

- Diet: eucalyptus leaves
- Eucalyptus leaves contain tannins ("phenolic compounds that form complexes with proteins and are resistant to degradation") (Aguilar, et al. 2007).
- Specialized gut bacteria breakdown tannins
- Antibiotics kill specialized gut bacteria and this causes dysbiosis
- Humans experience similar adverse side effects from antibiotic treatment
- The effects of antibiotics on the diversity of the human microbiome are shown in Figure 1 (Cotter et al. 2012).

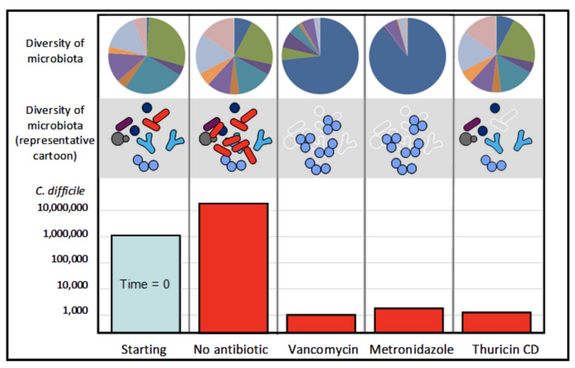


Figure 1. Impact of antimicrobials on gut bacteria
This figure shows the impact that antimicrobials have on the diversity of microbiota after 24 hours.

Hypothesis
If the doxycycline is given at a **higher dosage less frequently**, then the chlamydia infection will be successfully treated reducing the chance of the koala experiencing dysbiosis.

Nutritional Supplement

Lonepinella koalarum, a tannin-degrader, is associated with the health of the koalas comparing those who were treated with antibiotics and those who were not (Dahlhausen, et al. 2018).

Koalas given a soya-based dietary supplement during antibiotic treatment had a **higher survival and cure rate** (Osawa and Carrick, 1990)

Hypothesis
If a soya-based formula containing ***Lonepinella koalarum*** was fed to the koalas at the time of antibiotic treatment, then there will be **reduced** risk for antibiotic-related dysbiosis.

Methods

- Study site: Queensland, Australia
- Koala captures: 200 total
- Veterinary examinations
  - physical
  - diagnostic samples
  - radiographs
  - ultrasound
- Determination of 40 infected individuals

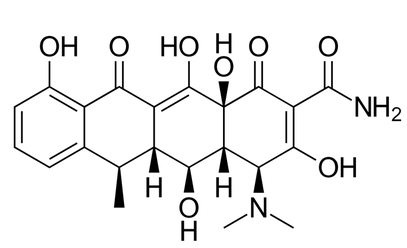
First Hypothesis

Antibiotic (Manufacturer)	Concentration	Dose rate	Frequency	Route	Duration	Comments	Ref
Azithromycin (Pfizer)	100 mg/mL	20 mg/kg	SID	IV	3 days	Diluted to a 2 mg/mL solution in sterile 0.9% saline and infused over 30 minutes in sedated koalas. Child dose rate.	[14]
Chloramphenicol (Ceva)	150 mg/mL	60 mg/kg	SID	S/C	28 days	Ready to use; rotated between sites; dose rate previously used to successfully treat chlamydiosis in koalas.	[15]
Doxycycline LA (Vetafarm)	50 mg/mL	5 mg/kg	Every 7 days	IM or S/C	28 days	Diluted 50:50 in saline; less tissue reaction if given s/c, rotated between sites; small animal dose rates.	[16]
Enrofloxacin (Bayer)	50 mg/mL	10 mg/kg loading dose; then 5 mg/kg	SID	S/C	28 days	Diluted 50:50 in saline; rotated between sites; small animal dose rates.	[17]
Florfenicol (Merck)	300 mg/mL	20 mg/kg	Every 2 days	S/C	28 days	Ready to use, domestic animal dose rate.	[18]

LA = long-acting formulation; SID = once daily, S/C = Subcutaneous, IV = Intravenous, IM = Intramuscular.

Figure 2. Antibiotic Administration Regimens for 5 Antibiotics Tested
This figure shows the 5 antibiotics that were tested in this study, their concentration, dose rate, frequency administered, route administered, and duration of administration. This was used as a reference for creating a higher dose rate and a different duration in the current study.

- Administer **doxycycline** in infected koalas **(200 mg/mL, 20 mg/kg)** SQ **once** per **28 days**.
- 4 groups of 10 koalas:
  - **Control** (no doxycycline)
  - **Low dose** (12.5 mg/mL, 1.25 mg/kg)
  - **Average dose** (50 mg/mL, 5 mg/kg)
  - **High dose** (200 mg/mL, 20 mg/mL)
- Fecal cultures obtained
- Side effects associated with the painful injection of such a high dosage (Booth and Nyari, 2020).



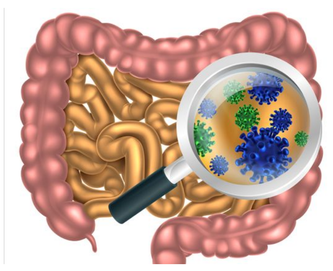
Second Hypothesis

- 2 groups of 25 koalas:
  - Control: no supplement
  - Soya-based supplement containing *Lonepinella koalarum.*
- Treat infected koalas with doxycycline at the average concentration and dosage (50 mg/mL, 5 mg/kg) for a standard amount of time (once every 7 days for 28 days)
- Administer supplement in 1 group
- Fecal cultures will be obtained

Expected Results

- **Reduced** chances of antibiotic-related dysbiosis and will need fewer follow-up treatments.
- **Lack of repeating treatments**, which has negative effects on the gut microbiota.

- Individuals receiving dietary supplements containing *Lonepinella koalarum* during the course of antibiotic treatment will show **reduced or no signs of antibiotic-related dysbiosis**
- **Higher survival rate** and koalas will be **successfully treated** for chlamydia.
- Dietary supplement **replenishes gut bacteria** that is killed by the antibiotics (Osawa and Carrick, 1990).



Conclusion

- Populations declines due to urbanization/deforestation, car collisions, dog attacks, and disease (Craig et al., 2014).
- Chlamydia: one leading causes of population decline
- For a conservation approach to be successful, all factors that affect population numbers should be considered.
- Population declines: slowing Figure 3. (Beyer et al., 2018).
- Absence of management interventions, within a decade local extinction is likely to occur.
- Extinction of koalas would affect Australia in many ways.
- The ecosystem would suffer cascade effects because koalas are herbivore specialists.
- Koalas are responsible for earning $3.2 billion per year in tourism ("Save the Koalas," 2015).

Cause of death	Year 1	Year 2	Year 3	Year 4	Total	Total (%)
Predation (total)	59	95	25	3	182	62.5
Predation, wild dog	35	68	14	0	117	40.2
Predation, carpet python	9	5	6	1	21	7.2
Predation, domestic dog	3	1	0	2	6	2.1
Suspected predation	12	21	5	0	38	13.1
Disease	32	26	19	7	84	28.9
Trauma, road	3	2	3	1	9	3.1
Trauma, rail	1	0	0	0	1	0.0
Trauma, intermale fighting	0	1	1	2	4	1.4
Other/unknown	2	3	3	3	11	3.8
Total	97	127	51	16	291	

Figure 3. Major Causes of Death in Koalas within Australia
This figure shows the major causes of death in koalas and highlights the percent of individuals affected over a 4-year period as a result of management intervention.

Limitations & Implications

- Advanced stage of infection
- Antibiotic administration over prolonged periods of time can change the clinical success of antibiotics
- Knowledge on antibiotic administration and the treatment of bacterial infections.
- Preventative vaccine for young koalas to prevent/reduce the chances of developing/spreading chlamydia
- Contribute to the research needed for the development of a human preventative chlamydia vaccine.

Acknowledgements

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