Science for Saving Species

Research findings factsheet

Project 2.3.5

Threatened Species Recovery Hub

National Environmental Science Programme

Understanding and treating a new Enterococcus bacterial infection affecting Christmas Island lizards

In brief

A deadly infection caused by a new and unusual bacterium was first observed in captive Critically Endangered and Extinct in the Wild Lister's geckos (*Lepidodactylus listeri*) on Christmas Island in late 2014. The infection was then identified in another captive Critically Endangered and Extinct in the Wild lizard species on Christmas Island, the blue-tailed skink (*Cryptoblepharus egeriae*), as well as in two species of invasive gecko, the Asian house gecko (*Hemidactylus frenatus*) and mute gecko (*Gehyra mutilata*), across much of the east of the island.

Conservation managers quarantined affected animals from the main population; however, they were not able to treat the affected lizards, as it was unknown which, if any, treatments would be effective. To fill this knowledge gap, we undertook carefully controlled experimental infection and treatment trials to discover how the disease is transmitted, what the incubation period is and the effectiveness of five different antibiotic treatments.

We found that infection likely happens due to bite wounds or other direct contact from infected lizards and that the incubation period is long. We trialled several antibiotics to determine if any would have an impact and be a potential means of treatment.

The findings will be of use to Christmas Island National Park and captive breeding staff as well as quarantine/biosecurity officers and wildlife disease and conservation scientists. Their application has the potential to reduce the threat, and possibly the spread of this deadly disease to the captive populations of Critically Endangered Christmas Island reptiles.

Background

The Australian external territory of Christmas Island, 1550 km off the north-western coast of mainland Australia, has reptile fauna that are found nowhere else. Although once common on the island, some of these species have become Extinct or Critically Endangered following the introduction of invasive species since settlement. In particular, the Asian wolf snake (*Lycodon capucinus*) and giant centipede (*Scolopendra subspinipes*), which were introduced to Christmas Island via international ships are likely to have had the most significant impact on native reptiles.

Around 10 years ago, out of concern for their dwindling populations, Parks Australia established captive breeding programs for the blue-tailed skink and Lister's gecko, which are now both Extinct in the Wild.

These captive breeding programs have become threatened by a recently emerged *Enterococcus* species of bacteria, known as *Enterococcus lacertideformus*. We identified the disease in October 2014, following an outbreak in the captive Lister's gecko population, which is likely to have resulted from direct contact with free-ranging invasive Asian house geckos and mute geckos.

Since 2014, there have been a number of outbreaks within the captive blue-tailed skink population that are housed in large open-aired pens called exclosures. These outbreaks have resulted in the death of an unknown number of animals and have been managed by isolating affected animals.

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Background (continued)

The disease causes visible facial deformation, with more advanced infection impacting bones, organs and tissues throughout the body. The progression of the disease varies widely among individuals and between species, but is ultimately fatal in geckos.

Treatment was not considered an option at that time, as it was unknown which, if any, antibiotics would be effective. Given that untreated infections result in the animals' certain death, conservation managers wanted to understand the pathology of the disease, and whether antibiotics present an effective option to treat infected individuals.

Following the initial outbreak in late 2014, we analysed the form, structure and genetic make-up of the bacterium to gain an understanding of the causes and effects of the disease. We found that the bacteria is contained within a kind of capsule that likely protects it from the immune response of the host and from some antibiotics.

We also discovered that the organism is genetically distinct; that is, it represents a novel species of *Enterococcus* bacteria. Because it is a unique bacterial species that we were not able to culture for antibiotic sensitivity tests, we had to experimentally infect and treat lizards to determine effective treatment protocols. We used highly invasive Asian house geckos collected in the wild on Christmas Island for these trials.

In order to better understand the on-going risk of infection we have also investigated where the disease was occurring on the island and how widespread it was. Island-wide surveys demonstrated that this bacterial disease continues to occur in both species of invasive geckos at multiple locations across the island, indicating that future outbreaks are likely.

Asian house gecko receiving an oral swab to confirm absence of E. lacertideformus *disease and enlistment into the experimental trial. Image: Jessica Agius*

Research aims

The aims of the experimental infection and treatment trial were to:

1. Identify the infection pathway
2. Identify the disease incubation period
3. Test the effectiveness of five potential antibiotic treatments
4. Determine whether any of the five antibiotic treatments have prophylactic application

What we did

The infection trial

The first step was to identify specific infection pathways in invasive free-ranging geckos on Christmas Island. We collected, euthanised and dissected diseased invasive geckos to obtain infected tissue. We then inoculated this infected tissue into 25 Asian house geckos randomly allocated to the following five groups (5 geckos per group).

- Subcutaneous (where we injected bacteria under the skin)
- Oral cavity (where we administered bacteria by mouth)
- Abdomen (where we injected bacteria into the abdominal space)
- Skin incision (where we placed bacteria onto a shallow skin incision)
- Mucosal incision (where we placed bacteria onto a shallow incision inside the moist lining of the mouth)

What we did (continued)

A control group was also used in the trial, five healthy Asian house geckos were injected with sterile saline solution instead of the bacterial inoculation.

We also wanted to investigate whether direct contact could cause infection, so we housed an additional five Asian house geckos individually with a diseased gecko. We then monitored all geckos in the trial twice weekly for three months to track the timing of the development of disease.

The treatment trial

Geckos that went on to develop disease in the infection trial were then randomly allocated to five groups, which were administered the following antibiotic treatments:

- Enrofloxacin
- Enrofloxacin combined with amoxicillin clavulanic acid
- Amoxicillin clavulanic acid
- Rifampicin
- Clarithromycin

A sample size of three animals per treatment group was used. All animals were dosed orally once a day for a total of 21 days, and the progress of disease was tracked by observing and photographing the treated geckos twice weekly. At the end of the treatment period, all geckos were examined to determine the effectiveness of the antibiotics.

To further analyse the effectiveness of enrofloxacin and our trialled dosage as an antibiotic treatment for this disease, we dosed 24 healthy invasive geckos once with enrofloxacin. We collected blood plasma at 0, 1, 2, 6, 12, 24, 48 and 72 hours following administration of the antibiotic to determine whether adequate concentrations of the antibiotic had been reached in the blood plasma to kill the bacteria.

Asian house gecko being examined to determine the effectiveness of antibiotic treatments during the trial. Image: Jessica Agius

Key findings

The infection trial

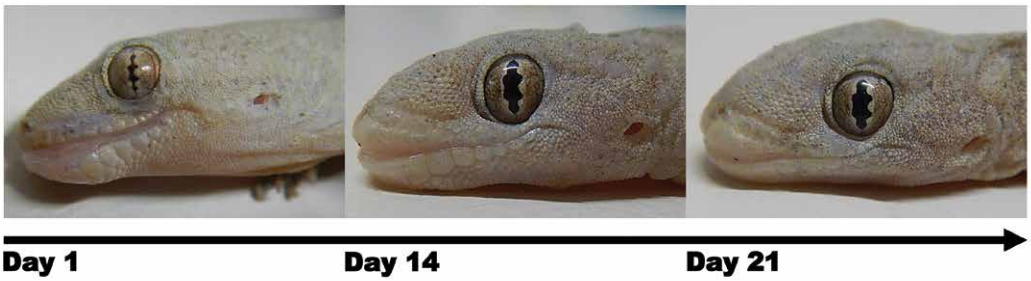
The experimental infection of geckos with the *Enterococcus* bacteria was successful, with all groups, except the control group and under-the-skin (subcutaneous) injection group, producing visible signs of disease. These results indicate that infection likely starts through bite wounds or some other form of direct contact.

The incubation period, or time taken for visible signs of disease to develop, varied significantly between infection groups, ranging from 50 to 100 days. This long incubation period indicates that this disease progresses slowly, with animals likely to be infected for quite long periods before they develop visible signs of disease.

Signs of disease occurred earlier in animals that were given the bacteria through incisions to the skin and oral mucosa (moist inner lining of the mouth), in contrast to those where the bacteria were dropped into the mouth or injected. This indicates that breaks in the skin or in the mucosal barriers may allow the bacteria to be more effective at establishing disease; and this likely also explains the shorter incubation periods we observed in animals from these groups.

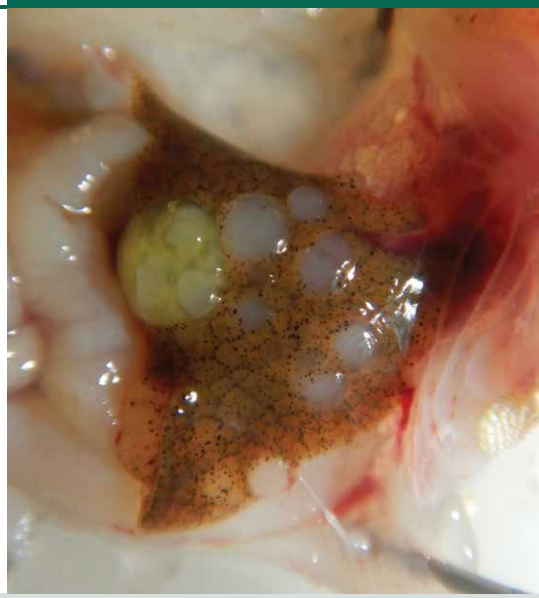
The treatment trial

The majority of the antibiotics we administered to geckos in this trial showed some degree of success. In geckos with advanced head lesions and evidence of disease spread throughout the organs, bones and/or tissues, 21 days of antibiotic treatment did not result in cure. Geckos with skin lesions at a less advanced state of the disease responded better to treatment, and were completely cured in one case. Although the majority of antibiotic treatments resulted in the signs of disease lessening following 21 days of treatment, complete resolution of the disease nevertheless was rare.



LEFT: Lesion regression of an Asian house gecko receiving antibiotic treatment for infection with E. lacertideformus. *Image: Jessica Agius*

BELOW: Necropsy of an Asian house gecko affected with E. lacertideformus. *A number of white lesions containing the bacteria throughout the liver are evident. Image: Jessica Agius*



Key findings (continued)

This finding may be to do with the capsule surrounding the bacteria. This capsule likely prevented the lizard's immune system from recognising the bacteria, and also prevented the antibiotics from inhibiting it.

On the basis of what we could observe visually of the disease, the antibiotic enrofloxacin was most successful, with the highest degree of the disease signs lessening or disappearing in one case. We conducted an analysis of this antibiotic in plasma over time, which revealed that the concentration we administered likely reached plasma concentrations that were sufficient to kill Enterococcus lacertideformus. However, the presence of the capsule surrounding the bacteria may hinder the effectiveness of enrofloxacin, and a higher dose or more frequent dosage may be required for cure.

Implications and recommendations for future work

The findings from this trial provide important information about the pathology and treatment of the novel *Enterococcus* bacterium that has led to the death of many Extinct in the Wild lizards on Christmas Island.

The trial indicated that antibiotic treatments may aid in regression or complete cure of the disease (in one animal), and that of the five antibiotics trialled, enrofloxacin was the most effective.

Although this trial did incorporate two antibiotics with a high Australian importance rating, only a small number of animals were treated, and the frequency and duration of antibiotic use ensured a full and effective course was given. Additionally, waste was incinerated and all animals were euthanised and were not released back into the wild, therefore, this trial did not pose a risk for the development of resistance."

These findings are of relevance to Christmas Island National Park and captive breeding program staff, in addition to quarantine/biosecurity officers and wildlife disease and conservation scientists.

By informing disease management and biosecurity protocols this new knowledge can reduce the threat of this disease to the captive population of Critically Endangered Christmas Island reptiles, and possibly reduce its spread to other regions. It is thus beneficial to the long-term conservation of these species.

Further analyses are underway to evaluate the total effectiveness of each antibiotic. This will involve microscopic examination of the tissues of animals in the trial following 21 days of antibiotic treatment to determine the extent of internal disease. This work will further add to our understanding of this disease and how to treat it.

Further Information

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Further reading

This factsheet summarises key findings from the following scientific papers. Please refer to the papers for more detail.

Rose, K., Agius, J., Hall, J., Thompson, P., Eden, J. S., et al. 2017. Emergent multisystemic *Enterococcus* infection threatens endangered Christmas Island reptile populations. *PLOS ONE* 12(7): e0181240. https://doi.org/10.1371/journal.pone.0181240

Agius, J., Rose, K., Emery, J-P., & Phalen, D. 2021. Experimental infection of Asian house geckos (*Hemidactylus frenatus*) with *Enterococcus lacertideformus* demonstrates multiple routes of disease transmission and the relative in-vivo efficacy of antibiotic treatments. *Scientific Reports*.

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