Amoxicillin Water Treatment for a *Corynebacterium bovis* Outbreak
It Takes a Team!

Christina N. Nestel[1], Chris Mullen-Fazekas[1], Holly R. Goold[1], Michael K. Fink[1, 2], Lauren M. Habenicht[1, 2], Derek L. Fong[1, 2], Jori K. Leszczynski[1,2] , Chris A. Manuel[1, 2]

Office of Laboratory Animal Resources[1], Department of Pathology[2], University of Colorado Anschutz Medical Campus, Aurora, CO

What happened?

Following 7 years of effort, *Corynebacterium bovis* was eliminated from the CU Anschutz Medical Campus. Five months later our *C. bovis* surveillance program detected 3 racks of *C. bovis* positive immunodeficient mice. These cages were located in a housing room that had recently been sterilized using VHP. This room contained approximately 27 principle investigators that used immunodeficient and immunocompetent mice. All immunodeficient mice were presumed to be exposed to *C. bovis* due to shared advanced imaging equipment.

PCR testing was used to detect and remove *C. bovis* shedding mice. A method was needed to prevent exposed mice from becoming infected and start shedding the bacteria. Thus, we selected antibiotic treatment over depopulation for all immunodeficient mice considered exposed. Immunocompetent mice were left untreated.

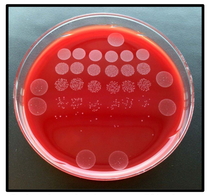
The goal of this poster is to demonstrate the teamwork needed to pull off a large scale and spontaneous prophylactic antibiotic treatment plan to contain a *C. bovis* outbreak. So, why did we treat exposed immunodeficient mice with antibiotics?

Antibiotics can be used to prevent *C. bovis* infections following exposure.
Antibiotics cannot be used to cure mice already infected with *C. bovis*.

Corynebacterium bovis

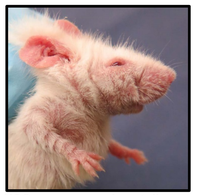
Corynebacterium bovis is an opportunistic bacterial pathogen that infects the skin of immunodeficient mice. Immunocompetent mice are rarely impacted by *C. bovis*.

- *C. bovis* has a negative impact of cancer mouse models
- 55% (38/69) of NCI's Cancer Centers have *C. bovis* infected mice
- 57% (28/50) of the top 50 NIH Funded academic institutions have *C. bovis*
- *C. bovis* is spread by equipment, supplies, and even frozen tumor tissue
- Infected mice create lots of bacteria that contaminate the vivarium environment
- *C. bovis* can survive on surfaces for >2.5 months
- Infected mice can look either normal, scaly, or very scruffy and sick



***C. bovis* colonies on a 5% sheep-blood agar plate after 72 hr.**

Athymic nude mice naturally infected with *C. bovis* at CU Anschutz campus.



NSG mouse experimentally infected with *C. bovis* after 8 wk.

Making Amoxicillin Water

Generic Amoxicillin for Oral Suspension ($0.04/water bottle)
Cost and availability of a generic powder for oral suspension resulting in significant savings without sacrificing efficacy. against *C. bovis* for this outbreak.
Concentration: ~0.25 mg/mL or 50 mg/kg/day for a mouse.

Clavamox™ (amoxicillin & clavulanic acid; $1.55/water bottle)
Historically the antibiotic of choice for the treatment of rodents by OLAR. Challenges with availability required reassessment.
Concentration: ~0.375 mg/mL or 75 mg/kg/day for a mouse.

Carboy

Carboy (4 gallon or 15.1 L). Add 75 mL of amoxicillin oral suspension (50 mg /mL) to 15.1 L of distilled water to get a final concentration of 0.25 mg/mL.

Bottle

Bottle (375 mL). Add 1 gram (~1 tea-spoon) of amoxicillin oral suspension powder to 375 mL of distilled water for a final concentration of 0.26 mg/mL.

Metrics

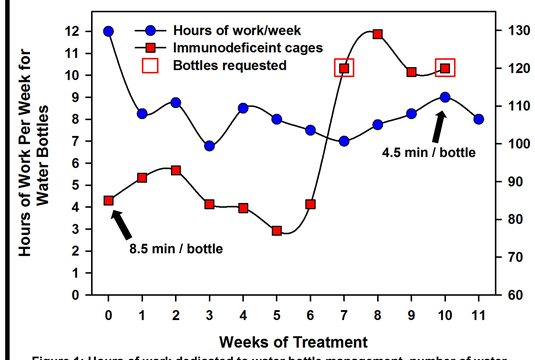


Figure 1: Hours of work dedicated to water bottle management, number of water bottles changed, or bottles requested in room #441 was recorded for the veterinary technician and animal care staff personnel that serviced the room.

How much time did water bottle management take per week?

Did the number of cages on antibiotics impact the work hours per week?

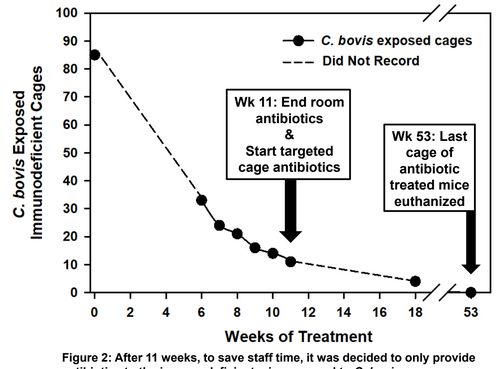


Figure 2: After 11 weeks, to save staff time, it was decided to only provide antibiotics to the immunodeficient mice exposed to *C. bovis*, or immunodeficient mice present at the time of initial infections.

How quickly did the 85 cages of *C. bovis* exposed immuno-deficient mice go away?

It takes a team!

Animal Care Techs

- Daily health checks to find leaking bottles and lixits
- Weekly change of amoxicillin water in coordination with vet techs
- Monitoring cages counts at the rack level for weekly water bottle requests
- Creation of additional individual amoxicillin bottles as needed for leaks or new cages
- Weekly coordination with cage wash about updated supply count
- Coordination and education of research staff about rack assignments for prophylactic antibiotic treatment

Vet Techs

- Weekly creation of amoxicillin water in carboys
- Weekly update of documentation and treatment flags
- Bi-weekly room check for individual amoxicillin water bottle placement for new cages
- Monitoring and replenishing amoxicillin supplies for duration of treatment
- Identify new cages of immunocompromised mice
- Coordination and education of research staff on water valve placement with and without antibiotic water

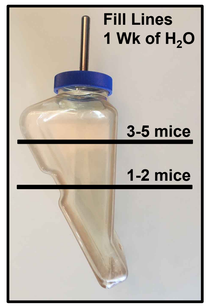
Cage Wash

- Obtain and sanitize water bottles and carboys weekly for delivery
- Collection and transportation of reverse osmosis water for amoxicillin water generation
- Communication with AC & vet techs about supply options and time estimates for supply availability
- Weekly collection and preparation of old amoxicillin for disposal

Vets & Admin

- Prophylactic antibiotic plan development
- Communication with research laboratories
- Timeline of implementation and duration
- Consultation with researchers concerning potential impact on research outcomes
- Amoxicillin dose calculation for drinking water
- Initial coordination with AC, vet techs, and cage wash
- Digital tracking of *C. bovis* exposed cages

Lessons Learned

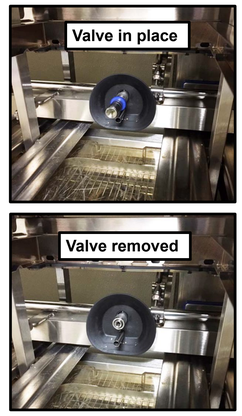


Reducing Antibiotic Water Waste

- 375 mL Allentown water bottle
- Bottles refreshed weekly
- Bottles were filled based on cage density

Water Valve Education for Research Staff

- Training of research staff to recognize:
  - a water valve
  - a missing water valve
- Continuous monitoring for researcher mistakes
  - No lixit and no water bottle
  - Lixit and water bottle



Moving Lots of Water and Water Bottles

- Water is heavy; 1 gal = 3.83 lbs
- Using 8 gal / week = 30 lbs
- Sterile, empty bottles provide in sanitized trash can
- Expired bottles with remaining antibiotic water were put into trash cans to roll to cage wash

Summary and The Future

- It worked at containing the outbreak!
- No animal deaths due to dehydration or amoxicillin water complications
- Prophylactic amoxicillin and environmental decontamination were 100% effective at preventing infection spread
- Time requirements: average of 8.3 hr/wk by the end of the project, 4.5 min/bottle for 2 staff members that were efficient at the task
- After 53 weeks, the last cage of immunodeficient mice exposed to *C. bovis* and on antibiotics was euthanized.

Medicated Water Positives

- It is effective and can be rapidly implemented within ~1 wk of lead time
- Ordering special manufactured amoxicillin food will take ~5 weeks of lead time which will include novel order delay, manufacturing, and shipping
- Amoxicillin water has less upfront costs than medicated feed

Medicated Water Negatives

- Time and labor intensive, significant coordination with the team, potential for human error resulting in animal death

Overall, the amoxicillin water project was effective and efficient and can be used in future *C. bovis* outbreaks for quick containment, and medicated feed should be considered based on the size of the outbreak. The source of *C. bovis* was traced back to new arrival, vendor-sourced immunodeficient mice.

Acknowledgements

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