Comparative Medicine
Copyright 2023
by the American Association for Laboratory Animal Science

Vol 73, No 6
December 2023
Pages 461–465

*Original Research*

# Antibiotic Treatment of *Corynebacterium bovis*-associated Clinical Disease in NSG Mice

**Elena M Pires,[1] Umarani Pugazhenthi,[2] Michael K Fink,[1,3] Lauren M Habenicht,[1,3] Derek L Fong,[1,3] Jori K Leszczynski,[1,3] Michael J Schurr,[4] and Christopher A Manuel[1,3,5,*]**

**Depending on the strain of immunodeficient mice, *Corynebacterium bovis* infection can be asymptomatic or cause transient or prolonged skin disease. *C. bovis* infection of NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NSG) mice results in clinical skin disease that progresses in severity. Amoxicillin metaphylaxic and prophylaxic therapy prevents transmission and infection of mice after exposure to *C. bovis* and inhibits the growth of *C. bovis* isolates at therapeutic doses that are clinically achievable in mice. Amoxicillin is not efficacious for treatment of transient clinical skin disease in athymic nude mice, but the efficacy of amoxicillin treatment has not previously been characterized in *C. bovis*-infected NSG mice. In the current study, NSG mice were treated with amoxicillin beginning at 5 wk after exposure to *C. bovis*, at which time they had well-established clinical signs of disease. Clinical signs were scored to assess disease progression, regression, and reappearance. Our results showed that amoxicillin treatment for 3 or 6 wk reduced the clinical scores of NSG mice with *C. bovis*-associated clinical disease. In addition, withdrawal of treatment led to the recurrence of clinical signs. Collectively, our data suggest that amoxicillin treatment is effective in alleviating the clinical signs associated with *C. bovis* infection for the duration of treatment in NSG mice. Clinical intervention with antibiotics for *C. bovis*-infected NSG mice can be an option for management of *C. bovis*-related clinical disease either before or during facility-wide remediation efforts.**

**Abbreviation and Acronym:** NSG, NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ

**DOI:** 10.30802/AALAS-CM-23-000039

## Introduction

NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ (NSG) mice are a valuable immunodeficient mouse strain for the study of oncology, immuno-oncology, and other areas. *Corynebacterium bovis* is a common gram-positive opportunistic bacterium that has been associated with skin disease in several immunodeficient rodent strains.[1,5,7,18,20,21] Pathogens such as *C. bovis* negatively affect research studies by contributing to poor patient-derived xenograft engraftment[17,21] and weight loss[13] and by concurrently contaminating the environment, thus making eradication of the agent difficult.[2,14,17] Specifically, in NSG mice, clinical signs of *C. bovis* infection involve decreased body condition and changes to the skin that include a rough haircoat, scaling, alopecia, conjunctivitis, and erythema of the pinnae. Associated behavioral changes include increased grooming and rapid head shaking.[13]

Previous studies on antibiotic treatment of *C. bovis*-infected mice have focused on prophylaxis and metaphylaxis. When amoxicillin is administered prophylactically, both NSG and athymic nude mice are resistant to *C. bovis* infection during treatment.[12] However, infection can occur after antibiotic treatment is discontinued.[1] Metaphylactic treatment of infected NSG mice with the antibiotic amoxicillin reduced their whole-body *C. bovis* burden, preventing bacterial shedding and transmission.[19] Furthermore, amoxicillin treatment of *C. bovis*-infected NSG mouse breeder pairs resulted in the weaning of *C. bovis*-free NSG offspring.[19] However, a successful antibiotic treatment regimen for *C. bovis*-infected NSG mice with clinical disease has not previously been characterized. Therefore, we assessed the therapeutic efficacy of oral amoxicillin treatment in NSG mice with *C. bovis*-associated clinical disease. After experimental exposure that generated the expected clinical signs, NSG mice were treated with amoxicillin, and their clinical signs were evaluated by using a recently described clinical scoring system.[13] Our findings support the use of amoxicillin treatment of *C. bovis*-infected NSG mice that show clinical signs. Treatment can be used to maintain irreplaceable NSG mice or those bearing unique tumor lines.

## Materials and Methods

**Mice, housing, and husbandry.** The study described here was approved by the IACUC of the University of Colorado Denver Anschutz Medical Campus, an AAALAC-accredited institution. Female NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ mice (NSG, strain number 005557; $n$ = 28; age, 7 wk) were ordered from Jackson Laboratories (Bar Harbor, ME). This strain was selected so that we could use a clinical scoring system that was developed in NSG mice[13] to evaluate the efficacy of antibiotic treatment on the clinical signs of *C. bovis* infection. Based on vendor report, the mice were free of endo- and ectoparasites and common viral and bacterial pathogens, including *C. bovis*. On arrival, mice were housed at 3 or 4 per cage in autoclaved JAG 75 cages

*Submitted: 21 Jun 2023. Revised: 08 Sep 2023. Accepted: 10 Oct 2023.*
*[1]Office of Laboratory Animal Resources, [2]Division of Endocrinology, Metabolism, and Diabetes, and the Departments of [3]Pathology and [4]Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, Colorado; and [5]University of Colorado Cancer Center, Aurora, Colorado*
**Corresponding author. Email: Chris.Manuel@cuanschutz.edu*

and placed on a 70-cage single-sided IVC rack (Micro Vent, Allentown Caging, Allentown, NJ) with 40 air changes per hour. The intracage environment included aspen chip bedding (Inotiv, Indianapolis, IN), a compressed cotton square (Ancare, Bellmore, NY), a Mouse Igloo (BioServ, Flemington, NJ) that was inverted to facilitate visual observation, irradiated rodent diet (2920X, Teklad Extruded Diet, Envigo, Inotiv), and reverse osmosis-purified, autoclaved water in 375-mL water bottles (Allentown Caging); chow and water were available ad libitum. The macroenvironment of the quarantine room in which the experiment was conducted was maintained at 22 ± 1 °C (72 °F) and 30 to 40% humidity with at least 12 complete air changes per hour and a controlled 14 h light cycle ending at 2000 hr. All mouse handling practices, including a specific glove-change technique developed to prevent cross-cage contamination when working with *C. bovis*-infected mice, were performed as described previously.[12,19]

***C. bovis* exposure and experimental design.** All mice were experimentally exposed to *C. bovis* through the transfer of 50 mL of soiled bedding from NSG mice demonstrating clinical signs, as described previously.[13] Clinical signs were scored weekly, as described below. The study comprised 2 experiments. Experiment 1 (11 wk) determined whether amoxicillin-medicated drinking water decreased clinical severity or eliminated clinical signs ($n$ = 4 mice). Once clinical resolution (i.e., a score of 4 [normal]), was observed, the duration of treatment was recorded, and the experiment was concluded. Experiment 2 monitored the clinical outcome when amoxicillin treatment was discontinued after 3 wk ($n$ = 4 mice) or 6 wk ($n$ = 4 mice) of continuous treatment. The time points of 3 and 6 wk were selected based on the outcome of experiment 1. The total duration of experiment 2 was 16 wk.

Amoxicillin-medicated drinking water was provided at a concentration of 0.26 mg/mL (equivalent to a dose of 50 mg/kg; amoxicillin trihydrate, 250 mg/5 mL for oral suspension, Hikma Pharmaceuticals USA, Eatontown, NJ). This dose of amoxicillin was determined based on our previous work on prophylactic and metaphylactic amoxicillin use and on published antimicrobial susceptibility results for *C. bovis* isolates collected from immunodeficient mice.[6,12,19,22] For all groups receiving amoxicillin, treatment began at 5 wk after exposure. At this time point, clinical signs would be visually obvious to both trained and novice observers, who clinically initiated treatment based on observation of these signs. For both experiments, positive controls comprised *C. bovis*-infected mice that had clinical signs but did not receive amoxicillin ($n$ = 12), and a single cohort of NSG mice was maintained *C. bovis*-free throughout both experiments study (*C. bovis*-negative control, $n$ = 4) to provide a visual example of clinically normal mice.

**Clinical scoring system.** Mice were evaluated weekly for 11 (experiment 1) or 16 wk (experiment 2) for the presence of clinical signs consistent with *C. bovis* infection based on a published clinical scoring system for NSG mice.[13] Briefly, mouse cages were removed from the IVC rack weekly, rotated in hand 180° and returned to a rack slot to allow the evaluator to view 3 of the 4 cage sides. Scoring was performed between 1600 and 1900 h. Mice were scored for the presence and severity of 6 clinical signs and behavioral changes, including ocular changes, aural changes, haircoat quality, grooming activity, hunched posture, and head shaking. These individual scores were summed to provide a total score that ranged from 4 (normal) to 18 (severe) (Figure 1). The same researcher, who was aware of the treatment groups, assessed and scored all mice.

**Photography.** At specific time points during both experiments 1 and 2, mice were photographed by using a Powershot G16 camera (12.1 megapixels; Canon USA, Melville, NY) using the automatic setting and TTL auto focus with auto white balance. Because anesthesia reduces blood pressure and results in blanching of skin hyperemia, mice were not anesthetized for image acquisition. JPG images were imported into Photoshop CS5 (Adobe, San Jose, CA) and were autocorrected for contrast, manually adjusted for brightness and color level, auto-sharpened, and maintained at their original native resolution of 180 dpi, as described previously.[13]

**Statistical analysis.** Six benchmark changes in the clinical condition of NSG mice occur incrementally with *C. bovis* infection.[13] Due to the robust validity of the previously published scoring system to capture these incremental changes,[13] the experimental power of part 1 was 0.97 for group sizes of 4 *C. bovis*-positive amoxicillin-treated mice and 6 *C. bovis*-positive untreated control mice. For experiment 2, we determined that the same number of treated mice and untreated controls used in experiment 1 was adequate to demonstrate a statistically significant clinical benefit of amoxicillin treatment. By design, experiment 2 also demonstrated reproducibility in clinical benefit of amoxicillin treatment and the rate of return of clinical signs. Because the scoring system generated nonparametric data, the Mann–Whitney rank sum test was performed to compare the clinical scores between treated and untreated groups at any given time point. All numerical figures are presented as the mean ± 1 SD of the combined clinical score for each experimental group at any given time point. A p value < 0.05 was considered significant. All statistical analyses were performed, and figures were generated by using SigmaPlot 11.2 (Systat Software, Point Richmond, CA).

| | Clinical Score and Criteria | | | | | |
|---|---|---|---|---|---|---|
| Parameter | 0 | 1 | 2 | 3 | 4 | 5 |
| **Eyes** | | Normal | Conjunctivitis, blepharitis, or blepharospasm | Periorbital alopecia | | |
| **Ears** | | Normal | Aural base hyperemia | Diffuse hyperemia, pinnae translucency | Hyperkeratosis on the pinnae | |
| **Haircoat** | | Normal | Mild unkemptness | Alopecia on face | Alopecia on forelimbs and ventral body | Muzzle swelling, physically thick skin† |
| **Grooming** | | Normal | Brief grooming | Prolonged grooming | Continuous grooming | |
| **Hunched** | Absent | Present | | | | |
| **Head shaking** | Absent | Present | | | | |

**Figure 1.** Summary of the clinical scoring system for NSG mice utilized in this study. †Assessment that may require direct animal manipulation to physically feel skin thickness during restraint. Minimum score of 4 and maximum score of 18. Republished and altered with permission from reference.[13]

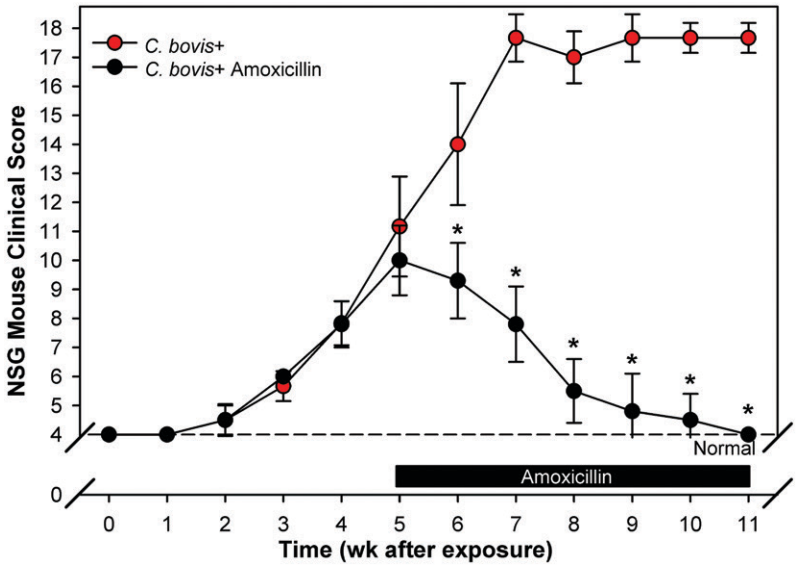


**Figure 2.** Cumulative clinical scores (mean ± 1 SD) of NSG mice exposed to *C. bovis* and treated with amoxicillin in the drinking water (0.26 mg/mL [50 mg/kg daily]) until clinical resolution ($n = 4$ per group) and untreated control mice ($n = 6$ per group) over 11 wk. NSG clinical scores are based on a progressive scoring system for *C. bovis*-infected, clinical NSG mice.[13] *, Significant difference ($P < 0.05$, Mann–Whitney rank sum test) between groups. The power level for these groups was 0.971.

## Results

**Amoxicillin treatment and clinical signs.** In experiment 1, to assess whether amoxicillin-medicated drinking water reduced or eliminated clinical signs, we compared treated and untreated *C. bovis*-infected NSG mice. Clinical scores were evaluated in NSG mice weekly for 11 wk from *C. bovis* exposure. At 5 wk after *C. bovis* exposure, clinical scores prior to treatment initiation were comparable ($P = 0.257$) between mice in the treated (score, 10.0 ± 1.41) and untreated (11.2 ± 1.7) groups. Beginning at 5 wk, antibiotic therapy was initiated in the treatment group until resolution of clinical signs. Mice in the positive control group were not treated during this time. Compared with the positive-control mice, treated mice showed that amoxicillin treatment was effective in reducing clinical scores within 1 wk of therapy initiation. After the first week of treatment, clinical scores had decreased in the treated mice (9.3 ± 2.5) but increased for the control group (14.0 ± 2.1; $P < 0.01$). Clinical scores of the treated mice had returned to normal (4.0 ± 0.0) after 6 wk of treatment, whereas scores for the untreated controls remained high (17.7 ± 0.5; $P < 0.01$; Figure 2).

**Amoxicillin withdrawal and clinical recurrence.** After confirming that 6 wk of exposure to amoxicillin in the drinking water can resolve clinical signs in *C. bovis*-infected NSG mice, we next wanted to observe the clinical outcome of discontinuing antibiotic treatment in experiment 2. We evaluated *C. bovis*-infected mice that received amoxicillin-containing drinking water for either 3 or 6 wk by comparing them with untreated *C. bovis*-positive controls, for 16 wk from exposure. At 5 wk after *C. bovis* exposure and immediately prior to initiation of treatment, clinical scores of the mice allocated for the untreated and

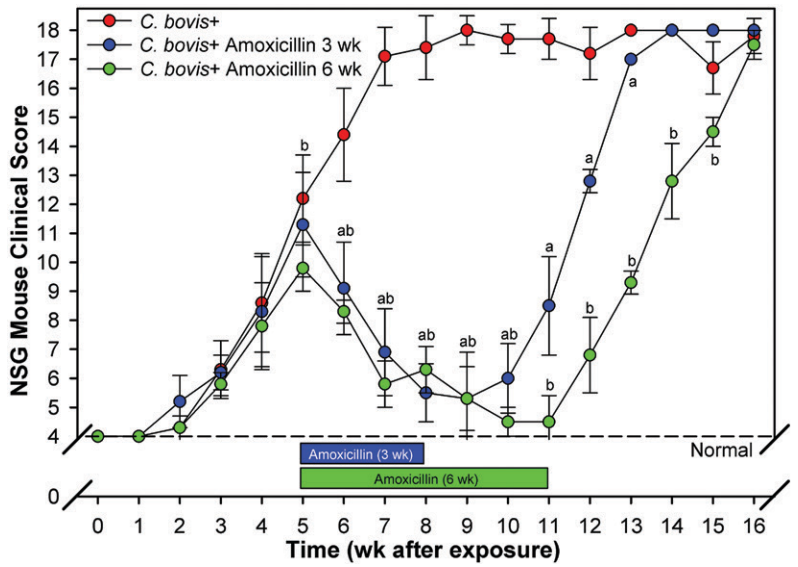


**Figure 3.** Cumulative clinical scores (mean ± 1 SD) of NSG mice exposed to *C. bovis* and treated with amoxicillin in the drinking water (0.26 mg/mL [50 mg/kg daily]) for either 3 or 6 wk ($n = 4$ per group) or untreated control mice ($n = 6$ per group) for 16 wk. NSG clinical scores are based on a progressive scoring system for *C. bovis*-infected clinical NSG mice. Significant difference ($P < 0.05$, Mann–Whitney rank sum test) between untreated controls and mice treated with amoxicillin for 3 wk (a) or 6 wk (b). The power level for these groups was 0.99.

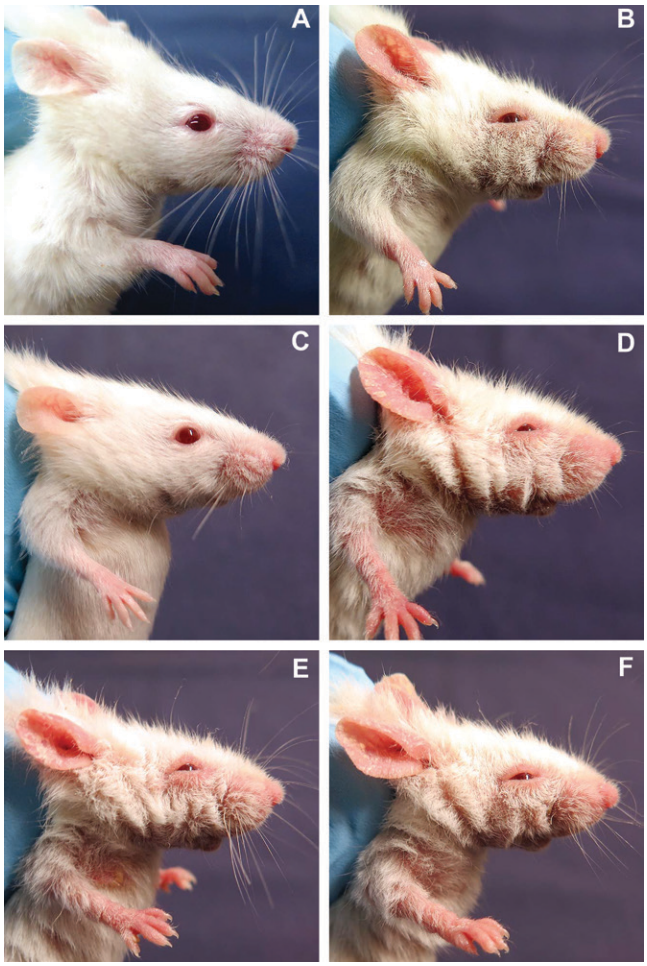


**Figure 4.** Photographs of the upper torso of NSG mice with and without amoxicillin treatment. (A) *C. bovis*-negative control mouse. (B) Mice at 5 wk after exposure to *C. bovis* and just prior to the start of amoxicillin treatment. Mice at 8 wk after exposure with (C) and without (D) 3 wk of amoxicillin treatment. (E) Withdrawal of treatment after either 3 or 6 wk results in the complete reappearance of clinical signs by 16 wk, as compared with (F) infected mice that were not treated.

3 wk treatment groups were (11.8 ± 0.7 and 11.8 ± 0.9, respectively ($P = 0.914$). The scores of mice allocated to the 6 wk treatment group were significantly ($P < 0.02$) lower (9.75 ± 0.9) than that of the *C. bovis*-positive controls (Figure 3). As in experiment 1, both groups of treated mice showed a significant ($P < 0.05$) reductions in clinical score after 1 wk of treatment. For the 3-wk treatment group, amoxicillin was discontinued at 8 wk after exposure and had a mean score of 5.5 ± 1.0 ($P < 0.01$) as compared with untreated controls (17.3 ± 1.6). For the 6-wk treatment group, amoxicillin was discontinued at 11 wk after exposure; at that time, mice had a mean score of 4.5 ± 1.0 ($P < 0.01$) as compared with controls (17.3 ± 1.6). After discontinuation of amoxicillin, clinical scores increased for both treated groups at approximately similar rates regardless of the duration of treatment. Following discontinuation of 3 and 6 wk of treatment, clinical scores no longer differed as compared to untreated controls after 5 and 4 wk respectively (Figure 3).

The same group of *C. bovis*-negative controls were used throughout both experiment 1 and 2 (Figure 4A). At 5 wk after exposure to *C. bovis*, early clinical signs were easily identified by animal care staff and researchers[6] (Figure 4B). Clinical signs had decreased noticeably after 3 wk of antibiotic treatment (Figure 4C) in treated as compared with untreated mice, whose clinical disease had progressed (Figure 4D). The clinical signs in both the 3 and 6 wk amoxicillin treatment groups (Figure 4E) were comparable to those of untreated mice at 16 wk after exposure (Figure 4F).

## Discussion

This study evaluated amoxicillin treatment of *C. bovis* infection and associated clinical disease in NSG mice. The results showed that NSG mice were responsive to continuous amoxicillin treatment (Figure 3), as demonstrated by reduced severity of clinical disease (Figure 4C) and the potential for clinical resolution after 6 wk of treatment (Figure 2). Our study also demonstrated that in addition to a decrease in clinical severity, continuous treatment with amoxicillin blocks clinical signs of *C. bovis* infection for as long as 6 wk in NSG mice (Figure 3). After discontinuation of treatment, rates of reemergence of clinical signs were similar (4-5 wk) and occurred regardless of treatment duration. Based on these results, we conclude that clinical signs will be controlled for up to 6 wk of continuous amoxicillin treatment and speculate that signs will be controlled as long as treatment continues. When antibiotics are discontinued, clinical signs will likely reemerge. Collectively, these data demonstrate the utility and the limitation of amoxicillin treatment for *C. bovis*-associated clinical disease in NSG mice.

Prior analyses of *C. bovis* isolates collected across the United States showed that all rodent isolates cluster genetically and distinctly from human and bovine *C. bovis* isolates.[4] Similarly, antimicrobial susceptibility testing of *C. bovis* isolates collected from rodents over a wide geographic and temporal range were all found to be susceptible to amoxicillin at a minimum inhibitory concentration of 0.25 µg/mL or less for 90% of the population.[6] Given that this measure is related to the efficacy of treatment, therapeutic blood plasma levels of amoxicillin can be achieved in mice with oral administration at reasonable concentrations in drinking water.[15] Despite clear evidence of antimicrobial susceptibility, the ability to achieve therapeutic plasma levels and clinical resolution with treatment, as demonstrated in our current study, introduces a conundrum due to the inability of amoxicillin to completely eliminate *C. bovis* from infected NSG mice. Previous studies have investigated the elimination of *C. bovis* with long-term, high-dose antibiotics, with little success. Prolonged treatment of subclinical *C. bovis* infected athymic nude mice with 4 or 8 wk of amoxicillin-containing diet resulted in 78% of mice as *C. bovis* culture-negative within 3 wk of treatment. However, only 13.5% of mice remained *C. bovis* culture-negative after discontinuation of treatment.[1] Another study performed by our group[19] examined prolonged treatment of *C. bovis*-positive NSG breeding pairs for approximately 7 wk with drinking water that contained both amoxicillin and clavulanic acid. Treatment began at the time of breeder pairing and continued until litters were weaned. Ten weeks after discontinuation of treatment, only 57% of breeders were *C. bovis* PCR-negative.

The outcomes of our current study's are encouraging because they provide evidence that maintenance of amoxicillin treatment can resolve clinical signs of *C. bovis* during the period of treatment. However, despite achieving the desired effect, antibiotics can have many off-target effects. As a result, the application of our results may be limited due to concern about antibiotic effects on the skin microbiota, based on our previous work which revealed loss of skin bacterial diversity in NSG and nude mice after oral administration of amoxicillin.[12] In addition,

antibiotics, including amoxicillin, influence the gut microbiome and alter bacterial diversity in immunodeficient CD1 mice.[10] In mouse tumor models, antibiotic-induced perturbation of the gut microbiota may affect tumor progression and the response to cancer therapy.[3,8,9,11,16] These issues should be weighed against the effects of untreated *C. bovis* in the context of weight loss and progressive clinical disease, as shown in NSG mice. We did not track *C. bovis* DNA skin burden by quantitative PCR analysis in the course of this study. Given previous reports[1, 19], we did not expect to achieve complete elimination of *C. bovis* from mice treated with oral amoxicillin, and a sequential decrease and increase in *C. bovis* DNA copies on the skin with amoxicillin based treatment and removal has already been demonstrated.[19] Rather than focusing on subclinical or asymptomatic infections mice, our goal in the current study was to focus on clinical signs of infection in conjunction with amoxicillin treatment.

*C. bovis* outbreaks in immunocompromised mice can negatively impact research and animal health. An efficient *C. bovis* surveillance program is critical to maintain the health of research mice and promote research validity. Our surveillance strategy involves routine monitoring of *C. bovis* in mice, followed by culling, isolating, and potentially treating positive cases in order to reduce or exclude the agent. Our findings here demonstrate the efficacy of amoxicillin-medicated drinking water, a simple and inexpensive treatment, to resolve and prevent reemergence of clinical signs associated with *C. bovis* infection in NSG mice during treatment. Collectively, our data provide veterinarians and researchers with an additional option for the management of *C. bovis*-infected mouse colonies and individual clinical cases.

## Acknowledgments

This project was funded in part by Grants for Laboratory Animal Science (GLAS) and the Office of Laboratory Animal Resources at the University of Colorado Anschutz Medical Campus. This project was completed as part of the University of Colorado Anschutz Medical Campus Internship in Laboratory Animal Medicine. We thank animal care technician Rachel Livingston and her colleagues for their compassionate care of the mice used in this study.

## References

1. **Burr HN, Lipman NS, White JR, Zheng J, Wolf FR.** 2011. Strategies to prevent, treat, and provoke *Corynebacterium*-associated hyperkeratosis in athymic nude mice. J Am Assoc Lab Anim Sci **50**:378–388.
2. **Burr HN, Wolf FR, Lipman NS.** 2012. *Corynebacterium bovis*: Epizootiologic features and environmental contamination in an enzootically infected rodent room. J Am Assoc Lab Anim Sci **51**:189–198.
3. **Chambers LM, Esakov Rhoades EL, Bharti R, Braley C, Tewari S, Trestan L, Alali Z, et al.** 2022. Disruption of the gut microbiota confers cisplatin resistance in epithelial ovarian cancer. Cancer Res **82**:4654–4669. https://doi.org/10.1158/0008-5472.CAN-22-0455.
4. **Cheleuitte-Nieves C, Gulvik CA, McQuiston JR, Humrighouse BW, Bell ME, Villarma A, Fischetti VA, et al.** 2018. Genotypic differences between strains of the opportunistic pathogen *Corynebacterium bovis* isolated from humans, cows, and rodents. PLoS One **13**:e0209231. https://doi.org/10.1371/journal.pone.0209231.
5. **Clifford CB, Walton BJ, Reed TH, Coyle MB, White WJ, Amyx HL.** 1995. Hyperkeratosis in athymic nude mice caused by a coryneform bacterium: Microbiology, transmission, clinical signs, and pathology. Lab Anim Sci **45**:131–139.
6. **Fagre AC, Pugazhenthi U, Cheleuitte-Nieves C, Crim MJ, Henderson KS, Fong DL, Leszczynski JK, et al.** 2021. Antimicrobial susceptibility of *Corynebacterium bovis* isolates from immunodeficient rodents. Comp Med **71**:210–214. https://doi.org/10.30802/AALAS-CM-20-000107.
7. **Gerardo Mendoza J, Cheleuitte-Nieves C, Lertpiriyapong K, Wipf JRK, Arbona RJR, Miranda IC, Lipman NS.** 2022. Establishing the median infectious dose ($ID_{50}$) and characterizing the clinical manifestations of mouse, rat, cow, and human *Corynebacterium bovis* isolates in select immunocompromised mouse strains. bioRxiv:2022.2011.2010.515978.
8. **Kim J, Lee HK.** 2021. The role of gut microbiota in modulating tumor growth and anticancer agent efficacy. Mol Cells **44**:356–362. https://doi.org/10.14348/molcells.2021.0032.
9. **Lee JG, Eun CS, Jo SV, Lee AR, Park CH, Han DS.** 2019. The impact of gut microbiota manipulation with antibiotics on colon tumorigenesis in a murine model. PLoS One **14**:e0226907. https://doi.org/10.1371/journal.pone.0226907.
10. **Lekang K, Shekhar S, Berild D, Petersen FC, Winther-Larsen HC.** 2022. Effects of different amoxicillin treatment durations on microbiome diversity and composition in the gut. PLoS One **17**:e0275737. https://doi.org/10.1371/journal.pone.0275737.
11. **Liu X, Chen Y, Zhang S, Dong L.** 2021. Gut microbiota-mediated immunomodulation in tumor. J Exp Clin Cancer Res **40**:221. https://doi.org/10.1186/s13046-021-01983-x.
12. **Manuel CA, Johnson LK, Pugazhenthi U, Fong DL, Fink M, Habenicht LM, Leszczynski JK, et al.** 2022. Effect of antimicrobial prophylaxis on *Corynebacterium bovis* infection and the skin microbiome of immunodeficient mice. Comp Med **72**:78–89. https://doi.org/10.30802/AALAS-CM-21-000082.
13. **Manuel CA, Pearson EC, Pugazhenthi U, Fink MK, Habenicht LM, Fong DL, Leszczynski JK, et al.** 2022. A clinical scoring systems for the evaluation of *Corynebacterium bovis*-associated disease in NSG mice. Comp Med **72**:386–393. https://doi.org/10.30802/AALAS-CM-22-000098.
14. **Manuel CA, Pugazhenthi U, Spiegel SP, Leszczynski JK.** 2017. Detection and elimination of *Corynebacterium bovis* from barrier rooms by using an environmental sampling surveillance program. J Am Assoc Lab Anim Sci **56**:202–209.
15. **Marx JO, Vudathala D, Murphy L, Rankin S, Hankenson FC.** 2014. Antibiotic administration in the drinking water of mice. J Am Assoc Lab Anim Sci **53**:301–306.
16. **McKee AM, Kirkup BM, Madgwick M, Fowler WJ, Price CA, Dreger SA, Ansorge R, et al.** 2021. Antibiotic-induced disturbances of the gut microbiota result in accelerated breast tumor growth. iScience **24**:103012. https://doi.org/10.1016/j.isci.2021.103012.
17. **Miedel EL, Ragland NH, Engelman RW.** 2018. Facility-wide eradication of *Corynebacterium bovis* by using PCR-validated vaporized hydrogen peroxide. J Am Assoc Lab Anim Sci **57**:465–476. https://doi.org/10.30802/AALAS-JAALAS-17-000135.
18. **Miedel EL, Ragland NH, Slate AR, Engelman RW.** 2020. Persistent *Corynebacterium bovis* infectious hyperkeratotic dermatitis in immunocompetent epidermal-mutant dep/dep mice. Vet Pathol **57**:586–589. https://doi.org/10.1177/0300985820922219.
19. **Pearson EC, Pugazhenthi U, Fong DL, Smith DE, Nicklawsky AG, Habenicht LM, Fink MK, et al.** 2020. Metaphylactic antibiotic treatment to prevent the transmission of *Corynebacterium bovis* to immunocompromised mouse offspring. J Am Assoc Lab Anim Sci **59**:712–718. https://doi.org/10.30802/AALAS-JAALAS-20-000005.
20. **Scanziani E, Gobbi A, Crippa L, Giusti AM, Pesenti E, Cavalletti E, Luini M.** 1998. Hyperkeratosis-associated coryneform infection in severe combined immunodeficient mice. Lab Anim **32**:330–336. https://doi.org/10.1258/002367798780559239.
21. **Vedder AR, Miedel EL, Ragland NH, Balasis ME, Letson CT, Engelman RW, Padron E.** 2019. Effects of *Corynebacterium bovis* on engraftment of patient-derived chronic myelomonocytic leukemia cells in NSGS mice. Comp Med **69**:276–282. https://doi.org/10.30802/AALAS-CM-18-000138.
22. **Watts JL, Rossbach S.** 2000. Susceptibilities of *Corynebacterium bovis* and *Corynebacterium amylocolatum* isolates from bovine mammary glands to 15 antimicrobial agents. Antimicrob Agents Chemother **44**:3476–3477. https://doi.org/10.1128/AAC.44.12.3476-3477.2000.