Journal of the American Association for Laboratory Animal Science
Copyright 2014
by the American Association for Laboratory Animal Science

Vol 53, No 5
September 2014
Pages 517–522

Elimination of *Pasteurella pneumotropica* from a Mouse Barrier Facility by Using a Modified Enrofloxacin Treatment Regimen

Justin W Towne,[1] April M Wagner,[1] Kurt J Griffin,[2] Adam S Buntzman,[2] Jeffrey A Frelinger,[2] and David G Besselsen[1,*]

Multiple NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}Tg(HLA-A2.1)Enge/Sz (NSG/A2) transgenic mice maintained in a mouse barrier facility were submitted for necropsy to determine the cause of facial alopecia, tachypnea, dyspnea, and sudden death. Pneumonia and soft-tissue abscesses were observed, and *Pasteurella pneumotropica* biotype Jawetz was consistently isolated from the upper respiratory tract, lung, and abscesses. Epidemiologic investigation within the facility revealed presence of this pathogen in mice generated or rederived by the intramural Genetically Engineered Mouse Model (GEMM) Core but not in mice procured from several approved commercial vendors. Epidemiologic data suggested the infection originated from female or vasectomized male ND4 mice obtained from a commercial vendor and then comingled by the GEMM Core to induce pseudopregnancy in female mice for embryo implantation. Enrofloxacin delivered in drinking water (85 mg/kg body weight daily) for 14 d was sufficient to clear bacterial infection in normal, breeding, and immune-deficient mice without the need to change the antibiotic water source. This modified treatment regimen was administered to 2400 cages of mice to eradicate *Pasteurella pneumotropica* from the facility. Follow-up PCR testing for *P. pneumotropica* biotype Jawetz remained uniformly negative at 2, 6, 12, and 52 wk after treatment in multiple strains of mice that were originally infected. Together, these data indicate that enrofloxacin can eradicate *P. pneumotropica* from infected mice in a less labor-intensive approach that does not require breeding cessation and that is easily adaptable to the standard biweekly cage change schedule for individually ventilated cages.

Abbreviations: GEMM, genetically engineered mouse model; IVC, individually ventilated cage; NSG, NOD SCID gamma.

Pasteurella pneumotropica is an opportunistic pathogen that is found in many research and commercial rodent colonies. This gram-negative coccobacillus infects a variety of rodents, including mice, rats, kangaroo rats, guinea pigs, hamsters, and gerbils.[3,22] Pathogenic strains include 2 biotypes, Heyl and Jawetz, with primary colonization in the oropharynx.[4,14] Although immunocompetent mice typically manifest a subclinical infection, *P. pneumotropica* can cause fatal clinical disease in immunocompromised mice. Clinical manifestations of *P. pneumotropica* infection include conjunctivitis, panophthalmitis, dacryoadenitis, orbital abscesses, pneumonia, otitis, mastitis, and genital tract infections.[1,3,4,6,13-15,22] Given the expanded use of genetically altered mice with undefined immune-system modulations, increased emphasis has been given to this potential pathogen in the research setting, particularly in mouse barrier facilities.

The primary route of transmission for *P. pneumotropica* is direct contact between animals.[3,4,22] Transmission of this pathogen indirectly to sentinel mice through exposure to soiled bedding is unreliable due to the organism's poor survival in the environment.[4,19] Therefore direct testing of colony mice may be required to accurately determine whether animals have been infected.[9] Bacterial isolation and PCR analysis are the most commonly used diagnostic tools to detect *P. pneumotropica* infections in research facilities.[4,17,18] *P. pneumotropica* grows optimally on blood agar media under aerobic conditions supplemented with 7% to 10% CO_2 at 37 °C and forms 0.5- to 1-mm, gray to yellow, smooth, nonhemolytic colonies.[4,18,22] In addition, Heyl and Jawetz biotypes can be differentiated with high sensitivity and specificity by PCR analysis using specific primer sets.[18]

Several methods have been used to eradicate *P. pneumotropica* from laboratory animal facilities.[5,8,10,12,13,20] Because of the possibility that pups might already have become infected in utero, Cesarean rederivation should be avoided as a solution.[4,12] Embryo transfer is a successful method to rid mice of bacterial and viral infections but is labor-intensive and costly.[5] Several prior reports have demonstrated that enrofloxacin (Baytril), a bactericidal, broad-spectrum, fluoroquinolone antibiotic that inhibits DNA gyrase, can eliminate *P. pneumotropica* from mice. Enrofloxacin administered in drinking water for 14 d at concentrations of 25.5 and 85 mg/kg body weight effectively eliminated *P. pneumotropica* from nonbreeding mice when the drinking water was changed daily.[8] Enrofloxacin administered in drinking water for 14 d at 40 to 50 mg/kg body weight followed by Cesarean rederivation effectively eliminated *P. pneumotropica* from infected mice.[12] In addition, antibiotic stability for 48 h in drinking water was demonstrated.[12] Enrofloxacin in acidified water (0.0125% concentration) provided to mice once weekly for 2 wk eliminated the detection of *P. pneumotropica* in experimentally infected mice for as long as 6 wk after treatment, and enrofloxacin in acidified or autoclaved water inhibited bacterial growth for as long as 14 d.[10] Enrofloxacin administered in drinking water for 14 d at 85 mg/kg body weight eliminated *P. pneumotropica* from a mouse colony when the water was changed once weekly; antibiotic stability was not directly assessed, and the breeding status of animals during treatment was not reported.[13] In each of these cited reports,[5,8,10,12,13] the treatment

Received: 02 Jan 2014. Revision requested: 31 Jan 2014. Accepted: 26 Feb 2014.
[1]University Animal Care and [2]Department of Immunobiology, University of Arizona, Tucson, Arizona.
[*]Corresponding author. Email: besselsd@email.arizona.edu

regimen was labor-intensive when applied to large populations of infected mice. The objective of the current study was to develop an easily implemented enrofloxacin treatment regimen that could reduce labor-intensiveness and cost when applied to elimination of *P. pneumotropica* from a large population of actively breeding mice of diverse strains and immunocompetency.

Materials and Methods

Barrier facility management. The BIO5 animal facility at the University of Arizona opened in December 2009 and is a production and research barrier facility for genetically engineered mouse (*Mus musculus*) models (GEMM). Stringent measures are in place to ensure that the facility stays pathogen free. Mouse strains can enter the facility in only 2 ways: 1) through approved commercial vendor sources (Harlan, Indianapolis, IN; The Jackson Laboratory, Bar Harbor, ME; Charles River Laboratories, Wilmington, MA; and Taconic, Hudson, NY), which provide comprehensive health reports that indicate negative status for all major pathogens including *Pasteurella pneumotropica*, or 2) by mandatory rederivation by embryo transfer through the GEMM Core of all mice from nonapproved sources (other research institutions). All mice are housed in sterile IVC with 30 to 35 intracage air exchanges hourly and are provided irradiated feed and hyperchlorinated, reverse-osmosis–purified water ad libitum from an automatic watering system. Animal holding rooms are maintained at 22 to 24 °C, at 40% to 60% humidity, and on a 14:10-h light:dark cycle. Cage changing occurs under HEPA-filtered workbenches (Lab Products, Seaford, DE) within the animal rooms on a biweekly schedule. Incoming supplies are either autoclaved or sterilized by vaporized hydrogen peroxide (VHP ARD System, Steris, Mentor, OH) in a sealable decontamination room.

Husbandry staff and researchers are required to enter the BIO5 facility prior to any other animal facility or areas where rodents are housed. Good hygiene is expected and a wet shower is required, either at home or within the facility, before the facility is entered each day. Personnel disrobe, put on freshly laundered scrubs and socks, enter through an air shower, and don facility-dedicated shoes. Personal protective equipment when working with mice consists of bonnet, surgical mask, disposable gown, and gloves. An integrated pest management program is in place to monitor for and eliminate vermin. A mouse health monitoring program is in place, with sentinel mice exposed to approximately 15 mL of soiled bedding from each cage at each biweekly cage change, with one sentinel cage per rack and each rack side changed on alternating weeks to maximize sentinel exposure. Sentinels are screened for common pathogens quarterly and undergo a comprehensive health assessment annually that includes histologic analysis and respiratory and enteric cultures. Excluded pathogens for this facility, which are targeted by the health monitoring program, include mouse hepatitis virus, minute virus of mice, mouse parvovirus, Sendai virus, Theiler mouse encephalomyelitis virus, epizootic diarrhea of infant mice, pneumonia virus of mice, reovirus, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus types 1 and 2, polyoma virus, mouse norovirus, mouse cytomegalovirus, *Mycoplasma pulmonis*, *Corynebacterium kutscheri*, *Pasteurella pneumotropica*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, cilia-associated respiratory bacillus, *Citrobacter rodentium*, *Clostridium piliforme*, *Salmonella* spp., *Helicobacter* spp., *Encephalitozoon cuniculi*, fur mites (*Radfordia*, *Myocoptes*), lice (*Polyplax*), pinworms (*Aspiculuris*, *Syphacia*), cestodes (*Rodentolepis*, *Taenia*), and coccidia (*Klossiella*). All procedures and treatments performed on mice involved in this case report were in accord with standard clinical veterinary care practices at the University of Arizona.

Microbiology. Mice displaying clinical signs were euthanized by CO_2 inhalation. Culture samples were collected from the nasopharynx and lesioned organs, streaked onto 5% sheep blood agar plates, and incubated at 37 °C in a 7% CO_2 environment. Isolates were gram-stained, and suspect colonies were identified by using the RapID NF Plus System biochemical assay (Thermo Fisher Scientific, Lenexa, KS). Isolates were frozen in cryogenic vials containing plastic beads and *Brucella* broth with 10% glycerol (Hardy Diagnostics, Santa Maria, CA) and were stored in a −80 °C freezer.

PCR and biotyping. DNA was extracted from the applicators used to sample the oropharyngeal region of the mice and analyzed by a *P. pneumotropica*-specific PCR assay as previously described.[18] Briefly, DNA was extracted (Ambion MagMAX Viral RNA Isolation Kit, Life Technologies, Grand Island, NY) by using a robotic extraction station (KingFisher, Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's recommendations. RT-PCR and PCR reactions were performed by using a thermocycler (GeneAmp 9700, Life Technologies). The reverse-transcription reaction consisted of 1× *Taq*Man buffer (50 mM KCl, 10 mM Tris-HCl [pH 8.3]); 5.5 mM $MgCl_2$; 500 µM each dATP, dCTP, dGTP, and dTTP; 8 U RNase inhibitor; 25 U MuLV reverse transcriptase; 2.5 µM random hexamers (Life Technologies); and 2 µL template DNA in a 20-µL reaction. Reaction conditions were 25 °C for 10 min for hexameric primer annealing and extension, reverse transcription at 48 °C for 30 min, and 95 °C for 5 min for reverse transcriptase inactivation. Each 25-µL PCR reaction consisted of 1× *Taq*Man buffer (50 mM KCl, 10 µM EDTA, 10 mM Tris-HCl [pH 8.3]) and 60 nM passive reference); 5.5 mM $MgCl_2$; 200 µM each dATP, dCTP, and dGTP; 400 µM dUTP; 300 nM each primer; 0.2 U of AmpErase uracil-N-glycosylase; 0.625 U Ampli*Taq* Gold polymerase (Life Technologies); and 2.5 µL cDNA from the reverse-transcription step. Thermal cycling conditions included polymerase activation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s. PCR products were analyzed by gel electrophoresis on an UV illuminator (Fotodyne, Harland, WI) with an imaging system (Kodak Gel Logic 100, Carestream Health, Rochester, NY) and accompanying software. Samples were considered positive when they produced a detectable band identical to the positive control (279 bp). Each PCR assay included a no-template control, oropharyngeal swab DNA from an uninfected mouse (negative control), and DNA extracted from a culture of *P. pneumotropica* biotype strain Jawetz (positive control).

To biotype the bacterial isolates, *P. pneumotropica* 16S DNA was PCR-amplified by using primer sequences kindly provided by Dr Robert Livingston (IDEXX BioResearch, Columbia, MO). The 533-bp amplicon was electrophoresed through a 2% NuSieve agarose gel, and the target band was eluted by using a QIAquick kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Amplicon DNA was sequenced by the University of Arizona Genetics Core, and the nucleotide sequence was aligned and compared with those from other *Pasteurella* spp. by using the ClustalW and Pretty software programs (Genetics Computer Group, Madison, WI). GenBank accession numbers for the *Pasteurella* sequences included in the analyses are: M75083.1 (Jawetz), GU809174 (Heyl), and AY465373 (*Pasteurella aerogenes*).

Antibiotic stability in drinking water. An automated water-packaging system (Hydropac AWS-2500, Lab Products, Seaford, DE) was used to prepare water pouches that contained 390 mL

of reverse-osmosis–purified water. Three water pouches were prepared to assess enrofloxacin stability: one injected with 2.25 mL of isotonic saline solution (negative control), one injected with 2.25 mL of 100 mg/mL enrofloxacin (equivalent to a daily dose of 85 mg/kg body weight) that was agitated daily, and one injected with 2.25 mL of 100 mg/mL enrofloxacin that was not disturbed for the duration of the stability experiment. Agitated and nonagitated water pouches were evaluated to determine whether the enrofloxacin eventually settled out of solution, affecting the efficacy of the treatment. The stability of antibiotics in water bottles was not tested, because the contaminated facility uses an automated water system, with water pouches used only as needed.

The original isolates of *P. pneumotropica* were reestablished from frozen stock and tested for purity. A 0.5 MacFarland standard was prepared, and a bacterial lawn was seeded onto Mueller–Hinton agar plates (Hardy Diagnostics). Each day, 1 mL of water was removed from each water pouch and placed in a test tube that contained a sterile, 6-mm blank paper disc (Becton-Dickinson, Sparks, MD). The disc was allowed to incubate at room temperature for 10 min. Impregnated discs were removed, blotted dry, and placed on a Mueller–Hinton agar plate that was split into quadrants. A disc impregnated with 5 µg ciprofloxacin (Hardy Diagnostics) was used as the positive control. Mueller–Hinton agar plates were placed in a 37 °C incubator with 7% CO_2 for optimal growth. After 24 h, plates were analyzed, and the zones of inhibition were measured and recorded (Figure 1). Sensitivity analysis was performed daily for 14 d and then every few days thereafter until a total of 21 d.

Evaluation of the modified enrofloxacin treatment regimen. After investigator consent was obtained, validation studies were performed on 3 *P. pneumotropica*-positive mouse colonies. One colony comprised immunocompetent, nonbreeding TRPV5 (Transient Receptor Potential Vanilloid) mice (n = 6 cages); another consisted of immunocompetent, breeding colonies of MMTV-myr-Akt1 and Atg5fl/fl;Aqp5-Cre mice (n = 25 cages); and the third was an immune-compromised, breeding colony of NSG mice transgenic for the human MHC-I A2 allele (n = 14 cages). An enrofloxacin dose of 85 mg/kg was chosen on the basis of previous publications.[8,13] To calculate an 85-mg/kg daily dosage, water consumption was approximated at 15 mL per 0.1 kg of body weight daily. Therefore, a 0.57-mg/mL dilution of enrofloxacin was produced by injecting 2.25 mL of Baytril 100 (Bayer Healthcare, Shawnee Mission, KS) into prepared water pouches containing 390 mL. Silicone patches (Lab Products) were used at injection sites to prevent leaking. Mice then received enrofloxacin-treated drinking water for 14 d without changing or agitating the pouches. The oropharyngeal region of one mouse from each cage was swabbed with polyester-tipped applicators (Puritan Medical Products, Guilford, ME) at 0, 2, 6, and 12 wk after initiation of treatment and stored at −80 °C until further analysis.

Barrier facility treatment. A 14-d enrofloxacin treatment period was selected on the basis of antibiotic stability and demonstrated efficacy against *P. pneumotropica* and to coincide with the standard 14-d IVC cage-change interval. Researchers with mice in the barrier facility were contacted to ensure that the proposed antibiotic treatment would not affect their research. One researcher, whose research focused on a specific commensal gut bacterium that could be negatively affected by enrofloxacin administration, requested an exemption from treatment. After oropharyngeal swabs collected from mice in each cage were verified as uniformly PCR-negative for *P. pneumotropica*, this researcher's mice were isolated on a separate rack and excluded from enrofloxacin treatment. Facility-wide treatment of 21 IVC racks containing approximately 2400 cages (7200 mice) was implemented over the course of a standard 14-d cage-change cycle, with approximately 2 racks started on treatment on each weekday within the cycle. As each IVC rack was changed, the automatic-watering lines were disconnected and drained to ensure that mice had access to treated water only. Medicated water pouches were prepared as described for the validation study within 24 h of their placement in the mouse cages. Each clean mouse cage was provided enrofloxacin-treated water (85 mg/kg body weight daily dosage) as the sole water source until the next scheduled cage change (14 d later). Cages established during the 14-d treatment period for mice that were separated or weaned were also provided with medicated water pouches to ensure completion of the entire 14-d regimen. The integration of treatment during a normally scheduled cage change minimized additional labor by the husbandry technicians. Treatment of the entire facility was completed in 4 wk.

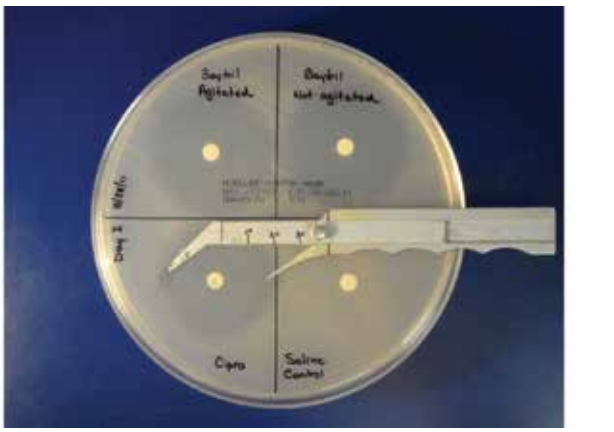

Figure 1. Mueller–Hinton agar plate split into quadrants for enrofloxacin sensitivity tests (day 2). Ciprofloxacin is the positive control, and saline is the negative control.

Case Report

Clinical presentation. Immunodeficient NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}Tg(HLA-A2.1)Enge/Sz (NSG/A2) mice that harbor the human MHC-I A2 transgene[21] were imported from another academic institution and were rederived by embryo transfer to enter the barrier facility in December 2010. The mice then underwent intravascular engraftment of MHC-matched human peripheral blood cells. Morbidity was first reported in April 2011, and affected mice were submitted for necropsy to determine the cause of facial alopecia, tachypnea, dyspnea, and sudden death. Gross necropsy and histopathology revealed suppurative pneumonia and pulmonary abscesses as the most common lesions in the mice that displayed respiratory difficulty or died suddenly. In addition, histopathology revealed lymphoplasmacytic interface dermatitis and vasculitis within internal solid organs, interpreted as evidence of graft-versus-host disease in these mice. Culturing of lesioned organs yielded a pure culture of small, light-gray bacterial colonies, shown to be gram-negative coccobacilli after staining. Similar colonies were consistently identified in nasopharyngeal cultures. These isolates biochemically speciated as *Pasteurella aerogenes* by the Remel RapID NF Plus System. Subsequent 16S ribotyping confirmed *P. pneumotropica* biotype Jawetz for all isolates, irrespective of the biochemical speciation. No additional pathogens were detected in necropsied mice. All sentinel health-monitoring reports since the facility opened in 2009 had been negative for all excluded pathogens, including *P. pneumotropica*.

Epidemiology. After the detection of *P. pneumotropica* in clinically affected NSG/A2 transgenic mice, additional NSG transgenic mice from the breeding colony were confirmed to be positive for *P. pneumotropica* according to oropharyngeal swab culture and PCR analysis. *P. pneumotropica* similarly was detected in 11 other mouse strains rederived or created by the GEMM Core during the same time period as when the NSG transgenic mice were rederived and thereafter. Nontransgenic NSG and other strains of mice directly imported from approved vendor sources were uniformly negative, including 4 mouse strains housed on the same racks that contained rederived mouse strains infected with *P. pneumotropica*. Ultimately a clear pattern was observed: strains that had been rederived by embryo transfer or generated by the GEMM Core were confirmed to be *Pasteurella*-positive, whereas all strains ordered directly from approved vendors were negative. Subsequently, *Pasteurella pneumotropica* biotype Jawetz was isolated by culture and detected by PCR in ND4 mice used routinely by the GEMM Core as pseuopregnant recipients for embryo implantation. Interestingly, *P. pneumotropica* was detected in 4 vasectomized ND4 male and 11 ND4 female mice that had been paired previously with the vasectomized male mice but not in 8 young ND4 female mice that had not been exposed to vasectomized male mice. Additional investigation revealed the GEMM Core continuously intermingled vasectomized male mice among available female mice to induce pseudopregnancy, with vasectomized male and female mice continuously ordered and replaced as needed. All ND4 mice used by the GEMM Core had been obtained from 3 different barriers of a single approved commercial vendor source. In addition, the animal holding room in which these mice were maintained was the first on the room order list for the facility, that is, it could not be reentered once another mouse room in the facility had been entered. Other mouse strains housed in the same room, C57BL/6Hsd (n = 6 cages) and B6D2F1/J (n = 3 cages) used for embryo/blastocyst production, were negative for *P. pneumotropica* by oropharyngeal culture and PCR analysis. ND4 vasectomized male and female mice (n = 6 mice) ordered from the same approved vendor used by the GEMM Core and tested directly for *P. pneumotropica* on arrival also were uniformly negative. A total of 27 mouse strains were tested to track the epidemiology of *P. pneumotropica*, with 60 of 198 cages confirmed to be PCR positive.

Results

Antibiotic stability of enrofloxacin in water. Water pouches were injected with 225 mg enrofloxacin per 390 mL drinking water (to provide 85 mg/kg body weight daily). Water samples removed from these pouches daily, adsorbed to sterile disks, and placed on a lawn of *P. pneumotropica* Jawetz on Mueller–Hinton plates successfully prevented bacterial growth in vitro for 14 d (Figure 2). There was no difference in efficacy between the agitated and nonagitated water pouches. Extended time points of 17 and 21 d were evaluated also and shown to be equally effective at inhibiting bacterial growth. According to manufacturer data, zones of inhibition of 21 mm or larger indicate that gram-negative bacteria are susceptible to enrofloxacin. The results of this experiment indicated antibiotic stability, with an average zone of inhibition of 40 mm that persisted for as long as 3 wk.

Evaluation of modified enrofloxacin treatment regimen. Three groups of mice were treated initially to test the efficacy of 14-d enrofloxacin treatment to eradicate *P. pneumotropica* from immunocompetent and immunodeficient breeding mice (Table 1). Group 1 consisted of immunocompetent, nonbreeding TRPV5 mice recently rederived by the GEMM Core. Group 2 was immunocompetent, actively breeding colonies of MMTV-myr-Akt1 and Atg5fl/fl Aqp5-Cre mice. The final group (group 3) was immunocompromised, actively breeding NSG/A2 transgenic mice and was the strain in which *P. pneumotropica* was detected initially within the barrier facility. All mice weaned during the treatment period were placed on enrofloxacin-treated water to complete the 14-d treatment period. A total of 34 (group 1, n = 6; group 2, n = 20; and group 3, n = 8), 30 (group 1, n = 6; group 2, n = 17; and group 3, n = 7), and 18 (group 1, n = 5; group 2, n = 6; and group 3, n = 7) cages of mice were evaluated at 2, 6, and 12 wk, respectively, after 14-d treatment with enrofloxacin in drinking water. The same cages of mice were tested at each time point, although sample numbers at later time points decreased due to colony attrition as animals were used in research experiments or culled without evidence of disease. DNA from oropharyngeal swabs from all cages and mice were evaluated for *P. pneumotropica* by PCR and were uniformly negative for the organism. Weanlings from these breeding cages (n = 36) were evaluated and were negative as well.

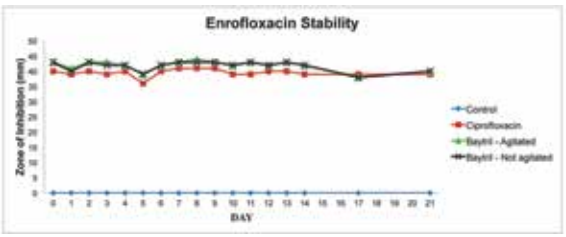

Figure 2. Inhibition zone measurements for enrofloxacin stability experiments.

Barrier facility treatment. The 11 GEMM strains previously confirmed to be positive for *P. pneumotropica* were evaluated at 2, 6, and 12 wk after enrofloxacin treatment was completed. All cages (n = 83) were uniformly negative by PCR analysis. At 12 wk after completing enrofloxacin treatment of the facility, random samples were collected from GEMM strains previously identified as positive for *P. pneumotropica* from all rooms in the barrier animal facility. No evidence of *P. pneumotropica* was detected by PCR analysis of the 100 cages tested. Oropharyngeal swab DNA collected from offspring of GEMM mouse strains originally identified as positive for *Pasteurella pneumotropica* (n = 75 cages) were evaluated at 52 wk after treatment and were uniformly negative by PCR analysis. Finally, after administration of the enrofloxacin treatment, no clinical symptoms or mortality have been observed in the NSG/A2 transgenic mouse colony infected originally.

Discussion

Despite many physical and operational safeguards, *P. pneumotropica* biotype Jawetz gained entry into a mouse barrier facility. The pathogen was not detected until clinical disease was observed in 'humanized' NSG transgenic mice. The source of infection was traced to ND4 vasectomized male and female mice used to generate pseudopregnant recipient female mice for embryo implantation. The intramural GEMM Core continuously ordered and replaced ND4 mice as needed, a practice that ultimately enabled amplification and transmission of *P. pneumotropica* by direct contact from one or a few infected mice to the entire ND4 mouse colony. Infected female mice used as recipients for embryo implantation led to a facility-wide epizootic through their use for production of new GEMM strains and embryo-transfer rederivation of mice from nonapproved sources. All ND4 mice used by the GEMM Core had been

Table 1. Treatment groups and time points for validation of modified enrofloxacin treatment

			After treatment			
Colony	Mice	Before treatment	2 wk	6 wk	12 wk	52 wk
Immunocompetent, nonbreeding	—	6/6[a]	0/6	0/6	0/5	0/8
Immunocompetent, breeding	Breeders	24/25	0/20	0/17	0/6	0/10[b]
	Weanlings	NT	0/12	NT	0/5	0/7
Immunodeficient, breeding	Breeders	11/14	0/8	0/7	0/7	0/7[b]
	Weanlings	NT	0/7	0/4	0/8	0/13

NT, not tested.
[a]No. of cages positive/no. of cages tested.
[b]Offspring of original breeders.

obtained from a single approved vendor source (3 different barriers, one surgical facility) throughout the Core's existence. The animal holding room that housed the GEMM Core's ND4 male and female mice was the first on the room-order list for the facility, that is, it could not be reentered after another mouse room in the facility had been entered. All other strains in the GEMM Core room were purchased from approved vendors, were housed on a separate IVC rack, and were not infected with *P. pneumotropica*. Additional epidemiologic investigation in the barrier facility revealed a pattern, with vendor-purchased mice uniformly negative and GEMM Core-derived mice uniformly positive for *P. pneumotropica*, even when mice from these different sources were housed in cages immediately adjacent on a rack. This cage-level correlation indicates that husbandry procedures maintained cage-level biosecurity and prevented cage-to-cage transmission of *P. pneumotropica* among mouse strains. Given the stringent physical and operational barriers in the facility and these epidemiologic data, we surmise that *P. pneumotropica* entered the facility through contaminated ND4 mice obtained from the approved vendor source. Unfortunately, this hypothesis could not be definitively proven through subsequent testing of purchased ND4 mice from the same vendor barriers or surgical facility. Regardless, several changes were implemented to minimize the potential for recurrence of pathogen entry into this barrier. These included a different vendor source for the mice used as recipients for embryo implantation; GEMM Core personnel now vasectomize the male mice used to induce pseudopregnancy in female recipient mice; and monthly health monitoring is performed on cull animals from the GEMM Core recipient mouse colony. An internal breeding colony that would supply mice to the GEMM Core for embryo implantation and produce sentinel mice for the health monitoring program is being established to maximize biosecurity.

P. pneumotropica is difficult to detect in contemporary mouse colonies for a variety of reasons. It is optimally transmitted by direct contact between mice; therefore transmission to sentinel mice via soiled bedding exposure is an unreliable method of detection, as exemplified by the current case. Although nasopharyngeal cultures are commonly used to efficiently detect a plethora of bacterial infections, nasopharyngeal cultures are less sensitive than are oropharyngeal cultures at detecting *Pasteurella pneumotropica*.[16] In addition, inherent problems exist with biochemical assays for microbial speciation, because atypical strains of *P. pneumotropica* may react variably to individual biochemical assays.[2] This characteristic leads to variable bacterial speciation, as noted in the current case, in which the isolates were speciated initially as *P. aerogenes* but subsequent ribotyping revealed them to be *P. pneumotropica* Jawetz. In addition, identification by using culture and biochemical methods is time consuming. PCR analysis is currently the most specific and sensitive diagnostic assay available for the detection of *P. pneumotropica* in laboratory mice and was used extensively in the current case for epidemiologic analysis and posttreatment assessment. A quantitative PCR assay sensitive and specific for the pathogenic Jawetz biotype of *P. pneumotropica* was evaluated initially[7] but failed to discriminate *P. pneumotropica* from *Proteus mirabilis*. An alternate gel-detection PCR assay subsequently evaluated was confirmed to be as sensitive and specific as described in the original publication.[18] In addition, the sample used as the source of DNA for PCR detection of *P. pneumotropica* is an important factor. PCR sensitivity increased 300-fold when oropharyngeal swab DNA was used as compared with fecal DNA,[16] a finding confirmed by the current investigation (data not shown). PCR analysis of oropharyngeal swab DNA was therefore used to detect *P. pneumotropica*-infected mice throughout the current study.

Previous reports have demonstrated the effectiveness of administering enrofloxacin in drinking water to eliminate *P. pneumotropica* infections when this treatment was combined with rederivation of animals or frequent replacement of antibiotic-treated water.[8,10,12,13,20] The current study validated the efficacy and stability of enrofloxacin in drinking water, at a concentration that would provide a daily dose of 85 mg/kg body weight, for as long as 21 d. Pilot studies demonstrated that mice remained negative for *P. pneumotropica* at 2, 6, and 12 wk after enrofloxacin treatment. Subsequent barrier-wide treatment of approximately 2400 cages (7200 mice) with actively breeding populations of mice and a variety of infected mouse strains, including several with severe immune deficiencies, was completed in 4 wk without changing medicated water pouches between biweekly cage changes. One year after treatment was completed, randomly sampled cages (2.5% of the total cage census in the barrier) that contained offspring of the mouse strains originally confirmed to be infected by *P. pneumotropica* remained negative for this pathogen. An untreated infected control group was not included in this case report given that the intent of the veterinary intervention was to eradicate the bacterium from the entire barrier facility. However it is highly unlikely that simply changing the mice from automated water to water pouches without the addition of enrofloxacin would have eliminated *P. pneumotropica* from the facility.

The modified enrofloxacin treatment regimen effectively eradicated *Pasteurella pneumotropica* from a mouse barrier facility that contained a large population of breeding, immunocompromised mice. Coordination with the normal cage change schedule allowed easy implementation and minimal inconvenience to the investigators who use the facility. As compared

with previously published reports,[8,10,13] the currently described enrofloxacin treatment regimen is less labor-intensive due to the decreased replacement frequency of medicated water pouches. An additional refinement could be applied to this enrofloxacin treatment regime if auto-water ventilated racks are available. A recent report described the use of a modified carboy attached to a ventilated rack to deliver ivermectin-treated water through the automated-watering system to eliminate pinworms.[11] Incorporating this novel approach to the delivery of medicated water could further reduce labor costs associated with enrofloxacin treatment of large mouse populations.

Acknowledgments

We thank Dr Robert Livingston for providing the *Pasteurella* spp. primer sequences, Dr Carlos Reggiardo for critical review of this paper, and Jessie Loganbill for technical advice and assistance.

References

1. **Artwohl JE, Flynn JC, Bunte RM, Angen O, Herold KC.** 2000. Outbreak of *Pasteurella pneumotropica* in a closed colony of STOCK-Cd28 (tm1Mak) Mice. Contemp Top Lab Anim Sci **39:**39–41.
2. **Baron EJ.** 1996. Classification. In: Baron S, editor. Medical microbiology, 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston.
3. **Brennan PC, Fritz TE, Flynn RJ.** 1969. Role of *Pasteurella pneumotropica* and *Mycoplasma pulmonis* in murine pneumonia. J Bacteriol **97:**337–349.
4. **Charles River Laboratories.** [Internet]. 2009. Technical sheet on *Pasteurella pneumotropica*. [Cited2 January 2014]. Available at: http://www.criver.com/SiteCollectionDocuments/rm_ld_r_Pasteurella_pneumotropica.pdf
5. **Charles River Laboratories.** [Internet]. 2009. Technical sheet on rederivation. [Cited 2 January 2014]. Available at: http://www.criver.com/en-us/prodserv/bytype/genengmodels/embryology/pages/ rederivation.aspx
6. **Dickie P, Mounts P, Purcell D.** 1996. Myopathy and spontaneous *Pasteurella pneumotropica*-induced abscess formation in an HIV1 transgenic mouse model. J Acquir Immune Defic Syndr Hum Retrovirol **13:**101–116.
7. **Dole VS, Banu LA, Fister RD, Nicklas W, Henderson KS.** 2010. Assessment of *rpoB* and *16S rRNA* genes as targets for PCR-based indentification of *Pasteurella pneumotropica.* Comp Med **60:**427–435.
8. **Goelz MF, Thigpen JE, Mahler J, Rogers WP, Locklear J, Weigler BJ, Forsythe DB.** 1996. Efficacy of various therapeutic regimens in eliminating *Pasteurella pneumotropica* from the mouse. Lab Anim Sci **46:**280–285.
9. **Hansen AK.** 2000. Handbook of laboratory animal bacteriology. Boca Raton (FL): CRC Press.
10. **Harlev M, Mujahed M, Waner T, Kalman R.** 2009. A novel protocol for treatment and elimination of *Pasteurella pneumotropica* infection from rodent colonies with subclinical latent infection. Israel J Vet Med **64:**108–113.
11. **Hickman D, Swan M, Hartman GP.** 2008. A cost-effective and efficacious method of pinworm treatment for large colonies of mice. Lab Anim (NY) **37:**308–312.
12. **Macy JD Jr, Weir EC, Compton SR, Shlomchik MJ, Brownstein DG.** 2000. Dual infection with *Pneumocystis carinii* and *Pasteurella pneumotropica* in B-cell–deficient mice: diagnosis and therapy. Comp Med **50:**49–55.
13. **Matsumiya LC, Lavoie C.** 2003. An outbreak of *Pasteurella pneumotropica* in genetically modified mice: treatment and elimination. Contemp Top Lab Anim Sci **42:**26–28.
14. **Mikazuki K, Hirasawa T, Chiba H, Takahashi K, Sakai Y, Ohhara S, Nenui H.** 1994. Colonization pattern of *Pasteurella pneumotropica* in mice with latent pasteurellosis. Jikken Dobutsu **43:**375–379.
15. **Moore GJ, Aldred P.** 1978. Treatment of *Pasteurella pneumotropica* abscesses syndrome in a mouse colony. Lab Anim **12:**227–228.
16. **Myles MH.** 2011. Quarantine challenges in identifying bacterial pathogens. In: 62nd Annual AALAS National Meeting Program. Memphis (TN): American Association for Laboratory Animal Science.
17. **Ouellet M, Cowan M, Laporte A, Faubert S, Heon H.** 2011. Implementation of a PCR assay of *Pasteurella pneumotropica* to accurately screen for contaminated laboratory mice. Lab Anim (NY) **40:**305–312.
18. **Patten CC, Myles MH, Franklin CL, Livingston RS.** 2010. Perterbations in cytokine gene expression after innoculation of C57BL/6 mice with *Pasteurella pneumotropica.* Comp Med **60:**18–24.
19. **Scharmann W, Heller A.** 2001. Survival and transmissibility of *Pasteurella pneumotropica.* Lab Anim **35:**163–166.
20. **Ueno Y, Shimizu R, Nozu R, Takahashi S, Yamamoto M, Sugiyama F, Takakura A, Itoh T, Yagami K.** 2002. Elimination of *Pasteurella pneumotropica* from a contaminated mouse colony by oral administration of enrofloxacin. Exp Anim **51:**401–405.
21. **Whitfield-Larry F, Young EF, Talmage G, Fudge E, Azam A, Patel S, Largay J, Byrd W, Buse J, Calikoglu AS, Shultz LD, Frelinger JA.** 2011. HLA-A2–matched peripheral blood mononuclear cells from type 1 diabetic patients, but not nondiabetic donors, transfer insulitis to NOD-*scid*/γc^{null}/HLA-A2 transgenic mice concurrent with the expansion of islet-specific CD8+ T cells. Diabetes **60:**1726–1733.
22. **Williford CL, Boivin GP.** 1994. *Pasteurella pneumotropica*, p 145. In: Manual of microbiologic monitoring of laboratory animals, 2nd ed. Bethesda (MD): National Institutes of Health.