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Outbreak of Otitis Media Caused by *Burkholderia gladioli* Infection in Immunocompromised Mice

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An athymic nude mouse with severe head tilt due to otitis media was identified. Within weeks of identification of this first case, immune-deficient mice of various genotypes from the same facility were similarly affected, and cases from other facilities were found within two months. Culture of ear exudate specimens from affected mice yielded bacteria that were initially identified as *Burkholderia cepacia*, a plant pathogen considered an important opportunistic pathogen in persons with cystic fibrosis or chronic granulomatous disease. Several of these isolates, however, were subsequently identified as *B. gladioli* on the basis of results of biochemical analysis and a species-specific polymerase chain reaction (PCR) assay. Genotyping analysis revealed clonality among the isolates, indicating a shared strain among affected mice. A 16S rDNA-based PCR assay specific for the genera *Burkholderia* and *Ralstonia,* and a selective culture medium were used in efforts to characterize the epidemiology of this outbreak. In addition to culture of specimens from the oropharyngeal cavity of affected mice, samples were obtained from the environment, feces, sipper tubes, drinking water, and soiled bedding from cages of affected individuals. *Burkholderia gladioli* was most consistently detected in oropharyngeal swab specimens from affected mice. The PCR assay was equivalent to selective culture in identifying mice in the carrier state that did not have clinical signs of infection. However, neither detection method had sufficient sensitivity to reliably identify all carrier mice, causing the organism to persist at low levels unless entire colonies of immune-deficient mice were removed. The organism was highly resistant to antibiotic therapy. The source and epidemiology of this organism remain unknown. This epizootic serves as an important reminder that immunocompromised rodent colonies may harbor important human opportunistic pathogens.

Burkholderia gladioli, an aerobic, motile, nonfermenting, gram-negative rod (11), was originally described in 1921 as a phytopathogen of gladioli and other flowers (21). Although initially thought to be a harmless commensal organism, *B. gladioli* has since been associated with human infection, particularly among persons with cystic fibrosis (1, 4, 5, 14-16, 33), or chronic granulomatous disease (13, 24). Pneumonia in immunocompromised patients (11), bacteremia (11, 28), cervical lymphadenitis (11), and most recently, keratitis/endophthalmitis (23), also have been reported. The pathogenesis of *B. gladioli* closely follows that of *B. cepacia*, which is responsible for severe respiratory failure in approximately 20% of infected cystic fibrosis patients and pneumonia and septicemia in persons with chronic granulomatous disease (8). Causative factors include immunosuppression and/or complement deficiencies.

Other than a single report of *B. cepacia* isolated from a herd of sheep with subclinical mastitis (2), infections caused by members of the *B. cepacia* complex have not been reported in animals. To date and to our knowledge, there are no published reports of *B. gladioli* infection in non-human species. Taxonomic identification of *Burkholderia*-like organisms is complex, and the genus itself now includes 22 species. Coenye and coworkers provided a phylogenetic tree that is based on 16S rRNA gene sequences, documenting the relationship of all *Burkholderia* species and representatives of related genera, such as *Ralstonia* and *Pandoraea* (8).

Head tilt due to otitis media is not an uncommon finding in immunocompromised mouse colonies, and is most often associated with opportunistic pathogens, such as *Pasteurella pneumotropica* and *Mycoplasma pulmonis* (10). Otitis media due to *Pseudomonas aeruginosa* also has been reported (18). At our institution, an athymic nude mouse was presented for routine necropsy due to a pronounced debilitating head tilt. The ear canal contained a caseated purulent exudate that was initially identified as *B. cepacia* at the University of Virginia Microbiology laboratory, and subsequently was identified as *B. gladioli* by the University of Michigan *Burkholderia cepacia* Research Laboratory and Repository (BCRLR). Other cases with similar presentation, all in immunocompromised mice, proceeded to appear. The outbreak reached a plateau of several mice a day being found with head tilt. Affected mice were scattered in different colonies and, within two months, cases were diagnosed in mice housed in two different buildings. Diagnosis and management of this unusual outbreak are described.

Materials and Methods

Colony management. All mice impacted by this epizootic were housed under barrier conditions consisting of autoclaved caging, corncob bedding, water, and irradiated rodent diet (Harlan Teklad 7912, Madison, Wis.). Personnel with access to the mice wore protective clothing and were required to handle

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the mice in HEPA-filtered laminar flow workstations or biosafety cabinets. Mice were transferred by use of forceps during cage changes, and staff applied disinfectant to gloves between cage handlings. Mice came from multiple sources, including several commercial vendors and in-house breeding colonies. There is no separation of these within the facility. All immunocompromised mice in these colonies are prophylactically treated for *Pneumocystis carinii*, using trimethoprim-sulfamethoxazole (Cotrimoxazole [Gensia Sicor Pharmaceuticals, Inc., Irvine, Calif.]; 37.5 mg of sulfamethoxazole and 7.5 mg of trimethoprim per ounce of drinking water). This is administered on an every-other-week basis. When no drugs are being given, acidified drinking water (pH 2.6 to 2.7) is administered.

The colony health status is monitored quarterly, using a sentinel program, and the colony has tested as being free of *M. pulmonis*, pneumonia virus of mice, Sendai virus, mouse parvovirus, mouse hepatitis virus, minute virus of mice, Theiler's murine encephalomyelitis virus, epizootic diarrhea of infant mice, pinworms, and fur mites. The colonies are additionally tested annually and been found negative for antibodies to ectromelia, mouse thymic virus, reovirus 3, lymphocytic choriomeningitis, mouse cytomegalovirus, polyoma, K-virus, and mouse adenovirus. The presence of bacterial opportunistic pathogens, such as *Pasteurella pneumotropica*, *Pseudomonas aeruginosa*, and *Helicobacter* spp., are not routinely monitored for as part of the health surveillance program.

Sentinel mice were typically ICR or Swiss-Webster mice purchased from a commercial vendor, and were group housed in cages without filter tops that received dirty bedding from other cages on a weekly or twice weekly basis. Autoclave performance is routinely monitored by verification of sufficient temperature (heat sensitive tape and strips), and by biological testing of water samples (Verify, Steris Corp., Mentor, Ohio).

Necropsy and microbiologic testing. Mice presenting with typical signs of disease (pronounced head tilt, circling when held by the tail) were euthanized. Sterile swabs were used to collect oropharyngeal and/or middle ear specimens for microbial analysis. The skin was wiped with 70% ethyl alcohol, then was aseptically dissected away to reveal the tympanic bullae. The outer part of the ear canal was transected, and the contents of the middle ear were visualized. A swab was then used to sample ear exudate. Swabs were plated on blood agar plates (5% sheep RBC) and *B. cepacia*-selective agar (BCSA). Plates were incubated at 35°C and examined for growth at 24, 48, and 72 h. Specimens were also sent to the University of Virginia Microbiology laboratory for identification and antibiotic susceptibility testing.

Composition of BCSA follows the recipe developed by Henry and co-workers (12). The following ingredients were combined to make BCSA (per liter of distilled water): 5.0 g of sodium chloride, 10.0 g of sucrose, and 10.0 g of lactose (Sigma Chemical Co., St. Louis, Mo.); 0.08 g of phenol red (Sigma Chemical Co.); 0.002 g of crystal violet (Sigma Chemical Co.); 10.0 g of trypticase peptone, 1.5 g of yeast extract, and 14.0 g of agar (Difco, Becton Dickinson, Sparks, Md.). The phenol red and crystal violet were prepared as 10× aqueous solutions, and 10 ml of each was added per liter. The pH of the medium was 7.0 ± 0.1. After autoclaving for 20 min at 15 lb/in^2, 600,000 U of polymyxin (Sigma Chemical Co.), 10 mg of gentamicin (Boehringer Ingelheim, St. Joseph, Mo.), and vancomycin (2.5 mg/L; Sigma Chemical Co.) were added.

Histopathologic findings. Tissues specimens from representative mice were collected from the ear canal, lungs, liver, kidney, and spleen, preserved in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. When indicated, tissues were decalcified by use of standard procedures. Some sections were additionally stained with Gomori methenamine silver (GMS) or silver stain.

Isolation of genomic DNA. Bedding samples were processed as follows. Bedding material was added to a 50-ml conical tube to the 10-ml mark, and 40 ml of sterile saline was then added. The tube was shaken for 90 sec. The contents of the tube were then poured through 4 × 4-in. gauze layers into another sterile tube. This solution was centrifuged at 13,000 ×*g* for 10 min, the supernatant was discarded, and DNA was collected from the pellet. Samples from cage surfaces and from the inside of sipper tubes were collected, using a calcium alginate swab, cutting the tip off into a sterile tube, and extracting DNA. Bacterial genomic DNA was purified, using DNeasy kits (Qiagen, Valencia, Calif.) and stored at –20°C until further use.

Genus- and species-specific polymerase chain reaction (PCR) assays. A series of previously described genus- and species-specific PCR assays were used. These included an assay using primers RHG-F and RHG-R, which target 16S ribosomal DNA sequences specific for species within the genera *Burkholderia*, *Pandoraea*, and *Ralstonia* (6, 19), as well as a *recA*-directed assay specific for the nine species within the *B. cepacia* complex (20). A PCR assay using primers LP1 and LP4, targeting *B. gladioli*-specific 23S rDNA sequences also was used as described (31).

Novel genus-specific PCR assay. An additional PCR assay specific for the 16S rRNA gene, generic for *Burkholderia and Ralstonia* spp., was developed. A primer pair with sequences GGGATTCATTTCCTTAGTAACG (RHG-F) and CTTAGAGTGCTCTTGCGTAGC (Bcepacia-R) was used. Each 50-µl PCR reaction contained 200 m*M* each deoxynucleotide triphosphate, two units of *Taq* polymerase (Qiagen, Valencia, Calif) 1× concentration of the buffer supplied with the enzyme, 1.5 m*M* Mg^{2+}, 0.5 µ*M* of each oligonucleotide primer, and 100 ng of DNA sample. Mouse genomic DNA isolated from tail biopsy specimens was used as a negative control. Purified genomic DNA from *B. cepacia* strain ATCC25416 was used as positive control. The assay was run on a Robocycler 9600 gradient (Statagene, La Jolla, Calif.) thermal cycler set with the following parameters: 95°C, 14 min and 15 sec (Hotstar, Qiagen Valencia, Calif.) one cycle; 95°C for 45 sec, 60°C for one minute, 72°C for one minute, 35 cycles; and a final extension at 72°C for five minutes. The PCR reactions were analyzed by use of horizontal slab gel electrophoresis with 1.0% agarose (Life Technologies/Invitrogen, Carlsbad, Calif.) and Tris acetate EDTA buffer containing 0.5 µg of ethidium bromide/ml. The PCR products were photographed. Amplification of a band with molecular mass of 270 bp was considered positive for *Burkholderia*/*Ralstonia* spp.

Sequence analysis of 16S rDNA. The DNA was prepared as described (7). The nearly complete sequence (corresponding to positions 9–1,500 in the *Escherichia coli* numbering system) of the 16S rRNA gene was amplified by use of PCR analysis with conserved primers UFPL (5'-AGTTTGATCCTGGCTCAG-3') and URPL (5'-GGTTACCTTGTTACGACTT-3') as described (19). The PCR product was purified, using the Promega Wizard PCR Preps kit (Promega, Madison, Wis.) according to the manufacturer's in-

structions. Sequence analysis was performed, using an Applied Biosystems 3700 DNA sequencer and the protocols of the manufacturer (PE Applied Biosystems, Foster City, Calif.), and the BigDye Terminator Cycle Sequencing Ready Reaction kit. The sequencing primers were UFPL, URPL, 16SF1 (5'-GCCTTTCGGGTTGTAAAGCAC-3'), 16SF2(5'CCTTACCTACCCTTGACA-3'), 16SB1(5'GCGCTCGTTGCGGGACT-3') and 16SB2(5'-GTATTACCGCGGCTGCTG-3'). Sequence assembly was performed, using EditSeq (DNAStar Inc., Madison, Wis.). Phylogenetic trees that are based on the neighbor-joining method were constructed using the MegAlign (DNAStar, Inc., Madison, Wis.) software package.

Isolate genotyping. Random amplified polymorphic DNA typing was done as described (7).

Nucleotide accession number. The 16S rDNA sequence determined in this study was deposited in the GenBank database under accession number AY297695.

Management of the outbreak. Samples were collected from bedding, water sipper tubes, feces, oropharyngeal swabs, and cage surfaces to determine the presence of *Burkholderia* spp. in carrier mice and the environment. Swabs were streaked onto BCSA for microbial analysis, and DNA was purified and assayed by use of PCR analysis for molecular identification.

Mice identified with head tilt were removed from the colony immediately and were euthanized. On the basis of culture results and PCR testing, it was determined that swabbing the oropharyngeal cavity was the most effective method of detecting the organism. Therefore, cagemates of affected mice were tested by collecting oropharyngeal swab specimens and culturing them on BCSA. These cagemates were either euthanized or removed to an isolation room designated positive for *B. gladioli*. In rooms where a culture-positive mouse was identified, all other mice in that room also were tested via culture of an oropharyngeal swab specimen. Any additional culture-positive animals were removed from the colony and euthanized. This testing procedure was repeated once every two weeks until results for all samples from animals in that specific animal room were negative.

Treatment was attempted on a limited basis, using a sequence of three-week trials, with antibiotic administered orally in the drinking water. These mice came from a single investigator's colony (C.B-*Igh-1*^{b}/GbmsTac-*Prkdc*^{scid}-*Lyst*^{bg}N7), and were either infected mice with head tilt or cagemates of identified infected mice. This group of mice was pulled from the main colony and isolated to a separate room while treatment was attempted. Antibiotics tested were trimethoprim-sulfamethoxazole (37.5 mg of sulfamethoxazole and 7.5 mg of trimethoprim/oz. of drinking water), enrofloxacin (2.8 mg/oz), and tobramycin (5 mg/oz) in that order. Water consumption was monitored by visual inspection of water bottles during the treatment phases.

Results

A variety of mouse genotypes were clinically affected, all immunodeficient in some manner. Strains impacted included C.B-*Igh-1*^{b}/GbmsTac-*Prkdc*^{scid}-*Lyst*^{bg}N7, .NOD/MrkBomTac-*Prkdc*^{scid}, C3SnSmn.CB17-*Prkdc*^{scid}/J, C57Bl/6J-*Rag1*^{tm1Mom}, B6.129S6-*Rag2*^{tm1Fwa}N12, BALB/cAnNCrl-*nu*BR, , B6.129P2-*Ptgs1*^{tm1Unc}, C57Bl/6J-*Tcra*^{tm1Mom}, and Tg[TCRzeta]. The outbreak spread rapidly within several weeks from identification of the first case. Affected animals always developed severe head tilt toward the affected ear as a clinical manifestation of the infection. Mice would spin intensely when lifted by the tail, and begin rolling to the side of the lesion when placed back in the cage. Typical examination at necropsy of advanced disease revealed variable degrees of purulent exudate in the middle ear (Fig. 1), whereas the ear canal appeared dry early in the disease. Exudate was always found only unilaterally on the side toward which the head tilted. Antibiotics chosen on the basis of in vitro susceptibility testing results included trimethoprim-sulfamethoxazole, enrofloxacin, and tobramycin, and these were formulated to provide therapeutic levels in the drinking water. These three treatment regimens were unsuccessful in either altering the course of the disease or impacting development of new cases in an isolated group of SCID.Bg mice, which were either clinically infected or were cage mates of infected mice. Eventually these mice were euthanized.

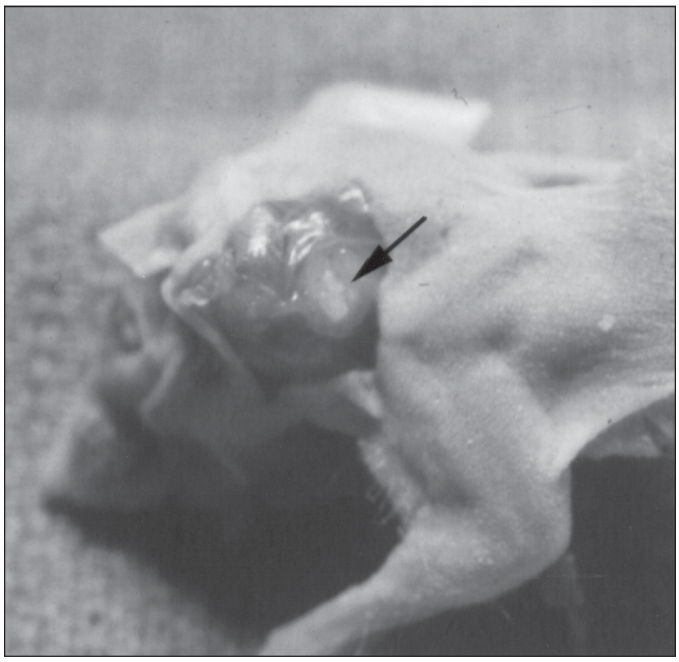

Figure 1. Athymic nude mouse at necropsy, with the outer portion of the ear canal removed to show the presence of a purulent exudate within the ear canal.

Detection of this contaminant in two facilities was determined to be due to movement of mice from the first site of infection to the second building, prior to detection of disease. Using the method of screening rooms by use of culture of oropharyngeal swab specimens from all mice present once every two weeks and euthanizing all culture-positive mice and their cagemates effectively reduced the infection to two colonies where the organism continued to persist at low levels. The swab specimens were cultured on BCSA, with colony growth evident after 72 h and an alkaline reaction evident by a pink color change of the phenol red in the agar. Management of the outbreak ultimately involved eliminating these two colonies (C57Bl/6J-*Rag1*^{tm1Mom} and SCID.Bg, one in each building), and replacing with breeding stock from commercial sources after thorough decontamination and disinfection of those rooms.

Isolate analysis. Five isolates from mouse middle ear specimen culture, including three from SCID.Bg, one from a SCID mouse, and one from a Cox-1 null mouse, underwent additional analysis. All 5 isolates grew on BCSA, were oxidase negative, ortho-nitrophenyl-D-galactopyranoside (ONPG) positive, lysine

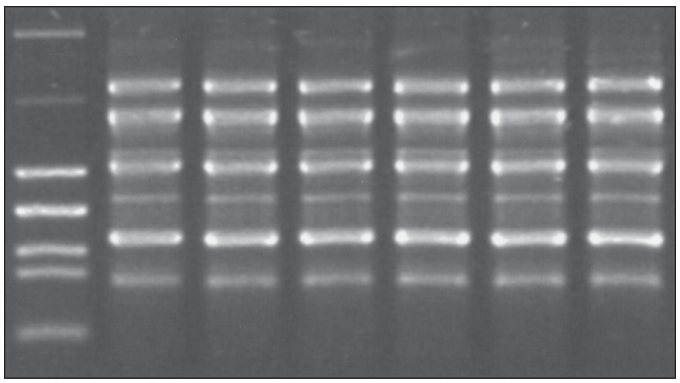

Figure 2. Five isolates were analyzed using random amplified polymorphic DNA typing and demonstrated clonality, indicating a shared strain of *Burkholderia gladioli*.

decarboxylase negative, did not produce acid from sucrose or lactose, and were positive by results of PCR assay specific for species within the genera *Burkholderia*, *Pandoraea*, or *Ralstonia*. These tested positive in a PCR assay specific for *B. gladioli*, but tested negative in an assay specific for species within the *B. cepacia* complex. Random amplified polymorphic DNA (RAPD) typing demonstrated clonality among the five isolates (Fig. 2).

Microbiologic and PCR analyses. During the peak of the outbreak, 16 of 16 cultured specimens from the middle ear of mice with head tilt were positively identified as gram-negative rods, most likely *B. gladioli*, at the University of Virginia Microbiology laboratory. Other organisms isolated included *Staphylococcus aureus*, and a coagulase-negative *Staphylococcus* sp. Culture of specimens from the throat yielded *Enterococcus faecalis*, and *Proteus mirabilis*.

In that same time period, more than 500 mouse boxes containing more than 1,500 mice were screened by culturing specimens from the throat of all immunodeficient mice and sentinels in those colonies. The number of culture-positive mice identified by use of this method was small (exact numbers not available) and there was no apparent pattern to the epidemiology of this epizootic. Often the cagemates of a culture-positive mouse were culture negative, even when that culture-positive mouse was clinically affected. Other culture-positive mice were often housed on a different rack or different shelf from the original source of infection. Only one sentinel (Tac:SW) mouse had positive throat swab culture results and was clinically normal.

Screening of the environment also did not yield insight into the method of spread of this organism. Fecal specimens, water sipper tube wipes, and bedding samples, did not consistently test positive in cages housing culture-positive mice. Table 1 shows culture results from a variety of mice, sipper tubes, and cage surfaces. Only one of six specimens from the oropharynx of clinically normal cagemates tested positive. Two of nine sipper tube and one of six cage surface wipe specimens were culture positive. Figure 3 shows positive PCR results for middle ear exudate specimens from six mice with head tilt. This PCR assay detected any organism of the *Burkholderia*/*Ralstonia* genera. The oropharynx also usually tested positive in mice with head tilt, whereas fecal specimens, and swab pecimens from the bedding and sipper tube tested negative (Fig. 4). The PCR assay was inconsistent in detecting the organism in oropharyngeal swab specimens taken from cagemates of mice with head tilt (data not shown).

Table 1. Results of culture of specimens on *Burkholderia cepacia*-specific agar (BCSA)

Identification	Genotype	Specimen	Results
Mouse with head tilt	TCRa -/-	Oropharynx	+
Cage mate 1	TCRα -/-	Oropharynx	-
Cage mate 2	TCRα -/-	Oropharynx	-
Mouse with head tilt	TCRa -/-	Oropharynx	+
Cage mate 1	TCRα -/-	Oropharynx	+
Cage mate 2	TCRα -/-	Oropharynx	-
Mouse with head tilt	SCIG.Bg	Oropharynx	+
Mouse with head tilt	SCIG.Bg	Middle ear	+
		Oropharynx	+
Mouse with head tilt	*Nu*/*nu*	Oropharynx	+
Cage mate1	*Nu*/*nu*	Oropharynx	-
Cage mate 2	*Nu*/*nu*	Oropharynx	-
Sipper tubes	N/A	Environmental	2/9 +
Cage surfaces	N/A	Environmental	1/6 +

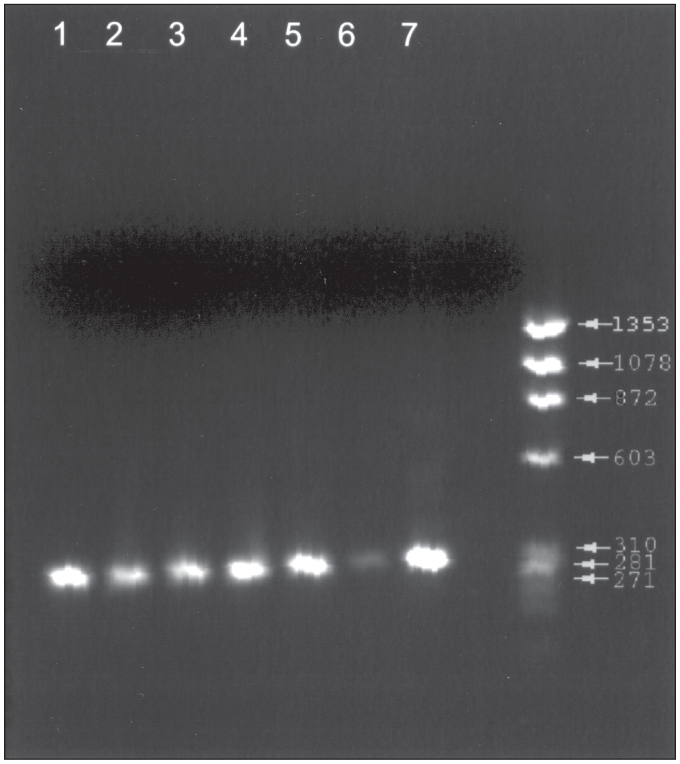

Figure 3. Polymerase chain reaction (PCR) assay results generic for *Burkholderia*/*Ralstonia* spp. Lanes 1–6 contain DNA isolated from culture of inner ear specimens obtained from six mice with head tilt. Lane 7 contains positive-control ATCC 25416. Lane 8 = water control.

Pathologic findings. Grossly, inner and middle ear were filled with caseated purulent material (Fig. 1). Other relevant lesions were not observed at necropsy. Histologic examination was performed on several mice with head tilt. Microscopic evaluation of the ear canal revealed suppurative otitis characterized by infiltration of many neutrophils, with fewer lymphocytes and only a few macrophages and plasma cells (Fig. 5). Vascular congestion also was seen. Inflammatory changes were seen in the dermis and subcutis. Brain abscessation was evident in one *nu*/*nu* mouse. Lesions in tissues other than the ear canal were not consistent, which parallels the findings in a murine infection model of *B. cepacia* (29). Liver lesions varied from no relevant lesions to mild subacute pericholangitis, to necrotizing suppurative hepatitis. Lung lesions included mild vascular congestion in several mice, and mild acute interstitial

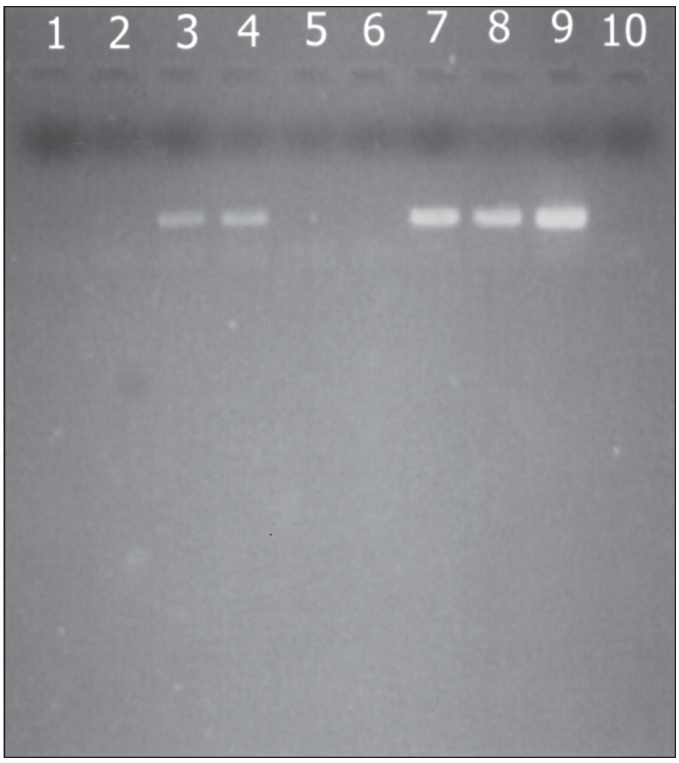

Figure 4. Results of PCR analysis of samples obtained from the sipper tube (lane1), the bedding (lane 2), oral cavity of two SCID.Bg mice with head tilt (lanes 3 and 4), fecal specimens from the same two mice (lanes 5 and 6), and middle ear of same two mice (lanes 7 and 8). Lane 9 = positive control, and lane 10 = negative control. The organism was only reliably detectable from the ear and the oral cavity of clinically ill mice.

pneumonia in one mouse. Tissues from mice with lung and/or liver lesions were further evaluated for the presence of *Pneumocystis carinii* and *Helicobacter* spp., respectively. Positive-staining cysts or organisms were not seen in GMS-stained lung and liver sections treated by use of Steiner's modification of the Warthin-Starry stain, and PCR analysis of samples from paraffin-embedded tissues yielded negative results for both organisms (data not shown). Findings for other tissues were either within normal limits, or there were lesions probably unrelated to the *Burkholderia* infection.

Discussion

We report here an unusual, and previously unreported, outbreak of *B. gladioli* infection in immunocompromised mice, housed under barrier conditions and affecting a variety of genotypes. Clinical signs of disease and associated pathologic changes indicated that this organism was associated with severe necrotizing otitis media. Pneumonia, the most common clinical condition seen in human infections was occasionally seen in these mice, but cannot conclusively be attributed to *B. gladioli*. A previous report of otitis media in mice due to *Pseudomonas aeruginosa* indicated similar histologic changes (18), and the organism was eliminated by acidifying the drinking water. Acidification of the water for our colonies did not impact *Burkholderia* infection. Indeed, the organism was isolated from two of nine sipper tubes that provided acidified drinking water.

Identification of the causative organism was initially difficult. The *B. cepacia* complex consists of several phenotypically similar, but genetically distinct species (or genomovars) (30). Commercial test systems frequently misidentify *B. gladioli* as *B. cepacia* (17, 27), and few systems include *B. gladioli* in their organism identification database. Not surprisingly then, the clinical microbiology laboratory at UVA initially identified the organism causing this outbreak as a *Pseudomonas* species. Subsequently, by use of further diagnostic testing, the organism was identified as *B. cepacia*. Not until the same isolates were sent to the BCRLR was the isolate correctly identified as *B. gladioli*.

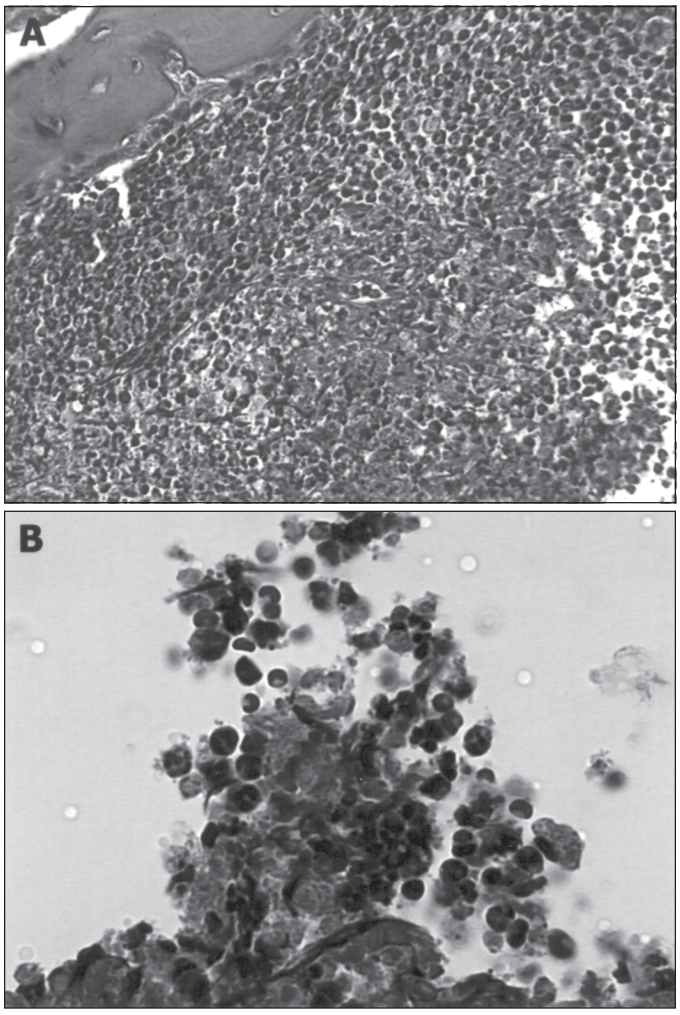

Figure 5. Photomicrograph of a section of the inner ear revealing severe necrotizing otitis with infiltration by neutrophils and mononuclear cells (A, original magnification of 400×; and B, magnification of 1,000×). Stained with hematoxylin and eosin.

Several animal models of *B. cepacia* have been developed to study the virulence and pathogenicity of this organism, particularly its predilection for cystic fibrosis patients and those with chronic granulomatous disease (3, 9, 25, 29). A model of chronic pneumonia was developed by instillation of a clinical isolate of *B. cepacia* into the lungs of cftr (m1 unc-/-) and cftr -/- mice (25). The CF transmembrane regulator knockout mice developed persistence of viable bacteria with chronic severe bronchopneumonia, whereas wild-type mice remained healthy. Histologic changes in lungs were characterized by infiltration of a mixed inflammatory cell population into the peribronchiolar and perivascular spaces.

In a mouse respiratory challenge model using intratracheal

inoculation of *B. cepacia*, the organism was capable of attaching and invading respiratory tract epithelial cells, and was able to translocate from the lung to liver and spleen. However, infection was cleared from all organs within seven days (3). In a different murine model, infection after intraperitoneal inoculation of *B. cepacia* was evaluated, and spleen infection persisted for two months (29). In that study, the sensitivity of various inbred mouse strains to *B. cepacia* also was evaluated. The BALB/c, A/J, and DBA/2 mice were similar in their ability to clear organisms from the spleen within 35 days. The C57Bl/6 mice were less able to resist infection and had recoverable infections from the spleen at postinoculation day 56. Gamma interferon knockout mice were not more susceptible to infection as was expected. In C3H/HeH mice (endotoxin resistant), a high dose of *B. cepacia* was lethal, and lower doses caused transient infection in a variety of organs (9).

Interestingly, none of these studies indicated clinical signs of otitis in experimentally infected mice. This may be related to route of infection or dose, or it may be that this clinical presentation is specific for *B. gladioli* infection in mice. It would be interesting to determine whether epithelial cells of the middle ear are rich in cytokeratin 13, a protein previously documented to act as a receptor in vitro for cable-piliated *B. cepacia* (26). *B. gladioli* infection in immunocompromised mice may prove useful as an animal model of otitis media.

Reported natural *Burkholderia* infection in animals or animal products is rare, and to our knowledge, there are no published reports of infection. A study of the prevalence of *B. cepacia* in food and water samples in Ireland indicated that 14 of 26 samples of raw unpasteurized bovine milk were test positive (22). Subclinical mastitis in a flock of 620 milking sheep in Spain was associated with *B. cepacia*. Those investigators were unable to identify the organism from milking equipment and other environmental sites, so the source of the infection remained unknown (2).

Emergence of currently unrecognized pathogens in laboratory rodents is on the increase. The exponential growth in experimental use of transgenic and knockout mouse strains, many with genetically altered immune systems, may lead to new opportunities for harboring potentially infective microorganisms. Routine use of antibiotics such as trimethoprim-sulfa to suppress growth of *P. carinii* may contribute to development of infections with antibiotic-resistant organisms. Despite the protections offered by barrier housing and husbandry, exposure to humans remains an avenue for transmission of new rodent infections. In the case of academic settings, where researchers are often providing clinical care to patients on the same day as handling research rodents, the possibility of carrying clinical isolates into the rodent colonies is something that must be considered. Other possible sources include feed, bedding, and the water supply.

It is clear from previous reports that opportunistic infections, known and unknown are a serious threat to our laboratory rodent colonies, in particular those strains that are immunodeficient, and that only pro-active colony management programs will be successful to detecting, characterizing, and controlling these infections (32). Determining appropriate strategies for bioexclusion will be based on many factors. In performing a risk assessment as described by White and co-workers (32), immunocompromised mice being used in experiments must be assigned the highest level of risk and be afforded the greatest degree of bio-exclusion possible on the basis of the institution's practices.

This report of *B. gladioli* infection in animals, documents a distinct clinical syndrome in affected mice; the only other known pathogenic activity of this organism is in humans where it causes a different clinical outcome. The only common thread appears to be that this organism is an opportunistic pathogen in both species. Although the source of this outbreak remains unknown, we believe that individuals working in a hospital setting with immunocompromised patients carry a real risk of transmitting unusual and rare microbial pathogens into the laboratory rodent environment.

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